Temporal differences in bladder dysfunction caused by diabetes, diuresis, and treated diabetes in mice

Firouz Daneshgari,1 Xiao Huang,1 Guiming Liu,1 James Bena,2 Lateef Saffore,1 and C. Thomas Powell1

1Glickman Urological Institute, Lerner Research Institute, and 2Department of Quantitative Health Sciences, The Cleveland Clinic Foundation, Cleveland, Ohio

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Diabetic bladder dysfunction (DBD) is among the most common and incapacitating complications of diabetes mellitus (DM), causing urinary incontinence and poor emptying of the bladder (7, 12, 16). DBD has been estimated to affect between 25 and 83% of diabetics (16). Although DBD is not life threatening, it affects the quality of life significantly. It has been reported that the impact of DBD symptoms on quality of life may equal or exceed the impact of acquired immunodeficiency syndrome (AIDS) (26).

Despite the prevalence of DBD, the natural history of this disease remains unclear. It is well recognized that the two major and distinct functions of the bladder are urine storage and urine voiding or emptying; therefore, classification of diseases of the bladder into storage or voiding problems is widely accepted (1, 29, 30). The classic symptoms of diabetic cystopathy are decreased bladder sensation, increased bladder capacity, and impaired bladder emptying with resultant elevated postvoid residual urine (14). Consequently, urinary incontinence in the diabetic patient has traditionally been attributed to overflow incontinence as a sign of voiding problems of the bladder. However, several studies dispute the classic picture by reporting evidence of storage problems of the bladder, such as increased frequency, urgency, and urge incontinence, in diabetic patients (15, 17, 27, 28). A more complete understanding of the natural history of DBD and variations in its pathophysiology has increasingly become important in view of recent large-scale studies indicating that diabetes is associated with 30–80% increased risk of urinary incontinence (5, 6, 9, 31). Relevant to the findings of our study is that, in clinical studies, the risk for urge incontinence (storage problem) was increased by 40–80% in multivariate analyses that controlled for stroke and other chronic medical conditions (6, 9, 31).

We have hypothesized previously that the reported inconsistency in the clinical and experimental manifestation of DBD is a reflection of time-dependent changes in the bladder under the diabetic condition (8). We were interested in examining the temporal changes in bladder function after induction of DM, as well as the disease-specific role of DM, beyond its diuretic effects, in causing bladder dysfunction. Therefore, we designed the current study to examine the temporal changes in bladder function in mice over the course of 20 wk of streptozotocin (STZ)-induced diabetes, with or without insulin treatment, in age-matched mice with induced diuresis, and in control mice. Mice were chosen to take advantage of future application of these results in small animal models used in the National Institutes of Health (NIH)-sponsored Animal Models of Diabetic Complications Consortium (AMDCC; www.amdcc.org). The goal of AMDCC is to employ transgenic and knockout technology to create animal models that replicate the organ-specific complications of diabetes. The C57BL/6 strain is widely used for genetic manipulation.

METHODS

Experimental animals. Four groups of eight male C57BL/6 mice (Charles River Laboratories; 36–45 days old) matched by date of birth were used at each time point in this study. Animals were housed in a 12:12-h light-dark facility with food and water provided ad libitum.
libitum. Animals were randomly assigned to one of four groups: 1) DM induced by STZ, 2) DM induced by STZ and treated with insulin, 3) diuresis induced by 5% sucrose, or 4) sham-treated control. Diabetes was induced, after a 24-h fast, by an intraperitoneal injection of 60 mg/kg STZ dissolved in 0.1 M citrate buffer. Control mice were treated identically except that a similar volume of buffer was injected instead of STZ. To confirm induction of DM (blood glucose >300 mg/dl), blood samples were taken from the tail 72 h after administration of STZ. Blood glucose levels were measured with the ACCU-CHEK advantage blood glucose monitoring system (Roche Diagnostics, Indianapolis, IN). Animals treated for induced DM received subcutaneous implantation of LinBit insulin pellets (LinShin Canada, Ontario, Canada) under the mid dorsal skin. The treatment with insulin began 1 wk after induction of diabetes. Diuresis was induced in mice by addition of 5% sucrose to the animals’ drinking water.

**Experiment design.** At 3, 9, 12, and 20 wk after induction of DM, the bladder function was evaluated by 24-h measurement of urine output, followed by conscious cystometry. After completion of cystometry, the animals were humanely killed with a single intraperitoneal injection of pentobarbital (200 mg/kg). All surgical procedures and experimental protocols were approved by the Cleveland Clinic Foundation Institutional Animal Care and Use Committee (Cleveland, OH).

**Urine output (24 h).** Mice were placed in individual metabolic cages (Nalgene, Nalge, NY), and the previous food, water, and light-dark conditions were maintained. After a 24-h familiarization period, the total voided volume during the next 24 h was measured.

**Suprapubic bladder catheter implantation.** Catheter implantation was performed 2 days before cystometry. In mice under ketamine anesthesia, a midline longitudinal abdominal incision was made 0.5 cm above the urethral meatus. The bladder was exposed, and a circular purse-string suture of 5-0 silk was placed on the bladder wall. A small incision was made in the bladder wall, and the catheter (PE-10 tubing with a flared tip) was implanted. The purse-string suture was tightened around the catheter. The catheter was tunneled subcutaneously and externalized at the back of the neck, out of reach of the animal. The distal end of the tubing was sealed, and the skin and abdominal incisions were closed separately.

**Conscious cystometry.** Two days after implantation of a bladder catheter, the animals were placed in specially modified metabolic cages so that conscious cystometry could be performed for a period of 2–5 h. Briefly, the implanted bladder catheter was attached via a stopcock to both a pressure transducer (BP-100; CB Sciences, Dover, NH) and a flow pump (Kent Scientific, Torrington, CT). The bladder was emptied through the suprapubic tube with a syringe. The bladder was then filled via the catheter with room temperature 0.9% saline (3 ml/h) while bladder pressure was recorded. The animal was awake and able to void and/or leak urine through the urethra during the study. Urine was collected in a beaker on a force transducer (FT-03 D; Grass Instrument, Quincy, MA) placed beneath each cage. The pressure and force transducers were connected to an amplifier (ETH-400; CB Sciences), and multiport controller software (Polyview; Grass Instrument) was used for data recording through a computer. The cumulative weight of the collected urine was monitored continuously. Saline infusion was continued until rhythmic bladder micturition contractions became stable, typically 30 min to 1 h. After the initial stabilization period, the data for at least 10–30 representative micturition cycles were collected to analyze all the cystometric parameters. The means of the collected data are reported for analysis. The bladder capacity was calculated by multiplying the time of infusion to the first void by the infusion rate. Voided volume is the volume expelled at micturition. The residual urine volume was calculated by subtracting the voided volume from the bladder capacity. The basal pressure was defined as the bladder pressure immediately before the start of filling for each micturition cycle. The mean threshold pressure was defined as the bladder pressure immediately before the start of each voiding contraction. The bladder compliance was calculated by dividing the bladder capacity by the difference between threshold and basal pressures [bladder capacity/threshold pressure − basal pressure], because the difference between the threshold pressure (at maximum bladder capacity) and the basal pressure should represent the change in the bladder pressure occurring with filling of the bladder. Peak voiding pressure (PVP) was measured at the peak of the detrusor contraction. Resting pressure was recorded after the first time voiding. The intercontraction interval between two successive contractions was calculated in each micturition cycle. Similar to urodynamic parameters in a human, the cystometric parameters in the animal represent storage and voiding functions of the bladder. For example, bladder capacity and compliance and basal and threshold bladder pressures reflect the ability of the bladder to store urine, whereas mean voided volume, PVP, and threshold pressure after voiding reflect the voiding function of the bladder in the animals.

**Statistical analysis.** Bladder and body weights, blood glucose, and cystometric variables were analyzed as outcome measures by using initial statistical models incorporating the main effects of the disease group (DM, insulin-treated DM, diabetic, or control), postinjection maintenance intervals (treated categorically), and their interactions. Variance was allowed to depend on disease status, weeks since injection, or both, based on the adjusted $R^2$ measures and residual plots from the suggested model. For all data, a logarithmic transformation of variables was performed to reduce the heterogeneity of the variance over time. Overall F-tests and F-tests of the interaction between treatment group and maintenance interval were performed for all variables. The reduced main effects model was used for inference when the interaction was nonsignificant. If the groups differed at a 0.05 significance level, we described distinct time trends for DM, treated DM, diabetic, and control mice and/or distinct disease status effects at postinjection times. When the interaction was statistically significant, differences between the DM and other treatment groups were evaluated at each maintenance interval. $P$ values from comparisons made at specific postinjection times were multiplicity adjusted, using the method of Edwards and Berry (10). Effects are described through adjusted (least squares) means. Outliers and influential observations were explored when warranted and checked for data error, but they were generally retained in analyses. An overall significance level of 0.05 was used for statistical comparisons. Statistical analysis was performed using SAS software (version 9.1; Cary, NC), and plots were made using S-Plus (version 6.2; Seattle, WA).

**RESULTS**

**Systemic diabetes impact.** A total of eight mice were included in each of the four groups for each time point; however, some animals were lost before examination in the DM group ($n = 7, 6, 7, 9, 12$, and 20 wk, respectively) and in the control and insulin-treated DM groups ($n = 7$ at 20 wk). General physical characteristics of the animals, including blood glucose concentrations, baseline and terminal body weights, and bladder weights, were determined at 3, 9, 12, and 20 wk after induction of diabetes and diuresis (Table 1). The blood glucose levels of the control mice were stable at all time points. The blood glucose levels of the DM mice were significantly higher than those of all other groups at all time points, escalating from 330% of the control group at 3 wk to 540% at 20 wk ($P < 0.001$ at all time points). Feeding 5% sucrose did not affect the blood glucose levels. In the present study, insulin treatment was effective but did not totally reverse the hyperglycemia. The blood glucose levels of the insulin-treated DM mice were lower than those of the DM mice but higher than those of the control mice at all time points ($P < 0.001$).
The weights of control and diuretic animals were similar to each other and did not change substantially from 3 to 12 wk but increased in both groups at 20 wk. The weights of DM mice did not change with time and were significantly less than those of the control mice at all time points except 12 wk. Insulin-treated DM mice weighed significantly more than DM mice at 12 and 20 wk (P < 0.02) but were similar in weight to control animals at all time points. The bladder weights of DM mice more than doubled between 3 and 12 wk before dropping by 35% at 20 wk. This differed from all other groups, where bladder weight consistently increased over time (interaction: P < 0.001). The bladder weight of the DM group increased from 18 to 94% larger than that of the control group at 3 to 12 wk before lapsing to 16% greater than that of controls at 20 wk. Bladder weight in the DM mice was significantly greater than the control and insulin-treated DM mice at 9 wk (P < 0.01) and compared with all other groups at 12 wk (P < 0.001).

**Urine output (24 h).** The 24-h urine outputs of DM and diuretic mice were more than seven times larger than those of the control group (P < 0.001 at all time points), although output increased in a generally parallel fashion in the diabetic and control groups (interaction: P = 0.36). The 24-h urine output of the diuretic mice increased from 3 to 9 wk and then was stable from 9 to 20 wk. The urine output of the DM group was significantly larger than that of all other groups at 20 wk (P < 0.001) and relative to the control and treated DM groups at all other time points (P < 0.001) (Fig. 1). Insulin replacement attenuated the increased 24-h urine output markedly. The 24-h urine output of the treated DM group increased at a slower rate than that of the DM group (interaction: P < 0.05) and was lower at all time points than in DM and diuretic animals.

**Cystometrogram.** Cystometrogram (CMG) tracings of DM, treated DM, diuretic, and control mice were obtained at 3, 9, 12, and 20 wk (Fig. 2). Bladder capacity (Fig. 3A) and compliance (Fig. 3B) were both greatly elevated in DM mice at all time points. The capacity of the DM group was significantly larger than that of the control and treated DM groups at all time points, with the greatest proportional difference occurring relative to the control group at 12 wk (P < 0.001). Bladder capacity also was elevated in the diuretic mice over both the control and treated DM groups at all time points, although it was significantly lower than in the DM mice only at 12 wk (P = 0.040). The DM and diuretic groups were not statistically different from one another at all other time points. At each time point, the DM and diuretic groups had significantly larger compliance levels than the treated DM and control groups (P < 0.001) but did not significantly differ from each other (P > 0.05) except at 20 wk (P = 0.025; Fig. 3B). Mean voided volume in diuretic mice was significantly greater than in DM mice at 9, 12, and 20 wk (interaction: P = 0.038; P < 0.001 at 9, 12, and 20 wk), but voided volume in DM mice was on average 43% larger than in control mice (P < 0.001 at 3 and 9 wk) and 54% larger than in insulin-treated DM mice (P < 0.05 at all time points) (Fig. 4A). As expected, residual urine was highly elevated in the DM group relative to all other groups (P < 0.001 for all groups and time points) (Fig. 4B).

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Table 1. General characteristics of age-matched DM, insulin-treated DM, diuretic, and control mice

<table>
<thead>
<tr>
<th>Time Postinduction, wk</th>
<th>Group</th>
<th>Blood Glucose mg/dl</th>
<th>Animal Weight, g</th>
<th>Bladder Weight, mg</th>
<th>Mean Bladder Weight/Mean Body Weight, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Control</td>
<td>107.0±6.15</td>
<td>23.5±0.3</td>
<td>45.5±1.6</td>
<td>1.9</td>
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<tr>
<td>DM</td>
<td>356.0±22.86</td>
<td>18.9±0.7</td>
<td>53.8±2.0</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>Treated DM</td>
<td>224.0±9.9</td>
<td>22.4±0.5</td>
<td>47.1±1.8</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>Diuretic</td>
<td>105.0±8.2</td>
<td>25.5±0.9</td>
<td>52.2±1.6</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Control</td>
<td>117.6±7.1</td>
<td>24.9±1.0</td>
<td>61.5±3.4</td>
<td>2.5</td>
</tr>
<tr>
<td>DM</td>
<td>581.8±15.4</td>
<td>18.6±1.2</td>
<td>88.1±4.5</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>Treated DM</td>
<td>215.1±8.4</td>
<td>22.4±0.8</td>
<td>63.7±2.3</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>Diuretic</td>
<td>101.2±9.0</td>
<td>22.8±0.6</td>
<td>79.8±3.8</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Control</td>
<td>105.8±4.9</td>
<td>25.1±1.1</td>
<td>66.3±3.3</td>
<td>2.6</td>
</tr>
<tr>
<td>DM</td>
<td>548.3±23.6</td>
<td>21.8±0.8</td>
<td>131.3±12.6</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>Treated DM</td>
<td>220.9±11.9</td>
<td>27.5±0.5</td>
<td>70.2±3.6</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>Diuretic</td>
<td>109.4±4.1</td>
<td>26.3±0.7</td>
<td>87.5±6.2</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Control</td>
<td>109.7±5.3</td>
<td>30.5±1.3</td>
<td>72.2±4.6</td>
<td>2.4</td>
</tr>
<tr>
<td>DM</td>
<td>590.7±5.0</td>
<td>19.0±1.5</td>
<td>83.9±3.7</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>Treated DM</td>
<td>284.9±9.0</td>
<td>26.0±0.7</td>
<td>78.5±2.6</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Diuretic</td>
<td>116.1±6.8</td>
<td>31.3±1.4</td>
<td>94.2±3.6</td>
<td>3.0</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE, except for ratio of mean bladder weight to mean body weight. Number of animals (n) = 8 except in the diabetes mellitus (DM) group at 9, 12, and 20 wk; where n = 7, 6, and 7, respectively, and in the control and treated DM groups at 20 wk, n = 7.
Insulin replacement prevented the increased bladder capacity, compliance, and voided and residual volume observed at all time points in DM mice. Basal bladder pressure changed similarly for diuretic and DM groups (interaction: \( P = 0.35 \)), although the diuretic group had on average 25% greater pressure than the DM group (Fig. 5A). The control and treated DM groups showed reductions in basal pressure from 12 to 20 wk, whereas both the DM and diuretic groups stabilized or increased in that last time interval (interaction: \( P = 0.042 \) for DM vs. control, \( P = 0.019 \) for DM vs. treated). Levels of threshold pressure for the DM, treated DM, and diuretic mice were not significantly different from those for controls, although the pattern of change in threshold pressure did differ between control and DM groups (Fig. 5B). The mean intercontraction interval for the control and treated DM mice increased or remained stable after 9 wk (Fig. 5C). The pattern in these groups differed from that in the DM and diuretic groups, which showed changes at each time point (interaction: \( P < 0.02 \) for DM vs. control and treated DM). At 9 wk, the DM mice had a significantly larger interval than the treated DM group. At all other time points, the levels of the interval for the DM mice did not differ significantly from those for the other three groups. Interpretation of the mean intercontraction interval data is complicated by the observation that before the main contraction in each cycle leading to emptying of the bladder, contractions of smaller magnitude occurred in both DM and diuretic animals. Such smaller contractions were not seen in the control and treated DM groups (Fig. 2).

Mean PVP changed over time differently for DM mice compared with all other groups (Fig. 6A). At 3 and 9 wk, the peak pressure increased in parallel in DM and diuretic mice and was higher than in control and insulin-treated DM mice (\( P < 0.05 \) vs. control and insulin-treated DM at 9 wk), but then it sharply diminished by 12 wk only in DM animals (interaction: \( P < 0.001 \) in DM relative to all other groups). In addition, the resting pressure in the DM mice increased over time and was on average 44% higher than that in the control group (\( P = 0.002 \) at 20 wk) (Fig. 6B). The diuretic and treated DM groups showed a significantly different change in resting pressure from the DM group (interaction: \( P < 0.001 \), with a decrease in resting pressure between 9 and 12 wk. Levels of the resting pressure in DM mice were significantly higher than in the diuretic mice at 12 and 20 wk (\( P < 0.001 \)) and significantly higher than in the treated DM mice at 20 wk (\( P = 0.002 \)).

DISCUSSION

The two major and distinct functions of the bladder are urine storage and urine emptying or voiding. Thus disorders of the bladder are commonly categorized as dysfunction of either the storage or the voiding mechanism (1, 29, 30). Evidence for both types of problems can be gathered using either clinical or urodynamic findings. Clinical/symptomatic manifestations of bladder storage problems may include increased urination frequency, urgency, or urinary incontinence (urge, stress or mixed), and urodynamic manifestations may include sensory urgency, restrictive compliance, and uninhibited detrusor mus-
cle contraction, also recognized as detrusor overactivity or instability. Clinical/symptomatic manifestations of bladder emptying or voiding problems may include hesitancy in start of urine flow, slow flow, poor emptying, elevated postvoid residual, and urinary overflow incontinence. Urodynamic manifestations of voiding problems are decreased voiding pressure, slow flow, and high postvoid residuals (1, 29, 30). Diabetic cystopathy is traditionally described as a triad of decreased sensation, increased capacity, and poor emptying, suggesting that pathology may result from voiding dysfunction (13, 14). This traditional view, however, has been challenged over the last few years by a number of investigators who have demonstrated the presence of both storage and voiding problems in patients with diabetes (15, 17, 27, 28).

The clinical picture of diabetic cystopathy is replicated in animal studies of diabetes. Currently, cystometry is the only objective in vivo measure of bladder dysfunction in animal models. Despite differences of micturition physiology between humans and animals, cystometric findings in the animals can adequately represent the same category of bladder dysfunction as urodynamic studies in humans. Altered bladder capacity and compliance, altered storage pressures (basal and threshold pressures), and altered intercontraction intervals reflect storage dysfunction of the bladder, whereas increased voiding pressures and increased resting pressure and residual urine reflect a voiding dysfunction of the bladder. Of the parameters that can be measured during the voiding phase of the micturition cycle in the animals, the PVP is probably the best indicator of the bladder’s ability to empty, because it represents the status of detrusor muscle contractility (25).

Animal studies of diabetic cystopathy show similarly mixed results. Studies of in vivo changes in the majority of small animal DM models (STZ- or alloxan-induced DM in rat or rabbit; spontaneously BB diabetic rats) consistently show increased bladder weight and capacity, and alterations of bladder compliance (3, 18, 20). Increased micturition frequency has been reported in these animals in both early and late diabetes (3, 18, 20).

To investigate the discrepancies reported in diabetic cystopathy in both clinical and experimental settings, we have previously examined the temporal dependency of changes that occur in the diabetic bladder in Sprague-Dawley rats with STZ-induced diabetes vs. nondiabetic controls (8). We observed that the bladder undergoes time-dependent adaptive changes, initially by an increase in the bladder’s voiding pressures during the first 9 wk after induction of diabetes. With
continuation of diabetes, the voiding pressure decreased after 12 wk of diabetes. This drop in the voiding pressure was associated with a decreased emptying ability and an increased postvoid residual in the rats (8).

In diabetic cystopathy, however, confounding effects of increased urine output, or diuresis, on bladder function also have been noted (11, 19). In DM, diuresis is caused by the hyperosmolarity of the urine, whereas nondiabetic diuresis may result from diuretic medications, diabetes insipidus, and any other condition that increases the urine output beyond the normal range. Diuresis causes a mild but significant increase in bladder weight. In animal models of 5% sucrose-induced diuresis, bladder hypertrophy and increased contractility, capacity, and compliance have been observed that are similar to what has been observed in diabetic rats (11). The similarities in diabetic and diuretic bladders suggest the possibility that the bladder hypertrophy and/or increase in bladder activity seen in the early phase of diabetes may simply result from physical adaptation of the bladder to increased urine production. Thus increased urine output, rather than hyperglycemia, may induce the changes in the bladder. In the current study, we aimed to identify the extent to which diuresis alters bladder function in mice.

The aim of this study was to examine the temporal effects of DM, insulin treatment of DM, and diuresis on bladder function in a mouse model. This allowed us to infer differences between

![Fig. 5. Basal bladder pressure (A), mean threshold pressure for leak (B), and reduction in mean intercontraction interval (C) in age-matched DM, insulin-treated DM, diabetic, and control mice. Data are observed means ± SE for each group at each time point, plotted with a log 2 scale ordinate. Significant differences in temporal trends (interaction) between the DM and other groups are as follows: in A, *P = 0.043 relative to control, +P = 0.019 relative to treated DM; in B, *P = 0.022 relative to control; and in C, *P = 0.018 relative to control, +P = 0.006 relative to treated DM.](image)

![Fig. 6. Mean peak voiding pressure (PVP, A) and resting pressure (B) in age-matched DM, insulin-treated DM, diabetic, and control mice. Data are observed means ± SE for each group at each time point, plotted with a log 2 scale ordinate. Significant differences in temporal trends (interaction) between the DM and other groups are as follows: in A, *P < 0.001 relative to control, treated DM, and diabetic groups; and in B, *P < 0.001 relative to treated DM group, +P < 0.001 relative to diabetic group.](image)
effects of DM and diuresis, as well as effects of treatment of DM on the time-dependent changes in the bladder at 3, 9, 12, and 20 wk after induction of diabetes or diuresis. Diabetes caused a decrease in animal weight that was reversed with treatment. Bladder weight increased foremost in DM mice and, to a lesser extent, in diuretic mice compared with treated DM and control mice. The bladder weights of the diabetic mice increased with time and reached the peak value at 12 wk. Both DM and diuretic mice produced much greater 24-h urine outputs than the control and treated DM groups. The output rates produced by DM mice were 130, 78, 135, and 184% greater than the output rates of the diuretic mice at 3, 9, 12, and 20 wk, respectively. The bladder in diabetic and diuretic animals adapted rapidly to the increased urine output by increasing the voided volume. Despite an overall higher rate of urine output in DM mice, the diuretic mice had a higher voided volume per void and much lower residual urine at all times, indicating a more efficient emptying ability in diuretic than in DM mice. Additional evidence for less efficient bladder emptying in the DM animals is shown in the significant drop in mean peak voiding pressure after 12 wk of DM. The significant rise in residual urine in DM mice compared with other groups, including the diuretic, may be the earliest evidence of inefficiency of the bladder’s voiding ability in DM. After insulin replacement, the increased bladder capacity and compliance and increased voided and residual volume were reversed, but 24-h urine output was only partially reversed.

The CMG data demonstrated an increase in bladder capacity at all time points in the DM and diuretic mice, with no significant differences between groups. These results suggest that the increase in bladder capacity in diabetes is indeed a reflection of the increase in urine production. A similar trend was seen in bladder compliance in the DM and diuretic mice, except at 20 wk, when compliance in the diuretic group started to decline. Of greatest interest, the mean PVP, which increased almost equally in DM and diuretic mice during the first 9 wk postinduction, sharply diverged between those two groups after 9 wk. In DM mice, the PVP dropped dramatically by 12 wk of DM to a level significantly lower than in the diuretic mice and slightly lower than in the control and insulin-treated DM mice. The sharp decline in PVP in the DM mice was associated with stable voided volume and increased residual urine; the latter was significantly higher than in the diuretic mice. Together, those data suggest that change in voiding ability of the bladder (as represented by PVP) is not related to urine volume production. This finding distinguishes the changes in the bladder induced by diabetes from those induced by diuresis.

As noted in the CMG tracings of Fig. 2, in both DM and diuretic animals, before each main contraction of the bladder initiating voiding, smaller contractions occur, in contrast to the smooth tracings of control and treated DM mice during the storage period. The representation of the overactivity mimicked the urgency and frequency seen in human subjects (28). This observation also differed from our results in STZ-induced diabetic rats, which exhibited regular intercontraction intervals that were 25% of the intervals in control rats at all time points from 3 to 20 wk, when cystometry was performed under urethane anesthesia (8). Precise interpretation of the intercontraction interval data may require execution of a detailed analysis of the bladder activities that do not lead to full contraction and emptying of the bladder. The overactivity might be altered during anesthetic cystometry.

The findings of the current study support the hypotheses that 1) the types of changes observed in DBD are time dependent; 2) a transition to increased bladder activity, or a compensatory function, in the first 9 wk of diabetes may be due to increased urine output, because it is seen in both DM and diuretic animals; and 3) the bladder’s function declines to a decompensated status after 9 wk of DM in the absence of treatment. Diuresis explains many of the diabetes-induced effects on the bladder, especially during the early phase (first 9 wk in mice) of diabetes, but other diabetes-induced effects are not observed in the diuretic group, especially at and after 12 wk in mice, and are presumed to result from metabolic alterations associated with diabetes. Long-term insulin replacement effectively reversed most changes in diabetes-induced bladder dysfunction. On the basis of these findings, we hypothesize that decompensation in the bladder function after 12 wk of DM may be due mainly to effects of diabetes on the myogenic and neurogenic components of the bladder function. This hypothesis varies from the traditional view in which the disturbance in autonomic neuropathy has been seen as the sole pathophysiological cause of DBD. Our hypothesis is supported by reports from other investigators (22, 32). Mannikarottu et al. (22) investigated the expression of the thin filament-associated proteins in the bladder smooth muscle of New Zealand White male rabbits 6 mo after induction of diabetes with alloxan. The results showed that increased expression of the thin filament proteins calponin, tropomyosin, and caldesmon in diabetic rabbits might alter the contractile and cytoskeletal structures of bladder myocytes. The overexpression of these thin filament-associated proteins, which suppresses actin-myosin interaction and actomyosin adenosine triphosphatase, and the enhancement of this suppression by tropomyosin, are likely to have an effect on the relationship between force and myosin light chain phosphorylation, requiring higher levels of phosphorylation in diabetic detrusor compared with that of control. The downstream metabolic effects of hyperglycemia (e.g., oxidative stress) appear to modulate the transcriptional regulation of thin filament-mediated regulatory proteins in bladder smooth muscle. In regard to disturbances in the neurogenic components of bladder function, studies have demonstrated large reductions in the number of myelinated fibers in the pelvic and hypogastric nerves of 3-mo alloxan-induced diabetic rats. Large- and medium-sized axons were rare or absent, whereas signs of de- and remyelination were present. Axonal sprouts and glycogen particles were noted under electron microscopy (23, 24). A number of mechanistic theories for such deterioration of both myogenic and neurogenic components of the bladder could be postulated, including the effects of accumulation of advanced glycation end products (2) and increased oxidative stress products in the autonomic innervation and detrusor muscle of the bladder (4).

Clinical translation of results of our study would further support the hypothesis that the transition of DM cystopathy into an atonic contractile bladder is a progressive process. If the risk factors for such progression are identified, it is plausible that they could potentially be prevented or stopped. Potential risk factors in humans may include duration of the diabetes for more than 10–15 yr, poorly controlled DM, and additional insults such as neuropathic and other damage to the
lower urinary tract caused by vaginal birth in women with DM. Identification of such risk factors are increasingly important in view of recent large-scale studies indicating that diabetes in men and women is associated with a 30–80% increased risk of urinary incontinence (5, 6, 9, 31). Relevant to the findings of our study is the finding in clinical studies that the risk for urge incontinence (storage problem) was increased by 40–80% in multivariate analysis that controlled for stroke and other chronic medical conditions (6, 9, 31).

The results of the current study are sufficiently intriguing to support further investigation into the molecular basis of the compensation/decompensation hypothesis in animal models. In addition, these data could justify initiation of a clinical study in which evidence about compensation/decompensation phenomena could be gathered. Performance of those studies is critical to provide a reliable and accurate description of diabetic cystopathy in humans.

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