Voiding function in obese and type 2 diabetic female rats

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1Research Service, Louis B. Stokes Veterans Affairs Medical Center, 2Department of Biomedical Engineering, Lerner Research Institute, and 3Glickman Urological and Kidney Institute, Cleveland Clinic, and 4Department of Urology, University Hospital, Case Western Reserve University, Cleveland, Ohio

Submitted 29 May 2009; accepted in final form 19 October 2009

Gasbarro G, Lin DL, Vurbic D, Quisno A, Kinley B, Daneshgari F, Damaser MS. Voiding function in obese and type 2 diabetic female rats. Am J Physiol Renal Physiol 298: F72–F77, 2010. First published November 4, 2009; doi:10.1152/ajprenal.00309.2009.—The effects of obesity and type 2 diabetes (DMII) on the lower urinary tract (LUT) were characterized by evaluating voiding function and anatomy in female Zucker diabetic fatty (ZDF) rats. Age-matched female virgin rats were separated into three experimental groups: Zucker lean rats (control; normal diet, n = 22), ZDF rats (obese+non-diabetic; low-fat diet, n = 22), and ZDF rats (obese+diabetic; high-fat diet, n = 20). Rats were placed on their specified diet for 10 wk before urodynamic LUT evaluation. A suprapubic catheter was implanted 2 days before urodynamic studies.Voiding function was evaluated by cystometric and leak point pressure (LPP) testing. The bladder, urethra, and vagina were immediately excised for qualitative histological evaluation. Compared with control rats, obese+non-diabetic and obese+diabetic rats had significantly decreased contraction pressure (P = 0.003) and increased cystometric filling volume (P < 0.001). Both obese groups exhibited significantly higher voided volumes (P = 0.003), less frequent urinary events (P < 0.001), and increased residual volumes (P = 0.039). LPP studies showed a nonsignificant decrease in LPP (P = 0.075) and baseline pressure (P = 0.168) in both obese groups compared with control. Histology of the external urethral sphincter in obese rats showed increased fibrosis, leading to disruption of the skeletal muscle structure compared with control. Additionally, the bladder wall of the obese+non-diabetic and obese+diabetic rats demonstrated edema and vasculopathy. Voiding dysfunction was evident in both obese groups but with no significant differences due to DMII, suggesting that voiding dysfunction in DMII may be attributable at least in part to chronic obesity.

diabetes mellitus type 2; urinary incontinence; urodynamics; urethra; bladder

THE PREVALENCE OF OBESITY, defined as a chronic condition of excess adipose tissue commonly characterized by a body mass index (BMI) greater than or equal to 30, has become a national and global epidemic. In 2000, an estimated 15–20% of the population in established market economies were clinically obese (BMI ≥ 30 kg/m²) (14). These rates have steadily risen over time, increasing the risk of comorbidities including type 2 diabetes mellitus (DMII), cardiovascular disease, and stroke (14). Diabetes alone was estimated to affect 135 million people in 1995 and is projected to affect 300 million by 2025 (28). Both obesity and diabetes have been established as independent risk factors for urinary incontinence (UI), the involuntary leakage of urine (3, 9). Considered to be a major social burden, the prevalence of weekly incontinence episodes among postmenopausal women has been shown to increase as a result of age, obesity, and diabetes (3).

Although UI is a complication of obesity and DMII, no previous investigation of voiding function in an obese rat model has been made. Such a model could be useful in evaluating the effects of obesity and its comorbidities. Previous animal models have focused on the effects of type 1 diabetes (DMI) on micturition behavior: streptozotocin (STZ) has been used to induce diabetes in both rats and mice (2, 8, 20, 32) while an additional model of spontaneous biobreeding rats has also been utilized (22). These studies have shown the increased bladder weight, capacity, and compliance typical of the clinical presentation of diabetes (17).

In the present study, we used obese female Zucker diabetic fatty (ZDF) rats, which feature a leptin receptor gene mutation and have been shown to develop DMII (27). Male ZDF rats develop DMII spontaneously, regardless of diet; in contrast, females only develop DMII when placed on a high fat diet (7). Our objective was to characterize lower urinary tract (LUT) function and anatomy in obese+non-diabetic and obese+diabetic animals compared with control. We hypothesized that obesity and its comorbidities detrimentally affect normal function of the LUT in ZDF rats, providing a model for further studies.

MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee of the Louisiana B. Stokes Veterans Affairs Medical Center. Sixty-four age-matched female virgin rats (5 wk old) were divided into three experimental groups: Zucker lean rats (control; n = 22) fed a normal lab diet (catalog no. 2018SX, Harlan Laboratories, Indianapolis, IN); ZDF rats (obese+non-diabetic; n = 22) fed a low-fat diet (12.3 kcal% fat, catalog no. C11000i, Research Diets, New Brunswick, NJ); and ZDF rats (obese+diabetic; n = 20) fed a high-fat diet (48 kcal% fat, catalog no. C13004i, Research Diets).

All rats were on their specified diet for 10 wk before urodynamic testing. Animal weight was recorded weekly, and blood glucose levels were recorded biweekly by tail vein blood sampling using an Accu-Chek Advantage glucometer (Roche Diagnostics, Basel, Switzerland) while the animal was gently restrained in a holder (7). Obese+diabetic animals whose blood glucose was <250 mg/dl were excluded from the study.

Catheter implantation. Two days before urodynamic testing, the animals underwent suprapubic bladder catheter implantation as previously reported (25). Rats were anesthetized by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). An abdominal incision was made ~1 cm above the urethral meatus, and a circular purse-string suture was placed on the bladder wall. A small incision was made in the dome of the bladder, and the catheter (PE-50 tubing with flared tip) was implanted. The catheter was subsequently tunneled subcutaneously to the back of the neck and through the skin, out of the reach of the animal. The catheter was capped and secured to the neck, and the incision was closed in two layers.

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Submitted 29 May 2009; accepted in final form 19 October 2009

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**Conscious cystometry.** Uninterrupted cystometry (CMG) was performed via the implanted suprapubic bladder catheter with rats placed in a modified metabolic cage as previously described (25). The bladder catheter was connected to both a syringe pump (model 200; KD Scientific, New Hope, PA) and a pressure transducer (model PT300; Grass Instruments, West Warwick, RI). Saline was infused into the bladder at 5 ml/h while pressure and volume voided were continuously recorded. Voiding contractions were defined by a bladder pressure increase related to urine loss, as detected by a force transducer (model FT10; Grass Instruments) calibrated to measure volume under the cage. At least three voiding events were recorded for each animal. Pressure and force signals were amplified (model P122; Grass Instruments) and digitized (10 samples/s) by a Dash8Xe (AstroMed, West Warwick, RI) data acquisition recorder. Following the uninterrupted cystometry, a period of interrupted cystometry was performed in which residual volume was determined after each void by detaching the bladder catheter from the tubing leading to the pressure transducer and lowering it to create a pressure differential, siphoning the residual urine into a preweighed vial. For each rat, the mean threshold pressure, contraction pressure, voiding frequency, volume voided, fill volume, void time, residual volume, and nonvoiding contractions were calculated. Threshold pressure was defined as the recorded pressure just before the onset of each micturition phase (16). Contraction pressure was calculated by subtracting threshold pressure from peak voiding pressure (maximum pressure recorded during micturition). Voiding frequency was derived entirely from periods of uninterrupted cystometry. A mean of three voiding events in each animal was calculated for each variable and used to calculate group means.

**Leak point pressure testing.** Following CMG, animals were anesthetized by an intraperitoneal injection of urethane (1.2 g/kg) and placed in a supine position at the level of reference pressure for leak point pressure (LPP) testing as previously described (25). The bladder catheter was connected to the pressure transducer and flow pump, and pressure data were amplified, digitized, and recorded as above. Each bladder was filled to approximately half capacity, and gentle pressure was applied to the abdomen to induce leakage. The external abdominal pressure was rapidly removed at the first sign of fluid leakage at the urethral meatus. Baseline pressure and peak pressure at leakage in the absence of detrusor contraction were recorded. An increase in pressure from baseline (LPP) was calculated as baseline pressure subtracted from peak pressure and used to assess urethral resistance to leakage (5, 6). Three independent LPP values were measured in each animal, and the mean was used for calculation of group means.

**Histology.** After LPP testing, the animals were euthanized and the urethra, vagina, and bladder were dissected for histological evaluation. Prior to excision, each animal underwent intracardiac perfusion with 0.9% saline as previously described (25). Tissues were subsequently immersed in 10% formalin, processed, embedded in paraffin, sectioned transversely (5 μm), and stained with Masson’s trichrome.

**Data analysis.** Quantitative variables are given as means ± SE. One-way analysis of variance on ranks followed by Dunn’s post hoc test were used to compare the three different groups. P < 0.05 was used to indicate significant differences between groups. Histology was analyzed qualitatively.

**RESULTS**

**Weight and blood glucose.** After 10 wk on the specified diet, at 15 wk of age, both obese + nondiabetic and obese + diabetic rats weighed significantly more (P < 0.001) than control rats (Table 1). Obese + diabetic rats had significantly increased blood glucose levels (P < 0.001) compared with both obese + nondiabetic and control rats (Table 1). These animals were evaluated on average 5.82 ± 0.54 wk after the development of diabetes as defined by blood glucose levels >250 mg/dl. Six obese + diabetic animals were excluded from the study due to blood glucose levels failing to reach this threshold.

<table>
<thead>
<tr>
<th>Weight, g</th>
<th>Control</th>
<th>Obese + Nondiabetic</th>
<th>Obese + Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose, mg/dl</td>
<td>100 ± 11.1</td>
<td>125 ± 12.4</td>
<td>385 ± 67.6*†</td>
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</tbody>
</table>

Values are means ± SE. *Significant difference compared with control (P < 0.001). †Significant difference compared with obese + nondiabetic (P < 0.001).

**Conscious cystometry and LPP testing.** Both voiding frequency (P < 0.001) and nonvoiding contractions (P < 0.001) were significantly decreased in both obese groups compared with control rats (Fig. 1; Table 2). There was also a significant decrease in void time (P = 0.006) of the obese + nondiabetic group compared with both the obese + diabetic and control groups (Table 2). Both obese + diabetic and obese + nondiabetic animals had a significant decrease in both contraction pressure (P = 0.002) and threshold pressure (P < 0.001) compared with the control group (Fig. 2). Fill volume (P < 0.001), volume voided (P < 0.001), and residual volume (P < 0.001) of both obese groups were significantly increased compared with control rats (Fig. 2). LPP in both obese groups was decreased (P = 0.075) compared with control rats, but this difference was not statistically significant (Fig. 2). There was no significant difference in baseline pressure between groups (P = 0.168; data not shown).

**Histology.** The external urethral sphincter in both obese groups demonstrated increased fibrosis and edema of the periurethral muscularis. This collagen infiltration led to marked disruption of the striated muscular structure compared with control rats (Fig. 3). The bladder wall of the obese + nondiabetic and obese + diabetic rats demonstrated edema and vasculopathy (Fig. 4). Within the detrusor muscle layer, vascular channels were dilated, which may contribute to the visible alterations in the microenvironment of the smooth muscle and connective tissue structure of the bladder (Fig. 4).

**DISCUSSION**

The prevalence of obesity has grown rapidly worldwide, increasing the occurrence of associated high-risk comorbidities and placing financial burdens on healthcare services. The World Health Organization estimates that by 2015, nearly 700 million adults will be chronically obese (15). In addition to increasing the risk of diabetes and cardiovascular disease, obesity has strongly been linked to UI. Studies have shown higher risks of UI among obese women, but there is currently no relationship suggesting that detrusor function is compromised (12, 24). Recent evidence has shown that obese patients on a weight loss program show a greater decrease in the frequency of stress UI episodes, but not of urge UI episodes compared with control (29). However, the relationship between weight loss and reduction of stress UI episodes cannot alone be attributed to lower BMI but from concurrent improvement of health associated with weight loss in these patients (29).

Increased risk of DMII has been strongly linked to chronic obesity. DMII is defined by the nonresponsive nature of target tissues to insulin levels in the blood (1). By 2025, an estimated
300 million people worldwide will suffer from diabetes, placing additional constraints on clinicians and health care providers attempting to deal with comorbidities of the disease (28). Recent evidence from The Nurses’ Health Study suggests that DMII increases the prevalence of UI, which correlates with other epidemiological studies (4, 10, 18). Current understanding correlates LUT dysfunction with changes in the detrusor muscle, urothelium, and central nervous system control of the bladder, but comprehensive risk factors for the development of UI remain unclear (1, 33). Clinical urodynamic studies suggest that the effects of DMII on voiding in women produces lower maximal flow rates and higher residual volume than age-matched controls, but conflicting data exist (17).

Animal models allow for investigation into the mechanisms of UI under controlled conditions. Previous animal studies of voiding dysfunction have focused on the effects of diabetes but have not characterized the role of obesity. Longhurst et al. (22) evaluated micturition behavior in insulin-treated spontaneously diabetic biobreeding rats. Compared with a nondiabetic control, these animals both consumed and excreted larger volumes of water. Bladder mass, as well as voiding frequency and volume, were significantly increased among the DMI rats (22). Andersson et al. (2) studied DMI rats induced by STZ injection. These animals exhibited increased voiding frequency, volume, bladder compliance, and total output over 24 h compared with a nondiabetic control group. Liu et al. (20) reported that STZ-induced DMI causes anatomic, in addition to functional abnormalities of the external urethral sphincter in nonobese female Sprague-Dawley rats. Diabetes was shown to increase the bladder weight, threshold volume, contraction duration, and residual volume compared with controls (20).

Table 2. Cystometric parameters

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Obese + Nondiabetic</th>
<th>Obese + Diabetic</th>
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<tbody>
<tr>
<td>Void time, s</td>
<td>19.1 ± 0.9</td>
<td>13.0 ± 1.5†‡</td>
<td>17.3 ± 2.1</td>
</tr>
<tr>
<td>Voiding frequency, voids/h</td>
<td>13.4 ± 0.9</td>
<td>6.9 ± 0.8†</td>
<td>5.4 ± 0.9†</td>
</tr>
<tr>
<td>Nonvoiding contractions, contractions/h</td>
<td>9.1 ± 2.0</td>
<td>0.7 ± 0.4†</td>
<td>1.0 ± 0.6†</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significant difference compared with control (P < 0.01). †Significant difference compared with control (P < 0.001). ‡Significant difference compared with obese + diabetic.
Yang et al. (32) also demonstrated reduced peak bladder pressure in STZ-induced rats. ZDF rats provide the opportunity to further understand alterations that obesity and DMII have on LUT function. This animal model could be used to develop a better understanding of UI and aid in determining clinical therapies for future patients. To date, no investigation into voiding function in obese rats has been made, although obesity has been strongly linked to UI (12, 24). However, the Zucker breed, including the lean animals, genetically suffers from hydronephrosis (23, 31) and therefore does not give an exact representation of the clinical conditions of obesity and DMII. Additionally, ZDF rats in the obese + diabetic group were fed a high-fat diet (catalog no. C13004i, Research Diets) for 10 wk to induce diabetes. An ingredient in this diet changed in 2007, although all of the animals included in this study were fed the original formula. This formula is presently unavailable, and its replacement (catalog no. C13004L, Research Diets) fails to induce diabetes.

Fig. 2. Urodynamic pressure and volume outcomes of control (open bars), obese + nondiabetic (hatched bars), and obese + diabetic (filled bars) animals at 15 wk of age after 10 wk on the diet. A: cystometric contraction pressure. B: cystometric threshold pressure. C: leak point pressure (LPP). D: cystometric fill volume. E: cystometric volume voided. F: cystometric residual volume. Values are means ± SE of control (n = 22), obese + nondiabetic (n = 22), and obese + diabetic (n = 20) rats. *Significant difference compared with control (P < 0.01). **Significant difference compared with control (P < 0.001).

Fig. 3. Sample cross sections of external urethral sphincter (A–C, ×10 magnification; D–F, ×40 magnification) from a control animal (A and D), an obese + nondiabetic animal (B and E), and an obese + diabetic animal (C and F) at 15 wk of age after 10 wk on the diet. The yellow arrow indicates areas of fibrosis infiltrating the perirectal muscularis, the black arrows show local edema, and the green arrow marks disruption of striated muscle structure. Sections are stained with Masson’s trichrome. Bars = 50 μm.
in female ZDF rats. This has been noted by several investigators, Charles River Laboratories, and Research Diets.

A number of DMII animal models have been developed, including common laboratory animals such as mice, rats, dogs, and nonhuman primates (26). Frequently used models include ob/ob mice, db/db mice, Goto-Kakizaki (GK) rats, and ZDF rats (13). Ob/ob mice exhibit leptin deficiency, while db/db mice have an autosomal recessive mutation of the diabetes (db) gene, leading to obesity and DMII. Mutated C57BL/KsJ mice present with early hyperinsulinemia and escalating hyperglycemia (13), developing DMII regardless of diet. The GK rat is a nonobese DMII model that fails to fully replicate the human metabolic syndrome. GK rats, created through selective breeding of Wistar rats, develop hyperglycemia without associated obesity and hyperlipidemia (13). The leptin-resistant ZDF rats, in contrast, more closely represent the complications of DMII in humans, including hyperglycemia, hyperlipidemia, and obesity. Furthermore, female ZDF rats only develop DMII when fed a high-fat diet, providing the opportunity to study obesity with and without the complications of DMII (7).

We examined LUT function in female ZDF rats to characterize the effects of obesity alone and with DMII comorbidity. Voiding frequency and nonvoiding contractions were significantly decreased in both obese+nonobese and obese+DMII animals, suggesting that obesity may reduce the activity of the bladder. This was further demonstrated by a significant decrease in contraction pressure in both obese groups, similar to the decrease seen in the STZ-induced DMII rats (32). In agreement with previous DMII studies (2, 8, 20, 22, 31), both fill volume and volume voided were significantly increased in both obese groups compared with control rats. These outcomes imply an increase in bladder capacity and, potentially, compliance as seen in the biobreeding and STZ rat models (2, 22). Significant increases in residual volume were observed in both obese+nonobese and obese+DMII groups as well, as previously described in STZ-induced diabetic animals (20). CMG outcomes suggest increased bladder capacity, higher voided volumes, and larger residual volumes in both obese groups. LPP studies showed a nonsignificant reduction of LPP and baseline pressure in both obese groups compared with control. That diabetes had no further effect is in agreement with previous work with STZ-induced diabetic rats (19).

The histological structure of the external urethral sphincter in obese rats revealed increased fibrosis, leading to disruption of the skeletal muscle structure (Fig. 3). This was similar to outcomes in STZ-induced diabetic animals, with reported smooth and striated muscle dysfunctions of the urethral outlet (30). Histology also revealed that the bladder wall of the obese+nonobese and obese+diabetic rats demonstrated edema and vasculopathy incrementally from control to obese+nonobese to obese+diabetic (Fig. 4). Overall collagen content was reduced as shown previously in STZ-induced diabetic animal bladders compared with control (11).

The ZDF animals in this study were evaluated ~6 wk after the onset of DMII. Hydronephrosis, in conjunction with the complications of diabetes, limits the ability to keep these animals alive after surgery over longer time points. Previous studies have used insulin treatment to keep spontaneously diabetic biobreeding rats alive for up to 6 mo (21), whereas most animals models of DMII range between 2 wk and 2 mo (2, 20, 22, 31). Future studies will address the temporal effects of obesity and DMII on voiding function to further characterize the functional and anatomic changes of the LUT.

The current study demonstrates that the function of the LUT is altered in obese animals when contrasted with controls, but with no significant differences in voiding outcomes due to DMII. This suggests that voiding dysfunction in DMII may be attributable in part to other aspects of chronic obesity, such as hyperlipidemia. Therefore, early onset of DMII may not present deleterious effects on LUT function additional to those attributed to obesity. Further development of this model and
other models of DMII are necessary to understand the underlying pathophysiological mechanisms of UI in obese and diabetic women.

ACKNOWLEDGMENTS

The authors thank Paul Fletter for technical assistance.

GRANTS

This work was supported in part by the Rehabilitation Research and Development Service of the Department of Veteran Affairs and the Cleveland Clinic.

DISCLOSURES

No conflicts of interest are declared by the authors.

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