Temporal Diabetes- and Diuresis-Induced Remodeling of the Urinary Bladder in the Rat

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Running head: Bladder remodeling in diabetic rat

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ABSTRACT

Diabetes mellitus causes remodeling of the urinary bladder, the natural history of which is poorly understood. In this study, we examined the temporal remodeling of the bladder in diabetic and diuretic rats. Male SD rats were divided into 3 groups: streptozotocin-induced diabetics, 5% sucrose-induced diuretics, and age-matched controls. Micturition and morphometric characteristics were evaluated using metabolic cages and examination of the bladder by light microscopy 4 days and 1, 2, 3 or 9 weeks after induction. Digital image analysis was used to quantify equatorial cross-sectional areas of bladder tissue and lumen, as well as relative content of the three primary tissue components: smooth muscle, urothelium, and collagen. Both diabetes and diuresis caused significant increases in fluid intake, urine output and bladder weight. In both groups, progressive increases were observed in the lumen area from 4 days to 3 weeks, and in the wall area from 2 to 3 weeks, after induction. The wall thickness decreased within the first 2 weeks in the diabetic and diuretic rats, but returned to control thickness at 3 and 9 weeks. As a percentage of the total cross sectional area, smooth muscle area increased, urothelium area was unchanged, and collagen area decreased in the diabetic and diuretic rats since 2 to 3 weeks compared with controls. In conclusion, diabetes and diuresis induced similar bladder remodeling. Diabetes-induced diuresis caused adaptive physical changes of the rat bladder by 4 days, and remodeling by 2 to 3 weeks after induction, which remained stable from 3 to 9 weeks.

Key words Streptozotocin; Morphology; Smooth muscle; Urothelium; Collagen
Diabetes mellitus (DM) is a metabolic disorder that is characterized by defects in insulin secretion and/or insulin action, resulting in hyperglycemia. The prevalence of DM rose from 4.9% in 1990 to 7.3% in 2000, an increase of 49% (23). DM seriously affects multiple organ systems, including the urinary bladder. In some series, 52% of randomly evaluated diabetic patients were found to have urologic symptoms, and even many hyperglycemic patients who reported no complications had unrecognized urologic symptoms (12). The classic symptoms associated with diabetic bladder dysfunction (DBD) include decreased bladder sensation, increased bladder capacity, and impaired detrusor contractility with resultant increased postvoid residual urine (7). In addition to these functional impairments, we and other investigators showed that diabetes induced alterations in bladder mass, tissue composition, capacity, compliance and response to pharmacological agents and electrical stimulation (3; 19; 27). Recently, Pitre, et al examined time-dependent morphological changes in rat bladder after induction of diabetes by streptozotocin (STZ). However, the full spectrum of morphological changes of the urinary bladder of diabetic rats and the role of diuresis still need to be determined.

Diuresis, induced by feeding 5% sucrose instead of water to animals, causes significant increases in bladder weight, but does not affect the body weight or serum glucose concentration. (20) Experimentally induced diuresis in both rats and rabbits causes bladder hypertrophy, increased contractility, increased capacity, and increased compliance that are similar to those changes observed in diabetic rats (3; 33). We and others have shown that diuresis and STZ-induced diabetes cause some of the same, as well as some different, changes in neurologic regulation of bladder contraction (17; 19). The similarities between the findings in diabetic and diuretic rats suggest that the bladder hypertrophy in diabetic animals may be a physical adaptation to increased urine production, and that the changes in the physical properties of the
bladder may be a significant factor in the development of vesicle dysfunction in diabetes. Therefore, the use of a diuretic group is crucial in distinguishing morphological changes produced by diabetes from those possibly induced by the pure effect of increased urine output. The extent to which diuresis alters the detailed morphology in diabetic animals is not known.

A more detailed characterization of organ remodeling in heart diseases has been studied. Cardiac hypertrophy occurs in response to long-term increases in haemodynamic load, but the pattern of hypertrophy differs, depending on the pathological conditions (6; 26). In pressure over-load hypertrophy (e.g. hypertension), an early stage of concentric hypertrophy, with an increase in left ventricular mass and an unchanged ventricular volume, is followed by late development of ventricular dilation. Volume overload hypertrophy (e.g. aortic regurgitation) is characterized mainly by eccentric ventricular hypertrophy. Conceptually, hypertrophy should provide functional benefits by increasing the numbers of sarcomeres per myocyte, and/or by decreasing wall stress. Quite surprisingly then, cardiac hypertrophy developing in these pathological conditions often progresses from a compensated stage, where cardiac function is well maintained, to a decompensated stage with clinical signs of heart failure. Heart failure is almost always accompanied by some degree of hypertrophy. So, hypertrophy itself may be a therapeutic target in heart failure (6; 26). Comparatively, time-dependent remodeling in the diabetic bladder has not been well documented.

The bladder is composed of urothelium, smooth muscle, and connective tissue mainly including collagen, and is capable of responding to mechanical stresses by increasing in mass through numerous cellular and structural changes. If the ratio between muscle and collagen is changed, bladder wall hypertrophy will result in an alteration in compliance. Therefore, it is
important to understand which tissue compartments (urothelium, detrusor muscle, or connective tissue) undergo hypertrophy, and at what stage of the disease.

The present study was designed to examine time-dependent (from 4 days to 9 weeks) remodeling of the bladder during diabetes, and define how much of these changes are attributable to diuresis.

**MATERIALS AND METHODS**

*Experimental animals*

Male Sprague-Dawley rats matched by date of birth (270 to 310 g, 10 weeks-old, Harlan), and housed in a 12-hour light/dark facility with food and water provided *ad libitum* were used in this study. The animals were randomly allocated to three groups: diabetics (n=30), diuretics (n=30), and age-matched controls (n=20). Diabetes was induced in the rats by intraperitoneal injection of STZ (65 mg/kg dissolved in 0.1 M citrate buffer, pH 4.5), and diuresis was induced by addition of 5% sucrose to their drinking water. Blood samples were taken 72 hours after administration of STZ and at the time of sacrifice to confirm diabetes (blood glucose >350 mg/dl). Blood glucose levels were measured with the ACCU-CHEK advantage blood glucose monitoring system (Roche Diagnostics Corporation, Indianapolis, IN). At 4 days, 1 week, 2 weeks, 3 weeks or 9 weeks after injection, micturition characteristics were evaluated using metabolic cages, then animals were sacrificed by a single intraperitoneal injection of pentobarbital (200 mg/kg). The urinary bladder was removed at the level of the bladder neck/proximal urethra caudally for the morphology study. Morphometric characteristics were evaluated by gross examination of the bladder (hematoxylin/eosin and Mason’s trichrome
staining). All experimental protocols and procedures were approved by the Cleveland Clinic Foundation Institutional Animal Care and Use Committee (Cleveland, OH).

**Drinking and micturition**

Drinking and micturition characteristics were measured for all rats. Rats were placed in individual metabolic cages (Nalgene, Nalge Company, New York) and the previous food, water, and light/dark conditions were maintained for a minimum of 24 hours. Following this familiarization period, a known volume of water or 5% sucrose was placed in the drinking bottles. Clean plastic beakers were used to collect urine. At the end of 24 hours, the volume of liquid remaining in the drinking bottles was measured. The volume consumed was calculated, and the voided volume was measured for each treatment groups.

**Bladder fixation and staining**

To characterize the morphological changes of the bladder in diabetic and diuretic rats, the bladders were equilibrated for 20 minutes at 37 °C in Krebs’ buffer aerated with 95% O₂ / 5% CO₂ to maintain pH 7.4. The composition of the Krebs solution was as follows (in mM): 133 NaCl, 4.7 KCl, 2.5 CaCl₂, 16.3 NaHCO₃, 1.35 NaH₂PO₄, 0.6 MgSO₄, and 7.8 Dextrose (9). After surrounding adipose tissue was removed, the bladder was sectioned at the equatorial midline and fixed in 10% neutral buffered formalin (pH 7.0). After fixation, tissues were dehydrated and paraffin-embedded. Serial 5 µm tissue sections were placed on microscope slides, dewaxed, and rehydrated for routine hematoxylin & eosin and Mason’s trichrome staining.

**Image analysis**

The stained slides were scanned (ArtixScan 4000tf, Microtek International, Inc., Carson, CA) and digital images of whole cross sections of the urinary bladder were saved for analysis. The
images were analyzed with Image-Pro Plus 5.1 image analysis software (Media Cybernetics, Silver Spring, MD). Hematoxylin and eosin stained slides were used to determine bladder tissue cross-sectional area. Fig. 2 A-C is the representative images of hematoxylin and eosin-stained equatorial sections of bladder in age-matched control (A), diabetic (B) and diuretic (C) rats 3 weeks after induction of diabetes or diuresis. Fig. 2 D-H illustrates the image analysis methods. The imaging analysis software can automatically trace the circumference of the bladder wall and calculate the internal area by counting the pixels and converting pixels to mm$^2$. The area within the outer circumference (including wall area and lumen area) and the inner circumference (only lumen area) of the bladder wall were measured by tracing the internal and external edges of the bladder wall separately, Fig. 2D and E delineated the outer and inner circumference, respectively, of the H&E stained tissue, and the green line is produced by the software automatically. The wall area was calculated as the difference between the area within the outer and the inter circumference of the wall. The thickness of the wall was measured at the 4 (L2), 8 (L3) and 12 (L1) o’clock positions and the mean was calculated to represent the wall thickness of this bladder (Fig. 2F). Mason’s trichrome stained slides (Fig. 2G) were used to determine three components (urothelium, collagen, and smooth muscle) of bladder tissues. The software can distinguish regions stained with different colors and accurately measure the areas. This color segmentation method was employed to determine the percentage of the tissue area that was stained “pink” (urothelium), “blue” (collagen), and “red” (smooth muscle). Fig 2H is a complex image based on the recognition of different color by the software. In all cases, the processing of images was performed by the same investigator unaware of treatment group assignments.
**Statistical analysis**

All data are expressed as the mean plus or minus standard error of the mean (SEM). Comparisons between multiple groups were done using 1-way ANOVA followed by Bonferroni post hoc test (GraphPad Prism 4.0, GraphPad Software, Inc. San Diego, CA). A probability of $p<0.05$ were considered significant.

**RESULTS**

**General characteristics**

General physical characteristics of the animals are shown in Table 1. The initial mean body weight was similar for all 3 groups, but the diabetic group weighed significantly lower than the diuretic and control groups at 4 days, 1 week, 2 weeks, 3 weeks and 9 weeks after induction ($p<0.05$). There were no significant differences between the body weights of the diuretic and control animals at the different time points ($p>0.05$). Blood glucose concentrations were significantly higher in the diabetic rats than in the control or diuretic groups by 4 days after STZ injection and were maintained throughout the study ($p<0.01$). The mean blood glucose levels of the diabetic rats were ~ 4 to 5 times higher than those of age-matched control and diuretic rats. There were no significant differences in blood glucose levels between control and diuretic animals ($p>0.05$). The bladder weights increased markedly in both the diabetic and diuretic rats compared with controls, and appeared to increase faster (4 days) in the diabetic rats, though there were no significant differences in bladder weights between the diabetic and diuretic animals at any of the time points. The weights of the diabetic and diuretic bladders reached a plateau at 3 weeks, a near doubling of control bladder weights. The ratio of bladder weight / body weight was
significant higher in diabetic group than in the age-matched control and diuretic groups at all time points \(p<0.01\).

**Fluid consumption and excretion**

In general, both diabetic and diuretic rats showed significantly increased 24-hr fluid consumption and urine output compared with controls \(p<0.01\). However, those increases occurred slightly more rapidly in the diabetic rats, which consumed and excreted 7-fold and 17-fold more volume, respectively, than controls 4 days after induction, compared to 4.2-fold and 7.7-fold more volume consumed and excreted by the diuretic rats relative to controls at that time point (Fig. 1 A and B). Fluid consumption and urine output peaked in the diabetic rats 1 week after induction, at 6.1-fold and 13.4-fold, respectively, greater than controls, while those measures peaked at 2 weeks in the diuretic rats, at 7.2-fold and 14.5-fold greater than controls. The amounts of fluid consumed and excreted by diabetic and diuretic rats declined slightly during the next 1 - 2 weeks, but remained stable from 3 to 9 weeks.

**Morphometric analysis**

Histologic examination using light microscopy showed bladder hypertrophy and lumen dilation in the diabetic and diuretic animals relative to controls (Fig. 2 A-C). Automated digital imaging was used to quantify the cross-sectional area and composition of bladder tissue at various times (Fig. 2 D-H).

The total cross-sectional bladder lumen area (at the equatorial midline) increased significantly as early as 4 days after induction in diabetic and diuretic rats relative to controls \(p<0.01\), then gradually increased until 3 weeks with no further increase at 9 weeks (Figure 3A). The lumen areas reached about 3.4 and 3.9 times greater than controls at 9 weeks. The total cross-sectional bladder wall areas (at the equatorial midline) of the diabetic and diuretic rats
increased by 2 weeks after induction and continued to increase through 3 and 9 weeks, reaching levels that were about 1.5 times controls (Figure 3B). The wall thickness in the diabetic and diuretic rats decreased within the first 2 weeks, but gradually increased back to control values at 3 and 9 weeks (Figure 3C). There were no significant differences between diabetic and diuretic rats in lumen area, wall area or wall thickness at any of the time points ($p>0.05$). These results show that the lumen enlargement and hypertrophy are two obvious responses to polyuria in the early stage of diabetes.

The three different components of the bladder wall changed in a time-dependent manner in diabetic and diuretic rats (Fig. 4). The absolute value of the urothelium cross-sectional area increased gradually in diabetic and diuretic rats and was significantly greater than controls at 3 and 9 weeks after induction ($p<0.05$, Fig. 4A). However, when expressed as percentage of the total tissue area, the urothelium area was not significantly higher during diabetes and diuresis progression ($p>0.05$). The collagen was mainly localized in lamina propria and within and between the muscle bundles (Fig. 2G). The actual collagen cross-sectional area did not change significantly during the progression of diabetes or diuresis, but decreased in both of those groups as a percentage of the total tissue area at 3 to 9 weeks ($p<0.05$). The smooth muscle of the bladder wall increased significantly at 2 weeks, and the percentage of the total tissue also showed a significant increase ($p<0.05$) at 2 weeks in the diabetic and diuretic animals compared to controls. There were no significant differences between diabetic and diuretic rats in any of the three tissue component areas at any of the time points ($p>0.05$). These results show that the significantly increased bladder wall areas in the diabetic and diuretic rats are due primarily to increased smooth muscle and urothelium.
DISCUSSION

Diabetic patients often demonstrate varied symptoms, including bladder hyperreflexia and areflexia, and indeterminate or normal bladder function (13; 31). The heterogenicity of the clinical symptoms of diabetic bladder dysfunction (DBD) may result from a variety of factors, including the duration or type of diabetes, or other associated risk factors such as gender, parity and level of hyperglycemia. DBD is associated with distinct morphological changes; however, the details and pathophysiological mechanisms of such changes are not well understood.

Diuresis alone can induce many of the effects associated with diabetes, including bladder hypertrophy, increased contractility, and increased capacity (3; 33). Therefore, in order to identify the disease-specific impact of diabetes on remodeling of the bladder, it is essential to consider the role of diuresis in that remodeling process.

The increase in the bladder weight is one of the most noticeable responses of the bladder to diabetes and diuresis. Our findings of increased bladder weight are in agreement with reports from other investigators who have examined rat bladders at times ranging from 2 to 16 weeks following STZ treatment (18; 35). In the present study, we demonstrated that a significant increase in bladder weight occurs as early as 4 days after induction of diabetes and 1 week after induction of diuresis in rats, and the bladder weight continued to increase up to 3 weeks in both groups. The growth process subsided after the first 3 weeks of diabetes or diuresis, probably due to sufficient adaptation of the bladder to the increased urine volume.

The increases in fluid intake and urinary output in the STZ-diabetic rats are also consistent with other functional studies in diabetic rats that have shown increased urine output, micturition volume, urinary capacity and bladder compliance (32). In our study, increases in fluid intake and urinary output occurred slightly sooner in the diabetic than in the diuretic rats. Such different
micturition patterns may contribute to the faster increase in bladder weight in diabetic rats than in diuretic rats. The exact stimulus that initiates the increase in bladder weight in response to polyuria is not known, but it is plausible that it is related to the alterations of bladder volume, rate of filling, and rate of stretch of the bladder. Whatever the reasons, changes in cell shape can stimulate DNA synthesis, which in turn results in increased protein synthesis, causing increased cell mass and hyperplasia. (5)

In the present study, bladder lumen dilation was observed in both diabetic and diuretic rats within 4 days after induction and continued to increase over the first 3 weeks, trailed by an increase in the bladder wall area. Consequently, lumen dilation was accompanied in both groups by an initial decrease in bladder wall thickness, which gradually recovered to control levels over the first 3 weeks after induction.

The mechanisms of the increased lumen area induced by polyuria are not very clear. The bladder rapidly adapts to increased urine production by increasing both micturition frequency and volume (21). It was reported that increased water intake can increase the bladder capacity within minutes (1). Such a resetting of the threshold volume for initiating a micturition may involve nervous mechanisms. A possible explanation would be a functionally disturbed sensory input from the bladder.

The micturition reflex is activated primarily by filling the bladder to a threshold volume at a physiological rate (natural filling). Afferent receptors and nerve endings are “calibrated” for natural filling, which is slow, intermittent and variable, normally averaging about 1 ml/kg/h in man (16). Many studies have demonstrated that the high rate of bladder filling during cystometry can affect cystometric features, particularly increasing the intravesical pressure and threshold volume (capacity) (11; 14-16; 29). High, non-physiological filling rates may induce a mechanical
trauma to the afferent pathway so that the frequency of action potentials, which gives desire to void, will occur at a larger than normal volume. In addition, rapid stretch may cause temporary functional disturbances in nerve endings, detrusor smooth muscle cells and cell junctions, leading to increased capacity. In the present study, within the first 4 days in diabetic and diuretic rats, urine excretion increased from 8 ml/24 hours to 136 and 61.25 ml/24 hours, respectively. The increased rate of urine formation might induce an increased bladder capacity (3; 22) and, at the same time, stimulate tissue hypertrophy and hyperplasia (30).

Although bladder hypertrophy and, specifically, growth of the detrusor in response to increased bladder work and distension have been reported (18; 35), the relative amounts and rates of growth of the different bladder tissue components are not well known. The present study showed that the three major components of the bladder wall (urothelium, connective tissue and smooth muscle) did not change significantly in control rats during the investigated period, but changed over that time in diabetic and diuretic rats. The absolute values of smooth muscle and urothelium areas increased between 1 and 3 weeks after induction of diabetes or diuresis, and the collagen area did not change significantly during the entire period studied. Since the smooth muscle and urothelium areas comprise high and very low percentages, respectively, of the total tissue area, the changes in area expressed as percentage of the total tissue area were an increase in smooth muscle, no change in urothelium and a decrease in collagen. Our results from diabetic relative to control rats differ slightly from a previous report (27), which showed similar changes, but with a later onset (5 weeks after induction with STZ). The reason may be that we used younger rats in our study, which may have been capable of generating a more vigorous growth response to polyuria. The present study showed that the primary source of the increased bladder weight was the smooth muscle, which progressively increased to comprise about 64% of bladder
wall in the diabetic and diuretic rats compared with 51% in the controls over the 9 weeks investigated. A previous study demonstrated that diabetes-induced diuresis stimulates DNA synthesis and cell proliferation initially and mainly in the urothelium, but is followed by transient proliferation of the smooth muscle and connective tissue compartments (4). The functional significance of smooth muscle and urothelium hypertrophy may be involved in the development of altered detrusor pressure as part of either a compensatory and/or pathophysiological response to the underlying disease process. The resulting hypertrophy may enable the bladder to adapt to the polyuria associated with diabetes.

The mechanisms involved in triggering tissue hypertrophy and hyperplasia are not fully understood. It has been reported that a high filling rate is a primary factor responsible for the induction of hyperplasia of the bladder urothelium, connective tissue and smooth muscle (30). Acute overdistention induced a 5-fold increase in $^3$H-thymidine incorporation in the bladder body and a 3-fold increase in the bladder base. Autoradiography of the overdistended bladders showed significant and substantial labelling which was confined to the urothelial basal cells (34). The proliferating urothelium might produce some factors that can modulate the proliferation of detrusor muscle cells (24; 25).

Collagen is the major constituent of the extracellular matrix in bladder. Bladder collagen has been suggested to influence the passive property of the bladder wall. Bladder compliance correlate with changes in relative amounts of collagen (2). An increase in detrusor muscle and a relative decrease in collagen density were evident in diabetic and diuretic bladders, and then the bladder becomes more compliant, which means bladder may increase in volume by two to three-fold without an increase in internal pressure. In addition, collagen fibrils in smooth muscle
probably also play an important role in intercellular transmission of active force. A changed collagen concentration might thus also affect the contractile properties of the smooth muscle.

It is well known that an important characteristic of an overloaded heart is progressive ventricle remodeling, which appears to be a key contributor to morbidity and mortality of congestive heart failure (10; 28). The angiotensin converting enzyme inhibitor (ACEI) and beta blocker (BB) therapy were proven to be remarkably effective in improving left ventricle (LV) remodeling (10) and reducing morbid events (28). Diabetic bladder remodeling might lead to some local neurogenic and myogenic changes that result in the altered bladder function. Obviously, controlling the blood glucose level is the best strategy in the treatment of diabetes. However, it is often difficult to maintain at a level that can completely prevent the diabetic cystopathy. It was reported that the prevalence rate of diabetic cystopathy in patients on oral hypoglycemic treatment is 25% (8). Therefore, it is necessary to use the processes leading to bladder dysfunction as a framework for designing novel therapeutic targets. Preventing or slowing the progressive remodeling of the bladder might be an effective strategy for the treatment of diabetic bladder dysfunction. The approaches may include timed voiding, intermittent catheterization, and even pharmacological inhibitors to attenuate the remodeling of the bladder.

In conclusion, STZ-induced diabetes and 5% sucrose-induced diuresis induced rapid, marked remodeling of the bladder wall, which included hypertrophy, lumen dilation and reorganization of the relative structural relationships among the 3 major tissue components. Morphology changed significantly within the first 3 weeks after induction. Time-dependent increases in smooth muscle and in total, but not relative, amounts of urothelium, and reductions in collagen
density were observed in both the diabetes and diuresis models. These data therefore suggest that diabetes-associated polyuria leads to remodeling of the bladder within the investigated 9 weeks.
ACKNOWLEDGEMENTS

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GRANTS

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Table 1. Body weight, bladder weight, and blood glucose levels of diabetic, diuretic and age-matched control rats.

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Group</th>
<th>Initial Weight (g)</th>
<th>Final Weight (g)</th>
<th>Blood Glucose (mg/dl)</th>
<th>Bladder Weight (mg)</th>
<th>Bladder to Body Weight Ratio (mg/g)</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>280.00±1.37</td>
<td>319.50±2.22</td>
<td>125.25±3.49</td>
<td>85.00±3.06</td>
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<td>4 days</td>
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<td>284.33±2.76</td>
<td>278.33±4.23*</td>
<td>587.33±2.40#</td>
<td>116.83±6.18*</td>
<td>0.42±0.02*</td>
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<td>Diuretic</td>
<td>275.33±2.23</td>
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<td>133.17±1.58</td>
<td>98.17±4.13</td>
<td>0.31±0.01</td>
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<td>295.75±2.04</td>
<td>342.50±2.55</td>
<td>117.50±4.18</td>
<td>86.25±4.10</td>
<td>0.25±0.01</td>
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<td>1 week</td>
<td>Diabetic</td>
<td>297.83±2.52</td>
<td>269.83±3.82#</td>
<td>564.50±10.19#</td>
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<td>302.17±4.92</td>
<td>340.67±3.74</td>
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<td>116.17±1.70*</td>
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<td>154.33±7.17*</td>
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<td>3 weeks</td>
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<td>298.67±2.53</td>
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Values are expressed as mean plus or minus standard error of mean of 4 to 6 individual rats.

* significantly different from corresponding value in age-matched control group (p < 0.05).

# significantly different from corresponding value in age-matched control and diuretic group (p < 0.05)
FIGURE LEGENDS

Figure 1. Temporal changes in consumed volume (A) and excreted volume (B) in age-matched control, diabetic and diuretic rats. Each point represents the mean ± standard error of the mean of 4 to 6 individual rats. *significantly different from the corresponding value in the age-matched control group (p <0.01). #significantly different from the corresponding values in the control and diuretic groups (p <0.05)

Figure 2. A-C. Representative images of hematoxylin and eosin-stained equatorial sections of bladder in age-matched control (A), diabetic (B) and diuretic (C) rats 3 weeks after induction of diabetes or diuresis. D-F. Image analysis of hematoxylin and eosin-stained equatorial sections of urinary bladders demonstrating the measurement of the area within the outer circumference (D), and the inner circumference (E), and the measurement of the thickness (F) of the bladder wall at the 12 (L1), 4 (L2) and 8 (L3) o’clock positions. G, H. Image analysis of a Mason’s trichrome-stained equatorial section of a urinary bladder, demonstrating measurement of the urothelium, collagen, and smooth muscle areas using the color segmentation method. (G) Different layers of the bladder wall divided into the red-colored smooth muscle area, blue-colored collagen area and pink-colored urothelium layer (Mason’s trichrome staining). (H) Software color segmentation performed on the Masson’s stained slides shows the pink-colored smooth muscle, the yellow-colored collagen and the green-colored urothelium areas that were recognized and captured by the automated digital image analyzer for measurement of the percentage of total tissue cross-section. Scale bar, 1 mm.
Figure 3. Temporal changes of lumen area (A), wall area (B), and wall thickness (C) in age-matched control, diabetic and diuretic rats. Each point represents the mean ± standard error of the mean of 4 to 6 individual rats. *significantly different from the corresponding value in the age-matched control group (p <0.05).

Figure 4. A-C. Temporal changes of the absolute value of urothelium (A), collagen (B), and smooth muscle (C) cross-sectional areas in age-matched control, diabetic and diuretic rats. D-F. Temporal changes of urothelium (D), collagen (E), and smooth muscle (F) areas as % of total tissue cross-sectional area. Each points represents the mean ± standard error of the mean of 4 to 6 individual rats. *significantly different from the corresponding value in age-matched control group (p <0.05).
Figure 1

A

Volume consumed (ml/24 hrs)

Time (weeks)

B

Volume excreted (ml/24 hrs)

Time (weeks)
Figure 2
Figure 3

A

B

C
Figure 4

A

B

C
Figure 4

D

![Graph D showing % of total tissue cross section over time for control, diabetic, and diuretic groups.](image)

E

![Graph E showing % of total tissue cross section over time for control, diabetic, and diuretic groups.](image)

F

![Graph F showing % of total tissue cross section over time for control, diabetic, and diuretic groups.](image)