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Voiding Dysfunction

Early Sequential Changes in Bladder Function after Partial Bladder Outlet Obstruction in Awake Sprague-Dawley Rats: Focus on the Decompensated Bladder

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Purpose: We investigated bladder function, with special focus on initial functional changes, by objective report of decompensated bladder according to the percentage of residual urine volume to bladder capacity in awake, obstructed rats.

Materials and Methods: Thirty rats were randomly subjected to sham operations (n=10) or partial bladder outlet obstruction (BOO, n=20). Cystometric investigations were performed without anesthesia 1 or 2 weeks after BOO surgery. To reduce the influence of confounding factors in awake cystometry, we used simultaneous recordings of intravesical and intraabdominal pressures. Decompensated bladder was defined as the bladder with more than 20% of residual volume compared with bladder capacity.

Results: Compared with that in sham animals, basal pressure was elevated in both BOO groups. Threshold pressure was higher in the 2 week BOO ($p<0.01$) group. Compliance was decreased in the 1 week BOO group ($p<0.01$) and increased in the 2 week BOO group ($p<0.001$). Bladder capacity was not increased in the 1 week BOO group, but was increased in the 2 week BOO group ($p<0.01$). Decompensation was found in 62.5% of the 1 week BOO group and in 33.3% of the 2 week BOO group.

Conclusions: From the earlier phase, the bladders exhibited serial changes in pressure and volume parameters, and decompensated bladders defined by the percentage of residual volume to bladder capacity could be seen. During the later phase, there was an increasing tendency of compensated bladders, accompanied by the bladders being enlarged and more compliant.

Key Words: *Detrusor, overactive; Urinary bladder; Urodynamics*

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INTRODUCTION

Bladder outlet obstruction (BOO) in men is a common clinical condition secondary to benign prostatic hyperplasia (BPH). Histologic BPH is a highly prevalent disease, starting in men in their 40s, and evidence of BPH can be found in 90% of men in their 80s [1,2]. The lower urinary tract symptoms (LUTS) caused by this BPH develop in men after their 60s [1]. In men older than 50 years of age, 81% will experience those symptoms and 5% to 25% will develop acute urinary retention. Many recent studies have provided strong evidence for the progressive nature of BPH [3,4]. There are still not enough clinical data, however, showing the pathophysiological serial changes in the bladder caused by BOO.

Experimental animal models with BOO have shown significant alterations in bladder structure and function [5,6]. It is known that the decompensatory changes in the bladder occur after the compensatory changes. Rabbits subjected to BOO will develop a rapid, progressive increase in bladder weight during the first 1 to 2 weeks (initial period) following surgical obstruction. The detrusor function during this phase shows normal or increased pressure generation, which is referred to as the compensated period. It

is known that the functional changes in the obstructed rat bladder are stabilized slowly and gradually by 2 weeks after the initiation of BOO. Around this period, those rats show variably different voiding functions, characterized by increased frequency, residual urine, and nonvoiding contractions (NVCs). After this, it is known that they enter the decompensated phase, which refers to a progressive loss of emptying detrusor function, resulting in increased residual urine [7-9]. However, information remains fragmentary on the initial functional changes during the acute obstruction.

The aim of this study was to study bladder function, with special focus on initial functional changes, by objective report of decompensated bladder according to residual urine volume in awake, obstructed rats. To reduce the influence of confounding factors, we used a simultaneous recording of intravesical pressure (IVP) and intraabdominal pressure (IAP).

MATERIALS AND METHODS

Experimental animals. Female Sprague Dawley rats (200 to 250 g) obtained from Orient Bio Inc. (Seongnam, Korea) were used in this study. They were maintained under standard 12:12 hour light:dark cycles with free access to water and food. The method for creating urethral constriction was identical to that previously described [10,11]. Sham-operated animals underwent similar surgery but no obstruction was created. After surgery, animals were caged individually and animal weights were recorded at the time of utilization. All procedures for animal handling and treatment were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health and were approved by the Ethics Committee of the Inha University College of Medicine.

Thirty rats were randomly subjected to sham operations (n=10) or partial BOO (n=20). Cystometric investigations were performed without anesthesia 1 week after BOO surgery in 10 rats in the BOO group (1 week BOO group) and 2 weeks after surgery in the sham group and in 10 rats in the BOO group (2 week BOO group). The catheters were implanted 3 days before the cystometries. Decompensated bladder was defined as a bladder with more than 20% of residual urine compared to bladder capacity.

On the basis of the cystometric criteria for the presence of detrusor overactivity (DO) during the filling phase, the rats were counted into subgroups showing DO (DO+) or not showing DO (DO-). DO in this study was defined as the occurrence of NVCs without simultaneous IAP elevation.

1. Surgical method

Rats were anesthetized with ketamine (Ketamine 50, Yuhan Corp., Seoul, Korea; 75 mg kg^{-1} intraperitoneally) and xylazine (Rompun®, Bayer Korea Ltd., Seoul, Korea; 15 mg kg^{-1} intraperitoneally). Through a lower midline incision, the bladder and the proximal urethra were freed from surrounding tissues. A 3/0 Novafil (monofilament polybutester, Davis & Geck, Wayne, NJ, USA) ligature was placed around the urethra and tied in the presence of an intraluminally placed steel rod with a diameter of 0.9 mm. After tying the knot, the steel rod was removed and the bladder was repositioned. The abdominal wall was closed.

2. Intra-vesical and intra-abdominal pressure catheter implantations

After an abdominal incision was made, a polyethylene catheter (PE-50; Becton Dickinson & Co., Franklin Lakes, NJ, USA) with a cuff was implanted into the dome of the bladder and held in place with a purse-string suture. To record IAP, a catheter with an abdominal balloon (Latex, Dawoo Medical, Incheon, Korea) was placed superior to the bladder, as described previously [12]. The catheters were tunneled subcutaneously and anchored to the skin of the back with a silk ligature. The free end of the catheter was sealed. Animals were allowed to recover for at least 24 hour.

3. Cystometric investigations

The conscious rats were placed in metabolic cages (Nalgene metabolic cage; Nalge Co., Rochester, NY, USA) without restraint. The indwelling bladder catheter was connected to a two-way valve that was connected to a pressure transducer (Research Grade Blood Pressure Transducer; Harvard Apparatus, Holliston, MA, USA) as well as an infusion pump (PHD 22/2000 Programmable syringe pump; Harvard Apparatus), and the indwelling IAP catheter was connected to another pressure transducer. Micturition volume was simultaneously recorded by using a fluid collector connected to a force displacement transducer (Research Isometric Transducer; Harvard Apparatus). Continuous bladder infusion was performed with room temperature, normal saline at a rate of 20 ml h^{-1} in all groups. Data were digitally stored and analyzed at a later time by using Acq Knowledge ver. 3.8.1 software (BIOPAC Systems Inc., Goleta, CA, USA) at a sampling rate of 100 Hz and an MP150 data acquisition system (BIOPAC Systems Inc.).

The values from three reproducible micturition cycles were used for evaluation. IAP was defined as the recorded balloon pressure corrected by subtracting the lowest balloon pressure in each voiding cycle for zeroing. The detrusor pressure (DP) was defined as the IVP minus the IAP. The NVCs during the filling phase were defined as increments of IVP that exceeded 2 cmH_2O from baseline without simultaneous changes in IAP and without fluid expulsion from the bladder.

The following cystometric parameters were investigated: 1) Cystometric pressure (by DP) and volume parameters, including basal pressure (the lowest pressure during filling), threshold pressure (pressure immediately before bladder contraction), maximal pressure (highest pressure during micturition), micturition volume, residual volume (remaining urine after voiding), bladder capacity (micturition volume+residual volume), residual volume percent (residual volume/bladder capacity x100), and micturition interval (interval between micturition contrac-

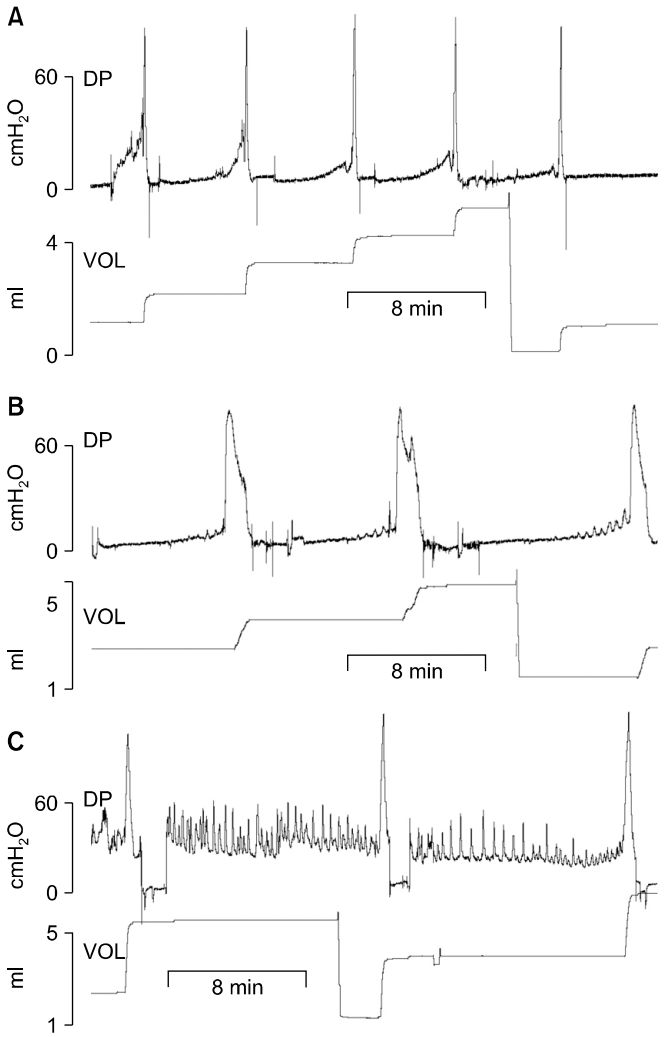

FIG. 1. Representative cystometrogram showing detrusor pressures and micturition volume in conscious sham rats (A), rats with bladder outlet obstruction (BOO) for 1 week (B), and rats with BOO for 2 weeks (C). The micturition interval significantly increased in rats with BOO for 2 weeks compared with those with BOO for 1 week. DP: detrusor pressure, VOL: volume.

tions). Compliance was calculated according to the formula (P2-P1)/(V2-V1), where P2 represents the pressure of the stable curve just before the bladder contracts and V2 the infused volume at that time. P1 represents the baseline pressure and V1 the corresponding infused volume. 2) Parameters to investigate DO during the filling phase, including time of filling phase (interval between the initiation of saline infusion and micturition), frequency of DO (per minute), and average pressure difference between the peak and the base of the DO (DO pressure).

Statistical analysis. The results are given as mean values±SEM. Normal distributions were confirmed by the Shapiro-Wilks' W test. Statistical significance was determined with unpaired Student's t-tests or a one-way ANOVA with the Tukey post-hoc test for multiple comparisons. All analyses were performed with GraphPad Prism, version 5.03 (Graph Pad Software, San Diego, CA, USA). Statistical significance was considered at $p < 0.05$.

RESULTS

During the course of the experiment, 2 (20%) rats in the 1 week BOO group died, 1 (10%) rat in the 2 week BOO group died, and 1 (10%) rat in the sham group died. Representative cystometric tracings from rats in the sham, 1 week BOO, and 2 week BOO groups are shown in Fig. 1. BOO was successfully produced in all surgically manipulated animals.

1. Body and bladder weights

There were no significant differences in body weight among any of the groups on the day of cystometry (data not shown). All of the groups with obstruction showed an increase in the ratio of bladder (mg) to body (g) weight compared with that in the sham group ($p < 0.001$). However, there were no significant differences in the ratio between the 1 week BOO and the 2 week BOO groups ($p > 0.05$) (Fig. 2A).

2. Urodynamic pressure- and volume-related parameters

Compared with that in sham animals, basal pressure was elevated in both 1 week BOO ($p < 0.05$) and 2 week BOO ($p < 0.05$) groups. There were no significant differences in the ratio between the 1 week BOO and 2 week BOO groups ($p > 0.05$). Compared with that in sham animals, threshold pressure was higher in the 2 week BOO group ($p < 0.01$) but not in the 1 week BOO group ($p > 0.05$). Neither BOO group showed any significant difference in maximal pressure compared with the sham group ($p > 0.05$) (Fig. 3).

Compliance was decreased in the 1 week BOO group ($p < 0.01$) and increased in the 2 week BOO group ($p < 0.001$) compared with the sham group (Fig. 2B). The 2 week BOO group showed increased compliance compared with the 1 week BOO group ($p < 0.001$) (Fig. 4). Compared with that in the sham group, the 1 week BOO ($p > 0.05$) group showed no significant difference in bladder capacity. This was due to increased residual volume ($p < 0.05$) and decreased micturition volume ($p < 0.05$) compared with that in the sham group. However, bladder capacity was increased in the 2 week BOO group ($p < 0.01$) compared with the sham group, which was associated with increased residual volume ($p < 0.01$) and no difference in micturition volume ($p > 0.05$) in the 2 week BOO group compared with the sham group. The micturition interval was increased in the 2 week BOO group compared with the sham ($p < 0.01$) and 1 week BOO ($p < 0.05$) groups. According to the definition of decompensated bladder in our study, 62.5% of the 1 week BOO group (5 of 8 bladders) and 33.3% (3 of 9 bladders) of the 2 week BOO group showed decompensation. The 1 week BOO group showed 37.9% of residual volume, and the 2 week BOO group showed 27.6% on average. There was no significant difference between the two groups (Fig. 5).

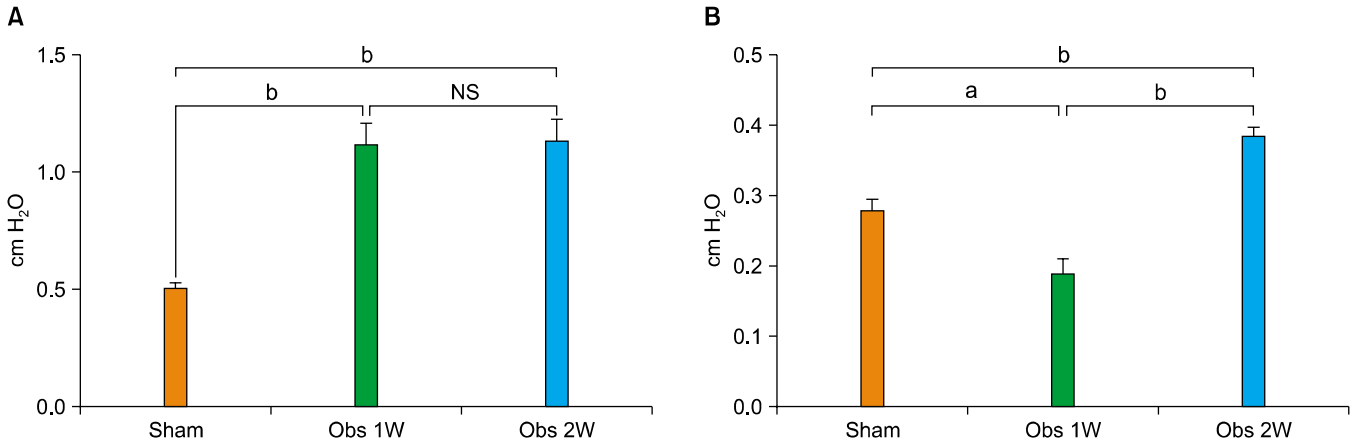

FIG. 2. Ratio of bladder weight to body weight (A) and compliance (B) in sham rats, rats with bladder outlet obstruction (BOO) for 1 week, and rats with BOO for 2 weeks. Rats with BOO showed significantly higher ratio of bladder weight to body weight than did sham rats. Compliance was significantly lower in rats at 1 week and higher in rats at 2 weeks compared with that in sham rats. Results are expressed as mean±SEM. NS: not significant, Obs: obstruction group. [a]$p<0.01$, [b]$p<0.001$ (unpaired Student's t-test).

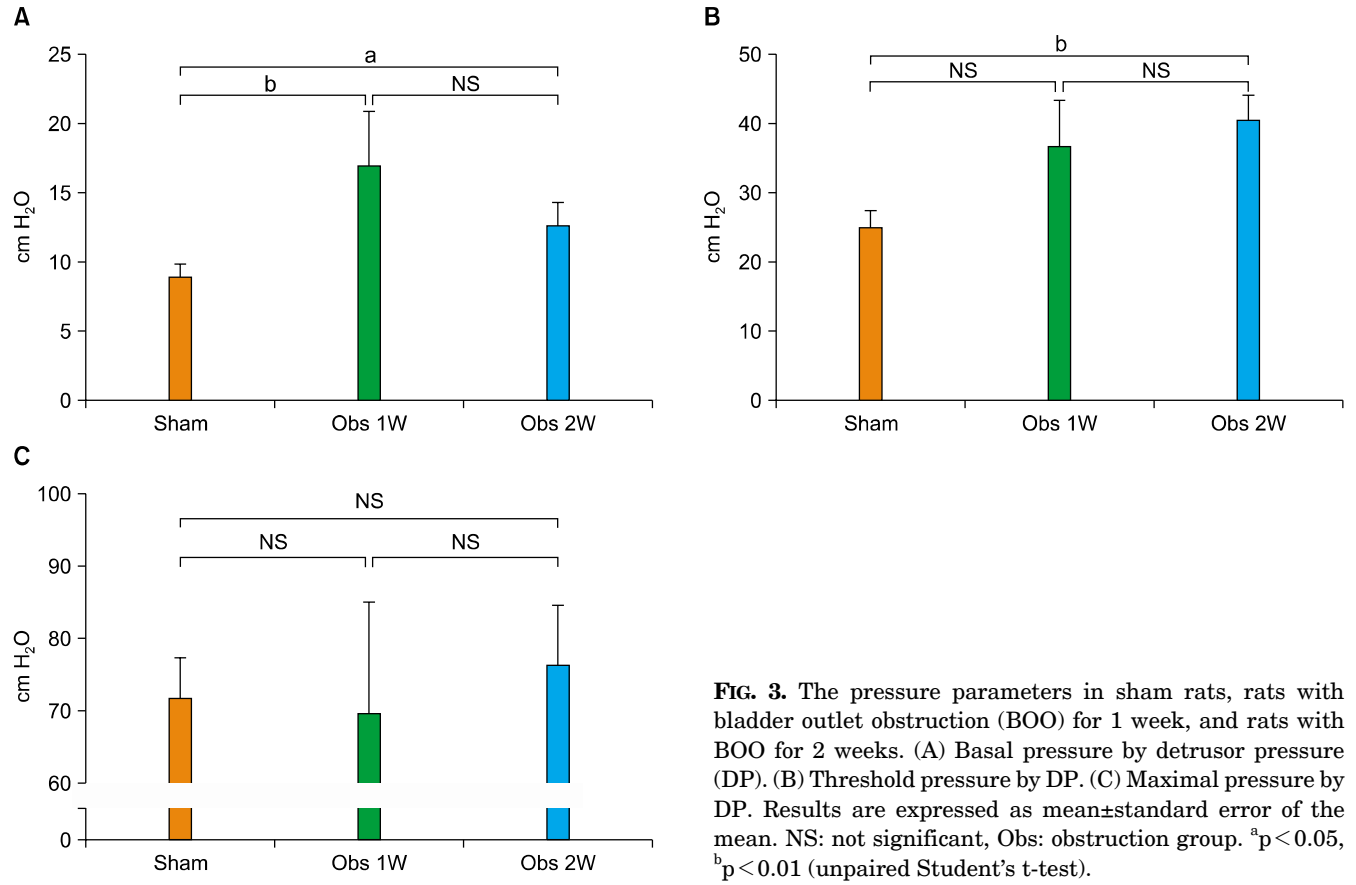

FIG. 3. The pressure parameters in sham rats, rats with bladder outlet obstruction (BOO) for 1 week, and rats with BOO for 2 weeks. (A) Basal pressure by detrusor pressure (DP). (B) Threshold pressure by DP. (C) Maximal pressure by DP. Results are expressed as mean±standard error of the mean. NS: not significant, Obs: obstruction group. [a]$p<0.05$, [b]$p<0.01$ (unpaired Student's t-test).

3. Parameters to investigate detrusor overactivity during the filling phase

Whereas the sham group showed no DO during the filling phase, the other two groups did. DO was demonstrated in all rats in both BOO groups (100%). However, the 1 week BOO group did not differ significantly from the 2 week BOO group in DO frequency or DO pressure (Fig. 6).

DISCUSSION

Our study showed that after the partial BOO, the rat bladders showed some sequential changes in bladder function in view of pressure and volume parameters and showed decompensation from the initial phase of 1 week after the surgery in view of the residual volume percentage. The decom-

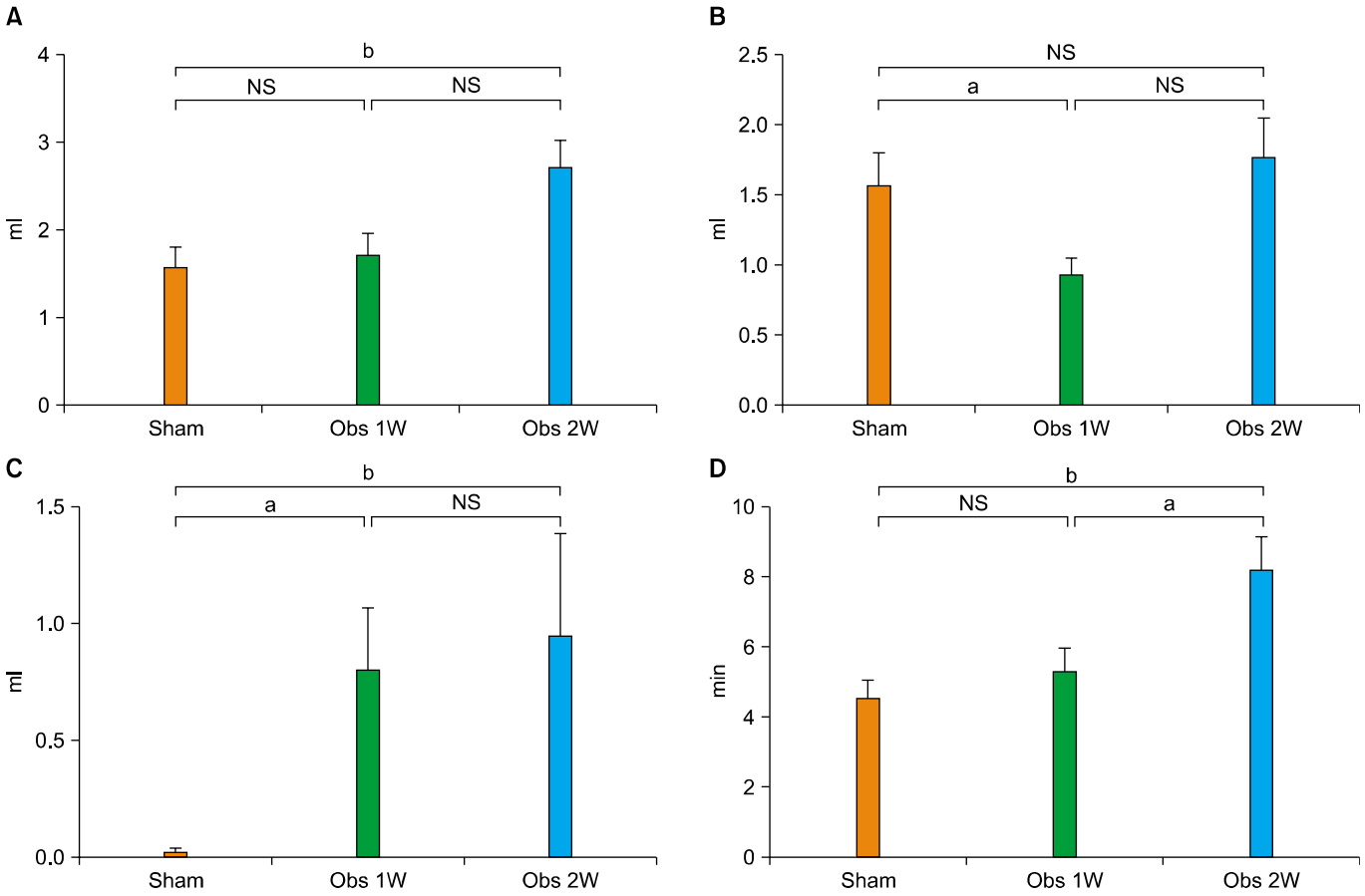

FIG. 4. The volume parameters in sham rats, rats with bladder outlet obstruction (BOO) for 1 week, and rats with BOO for 2 weeks. (A) Bladder capacity. (B) Micturition volume. (C) Residual volume. (D) Micturition interval. Results are expressed as mean±standard error of the mean. NS: not significant, Obs: obstruction group. [a]$p<0.05$, [b]$p<0.01$ (unpaired Student's t-test).

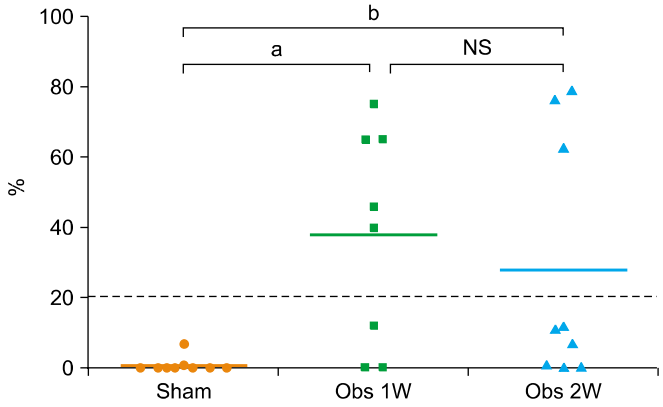

FIG. 5. The percentage of residual volume to bladder capacity in sham rats, rats with bladder outlet obstruction (BOO) for 1 week, and rats with BOO for 2 weeks. The rats with BOO showed significantly increased but variable values compared with the sham rats. Results are expressed as mean±standard error of the mean. NS: not significant, Obs: obstruction group. [a]$p<0.05$, [b]$p<0.01$ (unpaired Student's t-test). The dotted line indicates the criteria of decompensation as 20%.

pensatory changes in the bladder have been known to come after the compensatory changes, and those are stabilized by 2 weeks after the initiation of BOO [6,8]. However, our results suggest that decompensation can come before compensation, which can happen before 2 weeks after BOO.

Previous studies on awake animal models for partial BOO have provided clear evidence for the increased bladder weight, bladder capacity, residual volume, compliance, and NVCs [6,7,11]. However, many earlier studies could not provide clear evidence for the parameters of pressure and DO. In most urodynamic studies with awake rats with BOO, only IVP was measured, assuming that changes in the IVP curve reflect detrusor activity. Because a characteristic of an awake animal is to show instinctive and unpredictable physical activity that is difficult to control, however, movement-induced changes in IAP and IVP could limit the relevance of rats as a BOO model [12,13]. Our data with IAP measurement clearly showed that 1 week after the first obstructing surgery, the rats began to show the DO and increased basal pressure by DP during the filling phase. The bladders seem to increase their basal tensions in response to the increasing intravesical urine volume due to the increased residual volume during the filling phase, although they do not respond properly during the voiding phase. Two weeks after the surgery, they showed increased basal pressure and threshold pressure by DP. There could

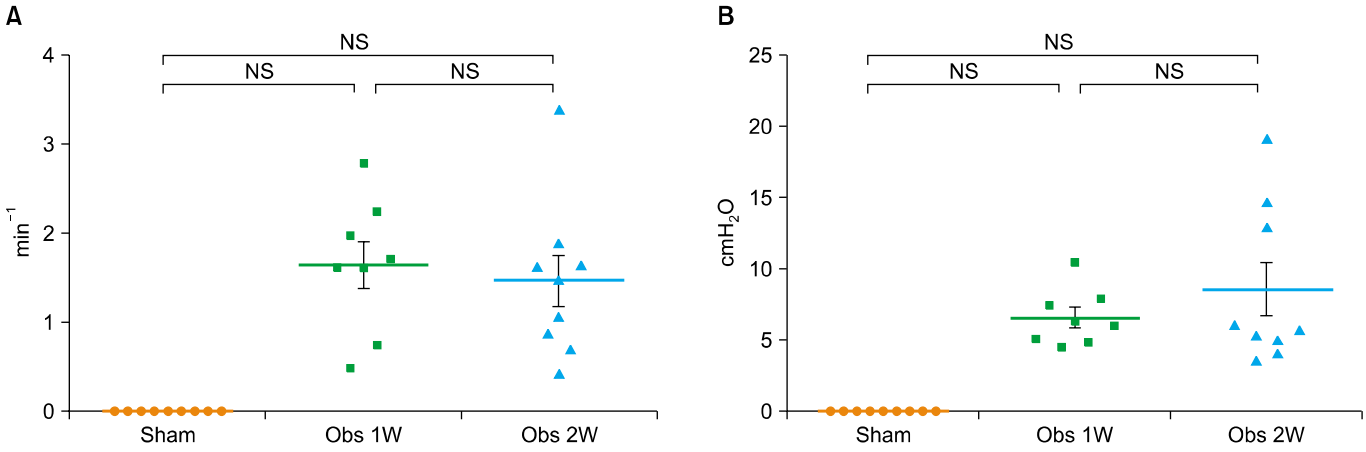

FIG. 6. Cystometric detrusor overactivity (DO)-related parameters during the filling phase in sham rats, rats with bladder outlet obstruction (BOO) for 1 week, and rats with BOO for 2 weeks. (A) DO frequency. (B) DO pressure. There were no significant differences in values among the groups. Results are expressed as mean±standard error of the mean. NS: not significant, Obs: obstruction group (unpaired Student's t-test).

be some changes in the voiding phase during this period, because the threshold pressure is a parameter for the function of the voiding phase. These results of increased basal pressure are consistent with the findings of Levin and associates, who showed a rapid increase in bladder contractile response during the initial stage of BOO [5,6,9].

The partial BOO in animal models is known to induce hypertrophy of the detrusor smooth muscle to adjust for the increased power required to expel urine against the obstruction [5,9]. Our data showed that 1 week after the first obstructing surgery, bladder capacity was not changed, possibly because of increased basal pressure and decreased compliance, despite the increased residual volume. Two weeks after the surgery, they showed increased bladder capacity with increased residual volume and compliance, and their bladder weight increased both at 1 and 2 weeks after the surgery. This might be due to the change in bladder wall characteristics such as the percentage composition of the muscle and collagen components in the increased bladder weight. Continued overdistension has been demonstrated to impair the blood flow of the wall [14-17], resulting in ischemia. This condition can induce tissue damage in the bladder wall and major changes in its characteristics. Thus, the bladder at 2 weeks after the surgery seems to increase bladder capacity with increased compliance, although the bladder weight is not different from that at 1 week.

The bladders from the animals with BOO would try to change their contractility required to overcome the increased outlet resistance during micturition. Some bladders show successful changes referred to as compensation, but other bladders show failure of micturition revealing increased residual urine, referred to as decompensation [6-8,18]. Clinically, the responses of the bladder to partial BOO, as seen with benign prostatic hyperplasia, also result in characteristic changes including the compensatory and decompensatory conditions [5]. The decompensated bladder refers to the condition with the impaired ability to empty efficiently and completely, which seems to be very vague and without scientific criteria. Thus, we defined the decompensated bladder in our experiment as the bladder showing more than 20% of residual volume in awake cystometry. Applying this definition, most bladders during the earlier phase, such as 1 week after the surgery, already showed decompensatory changes, and the incidence was higher than that of 2 weeks. This suggests that in view of the residual volume percentage, the rats' bladder can undergo the decompensatory condition first, and then some bladders can achieve the compensatory function after overcoming the obstruction via structural modifications of the bladder wall, such as becoming enlarged and more compliant. This variable incidence of compensation and decompensation in the bladder at some period might depend on differences in individual biochemical characteristics. It will be important to determine the major players among the biomolecules critical for this determination of these two conditions in the treatment of BPH.

After the BOO, the rats showed variably different voiding functions, characterized by increased frequency, residual urine, and NVCs. The NVCs may develop as the result of several factors. One is the surgical trauma of the abdominal or urethral surgery, but this occurrence seems to be associated with BOO, because sham animals did not show any NVC. Furthermore, in our study, the NVCs were determined as DO or abdominal straining, based on the presence of simultaneous changes on the IAP curve and IVP curve. Since 1 week after the surgery, DO was found in the rats with BOO, and there were no significant differences between the 1 week and 2 week groups.

CONCLUSIONS

In obstructed rats, we observed changes in bladder function and DO characteristics until the obstructed bladders

were stabilized [5]. From the earlier phase, the bladders exhibited serial changes in pressure parameters from the filling to the voiding phase. There were no changes in bladder capacity during the earlier phase, although the bladders showed increased residual volume and decreased micturition volume/compliance. We also confirmed that decompensated bladders in view of residual urine could be seen from the earlier phase, and during the later phase, there was an increased tendency for compensated bladders, which were accompanied by the bladders being enlarged and more compliant.

Conflicts of Interest

The authors have nothing to disclose.

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