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# Minimal Invasive Cystometry and Intra-Abdominal Pressure Assessments in Rodents: A Novel Animal Study

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Data Collection B  
Statistical Analysis C  
Data Interpretation D  
Manuscript Preparation E  
Literature Search F  
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**Background:** The abdominal straining pattern can act as a novel parameter for improving the prediction of bladder outlet obstruction (BOO). To preserve detrusor function in the early stage of urinary system impairment, such as BOO, we establish a novel method for cystometry and Intra-abdominal pressure (IAP) assessments in rodents without cystostomy.

**Material/Methods:** Twenty mice and rats were divided into three groups (control, sham-operated and BOO group) respectively. The cystometry and IAP assessments were measured by the pediatric venous indwelling sheath and coronary dilatation catheter connected to Laborie urodynamic system on postoperative day 7. Data was collected simultaneously through urethra and rectum in each group. In addition, bladder histology was assessed to confirm BOO.

**Results:** The novel method can collect the urodynamic parameters successfully, including the BLPP, IAP, MBC, etc. IAP was elevated in BOO rats, but no significantly difference was found between the sham-operated rats and the control rats. The hypertrophy of detrusor muscle in bladder section was observed by Masson trichrome staining in BOO group compared with other groups.

**Conclusions:** Our novel method based on innovative research implement for cystometry and IAP assessments in rodents is a reliable and replicable approach for evaluating the lower urinary tract function. Especially it provides detailed information to evaluate lower urinary tract structures and function in the early stage of BOO.

**MeSH Keywords:** **Lower Urinary Tract Symptoms • Rodentia • Urinary Bladder • Urinary Bladder Neck Obstruction • Urodynamics**

**Abbreviations:** **IAP** – Intra-abdominal pressure; **BOO** – bladder outlet obstruction; **TURP** – transurethral resection of prostate; **ALPP** – abdomen leak point pressure; **BLPP** – bladder leak point pressure; **MBC** – maximum bladder capacity; **PVR** – post-void residual urine volume; **VV** – voiding volume; **BC** – bladder compliance; **ICC** – interstitial cells of Cajal

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## Background

Cystometry, also named flow cystometry, is one of the most important clinical diagnostic procedure used to investigate bladder function in storage and voiding stage. The key content of urodynamic is filling cystometry and pressure flow measurement. It also measures contractile force of the bladder when voiding [1]. Because of the lack of tiny catheter, cystostomy and inserting a piezometric tube for cystometric investigation were widely performed on rodents since 1987 [2,3]. However, this method destroys bladder muscle and differs from that in human significantly. It also cannot collect data of abdominal pressure, which is the key factor for diagnose of BOO [4].

BOO, a common urological disease, usually results from benign prostatic hyperplasia (BPH), posterior urethral valves and urethral stricture [5,6]. The previous view has long recognized that pressure-flow studies (PFS) are the gold standard for BOO with obstructive lower urinary tract symptoms, characterized by a high detrusor pressure and low flow type of voiding pattern [7]. However, high pressures and low flow or even retention as the indication for transurethral resection of prostate (TURP) markedly increased the possibility of detrusor failure, which is associated with long-term catheter (LTC) or intermittent self-catheterization (ISC) [8,9]. Therefore, the perfect urodynamic method should include more parameters to diagnose BOO in early stage.

Few studies [10,11] has been investigated the abdominal pressure in BOO rodents model, While the latest research of Han JH et al. has reported that the change in abdominal pressure of patients was correlated with endoscopically-proven obstruction. The abdominal straining pattern can act as a novel parameter for improving the prediction of BOO [12]. The concrete mechanisms responsible for the effects of IAP on auxiliary micturition are not completely clear. Therefore, in the current study, we hypothesized that the increased intravesical pressure derived from abdominal straining in rodents can be detectable in a convenient and minimal invasive way which may provide a new model to study the unknown mechanism.

For the first time, we imitate human urodynamic testing to measure the effects of IAP and other urodynamic parameters in rodents. In order to evaluate the efficacy of IAP, we used the Balloon Dilatation Catheter (Medtronic, MN, USA) connect to a pressure sensor in order to mimic the method in human, which allowed for easy transrectal catheterization. We also use a pediatric intravenous catheter as urethral catheterization for rodents which allow good intravesical pressure transmission. This modification is important in the light of the still unknown mechanism of the complex interactions between intravesical pressure (IVP), IAP and bladder activity.

## Material and Methods

### Animals

Twenty female Sprague-Dawley rats (8–12 weeks, 250–280 g) and twenty female C57BL/6J (8–10 weeks, 22–25 g) mice were purchased from the Dashuo Laboratory Animal Technology Co, Chengdu, China. The rodents were all maintained under a certified facility with a humidity and temperature controlled environment and a 12: 12h light: dark cycle. They were maintained 3 to 4 per cage with free access to food and water under standard laboratory conditions. All the experimental procedures and the experimental designs were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The ethics committee of the Affiliated Hospital of Chengdu University, approved all animal experiments (Permit Number: LL/KY-201502-03). All surgeries were performed under urethane anesthesia, and all efforts were made to minimize animal suffering.

### Experimental design

Rats and mice were divided randomly into three groups respectively: the control group (n=5), sham-operated group (n=5), BOO model group (n=10). Briefly, in the BOO group, each rodent was anesthetized via intraperitoneal injection of 1.2 g/kg urethane (Sigma-Aldrich, St. Louis, MO, USA). The procedure to establish a model of BOO was induced as Kim SJ described [13]. In the sham group, an operation was performed under similar circumstances without the procedure of tying the ligature.

### Cystometric investigations

The cystometry was performed on the 7<sup>th</sup> postoperative day. Light sedation was induced with 1.0 g/kg urethane in rodents. Spontaneous breathing and corneal reflex was maintained, and the optimum depth of sedation is the rodents too weak to stand up but can lift their heads. Placed the animal into the supine position on a foam plastic board, then the pediatric venous indwelling catheter (26-G, i.d.=0.4 mm, o.d.=0.6 mm, 16 mm length, Weigao Medical Products Co, Shangdong, China) was used for urethral catheterization on female rodents (Figure 1). It has a trocar structure consisting of two parts, namely a sleeve and a tube core needle. Then pulling out the needle and adopting normal saline to exhaust the air in the sleeve, lubricated it by the Pontocaine plasmagel. The catheter was inserted into the urethra to act as cystometry tube. The tube was connected to a vesical pressure channel transducer of the urodynamic system (Bonito XL, Laborie Medical Technologies Inc, Canada) and a syringe pump (Jianyuan Medical Technology Co, Changsha, China) via a 3-way stopcock for saline instillation and pressure recording. Cystometry was performed infusing warm normal saline (37–38°C) at a rate of 3ml/h in mice



**Figure 1.** Cystometry and intra-abdominal pressure (IAP) assessment set-up. Light sedation was induced with urethane in mouse (A). Urethral catheterization and anal intubation placed on the mouse, the cystometry tube connect to a three-way stopcock, then connected to a pressure transducer and an infusion pump, and the rectal manometry tube was connected to a pressure transducer simultaneously (B-D). A computer with the data acquisition system record the pressure change in real time (E).

**Table 1.** Comparison of urodynamic parameters in the control rodents and in rats subjected to sham-operated or bladder outlet obstruction (BOO).

Group	BLPP (cmH <sub>2</sub> O)	IAP (cmH <sub>2</sub> O)	MBC (ml)	PVR (ml)	VV (ml)	BC (mL/cm cmH <sub>2</sub> O)
Group I (n=5)	23.2±3.49	1.27±1.14	1.23±0.14	0	1.23±0.14	0.054±0.010
Group II (n=5)	24.87±3.9	0.73±2.07	1.13±0.18	0.27±0.04	0.86±0.16	0.046±0.011
Group III (n=10)	42.37±5.2*	7.70±3.04*	1.65±0.19	0.37±0.10	1.28±0.22	0.039±0.005
Group IV (n=5)	36.67±3.1	/	0.12±0.03	0.05±0.02	0.07±0.03	0.004±0.001

Values are presented as mean ± standard error of the mean. BLPP – bladder leak point pressure; IAP – intra-abdominal pressure; MBC – maximum bladder capacity; PVR – post-void residual urine volume; VV – voiding volume; BC – bladder compliance.

Group I: control rats with two catheters in the bladder and rectal; group II: sham-operated rats; group III: BOO rats; group IV: control mouse with only one catheter in bladder. Comparisons were made among the first three groups, but there were no significant differences except one (unpaired Student t-test). \* P<0.05 vs. group I and group II.

and 6 ml/h in rats respectively to stimulate the process in the formation of urine. Infusion was terminated after the leakage of urine was detected around the tube, at least 3 micturition cycle repeated, and the results of cystometry were recorded.

The IAP was measured simultaneously during the period of cystometry. We used a coronary balloon dilatation catheter, which is a common surgical product used for percutaneous transluminal coronary angioplasty (PTCA), to connect with an external pressure transducer. The catheter was inserted into the rectum to detect the IAP (Figure 1). At the beginning of cystometry, it requires to empty the bladder via the bladder catheter.

#### Urodynamic parameters and data collection

Urodynamic parameters were investigated, including the abdomen leak point pressure (ALPP), bladder leak point pressure (BLPP), maximum bladder capacity (MBC), and post-void residual urine volume (PVR), voiding volume (VV) and bladder compliance (BC). The VV is shown by subtracting the PVR from measured MBC, and BC is MBC divided by BLPP. Continuous cystometry was performed, and at least 3 reproducible micturition cycles were analyzed.

#### Histological preparation

After the examination, all animals were anesthetized with urethane and sacrificed. Urinary bladders were removed rapidly and fixed in 4% paraformaldehyde phosphate buffer (Biosharp, Hefei, China) for histological studies. The fixed bladders were embedded in paraffin and longitudinally cut at 3 mm thickness. The sections were deparaffinized and stained with Masson trichrome staining.

#### Statistical analysis

Data were shown as the mean ±SD. Statistical significance was analyzed by the Tukey's multiple comparison test with p<0.05 considered statistically significant.

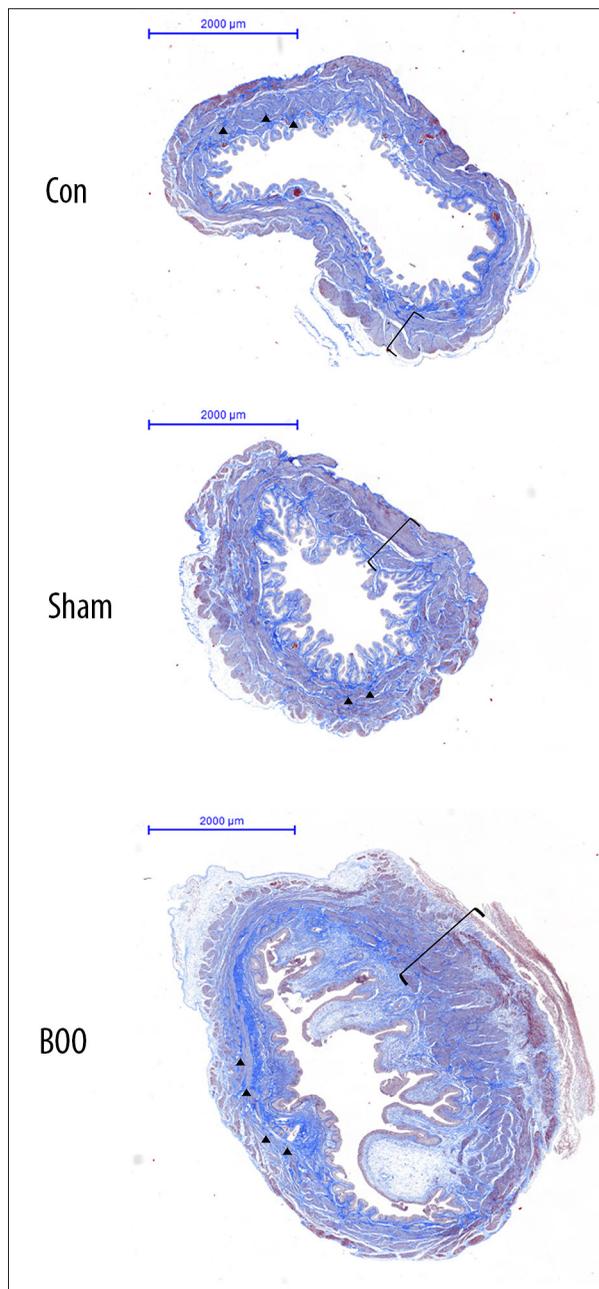
## Results

#### General observation

One mouse dead from urine retention in the BOO group during the period of the experiment, and the rodents tolerated the catheter very well. During the period of urodynamic test, the rats adapted easily to the laboratory circumstances without any signs of unusual or discomfort. Additionally, none of the catheters exist the occlusion or twisting in the whole process of experiment. However, some mice in BOO group suffered from retention during urodynamic test. Therefore, we only finished the subsequent test in rats and normal mice.

#### Urodynamic investigation in the control rodents

The control rats (n=5) and mice (n=5) were performed the urodynamic respectively. The urodynamic and IAP results are given in Table 1. No significant difference in urodynamic results was observed between the control and sham-operated rats. However, the micturition of mice was hindered by the coronary balloon dilatation catheter, which was for IAP measurement. It was found that the diameter of the catheter was exceed the size of mouse's rectum. And it caused obstruction leading the mouse cannot voiding. Therefore, we obtained cystometry parameters in mouse with only catheter in bladder.



**Figure 2.** Histological changes in rats of control, sham-operated and bladder outlet obstruction (BOO) groups. Arrow indicates collagen deposition. Brackets indicate detrusor muscle.

### Masson trichrome staining revealed the success of BOO

In BOO group, as observed in Masson trichrome staining, the collagen deposition in partial detrusor muscle bundles increased compared to the control and sham-operated group. Moreover, progressive disruption of bladder wall architecture was observed in BOO rat (Figure 2). Profound disruption of the bladder wall architecture with smooth muscle hypertrophy and

increased collagen deposition between smooth muscle bundles in obstructed bladders were found, and these findings verified the success of BOO model. It also indicated that was in the state of early stage of BOO without large number of collagen deposition.

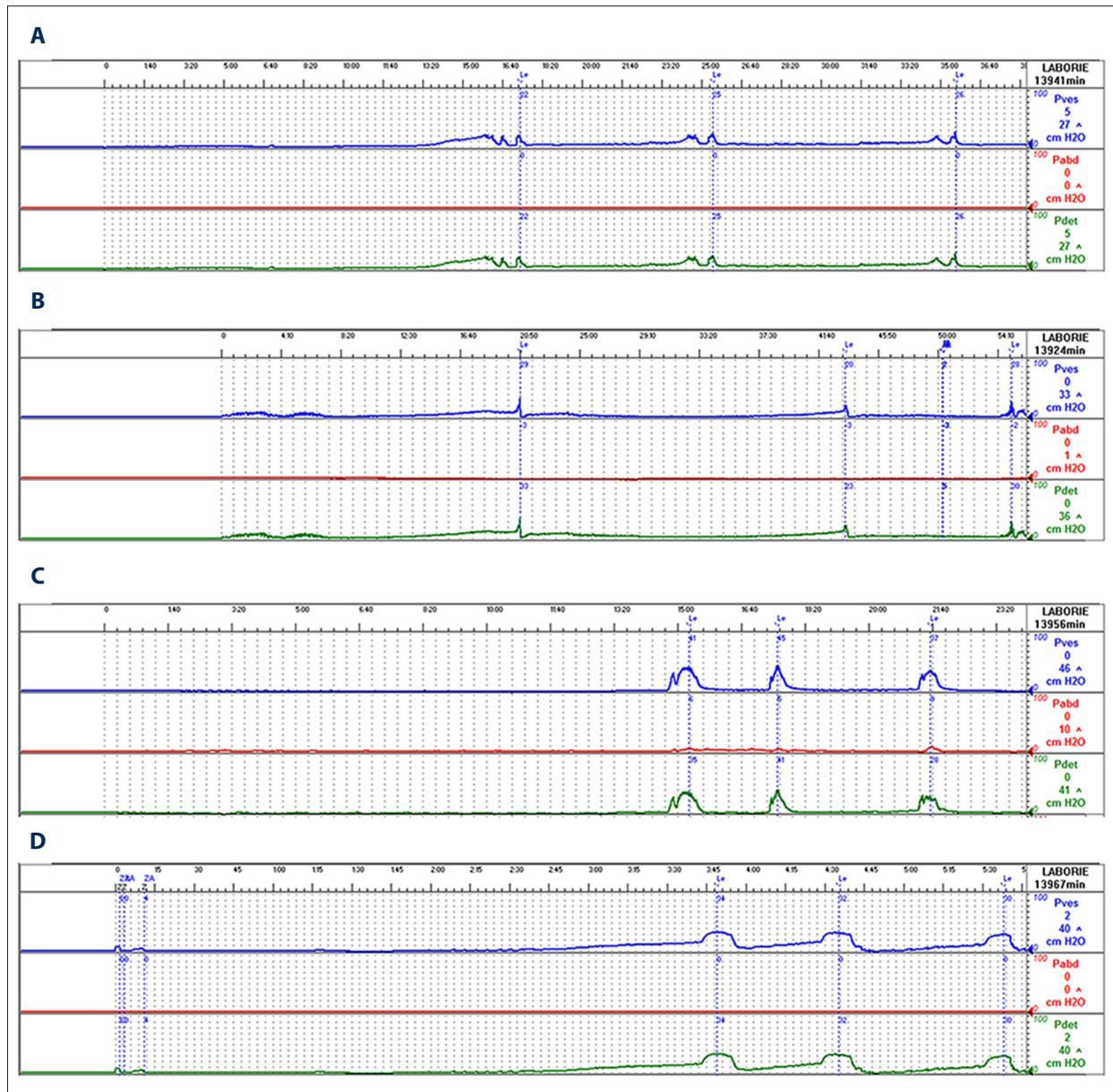
### Urodynamic investigation in BOO rats

Compare of the pressure-related parameters among the 3 groups in rats, the BOO group showed higher BLPP ( $42.37 \pm 5.20$  cmH<sub>2</sub>O,  $P < 0.05$ ) than the control ( $23.20 \pm 3.49$  cmH<sub>2</sub>O) and sham group ( $24.87 \pm 3.88$  cmH<sub>2</sub>O). The IAP was  $7.73 \pm 3.04$  cmH<sub>2</sub>O ( $P < 0.05$ ) in BOO group, which was changed simultaneously with abdominal straining. Recorded as significant increases in IAP, the BOO rats exhibited increased abdominal strain during every voiding. No significant difference was found between the sham ( $1.73 \pm 2.07$  cmH<sub>2</sub>O) and control ( $1.27 \pm 1.14$  cmH<sub>2</sub>O) groups in IAP. (Figures 3 and 4)

### Discussion

In the present study, we investigate whether the pediatric venous indwelling sheath and coronary balloon dilatation catheter is appropriate to perform the cystometry and IAP assessment, as well as the abdominal straining has a potentially promote effect on BOO model. To the best of our knowledge, this is the first presentation of cystometry and IAP which performed in rats can approximately imitate human urodynamic investigation by a minimal invasive method, and it is can generally recapitulate symptoms observed in clinic. The result of urodynamic testing clarifies the effect of IAP on BOO model. In general, our data indicate that the novel innovation have been implemented on rodents can simulate the human body to acquire rodent urodynamic data through the urethra catheter and rectal catheter successfully.

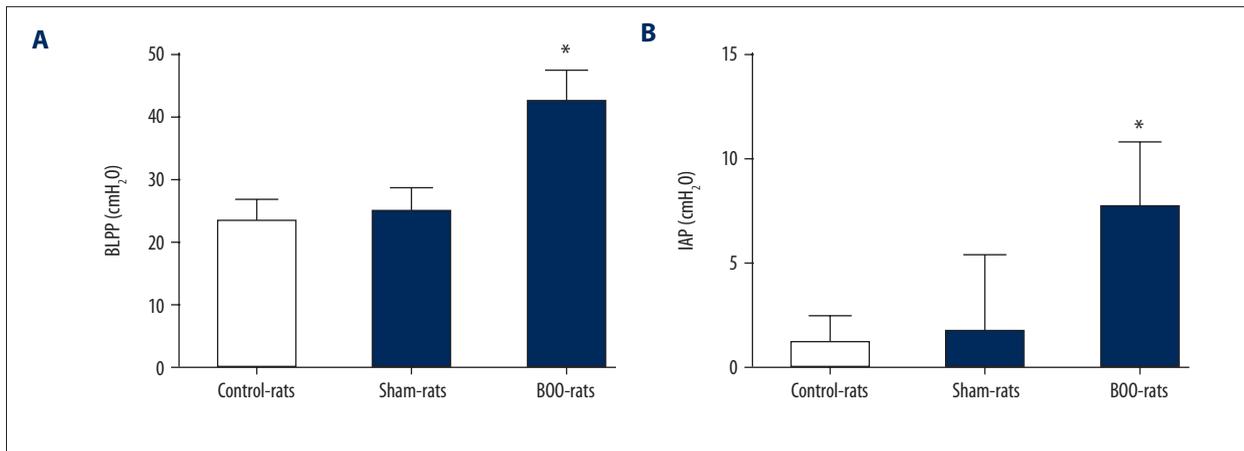
Cystostomy is a traditional method for detecting urodynamic indexes in rodents, which not only destroys the continuity of the bladder, but also differs from the clinically used transurethral method. Our study has developed an appropriate urethral catheter to investigate the advantages and application value of transurethral method for urodynamic test. As shown in Figure 3, according to the urodynamic results, the novel method performed on the rodents can transduce the pressure sensitively, which was in accordance with the previous study by the traditional method [14]. Likewise, we quantitated the urodynamic parameters, and the results confirms that the modification is feasible in mice and rats. The novel implementations of the pediatric venous indwelling catheter were adopted because of the mice urethra size is small and narrow. In previous study [15], transurethral cystometry via the PE50 tube (i.d.=0.58 mm, o.d.=0.96 mm) appeared to be obstructive



**Figure 3.** Cystometrogram showing bladder pressure, IAP and micturition interval in control rats (A), sham-operated rats (B), bladder outlet obstruction (BOO) rats (C) and control mice (D).

and may active nociceptive reflexes, which mainly due to it is larger diameter and thicker wall contrast with pediatric venous indwelling catheter. Moreover, the previous method of cystometry [16] has failed to consider the effect of interstitial cells of Cajal (ICC) which are mainly located in the dome of the bladder [17,18]. Researchers has indicated that the role of ICC [19], as neuromodulators and pacemakers, may be involved in detrusor overactive induced by BOO. The functional network formed by ICC, which are contribute to the spontaneous electrical activity and mediate the neural drive to smooth muscle [20], may be damaged by the conventional invasive examination.

In BOO model (Figure 1), we modified the previous method [10], instead of use an latex balloon filled with distilled water around the cuff of a catheter tip which was placed proximal to the bladder through the abdominal incision. The coronary balloon dilatation catheter was inserted into the rectum. Our model of IAP assessment shows characteristic similar to the previous result in human [21] (Figure 3). Cystometrograms were obtained by use of simultaneous recording of intravesical and intra-abdominal pressure shows that abdominal straining can increase the IAP. This suggest that IAP can be detected and help the micturition to a certain extent. Our findings are corroborated by studies of BOO caused by BPH in human [22].



**Figure 4.** Comparisons were made among the control, sham, and bladder outlet obstruction (BOO) rats. **(A)** BLPP **(B)** IAP. BLPP, bladder leak point pressure. IAP, Intra-abdominal pressure. Results are expressed as the mean±standard error of the mean. Asterisk indicates  $p < 0.05$  vs. the control and sham group. All other differences were not significant.

We also found IAP increasing in accordance with bladder muscle hypertrophy with a small number of collagen deposition which indicating early stage of BOO (Figure 2). Combining our research and the two survey on TURP [8,9], we suggest the IAP be used as one of the new BOO Index indicating TURP to avoid large number of collagen deposition in detrusor and to protect bladder function.

Our novel method performed on the BOO model can be used repeatedly, and the rodents can be long-term survival after the urodynamic test. The traditional method or the latest modified method [23] for urodynamic test of rodents are underwent suprapubic cystostomy, which were approximately equal to relieve the obstruction in varying degrees, and it may destroy the natural history and pathophysiology of BOO. At the same time, it also increase the exposure risk to infectious pathogens after the suprapubic cystostomy [24]. In the next step, we are going to study the cellular signaling pathways, for example, the expression of PI3K (phosphoinositide 3-kinase) and SGK1 (serum-glucocorticoid regulated kinase 1), which we found *in vitro* previously [25], to test and verify it in BOO model. On the basis of this study, the novel method can be used for real-time dynamic monitoring and have a better understanding of the correlations between BOO, PI3K/SGK1 pathway and urodynamic results. As a primary methodological investigation, several limitations are existed in the study. First, the results of IAP was limited in the BOO model, so it is remains unknown whether our results are applicable to other kinds of disease model. Further study with additional sample

data and more disease model are needed. Another limitation is that the efficiency of voiding (VV divided by MBC and multiplied by 100%) in control mice concluded in this study are at a lower level. With a range from 33% to 75%. This may indicate that the post-void residual urine volume is high. It has been widely accepted that the residual urine volume should not more than 20% of the MBC, otherwise it shows the existence of BOO [26]. Obviously, more fine-structure catheter for mice remains to be developed. Finally, in order to relieve the discomfort and avoid restraint, we use a low dose of urethane to sedate the rodents during the test, which differ from the method in human.

## Conclusions

Our novel urodynamic investigation is a reliable and replicable measurement. The experimental research results will hopefully serve as useful feedback information for improvements to perform urodynamic investigation in rodents. Especially, it demonstrates that IAP was sensitively detected and substantially reflect detailed pressure changes at different points of IAP in BOO model. Further study based on this method is needed to explore BOO and other functional diseases of the bladder.

## Conflict of interest

None declared.

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