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RESEARCH ARTICLE

The effects of neuromodulation in a novel obese-prone rat model of detrusor underactivity

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Gonzalez EJ, Grill WM. The effects of neuromodulation in a novel obese-prone rat model of detrusor underactivity. *Am J Physiol Renal Physiol* 313: F815–F825, 2017. First published June 21, 2017; doi:10.1152/ajprenal.00242.2017.—Obesity is a global epidemic associated with an increased risk for lower urinary tract dysfunction. Inefficient voiding and urinary retention may arise in late-stage obesity when the expulsive force of the detrusor smooth muscle cannot overcome outlet resistance. Detrusor underactivity (DUA) and impaired contractility may contribute to the pathogenesis of nonobstructive urinary retention. We used cystometry and electrical stimulation of peripheral nerves (pudendal and pelvic nerves) to characterize and improve bladder function in urethane-anesthetized obese-prone (OP) and obese-resistant (OR) rats following diet-induced obesity (DIO). OP rats exhibited urinary retention and impaired detrusor contractility following DIO, reflected as increased volume threshold, decreased peak micturition pressure, and decreased voiding efficiency (VE) compared with OR rats. Electrical stimulation of the sensory branch of the pudendal nerve did not increase VE, whereas patterned bursting stimulation of the motor branch of the pudendal nerve increased VE twofold in OP rats. OP rats required increased amplitude of electrical stimulation of the pelvic nerve to elicit bladder contractions, and maximum evoked bladder contraction amplitudes were decreased relative to OR rats. Collectively, these studies characterize a novel animal model of DUA that can be used to determine pathophysiology and suggest that neuromodulation is a potential management option for DUA.

detrusor underactivity; obesity; electrical stimulation; voiding efficiency

OBESITY is a global health epidemic associated with an increased risk for diabetes, cardiovascular diseases, stroke, and cancer (22). More recently, obesity was identified as a risk factor for the onset and progression of lower urinary tract symptoms (23). Storage and voiding dysfunction may occur from visceral fat deposition, tissue remodeling, circulating metabolites, and/or peripheral neuropathy that can collectively contribute to an overactive or underactive bladder symptom complex (17, 30). The associations between overactive bladder and obesity have been extensively covered in women (12); however, given the chronicity of obesity, the dysfunctional bladder may also progress to an underactive phenotype (7). Inefficient voiding and urinary retention arise when intravesical pressure in the urinary bladder cannot overcome the resistance through the urethra and occurs with increased outlet resistance and/or detrusor underactivity (DUA).

DUA is an understudied health concern that affects between 10 and 45% of men and women in secondary care (19, 32). DUA is defined by the International Continence Society as a contraction of reduced strength and/or duration that results in prolonged and/or incomplete bladder emptying (1). The clinical management of DUA is inadequate and fails to improve quality of life (14). The limited availability of animal models that exhibit the integrated pathophysiology of DUA impedes the development of new therapeutic approaches (14). To address this limitation, we characterized bladder dysfunction of an animal model of DUA that exhibited urinary retention following diet-induced obesity (DIO).

Considering that pharmacological therapies to increase bladder contractility are ineffective in DUA (32), we also investigated neuromodulation approaches to increase voiding efficiency (VE). We sought to recover efficient voiding by *1*) increasing sensory feedback from the urethra through electrical stimulation of the sensory branch of the pudendal nerve, *2*) reintroducing phasic EUS bursting activity by patterned electrical stimulation of the motor branch of the pudendal nerve, or *3*) evoking urinary bladder contractions through electrical stimulation of the pelvic nerve. If effective, targeting peripheral bladder nerves with electrical stimulation offers an alternative therapeutic approach to manage DUA.

MATERIALS AND METHODS

Animals

Female obese-prone (OP, 214–240 g) and obese-resistant (OR, 133–167 g) rats purchased at 8 wk old from Charles River Laboratories (Boston, MA) were housed two per cage and maintained in standard laboratory conditions with food and water available ad libitum. Animal care and experimental procedures were approved by the Duke University Institutional Animal Care and Use Committee and experimentation was in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (8th ed.).

High-Fat Feeding and Blood Sampling

OP and OR rats were fed a 45 kcal% fat diet (D12451, Research Diets) from 9 to 21 wk old and a 60 kcal% fat diet (D12492, Research Diets) from 21 to 24 wk old. Blood samples (1 ml) were collected from the tail vein during the light cycle of fasted (5 h) OP and OR rats at 8 and 24 wk old. Blood glucose (mg/dl) was immediately assayed with a monitoring system kit (AlphaTRAK 2, Abbott Animal Health).

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The remaining whole blood was allowed to clot for 45 min at room temperature and then centrifuged (1,200 RCF, 15 min). The resulting supernatant (serum) was aspirated and stored at −80°C until processing. Serum insulin (μIU/ml), blood urea nitrogen (mg/dl), creatinine (mg/dl), and a lipid profile were assayed by IDEXX BioResearch (North Grafton, MA).

Surgical Procedures

At 24 wk old, OP and OR rats were anesthetized with urethane (1.2 g/kg sc and supplemented as needed) 1 h before surgical procedures for acute terminal experiments. Body temperature was monitored and adjusted to 37°C by a thermostatically controlled warming pad (ATC2000, World Precision Instruments, Sarasota, FL). Heart rate and arterial blood oxygen saturation levels were monitored by a pulse oximeter (2500A, Nonin Medical, Plymouth, MN).

With the animal in a prone position, the motor and sensory branches of the pudendal nerve were accessed using a posterior approach as previously described (26). Briefly, the gluteus muscles were incised, the ilium and sacrum were separated, and the pudendal nerves were isolated from connective tissue and vasculature. A bipolar cuff electrode (3 mm length, 0.3 mm inner diameter, CorTec, Freiburg, Germany) was placed on the motor or sensory branch of the pudendal nerve for electrical stimulation. The leads of the nerve cuff were secured to the skin and the incision was closed with 3–0 silk suture.

After placement of the nerve cuff, the animal was turned to a supine position and the urinary bladder was exposed through a lower midline abdominal incision. In experiments with electrical stimulation of the pelvic nerve, the nerve was isolated ~5 mm distal to the bladder and a bipolar cuff electrode (2 mm length, 0.2 mm inner diameter, CorTec) was placed. After the nerve cuff was secured, a flared PE-60 polyethylene catheter was inserted into the bladder lumen through an incision in the bladder dome and secured with 6–0 silk suture. The catheter was connected by a three-way stopcock to an infusion pump (PHD 4400, Harvard Apparatus, Holliston, MA) and to a pressure transducer and amplifier (ETH-255, CB Sciences) to measure intravesical pressure. A silicone paddle with two platinum-iridium contacts (Micro-Leads, Boston, MA) was also placed between the pubic symphysis and the external urethral sphincter (EUS) and connected to an amplifier (ETH-255) to measure EUS electromyogram (EMG). Pressure and EMG signals were amplified, filtered, and sampled at 400 or 4,000 Hz, respectively, by a PowerLab 8/30 recording unit (AD Instruments, Colorado Springs, CO) and displayed by LabChart 7 Pro (v7.3.7, AD Instruments) for off-line analysis (26). At the conclusion of the experiment, animals were euthanized (500 mg Euthasol ip) and the urinary bladder and urethra were excised and weighed.

Cystometry

Before the collection of data, the urinary bladder was allowed to recover for 45 min with a continuous infusion of 0.9% sodium chloride injection, USP (3–8 ml/h) and the urethra open. The rate of saline infusion was adjusted in each animal to achieve 8 min intermicturition intervals. Voided volumes were collected and measured with a 1-ml syringe after each voiding event.

Sensory branch of the pudendal nerve. Following the control period, the bladder was emptied and the current threshold to evoke a reflex response in the EUS from electrical stimulation of the sensory branch of the pudendal nerve was determined at 1 Hz. Single-trial cystometry ($n = 4$ OP and $n = 3$ OR rats) then began by filling (3–7 ml/h) the bladder until a voiding event, stopping the infusion pump, recording infused, residual, and voided volumes, and waiting 3 min before the next trial. Regulated current, biphasic stimulus pulses were applied with a pulse width of 0.1 ms, frequency between 5 and 20 Hz, and 0.125–0.5 times the EUS reflex threshold amplitude. An increase in bladder contraction area was observed in response to low-intensity (0.025–0.2 mA) stimulation suggesting larger-diameter nerve fibers contributed to this excitatory response, whereas higher amplitudes produced inhibition of the bladder (9, 34). Therefore, we chose to span frequencies (5–20 Hz) and amplitudes (<1 T). Electrical stimulation trials ($n = 46$ trials for OP, $n = 53$ trials for OR) were randomized as either continuous stimulation throughout the filling phase or conditional stimulation begun at the onset of the voiding event and many of the parameters had at least 2 replicates that were averaged within animals. The infusion pump and electrical stimulation continued for 60 s after signs of the first leak in trials of OP rats to ensure a bladder contraction would not occur.

Motor branch of the pudendal nerve. Similar to the preparation above, the bladder was emptied and the current threshold to evoke a maximum EUS response from electrical stimulation of the motor branch of the pudendal nerve was determined at 1 Hz. Based upon prior work quantifying pudendal efferent bursting activity during voiding (11), biphasic pulses to the motor branch of the pudendal nerve were patterned to evoke 3 maximum EUS EMG responses at a frequency of 40 Hz with a 160 ms interburst frequency (26). Single-trial cystometry ($n = 4$ OP and $n = 4$ OR rats) began by filling (3–6 ml/h) the bladder until a voiding event, initiating electrical stimulation for 10–15 s (OR rats) or 90 s (OP rats), stopping the infusion pump, recording infused, residual, and voided volumes, and waiting 3 min before the next trial. The trials ($n = 47$ trials for OP, $n = 65$ trials for OR) were randomized within animals, and at least six repeated measurements were averaged for each condition per animal. The time after a voiding event in OP rats was consistent across the no-stimulation and stimulation trials (i.e., 90 s after a void with no stimulation before ending the trial).

Pelvic nerve. Electrical stimulation of the pelvic nerve was assessed at the conclusion of pudendal nerve (sensory or motor branches) stimulation trials in OP rats. Carry-over effects were not observed from the previous stimulation trials. As in the previous preparations, the bladder was emptied and the current threshold to evoke an EUS reflex from electrical stimulation of the pelvic nerve was determined at 1 Hz. Single-trial cystometry ($n = 7$ OP rats) began by filling (4–8 ml/h) the bladder until a voiding event, applying electrical stimulation to the pelvic nerve for 10 s, stopping the pump, recording infused, residual, and voided volumes, and waiting 3 min before the next trial. Biphasic stimulus pulses applied to the pelvic nerve had a pulse width of 0.1 ms, frequency of 40 Hz, and 4–10 times the EUS reflex threshold amplitude. Stimulation trials ($n = 67$ trials for OP) were randomized within animals and many of the parameters had at least 2 replicates that were averaged within animals. At the conclusion of single-trial cystometric studies ($n = 7$ OP and $n = 7$ OR rats), a bolus to 50% of bladder capacity was instilled into the bladder. Once intravesical pressure stabilized, biphasic stimulus pulses were applied to the pelvic nerve for 10 s with a frequency between 10 and 40 Hz and 1–10 times the EUS reflex threshold amplitude. For each animal, the trials ($n = 160$ trials for OP, $n = 243$ trials for OR) were randomized into blocks spanning the frequency range of 10–40 Hz and blocks were repeated at least 2–3 times with measurements averaged within animals.

Data Analysis

The Homeostasis Model Assessment (HOMA) and the Quantitative Insulin Sensitivity Check Index (QUICKI) were calculated as indexes of insulin sensitivity. HOMA was calculated as (fasting glucose × fasting insulin)/2,430 and QUICKI was calculated as 1/[log(fasting glucose) + log(fasting insulin)] (5). Cystometrograms were analyzed using the following parameters: pressure at volume threshold (cmH_2O), defined by the user at the first deflection of pressure that occurred during a voiding event; filling pressure (cmH_2O), the mean value of the data points between the start of the infusion pump and the

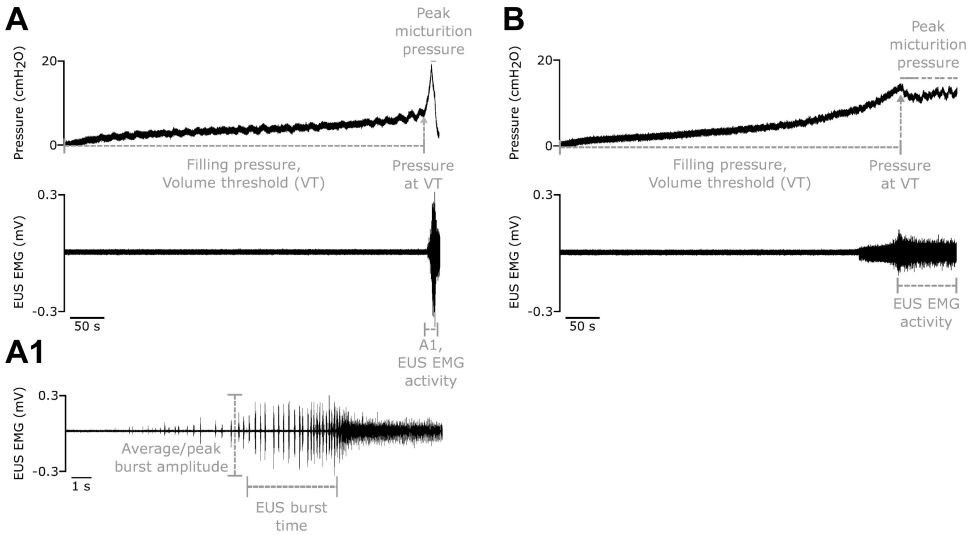

Fig. 1. Single-trial cystometrogram parameters evaluated in obese-resistant and obese-prone rats. Representative traces of intravesical pressure and external urethral sphincter (EUS) EMG measuring filling pressure (cmH_2O), volume threshold (ml), pressure at volume threshold (cmH_2O), peak micturition pressure (cmH_2O), and EUS EMG activity (spikes/s) in obese-resistant (*A*) and obese-prone (*B*) rats. *A1*: an expanded EUS EMG trace from *A*, measuring average and peak bursting amplitude (mV) and bursting time (s).

user-defined pressure at volume threshold; peak micturition pressure (cmH_2O), the value of the largest data point within opening or closing pressure; volume threshold (ml), the volume instilled into the bladder from the start of the infusion pump to the user-defined pressure at volume threshold; evoked contraction amplitude (cmH_2O), the difference between the largest and smallest data points within the selected bladder contraction; EUS EMG activity during voiding, the number of spikes per second above the user-defined noise threshold between the bladder contraction start and bladder contraction end; average EUS bursting amplitude (mV), the average amplitude of EUS bursting spikes; peak EUS bursting amplitude (mV), the largest data point in EUS bursting activity; EUS bursting time (s), the average time of EUS bursting activity per single-trial cystometrogram; and VE (%), expressed as the percentage of voided volume divided by the sum of the voided volume and residual volume (see Fig. 1, *A* and *B*).

All values are reported means ± SE. Body weight data were compared with two-way repeated-measures analysis of variance (ANOVA), VE were compared with one-way or two-way ANOVA or two-tailed Student's paired t-test, and the remaining data were compared with two-tailed Student's unpaired or paired *t*-test as appropriate. When *F*-test statistic exceeded the critical value at $\alpha = 0.05$, the Bonferroni multiple comparisons test was used to compare group means.

Table 1. *Body and serum measurements pre and post diet-induced obesity (DIO)*

	Pre-DIO	Two-Tailed Unpaired *t*-Test	Post-DIO	Two-Tailed Unpaired *t*-Test
Obese-resistant				
Body weight, g	154 ± 4.28		270 ± 6.70	
Bladder weight, g			0.14 ± 0.01	
Glucose, mg/dl	154 ± 8.63		177 ± 6.09	
Insulin, μIU/ml	6.335 ± 1.374		8.946 ± 2.839	
Triglycerides, mg/dl	46 ± 4.31		112 ± 64.8	
Cholesterol, mg/dl	72.6 ± 2.35		101 ± 3.33	
HDL cholesterol, mg/dl	29 ± 1.3		40 ± 1.1	
LDL cholesterol, mg/dl	7 ± 0		7 ± 0	
BUN, mg/dl	14 ± 0.6		13 ± 0.4	
Creatinine, mg/dl	0.2 ± 0.01		0.2 ± 0	
HOMA	0.3888 ± 0.0831		0.6236 ± 0.1732	
QUICKI	0.3465 ± 0.0111		0.3259 ± 0.0102	
Obese-prone				
Body weight, g	230 ± 2.59****	$P \leq 0.0001$	450 ± 11.1****	$P \leq 0.0001$
Bladder weight, g			0.14 ± 0.01	$P = 0.9558$
Glucose, mg/dl	136 ± 11.7	$P = 0.276$	169 ± 7.11	$P = 0.4544$
Insulin, μIU/ml	9.202 ± 2.091	$P = 0.2826$	36.54 ± 10.81*	$P = 0.041$
Triglycerides, mg/dl	74.7 ± 9.49*	$P = 0.0223$	343 ± 67.9**	$P = 0.0098$
Cholesterol, mg/dl	66 ± 2.36	$P = 0.067$	90.5 ± 6.06	$P = 0.1626$
HDL cholesterol, mg/dl	28 ± 1.0	$P = 0.7476$	32 ± 1.2****	$P \leq 0.0001$
LDL cholesterol, mg/dl	7 ± 0		8 ± 0.7	$P = 0.1362$
BUN, mg/dl	18 ± 0.6***	$P = 0.0007$	15 ± 0.7	$P = 0.2166$
Creatinine, mg/dl	0.3 ± 0.01**	$P = 0.0023$	0.2 ± 0.01	$P = 0.2011$
HOMA	0.5482 ± 0.1552		2.465 ± 0.6664	
QUICKI	0.3361 ± 0.012		0.2867 ± 0.0147	

All values are reported as means ± SE. Following DIO, obese-prone (OP) rats exhibited decreased HDL cholesterol relative to obese-resistant (OR) rats and developed hyperinsulinemia and hypertriglyceridemia. BUN, blood urea nitrogen; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; LDL, low-density lipoprotein; QUICKI, Quantitative Insulin Sensitivity Check Index. n = 7–8 for OR, n = 7–10 for OP. Significant differences by 2-tailed unpaired *t*-test comparing OR to OP rats in either pre- or post-DIO: *$P \leq 0.05$ (specific P values shown in table), **$P \leq 0.01$ (specific P values shown in table), ***$P \leq 0.001$ (specific P values shown in table), ****$P \leq 0.0001$.

RESULTS

OP Rats Developed Diet-Induced Obesity with Hyperinsulinemia and Hypertriglyceridemia

Before diet-induced obesity. At 8 wk old, OP rats weighed more than OR rats ($P \leq 0.0001$) (Table 1). The blood panel determined that whole blood glucose, serum insulin, and total serum cholesterol were similar between OP and OR rats (Table 1). However, OP rats had elevated levels of triglycerides ($P = 0.0223$), blood urea nitrogen (BUN, $P = 0.0007$), and creatinine ($P = 0.0023$) when compared with OR rats even though the BUN/creatinine ratios were similar between groups (Table 1). Additionally, neither index of insulin sensitivity, HOMA ($x > 1.716$) or QUICKI ($x < 0.2765$), met the cutoff values for either OP or OR rats (Table 1) (5).

After diet-induced obesity. Following 15 wk of high-fat feeding, OP rats gained more body weight than OR rats ($P \leq 0.0001$) (Table 1). Despite these changes in body composition, urinary bladder weight was similar between OP and OR rats (Table 1). Whole blood glucose, BUN, and creatinine were unchanged in OP and OR rats (Table 1). Total serum cholesterol was also unchanged between OP and OR rats; however, serum HDL cholesterol was lower in OP rats ($P \leq 0.0001$) (Table 1). OP rats continued to have elevated levels of triglycerides ($P = 0.0098$), in addition to increased serum insulin ($P = 0.041$) (Table 1). OP rats exhibited mildly decreased insulin sensitivity as reflected by meeting the proposed cutoff value of HOMA ($2.465 > 1.716$), but not the cutoff of QUICKI ($0.2867 > 0.2765$) (Table 1) (5).

OP Rats Exhibited Detrusor Underactivity and Impaired Contractility Following DIO

OP rats had average filling pressures similar to OR rats in single-trial cystometry ($P = 0.4823$) (Fig. 2, *A* and *B*; Table 2). Additionally, whereas pressures at volume threshold were similar between OR and OP rats ($P = 0.5903$), the volume threshold increased in OP rats ($P = 0.0177$) (Fig. 2*E*, Table 2). Once reaching the volume threshold, OP rats did not initiate adequate bladder contractions as evidenced by decreased peak micturition pressure relative to OR rats ($P = 0.0005$) (Fig. 2, *C–E*, Table 2). Impaired contractility of the urinary bladder in OP rats resulted in decreased voided volumes (0.19 ± 0.02 vs. 0.07 ± 0.01 ml, $P = 0.0004$) and increased residual volumes (0.30 ± 0.04 vs. 0.74 ± 0.08 ml, $P = 0.0006$), reflected in decreased VE in OP rats when compared with OR rats ($P \leq 0.0001$) (Fig. 2*E*, Table 2). During the voiding event, overall EUS EMG activity was decreased in OP rats (102 ± 16.5 vs. 36.4 ± 7.33 spikes/s, $P = 0.0015$) (Fig. 2*E*). Phasic bursting activity in the EUS was preserved only in OR rats with an average bursting amplitude of 0.03 ± 0.01 mV, a peak bursting amplitude of 0.27 ± 0.05 mV, and an average bursting time of 4.29 ± 0.39 s (Fig. 2*C*).

Electrical Stimulation of the Sensory Branch of the Pudendal Nerve Did Not Recover Efficient Voiding in OP Rats

The minimum current amplitude to evoke an EUS reflex by stimulation of the sensory branch of the pudendal nerve was increased in OP rats relative to OR rats (0.11 ± 0.05 vs.

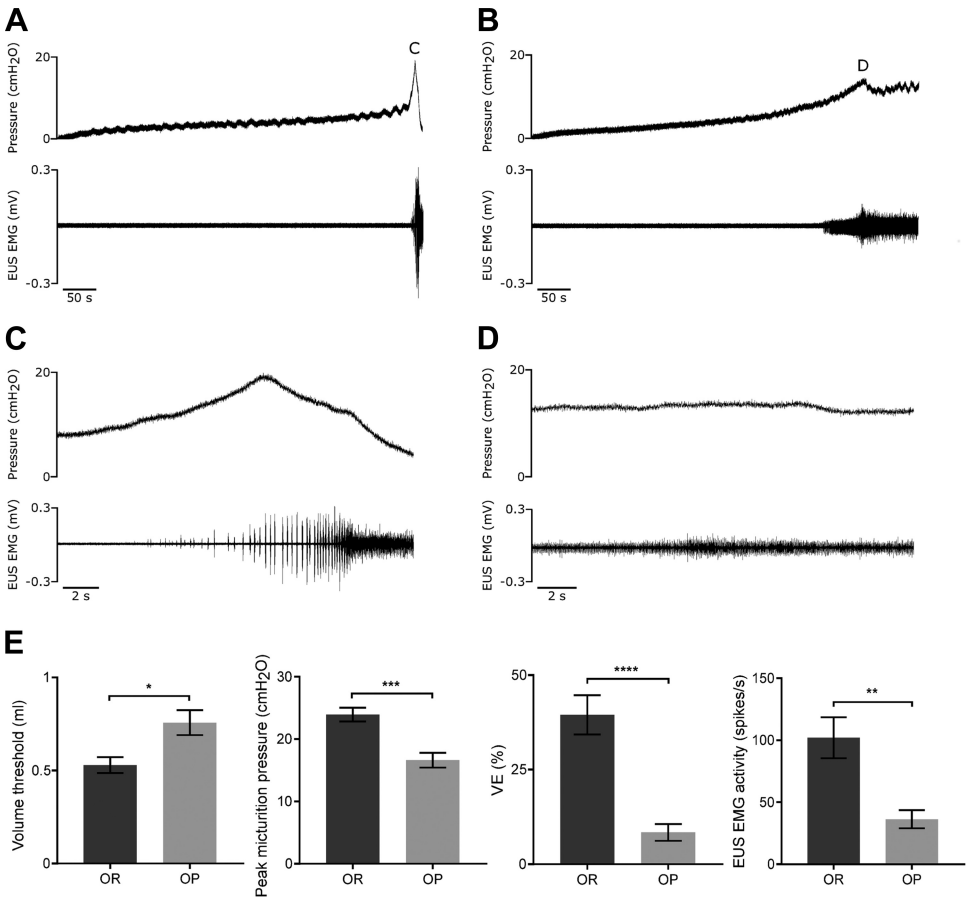

Fig. 2. Obese-prone rats exhibited detrusor underactivity and impaired contractility following diet-induced obesity (DIO). Representative cystometrogram traces of intravesical pressure and external urethral sphincter (EUS) EMG activity in obese-resistant (*A*, OR) and obese-prone (*B*, OP) rats. *C*: an expanded trace from OR rats (*A*) initiating a bladder contraction and EUS bursting during a void. *D*: an expanded trace from OP rats (*B*) unable to initiate a bladder contraction or phasic bursting activity in the EUS. *E*: during single-fill cystometry, obese-prone rats had increased volume threshold, decreased peak micturition pressure, decreased voiding efficiency, and decreased EUS EMG activity compared with obese-resistant rats. $n = 7$ for OR, $n = 9$ for OP: $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, $^{****}P \leq 0.0001$, two-tailed Student's unpaired *t*-test.

Table 2. *Cystometrogram measurements for obese-resistant and obese-prone rats*

	Average Filling Pressure, cmH_2O	Pressure at Volume Threshold, cmH_2O	Peak Micturition Pressure, cmH_2O	Volume Threshold, ml	Voiding Efficiency, %
Obese-resistant					
Baseline	5.07 ± 0.5	12.1 ± 0.77	23.9 ± 1.1	0.53 ± 0.04	40 ± 5.2
Pudendal nerve, sensory					
5 Hz, 0.125T, cont.	4.32 ± 0.56	9.87 ± 1.6	21.9 ± 2.3	0.64 ± 0.03	36 ± 9.1
10 Hz, 0.125T, cont.	3.77 ± 0.65	8.59 ± 1.1	21.9 ± 1.6	0.55 ± 0.04	45 ± 11
5 Hz, 0.25T, cont.	4.5 ± 0.51	11.2 ± 2.4	22.7 ± 1.3	0.71 ± 0.04	35 ± 8
10 Hz, 0.25T, cont.	4.9 ± 0.95	14.5 ± 3.9	21.9 ± 2.4	0.71 ± 0.08	26 ± 6
20 Hz, 0.25T, cont.	4.86 ± 0.37	13.2 ± 0.18	23.6 ± 1.8	0.75 ± 0.09	24 ± 3
10 Hz, 0.125T, cond.	4.48 ± 1.4	9.5 ± 2.9	22.4 ± 1.6	0.6 ± 0.06	45 ± 5.2
Pudendal nerve, motor					
40 Hz, bursting	5.49 ± 0.39	13 ± 1	25.1 ± 1.4	0.49 ± 0.07	40 ± 5.6
Obese-prone					
Baseline	5.48 ± 0.33	12.9 ± 1.1	16.6 ± 1.2***	0.76 ± 0.07*	8.4 ± 2.2****
Pudendal nerve, sensory					
10 Hz, 0.25T, cont.	4.42 ± 0.15	8.87 ± 0.69	12.4 ± 1.1	0.65 ± 0.16	6.5 ± 1.6
20 Hz, 0.25T, cont.	4.12 ± 0.28	10.1 ± 0.86	12.6 ± 0.7	0.76 ± 0.22	5.4 ± 1.6
10 Hz, 0.5T, cont.	4.49 ± 0.49	10.1 ± 0.73	13.2 ± 1.5	0.52 ± 0.06	4.7 ± 0.02
20 Hz, 0.5T, cont.	5.08 ± 0.44	12.3 ± 2	14.4 ± 1.9	0.58 ± 0.1	3.2 ± 0.99
10 Hz, 0.25T, cond.	4.94 ± 0.78	12.1 ± 1.2	14.5 ± 1.3	0.6 ± 0.06	8.4 ± 4.1
10 Hz, 0.5T, cond.	4.89 ± 0.62	11.5 ± 1.5	13.7 ± 1.7	0.62 ± 0.07	9.8 ± 4.3
Pudendal nerve, motor					
40 Hz, bursting	5.31 ± 0.37	14.5 ± 1.8	18.3 ± 2	0.72 ± 0.08	21 ± 6.3†
Pelvic nerve					
40 Hz, 4T	4.82 ± 0.51	10.6 ± 1.2	15.1 ± 2.8	0.71 ± 0.05	3.1 ± 2.2
40 Hz, 6T	5.26 ± 0.35	12.2 ± 0.85	18 ± 1.2	0.79 ± 0.1	9.7 ± 3.5
40 Hz, 8T	5.59 ± 0.43	12.6 ± 1	20.2 ± 1	0.81 ± 0.09	6.7 ± 2
40 Hz, 10T	5.07 ± 0.6	12.2 ± 1.8	19.8 ± 2.5	0.81 ± 0.19	15 ± 6.7

All values are reported as means ± SE. Obese-prone (OP) rats exhibited decreased peak micturition pressure, increased volume threshold, and decreased voiding efficiency relative to obese-resistant (OR) rats. Voiding efficiency increased twofold in OP rats following patterned electrical stimulation of the motor branch of the pudendal nerve. Abbreviations: cont. and cond. refer to continuous and conditional low-amplitude stimulation, respectively. $n = 7$ for OR; $n = 9$ for OP. *$P = 0.0177$, ***$P = 0.0005$, ****$P \leq 0.0001$ compared with OR baseline with two-tailed unpaired t-test. $n = 4$ for OP, †$P = 0.0404$ compared with OP baseline with two-tailed paired t-test.

0.33 ± 0.03 mA, $P = 0.0105$). Continuous, low-amplitude (0.25–0.5T) stimulation of the sensory branch of the pudendal nerve did not increase VE in OP rats ($P = 0.4048$), and in most cases, VE was reduced by stimulation (Fig. 3*D*, Table 2). Conditional, low-amplitude (0.25–0.5T) stimulation beginning at the onset of voiding also did not increase VE in OP rats ($P = 0.9975$) (Fig. 3*D*, Table 2). Other cystometric parameters, including average filling pressure ($P = 0.4152$), pressure at volume threshold ($P = 0.442$), peak micturition pressure ($P = 0.4045$), and volume threshold ($P = 0.5049$), were unchanged with continuous or conditional low-amplitude stimulation (Fig. 3*B*, Table 2).

Given that the sensory pudendal nerve stimulation in OP rats appeared ineffective, lower frequencies (5–20 Hz) and amplitudes (0.125–0.25T) were attempted to improve efficient voiding in OR rats. Similar to OP rats, continuous or conditional low-amplitude stimulation did not alter VE in OR rats ($P = 0.4387$) (Fig. 3*C*, Table 2). Additional cystometrogram parameters, including average filling pressure ($P = 0.8455$), pressure at volume threshold ($P = 0.3646$), peak micturition pressure ($P = 0.2592$), and volume threshold ($P = 0.0612$), were also unchanged with continuous or conditional low-amplitude stimulation (Fig. 3*A*, Table 2).

Patterned Electrical Stimulation of the Motor Branch of the Pudendal Nerve Increased Efficient Voiding in OP Rats

The current amplitude to evoke a maximum EUS EMG response by stimulation of the motor branch of the pudendal nerve was similar between OR and OP rats (0.05 ± 0.01 vs. 0.16 ± 0.08 mA, $P = 0.2387$). Phasic bursting stimulation of the motor branch of the pudendal nerve in OP rats increased VE twofold ($P = 0.0404$) (Fig. 4*E*, Table 2). Phasic bursting stimulation in OP rats, however, did not alter cystometrogram parameters including average filling pressure ($P = 0.1845$), pressure at volume threshold ($P = 0.936$), peak micturition pressure ($P = 0.2683$), or volume threshold ($P = 0.7605$) (Fig. 4, *A–D*, Table 2).

Phasic bursting stimulation in OR rats, which already exhibited EUS bursting, did not alter VE ($P = 0.8801$), average filling pressure ($P = 0.4225$), pressure at volume threshold ($P = 0.7175$), peak micturition pressure ($P = 0.742$), or volume threshold ($P = 0.1926$) (Fig. 5, *A–E*, Table 2).

OP Rats Exhibit Impaired Responses to Electrical Stimulation of the Pelvic Nerve

The minimum current amplitude to evoke an EUS reflex by stimulation of the pelvic nerve was similar between OR and OP rats (0.1 ± 0.02 vs. 0.06 ± 0.02 mA, $P = 0.203$). During single-trial cystometry in OP rats, evoking a bladder contraction through electrical stimulation (40 Hz, 4–10T) of the pelvic nerve increased EUS EMG activity during voiding ($P = 0.0007$), but did not alter VE ($P = 0.1945$), average filling pressure ($P = 0.504$), pressure at volume threshold ($P = 0.4441$), peak micturition pressure ($P = 0.2437$), or volume threshold ($P = 0.8669$) (Fig. 6, *A* and *B*; Table 2).

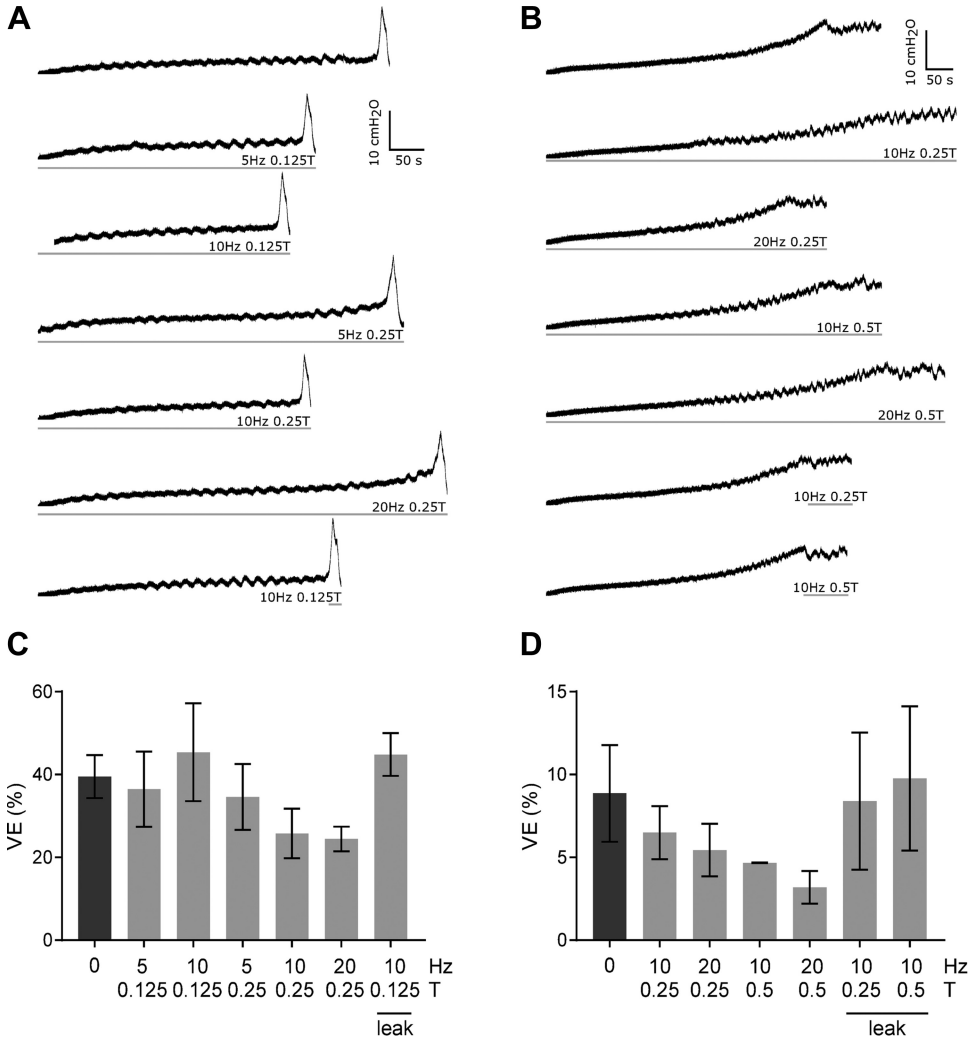

Fig. 3. Electrical stimulation of the sensory branch of the pudendal nerve did not alter voiding efficiency in obese-resistant or obese-prone rats following DIO. Representative cystometrogram traces of intravesical pressure in obese-resistant (*A*, OR) and obese-prone (*B*, OP) rats. Continuous (stimulation throughout the filling and voiding event) or conditional (stimulation at the voiding event), low-amplitude stimulation did not alter cystometrogram parameters. Voiding efficiency (VE) in OR (*C*) or OP (*D*) rats was not affected by varying electrical stimulation parameters. $n = 3$ for OR, $n = 4$ for OP. $P \geq 0.05$, one-way ANOVA, post hoc comparisons with Bonferroni correction. $n = 2$ for 10 Hz, 0.125T (leak, OR) and 10 Hz, 0.5T (OP).

To assess contractility of the urinary bladder following single-trial cystometry, a bolus to 50% of bladder capacity was instilled intravesically and the pelvic nerve was stimulated. The average current amplitude necessary to evoke a bladder contraction over 5 cmH_2O was increased in OP rats relative to OR rats ($P \leq 0.0001$) across stimulation frequencies (Fig. 6, *C–F*). Electrical stimulation of the pelvic nerve evoked maximum bladder contraction amplitudes at 8T in OP rats and 4T in OR rats. At these current amplitudes, the bladder contraction amplitudes were consistently lower in OP rats ($P \leq 0.0006$) at 20 Hz and 40 Hz, whereas evoked contraction amplitudes were similar between OR and OP rats at 10 Hz (Fig. 6, *D* and *F*). Not surprisingly, when we compared OR to OP rats at 4T, the evoked bladder contraction amplitude was also decreased in OP rats ($P \leq 0.0001$) at 10, 20, and 40 Hz (Fig. 6, *D* and *F*).

DISCUSSION

These studies established an animal model of DUA that exhibited impaired contractility and urinary retention following DIO. Peak micturition pressure and bladder contraction amplitudes evoked by pelvic nerve stimulation were reduced in OP rats, consistent with a reduction in bladder contractility. Patterned electrical stimulation of the motor branch of the pudendal nerve, leading to phasic activation of the EUS, more than doubled VE in OP rats. These results suggest that neuromodulation may be a promising approach to manage at least some forms of DUA.

Obesity and Serum Panel

High-fat feeding in OP rats was previously associated with increased body weight, elevated circulating lipids (28), insulin resistance (36), and the development of prediabetes (36). Following DIO in our studies, OP rats gained more weight than OR rats, exhibited decreased HDL cholesterol relative to OR rats, and developed hyperinsulinemia and hypertriglyceridemia. Hyperinsulinemia is likely a consequence of insulin resistance in OP rats as they met the proposed HOMA-IR cutoff value (5). Insulin resistance with obesity has also been associated with dyslipidemia. The increased intra-abdominal fat in OP rats may contribute to insulin resistance and increased portal vein free fatty acids (24). Hyperinsulinemia and elevated free fatty acids may then increase hepatic lipase activity resulting in hypertriglyceridemia and lipid removal from LDL and HDL cholesterol that was observed following DIO (24).

Obesity is associated with autonomic nervous system dysfunction. Hyperinsulinemia with obesity may increase sympa-

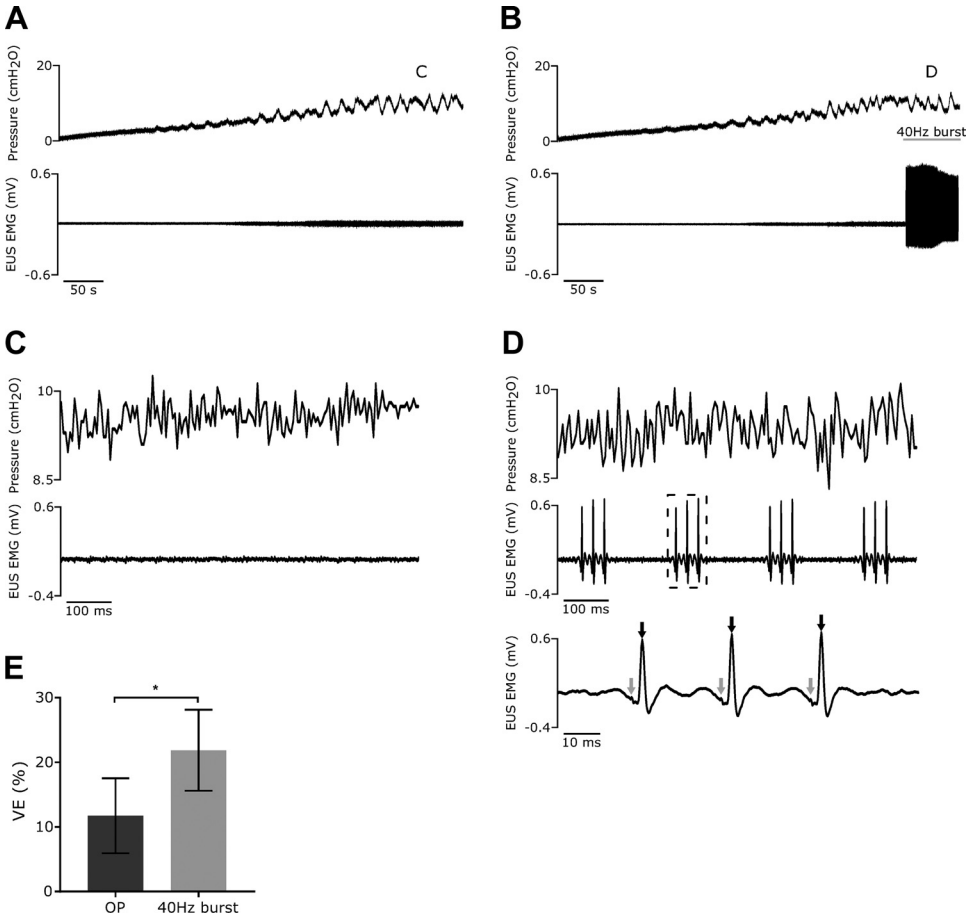

Fig. 4. Electrical stimulation of the motor branch of the pudendal nerve increased voiding efficiency in obese-prone rats following DIO. Representative cystometrogram traces of intravesical pressure and external urethral sphincter (EUS) EMG activity before (*A*) and after (*B*) electrical stimulation. Patterned phasic stimulation was introduced at the voiding event for 90 s. *C*: an expanded trace from *A*, demonstrating the absence of phasic bursting activation of the EUS in obese-prone (OP) rats. *D*: an expanded trace from *B*, demonstrating evoked motor responses from phasic electrical stimulation of the motor branch of the pudendal nerve. Gray arrows indicate stimulation artifacts, whereas black arrows indicate evoked motor responses. *E*: reintroducing EUS bursting with patterned phasic stimulation increased voiding efficiency in OP rats. Baseline values are from the four paired OP rats. $n = 4$ for OP: $^*P \leq 0.05$, 2-tailed Student's paired *t*-test.

thetic tone to limit dietary intake by stimulating energy consumption and expenditure (25). The resulting sympathetic overactivity may contribute to the pathophysiology of hypertension, insulin resistance, and target organ complications (15). More specifically in OP rats, elevated sympathetic outflow was previously demonstrated to underlie increased blood pressure (40). In addition to hypertension, perturbations to sympathetic outflow may also contribute to bladder dysfunction given the autonomic neural control of the lower urinary tract. Although this is an intriguing possibility, further studies are needed to determine the contribution of the sympathetic nervous system to DUA in OP rats.

Detrusor Underactivity

DUA is the urodynamic observation of inefficient bladder emptying from decreased strength and/or duration of a detrusor contraction (1). Clinical signs and symptoms, now termed the underactive bladder (UAB), that are suggestive of DUA include reduced or prolonged flow, hesitancy, intermittency, sensation of incomplete bladder emptying, and increased postvoid residual (39). UAB symptoms may be observed in individuals with bladder outlet obstruction, diabetes mellitus, Parkinson's disease, spinal cord injury, pelvic surgery, and in the aged population (42). Given the multifactorial pathogenesis of UAB and DUA, current animal models attempt to reproduce altered micturition parameters through myogenic or neurogenic etiologic hypotheses. Models that express the voiding patterns of DUA (42) include aged rodents, streptozotocin-induced diabetes, bladder outlet obstruction, spinal cord injury, and transgenic knockout mice.

The current studies introduce another animal model of urinary retention and provide a foundation to characterize further the etiology of DUA in OP rats following DIO. Our first observation was that OP rats had increased filling capacity despite having reduced bladder weight-to-body weight ratios when compared with OR rats. Additionally, OP rats exhibited decreased voided volume and increased residual volume, and both the reduced amplitude of voiding bladder contractions and the lack of EUS phasic bursting activity contributed to poor VE. Stimulation of the pudendal motor nerve to restore phasic burst activity in the EUS increased VE, whereas stimulation of the pelvic nerve did not increase bladder pressure or VE. These results suggest that deficiencies in smooth muscle function, neurotransmitter release, or other cellular changes limited detrusor contractility in OP rats. It is also possible that tissue reorganization due to bladder outlet obstruction contributed to low VE; however, gross bladder weight, bladder pressure traces, and EUS EMG traces do not suggest this. Further studies are warranted to determine the histopathological alterations that may underlie retention in OP rats.

The 40% VE of OR rats following high-fat feeding was lower than the 60–77% VE reported in control, urethane-anesthetized rats (27, 34, 35) and 97–99% VE reported in conscious rats (18). The lower VE observed in OR rats compared with control, urethane-anesthetized rats may be due to the high-fat feeding protocol, intrinsic differences in bladder

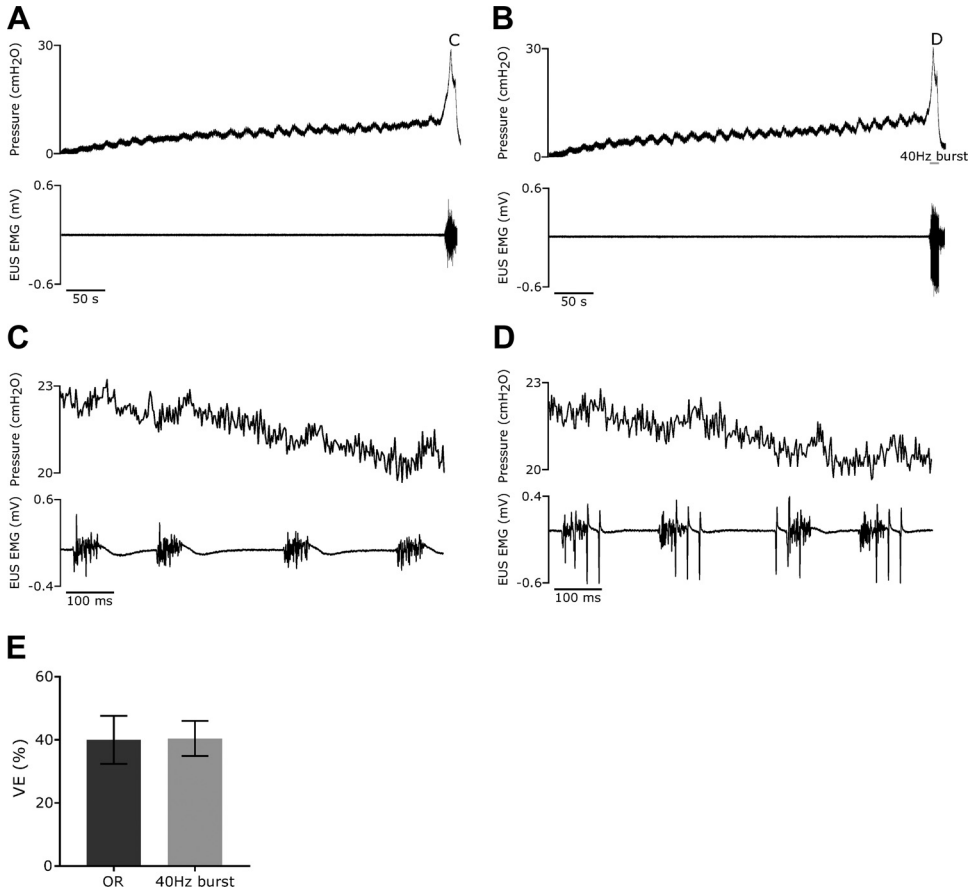

Fig. 5. Electrical stimulation of the motor branch of the pudendal nerve did not alter voiding efficiency in obese-resistant rats following DIO. Representative cystometrogram traces of intravesical pressure and external urethral sphincter (EUS) EMG activity before (*A*) and after (*B*) electrical stimulation. Patterned phasic stimulation is introduced at the voiding event for 10–15 s. *C*: an expanded trace from *A*, demonstrating phasic bursting activation of the EUS in obese-resistant (OR) rats. *D*: an expanded trace from *B*, demonstrating stimulation evoked motor responses introduced on top of EUS bursting. *E*: patterned phasic stimulation did not alter voiding efficiency in OR rats. $n = 4$ for OR. $P \geq 0.05$, 2-tailed Student's paired *t*-test.

function, and/or the variation in methodology (e.g., continuous vs. single-trial cystometry). Even though OR rats had lower VE compared with normal rats, OR rats were chosen as the control group because they did not develop DIO and could still initiate bladder contractions following high-fat feeding. Cystometric parameters are also sensitive to anesthesia, including urethane, and this may account for the low VE among urethane-anesthetized rats (6, 33). Despite these limitations, urethane is the preferred anesthetic agent to use in urodynamic studies as the micturition reflex with EUS EMG activity is preserved (8, 29).

Pudendal Nerve Stimulation

Pudendal afferent nerve discharge evoked by fluid flow through the urethra initiates and enhances bladder contractions (2, 3, 13). In addition, stimulating the sensory branches of the pudendal nerve increases bladder pressure and promotes voiding, whereas transection of the pudendal nerve reduces VE in the rat (34). Similarly, reducing urethral sensory nerve activity with topical lidocaine anesthesia also decreased contraction amplitude and reduced VE in rats and humans (21, 37). Taken together, these results suggest that sensory feedback from the pudendal nerve is necessary to maintain the amplitude and duration of bladder contractions required for efficient voiding. We hypothesized that decreased sensory feedback from the urethra might contribute to voiding dysfunction in OP rats and that electrical stimulation of the sensory branch of the pudendal nerve may mitigate these deficiencies. However, low-amplitude stimulation of the sensory branch of the pudendal nerve did not influence VE in either OP or OR rats, suggesting that altered urethral afferent feedback may not contribute to DUA following DIO.

Phasic bursting activity of the EUS occurs during voiding in rats and dogs (11, 31) and is a critical component for bladder emptying because transection of the motor branch of the pudendal nerve or neuromuscular blockade decreases efficient voiding (26, 31, 43). OP rats lacked phasic EUS bursting suggesting that impaired motor activity may contribute to poor voiding. We hypothesized that reintroducing EUS bursting activity by electrical stimulation would restore efficient bladder emptying in OP rats. Patterned electrical stimulation of the motor branch of the pudendal nerve generated a twofold increase in VE in OP rats but did not restore VE to the levels observed in OR rats. These studies demonstrate the role of EUS bursting activity in efficient voiding and suggest electrical stimulation of the motor, rather than sensory, pathways as a novel approach to manage retention in rats presenting with DUA.

Pelvic Nerve Stimulation

OP rats exhibited DUA, and we hypothesized that detrusor contractility may be impaired in OP rats because of decreased efferent discharge in the pelvic nerve. Electrical stimulation of the pelvic nerve elicited a bladder contraction during cystometry; however, VE was unchanged. Poor emptying could be explained by the emergence of detrusor sphincter dyssynergia with pelvic nerve stimulation, and we observed increased EUS EMG activity without phasic bursting during pelvic nerve stimulation (10, 20, 44). Despite direct electrical stimulation of

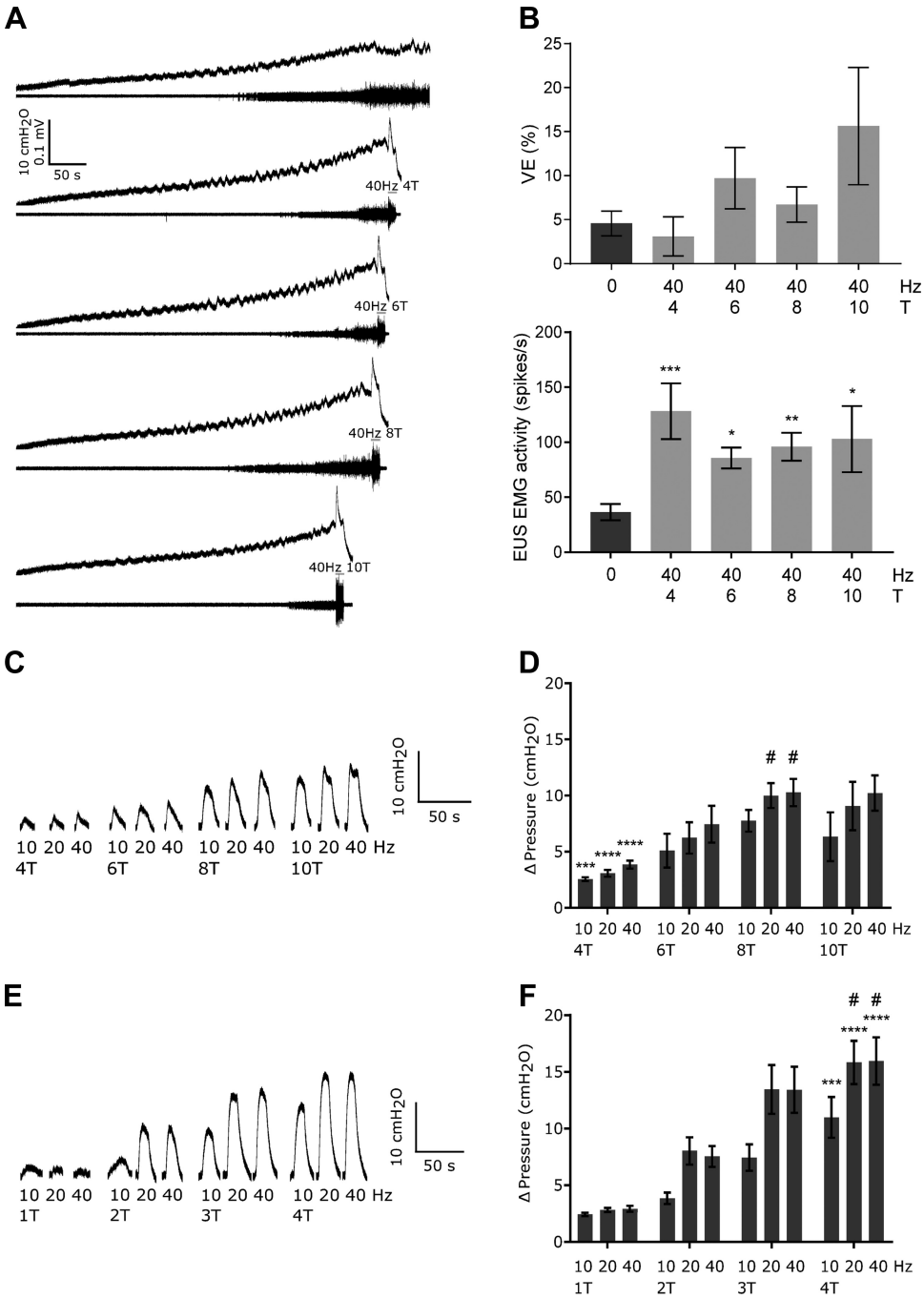

Fig. 6. Obese-prone rats exhibited impaired contractility following DIO and electrical stimulation of the pelvic nerve. *A*: representative cystometrogram traces of intravesical pressure in obese-prone (OP) rats and electrical stimulation of the pelvic nerve. *B*: pelvic nerve stimulation increased EUS EMG activity but did not affect voiding efficiency in OP rats following DIO. *C* and *E*: representative intravesical pressure traces of contraction amplitude assessment in OP (*C*) and obese-resistant (*E*, OR) rats. Compared with OR rats (*F*), the current amplitude to evoke a detrusor contraction was increased and the maximum evoked detrusor contraction amplitude was decreased in OP rats (*D*). $n = 7$ for OR, $n = 7$ for OP. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, $^{****}P \leq 0.0001$, 2-way ANOVA, post hoc comparisons with Bonferroni correction. $\#P \leq 0.05$, 2-way ANOVA, post hoc comparisons with Bonferroni correction.

the pelvic nerve, OP rats had reduced peak micturition pressures, required increased current amplitudes to elicit bladder contractions, and exhibited reduced maximum evoked bladder contraction amplitudes compared with OR rats. These results suggest impaired contractility of the bladder and may be due to reductions in the excitability of innervating efferent neurons, changes in innervation density, smooth muscle dysfunction, or other biochemical and cellular events that contribute to reduced detrusor responsiveness (4, 16, 38, 41). Further work is required to differentiate the myogenic and neurogenic contributions to the reduced responsiveness of the bladder in OP rats.

Conclusion

DUA is an underappreciated health concern with limited and largely ineffective management options. The paucity of animal models exhibiting DUA makes it challenging to understand pathophysiology and develop novel approaches to treatment. The current studies address this limitation by characterizing a new animal model of DUA that may contribute to identifying the initiation and progression of impaired voiding. We characterized alterations in systemic metabolism following DIO and made urodynamic observations similar to the clinical presentation of DUA. We also established the potential of neuro-

modulation of peripheral nerves to manage DUA in OP rats. An understanding of alterations in lower urinary tract function in DUA will enable clinicians to identify novel management options and potentially improve patient outcomes.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

E.J.G. and W.M.G. conceived and designed research; E.J.G. performed experiments; E.J.G. analyzed data; E.J.G. and W.M.G. interpreted results of experiments; E.J.G. prepared figures; E.J.G. drafted manuscript; E.J.G. and W.M.G. edited and revised manuscript; E.J.G. and W.M.G. approved final version of manuscript.

REFERENCES

1. **Abrams P, Cardozo L, Fall M, Griffiths D, Rosier P, Ulmsten U, van Kerrebroeck P, Victor A, Wein A; Standardisation Sub-committee of the International Continence Society.** The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. *Neurourol Urodyn* 21: 167–178, 2002. doi:10.1002/nau.10052.
2. **Barrington FJF.** The component reflexes of micturition in the cat. Part III. *Brain* 64: 239–243, 1941. doi:10.1093/brain/64.4.239.
3. **Barrington FJF.** The component reflexes of micturition in the cat. Parts I and II. *Brain* 54: 177–188, 1931. doi:10.1093/brain/54.2.177.
4. **Brierly RD, Hindley RG, McLarty E, Harding DM, Thomas PJ.** A prospective controlled quantitative study of ultrastructural changes in the underactive detrusor. *J Urol* 169: 1374–1378, 2003. doi:10.1097/01.ju.0000055781.07630.aa.
5. **Cacho J, Sevillano J, de Castro J, Herrera E, Ramos MP.** Validation of simple indexes to assess insulin sensitivity during pregnancy in Wistar and Sprague-Dawley rats. *Am J Physiol Endocrinol Metab* 295: E1269–E1276, 2008. doi:10.1152/ajpendo.90207.2008.
6. **Cannon TW, Damaser MS.** Effects of anesthesia on cystometry and leak point pressure of the female rat. *Life Sci* 69: 1193–1202, 2001. doi:10.1016/S0024-3205(01)01182-1.
7. **Chancellor MB.** The overactive bladder progression to underactive bladder hypothesis. *Int Urol Nephrol* 46, *Suppl* 1: S23–S27, 2014. doi:10.1007/s11255-014-0778-y.
8. **Chang HY, Havton LA.** Differential effects of urethane and isoflurane on external urethral sphincter electromyography and cystometry in rats. *Am J Physiol Renal Physiol* 295: F1248–F1253, 2008. doi:10.1152/ajprenal.90259.2008.
9. **Chen SC, Grill WM, Fan WJ, Kou YR, Lin YS, Lai CH, Peng CW.** Bilateral pudendal afferent stimulation improves bladder emptying in rats with urinary retention. *BJU Int* 109: 1051–1058, 2012. doi:10.1111/j.1464-410X.2011.10526.x.
10. **Creed KE, Van der Werf BA.** The innervation and properties of the urethral striated muscle. *Scand J Urol Nephrol Suppl* 207: 8–11, 2001.
11. **D'Amico SC, Collins WF III.** External urethral sphincter motor unit recruitment patterns during micturition in the spinally intact and transected adult rat. *J Neurophysiol* 108: 2554–2567, 2012. doi:10.1152/jn.00927.2011.
12. **Dallosso HM, McGrother CW, Matthews RJ, Donaldson MM; Leicestershire MRC Incontinence Study Group.** The association of diet and other lifestyle factors with overactive bladder and stress incontinence: a longitudinal study in women. *BJU Int* 92: 69–77, 2003. doi:10.1046/j.1464-410X.2003.04271.x.
13. **Danziger ZC, Grill WM.** Dynamics of the sensory response to urethral flow over multiple time scales in rat. *J Physiol* 593: 3351–3371, 2015. doi:10.1113/JP270911.
14. **Drake MJ, Williams J, Bijos DA.** Voiding dysfunction due to detrusor underactivity: an overview. *Nat Rev Urol* 11: 454–464, 2014. doi:10.1038/nrurol.2014.156.
15. **Egan BM.** Insulin resistance and the sympathetic nervous system. *Curr Hypertens Rep* 5: 247–254, 2003. doi:10.1007/s11906-003-0028-7.
16. **Elbadawi A, Yalla SV, Resnick NM.** Structural basis of geriatric voiding dysfunction. II. Aging detrusor: normal versus impaired contractility. *J Urol* 150: 1657–1667, 1993. doi:10.1016/S0022-5347(17)35867-6.
17. **Fry CH.** Obesity and the overactive bladder. *Curr Bladder Dysfunct Rep* 8: 62–68, 2013. doi:10.1007/s11884-012-0172-5.
18. **Gonzalez EJ, Girard BM, Vizzard MA.** Expression and function of transforming growth factor-β isoforms and cognate receptors in the rat urinary bladder following cyclophosphamide-induced cystitis. *Am J Physiol Renal Physiol* 305: F1265–F1276, 2013. doi:10.1152/ajprenal.00042.2013.
19. **Jeong SJ, Kim HJ, Lee YJ, Lee JK, Lee BK, Choo YM, Oh JJ, Lee SC, Jeong CW, Yoon CY, Hong SK, Byun SS, Lee SE.** Prevalence and clinical features of detrusor underactivity among elderly with lower urinary tract symptoms: a comparison between men and women. *Korean J Urol* 53: 342–348, 2012. doi:10.4111/kju.2012.53.5.342.
20. **Jung J, Ahn HK, Huh Y.** Clinical and functional anatomy of the urethral sphincter. *Int Neurourol J* 16: 102–106, 2012. doi:10.5213/inj.2012.16.3.102.
21. **Jung SY, Fraser MO, Ozawa H, Yokoyama O, Yoshiyama M, De Groat WC, Chancellor MB.** Urethral afferent nerve activity affects the micturition reflex; implication for the relationship between stress incontinence and detrusor instability. *J Urol* 162: 204–212, 1999. doi:10.1097/00005392-199907000-00069.
22. **Khaodhiar L, McCowen KC, Blackburn GL.** Obesity and its comorbid conditions. *Clin Cornerstone* 2: 17–31, 1999. doi:10.1016/S1098-3597(99)90002-9.
23. **Kim JH, Sun HY, Park SY, Soh MJ, Kim YJ, Song YS.** Association between obesity and lower urinary tract symptoms: propensity score matching study between healthy controls and obese patients seeking bariatric surgery. *Surg Obes Relat Dis* 12: 1585–1593, 2016. doi:10.1016/j.soard.2016.04.027.
24. **Klop B, Elte JW, Cabezas MC.** Dyslipidemia in obesity: mechanisms and potential targets. *Nutrients* 5: 1218–1240, 2013. doi:10.3390/nu5041218.
25. **Landsberg L.** Diet, obesity and hypertension: an hypothesis involving insulin, the sympathetic nervous system, and adaptive thermogenesis. *Q J Med* 61: 1081–1090, 1986.
26. **Langdale CL, Grill WM.** Phasic activation of the external urethral sphincter increases voiding efficiency in the rat and the cat. *Exp Neurol* 285, *Pt B*: 173–181, 2016. doi:10.1016/j.expneurol.2016.05.030.
27. **Lin Y-T, Lai C-H, Kuo T-S, Chen C-C, Chen Y-L, Young S-T, Chen S-C, Lai J-S, Hsieh T-H, Peng CW.** Dual-channel neuromodulation of pudendal nerve with closed-loop control strategy to improve bladder functions. *J Med Biol Eng* 34: 82–89, 2014. doi:10.5405/jmbe.1247.
28. **Liu TW, Heden TD, Matthew Morris E, Fritsche KL, Vieira-Potter VJ, Thyfault JP.** High-fat diet alters serum fatty acid profiles in obesity prone rats: implications for in vitro studies. *Lipids* 50: 997–1008, 2015. doi:10.1007/s11745-015-4061-5.
29. **Matsuura S, Downie JW.** Effect of anesthetics on reflex micturition in the chronic cannula-implanted rat. *Neurourol Urodyn* 19: 87–99, 2000. doi:10.1002/(SICI)1520-6777(2000)19:1<87::AID-NAU9>3.0.CO;2-O.
30. **Miscio G, Guastamacchia G, Brunani A, Priano L, Baudo S, Mauro A.** Obesity and peripheral neuropathy risk: a dangerous liaison. *J Peripher Nerv Syst* 10: 354–358, 2005. doi:10.1111/j.1085-9489.2005.00047.x.
31. **Nishizawa O, Satoh S, Harada T, Nakamura H, Fukuda T, Tsukada T, Tsuchida S.** Role of the pudendal nerves on the dynamics of micturition in the dog evaluated by pressure flow EMG and pressure flow plot studies. *J Urol* 132: 1036–1039, 1984. doi:10.1016/S0022-5347(17)49994-0.
32. **Osman NI, Chapple CR, Abrams P, Dmochowski R, Haab F, Nitti V, Koelbl H, van Kerrebroeck P, Wein AJ.** Detrusor underactivity and the underactive bladder: a new clinical entity? A review of current terminology, definitions, epidemiology, aetiology, and diagnosis. *Eur Urol* 65: 389–398, 2014. doi:10.1016/j.eururo.2013.10.015.

33. **Ozkurkcugil C, Ozkan L.** Effects of anesthetics on cystometric parameters in female rats. *Int Urol Nephrol* 42: 909–913, 2010. doi:10.1007/s11255-010-9745-4.
34. **Peng CW, Chen JJ, Cheng CL, Grill WM.** Improved bladder emptying in urinary retention by electrical stimulation of pudendal afferents. *J Neural Eng* 5: 144–154, 2008. doi:10.1088/1741-2560/5/2/005.
35. **Peng CW, Chen JJ, Cheng CL, Grill WM.** Role of pudendal afferents in voiding efficiency in the rat. *Am J Physiol Regul Integr Comp Physiol* 294: R660–R672, 2008. doi:10.1152/ajpregu.00270.2007.
36. **Picklo MJ Sr, Newman JW.** Antioxidant supplementation and obesity have independent effects on hepatic oxylipin profiles in insulin-resistant, obesity-prone rats. *Free Radic Biol Med* 89: 182–191, 2015. doi:10.1016/j.freeradbiomed.2015.07.152.
37. **Shafik A, Shafik AA, El-Sibai O, Ahmed I.** Role of positive urethrovesical feedback in vesical evacuation. The concept of a second micturition reflex: the urethrovesical reflex. *World J Urol* 21: 167–170, 2003. doi:10.1007/s00345-003-0340-5.
38. **Smith PP.** Aging and the underactive detrusor: a failure of activity or activation? *Neurourol Urodyn* 29: 408–412, 2010. doi:10.1002/nau.20765.
39. **Smith PP, Birder LA, Abrams P, Wein AJ, Chapple CR.** Detrusor underactivity and the underactive bladder: Symptoms, function, cause-what do we mean? ICI-RS think tank 2014. *Neurourol Urodyn* 35: 312–317, 2016. doi:10.1002/nau.22807.
40. **Stocker SD, Meador R, Adams JM.** Neurons of the rostral ventrolateral medulla contribute to obesity-induced hypertension in rats. *Hypertension* 49: 640–646, 2007. doi:10.1161/01.HYP.0000254828.71253.dc.
41. **Suskind AM, Smith PP.** A new look at detrusor underactivity: impaired contractility versus afferent dysfunction. *Curr Urol Rep* 10: 347–351, 2009. doi:10.1007/s11934-009-0055-2.
42. **Tyagi P, Smith PP, Kuchel GA, de Groat WC, Birder LA, Chermansky CJ, Adam RM, Tse V, Chancellor MB, Yoshimura N.** Pathophysiology and animal modeling of underactive bladder. *Int Urol Nephrol* 46, *Suppl* 1: S11–S21, 2014. doi:10.1007/s11255-014-0808-9.
43. **Yoshiyama M, deGroat WC, Fraser MO.** Influences of external urethral sphincter relaxation induced by alpha-bungarotoxin, a neuromuscular junction blocking agent, on voiding dysfunction in the rat with spinal cord injury. *Urology* 55: 956–960, 2000. doi:10.1016/S0090-4295(00)00474-X.
44. **Zhang X, Alwaal A, Lin G, Li H, Zaid UB, Wang G, Wang L, Banie L, Ning H, Lin CS, Guo Y, Zhou L, Lue TF.** Urethral musculature and innervation in the female rat. *Neurourol Urodyn* 35: 382–389, 2016. doi:10.1002/nau.22722.