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Dysfunctional voiding behavior and impaired muscle contractility in a rat model of detrusor underactivity

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Abstract

Aims: Detrusor underactivity (DU) is an understudied health concern with inadequate clinical management. The pathophysiology of DU is unclear, and current therapies fail to improve symptoms. The current studies characterized voiding function and contractility of bladder and urethral tissues in a novel rat model of DU.

Methods: Female obese prone (OP) and obese resistant (OR) rats were fed a 60 kcal% fat diet at 8 weeks old. A subset of rats ($n = 4$/strain) underwent uroflowmetry biweekly for 18 weeks in metabolic cages. At 40–56 weeks old, rats ($n = 9$–10/strain) underwent instrumented cystometry under urethane anesthesia. Following cystometry, bladder and urethral tissues ($n = 8$–9/strain) were harvested for in vitro assessments of contractility in response to carbachol, electric field stimulation, atropine, alpha, beta-methylene ATP, and caffeine.

Results: OP rats exhibited increased urinary frequency ($p = 0.0031$), decreased voided volume ($p = 0.0093$), and urine flow rate ($p = 0.0064$) compared to OR rats during uroflowmetry. Bethanechol (10 mg/kg) did not alter uroflowmetry parameters. During cystometry, OP rats exhibited decreased bladder emptying efficiency ($p < 0.0001$), decreased pressure to generate a void ($p < 0.0001$), and increased EUS activity during filling ($p = 0.0011$). Bladder contractility was decreased in OP rats when exposed to carbachol ($p < 0.0003$) and ATP ($p = 0.0004$), whereas middle urethral contractility was increased when exposed to carbachol ($p = 0.0014$), EFS ($p = 0.0289$), and caffeine ($p = 0.0031$).

Conclusion: Impaired cholinergic and purinergic signaling in the bladder may contribute to poor voiding function in OP rats. In addition, increased urethral activity may engage a guarding reflex to augment continence and exacerbate incomplete emptying.

KEYWORDS
detrusor underactivity, myography, urethra, urodynamics

1 | INTRODUCTION

Incomplete emptying due to underactive bladder (UAB) is a poorly understood health concern that affects up to 40% of the aged population.[1,2] UAB is a symptom complex that may involve reduced motor drive (detrusor underactivity, DU) during bladder emptying.[3] DU is diagnosed through urodynamics and is defined as a contraction of reduced strength and/or duration, resulting in increased bladder emptying time and/or incomplete emptying.[4] Patients with DU report decreased urinary stream, hesitancy, feeling of incomplete emptying, and decreased sensation.[5]

Despite its prevalence, the pathophysiology of DU remains unclear and may involve neurogenic, myogenic, mixed, or idiopathic sources. Efferent denervation,[6] peripheral and central afferent dysfunction,[7] smooth muscle degeneration,[8] and neurotransmission dysregulation[9] have all been suggested to contribute to DU lower urinary tract symptoms. Preclinical models that exhibit poor voiding function may help to clarify underlying mechanisms. The obese prone (OP) rat exhibits decreased bladder emptying efficiency and reduced bladder contractions evoked by pelvic nerve stimulation following diet-induced obesity (DIO).[10]

The current study sought to expand understanding of DU by investigating the functional mechanism(s) that underlie impaired emptying in OP rats. We examined (1) the onset, progression, and treatment of urinary frequency and flow rate dysfunction with uroflowmetry, (2) bladder pressure deficits and postvoid residual volumes with cystometry, and (3) myogenic and neurogenic contributions to bladder and urethral muscle contractility with in vitro myography. This comprehensive characterization of voiding and muscle dysfunction in a preclinical rat model of DU may inform etiology and inspire alternative therapeutic approaches.

2 | MATERIALS AND METHODS

2.1 | Animals

Female OP ($n = 10$) and obese resistant (OR, $n = 9$) rats were bred by the Duke University DLAR Breeding Core (original breeding pair from Charles River) and transferred at 4 weeks old. Rats were housed two per cage with species-appropriate enrichment and maintained on a regular diurnal cycle (12:12 light:dark) with ad libitum access to food and water. OP and OR rats were fed a 60 kcal% fat diet (D12492, Research Diets, Inc.) from 8 weeks old to end of the study. Animal care and experimental procedures were approved by the Duke University IACUC.

2.2 | Uroflowmetry

Conscious, un-instrumented uroflowmetry began in OP ($n = 4$, 337 ± 6 g) and OR ($n = 4$, 231 ± 12 g) rats at 19 weeks old. Rats were single-housed in metabolic cages (40618-R, Lab Products, Inc.) for 24 h twice per week. Habituation to metabolic cages was allowed for the first week of uroflowmetry, and then experimental data were collected biweekly for 18 weeks. Bethanechol (10 mg/kg, po, Sigma) was administered in 50 ml drinking water during uroflowmetry from 16 to 18 weeks.[11] Water and normal chow were available ad libitum and were measured before and after each session. Voided volume was captured through a mesh floor by a collection pan atop a calibrated scale (GF-1200, Central Carolina Scales). Experimental data were acquired with Notocord-hem software (4.3.0.74) and urinary events were manually identified offline.

2.3 | Cystometry

Instrumented cystometry began in OP ($n = 10$, 567 ± 30 g) and OR ($n = 9$, 308 ± 16 g) rats at 40–56 weeks old after 2 weeks of washout from bethanechol. Rats previously undergoing uroflowmetry were included in this cohort. Rats were anesthetized with urethane (1.2 g/kg sc, supplemented as needed) 1 h before surgical procedures. As described previously,[10] a catheter was inserted into the urinary bladder dome and connected to an infusion pump (PHD 4400, Harvard Apparatus) and to a pressure transducer and amplifier (ETH-255, CB Sciences, Inc.) to measure intravesical pressure. A bipolar paddle electrode (Micro-Leads) was placed on the external urethral sphincter (EUS) to measure electromyogram (EMG). The abdominal cavity was remained open during testing and was covered with polyethylene. Pressure and EMG signals were amplified, filtered, and sampled at 400 and 4000 Hz, respectively (PowerLab 8/30, AD Instruments) and displayed by LabChart (v7.3.7, AD Instruments) for offline analysis.[10]

The urinary bladder recovered from instrumentation during 45 min of continuous infusion of physiological saline. The infusion rate was adjusted in each animal to achieve 10 min intermicturition intervals and was not different between strains (8 ± 0.5 vs. 9 ± 0.9 ml/h, $p = 0.1624$). Infused, residual, and voided volumes were manually measured for each voiding event. Each animal was considered an experimental unit and had at least two replicates that were averaged over a total of $n = 40$ OP trials and $n = 39$ OR trials. Following cystometry, blood samples were collected from the tail vein and blood glucose (mg/dl) was assayed (AlphaTRAK 2, Abbott

Animal Health). Animals were euthanized with Euthasol (250 mg/kg, ip) and bilateral thoracotomy, and tissues were harvested for myography.

2.4 | Myography

Full-thickness longitudinal strips of bladder tissue ($2 \times 10\,mm^2$) and circumferential 2 mm rings of urethral tissue ($n = 9$ OP, $n = 8$ OR) with the mucosa intact were cut and mounted in chambers (820M, 620M, Danish Myo Technology A/S) filled with Krebs (in mM: 130 NaCl, 4.7 KCl, 14.9 $NaHCO_3$, 5.6 dextrose, 1.18 KH_2PO_4, 1.18 $MgSO_4$, 1.56 $CaCl_2$) aerated with 37°C 95% O_2 and 5% CO_2. Baseline tension was applied (2–4 mN). Force generation was quantified with a force transducer and acquired by a PowerLab recording unit and LabChart for offline analysis. Tissue viability was determined with 120 mM KCl.

Bladder strip contractile force was measured to escalating concentrations of carbamylcholine chloride (carbachol, 10^{-8}–$10^{-4.5}$ M, Sigma-Aldrich).[12] Force responses to electric field stimulation (EFS) were measured using square-wave pulses at a frequency of 1–32 Hz, a pulse width of 0.3 ms, and an amplitude of 20 V (Grass Instruments).[12] Following baseline EFS, bladder tissue was incubated with atropine (1 μM, Sigma-Aldrich) or alpha, beta-methylene ATP (ABMA, 10 μM, Sigma) and EFS repeated.[13] Atropine and ABMA were added to the tissue bath 20 min or immediately before EFS, respectively. Tetrodotoxin (TTX, 0.5 μM, 10 min, Tocris) was applied at the end of the study and EFS stimulation at 32 Hz showed no direct muscle activation confirming that EFS responses were via nerve stimulation (data not shown).

Urethral tissue was dissected into proximal (0–5 mm from bladder neck) and middle (5–10 mm) ring sections. Contraction force in the urethra was measured in response to both increasing concentrations or frequencies of carbachol and EFS (30 V amplitude) as described above. Caffeine (40 mM, Tocris) was administered to probe calcium pathways, and isometric tension was measured for 10 min.[14]

2.5 | Analysis

All values are reported as mean ± standard error. Statistical computations were performed in MATLAB (R2019b, Mathworks) and R 3.6.3/RStudio 1.4.1103. Reported p values and confidence intervals were corrected for multiple comparisons with a one-step multiplicity adjustment from a multivariate t distribution.

Parameters measured in uroflowmetry include urinary frequency, the sum of all voids per week, voided volume (ml), the average volume of each void per week, urine flow rate (ml/s), the average of volume divided by time of each void per week, water consumption (ml), the sum of all water consumed per week, and body weight (g). Uroflowmetry was analyzed with a linear mixed model (nlme::lme) containing effects of water consumption, bethanechol treatment, strain and time interaction, and a varying slope and intercept per animal. Parameters measured in cystometry were previously defined[10,15] and include volume threshold capacity (ml), voiding efficiency (%), pressure during filling (cmH_2O), pressure to generate a void (cmH_2O), the difference between pressure at volume threshold and maximum void pressure, EUS activity during filling (μV), the average value of rectified EMG signal, and EUS bursting time (s). Cystometry was analyzed with a linear model using generalized least squares (nlme::gls) with unequal variance in strain. Muscle contractility was measured as the maximum – minimum baseline force (mN). Myography was analyzed with a linear mixed model (nlme::lme) containing effects of strain and frequency or strain and dose interaction, and a varying slope and intercept per animal. The percent change from paired baseline measures was calculated and analyzed for atropine and ABMA. Area under the curve (mN*s) with caffeine and force (mN) response to ABMA were compared (nlme::lme) between strains.

3 | RESULTS

3.1 | OP rats exhibited increased urinary frequency and decreased voided volume and urine flow rate during awake uroflowmetry

3.1.1 | Urinary frequency

Urinary frequency increased in OP compared to OR rats when adjusted for water consumption ($p = 0.0031$; -Figure 1A). Increased urinary frequency was observed from Week 1 ($p = 0.0193$) and persisted to Week 15 ($p = 0.021$; -Figure 1A). Bethanechol did not change urinary frequency ($p = 0.5636$), and increased frequency remained in OP rats from Week 16 ($p = 0.0249$) through Week 18 ($p = 0.0337$; Figure 1A).

3.1.2 | Voided volume

The rate of change of voided volume over time varied between OP and OR rats when adjusted for water consumption ($p = 0.0077$; Figure 1B). Voided volume increased at a faster rate in OR rats with a difference of 0.055 ml per week ($p = 0.0087$; Figure 1B). Voided volume was also decreased

in OP compared to OR rats ($p = 0.0093$; Figure 1B), and decreased voided volume was observed from Week 2 ($p = 0.0328$) and persisted to Week 15 ($p = 0.0355$; -Figure 1B). Bethanechol did not change voided volume ($p = 0.2514$), and decreased volume remained in OP rats from Week 16 ($p = .0374$) through Week 18 ($p = 0.0409$; Figure 1B).

3.1.3 | Urine flow rate

The rate of change of urine flow rate over time varied between OP and OR rats when adjusted for water consumption ($p = 0.0013$; Figure 1C). Urine flow rate increased at a faster rate in OR rats with a difference of 0.006 ml/s per week ($p = 0.0017$; Figure 1C). The urine flow rate was also decreased in OP compared to OR rats ($p = 0.0064$; Figure 1C), and decreased urine flow rate was observed from Week 4 ($p = 0.0487$) and persisted to Week 15 ($p = 0.0096$; Figure 1C). Bethanechol did not change urine flow rate ($p = 0.9617$), and decreased flow rate remained in OP rats from Week 16 ($p = 0.0096$) through Week 18 ($p = 0.0097$; Figure 1C).

3.1.4 | Other parameters

Water consumption was not different between OP and OR rats ($p = 0.0743$; Figure 1D). OP rats weighed more than OR rats ($p = 0.0029$), and increased weight was observed from Week 1 ($p = 0.0031$) through Week 18 ($p = 0.0118$; Figure 1E).

3.2 | OP rats exhibited decreased voiding efficiency, decreased pressure to generate a void, and increased EUS filling activity during anesthetized cystometry

At the time of instrumented cystometry, OP rats weighed more than OR rats (567 ± 30 vs. 308 ± 16 g, $p < 0.0001$) but exhibited no difference in whole blood glucose (153 ± 18 vs. 152 ± 12 mg/dl, $p = 0.9705$). OP rats had similar volume threshold capacity to OR rats ($p = 0.5807$) but were unable to empty efficiently their bladder, as reflected in a 31% decrease in voiding efficiency ($p < 0.0001$, VV: 0.07 ± 0.01 vs. 0.5 ± 0.07, PVR: 1.5 ± 0.25 vs. 0.9 ± 0.14 ml; Figure 2B,C). OP and OR rats also had similar bladder pressure during filling ($p = .0.2587$), but OP rats were unable to generate robust voiding contractions and exhibited 9.8 cmH_2O lower voiding pressures than OR rats ($p < 0.0001$); Figure 2D,E). EUS activity was also altered in OP rats during filling and voiding, and OP rats exhibited an increase in EUS EMG activity

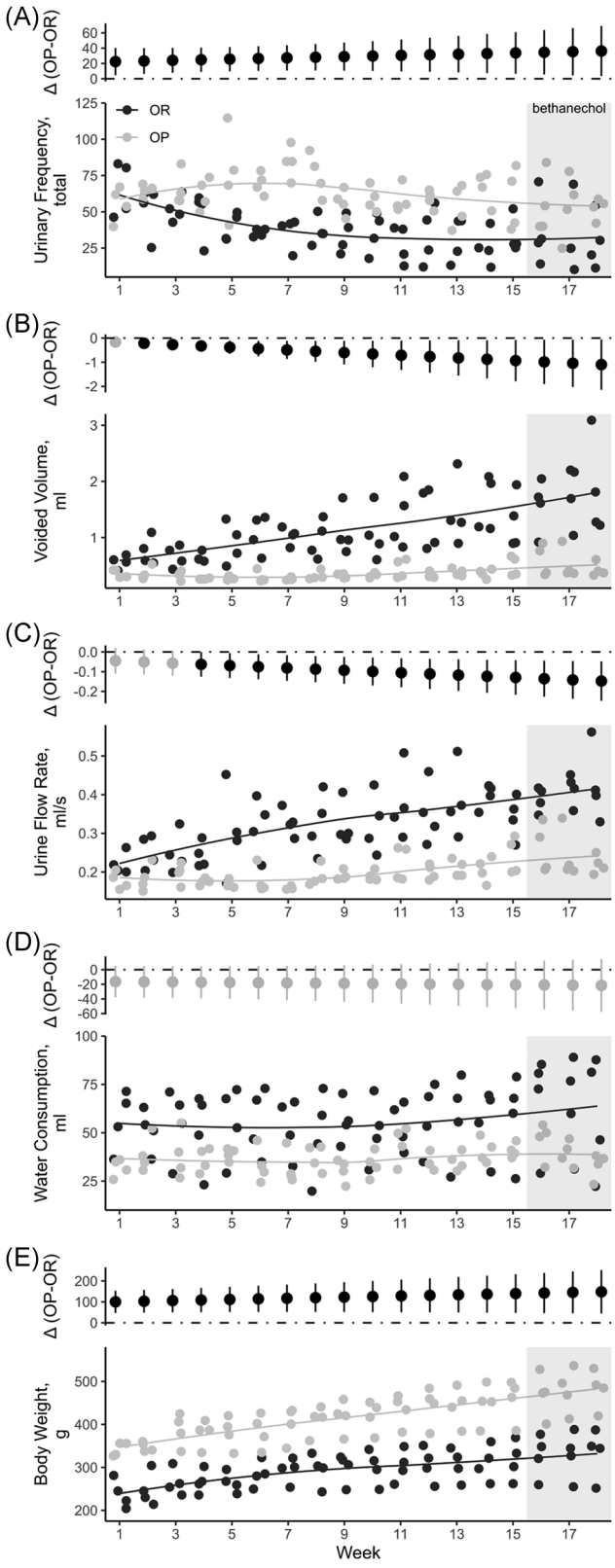

FIGURE 1 (See caption on next page)

during filling ($p = 0.0011$), as well as a decrease in EUS bursting time ($p = 0.0175$; Figure 2F,G).

3.3 | OP rats exhibited decreased bladder contraction strength when exposed to carbachol and ATP, and increased middle urethral contraction strength when exposed to carbachol, EFS, and caffeine

Contraction strength to KCl was not different between OP and OR rats in the bladder (23 ± 1.9 vs. 21 ± 2.1 mN, $p = 0.9835$), proximal urethra (5.6 ± 2.7 vs. 4.4 ± 2.9, $p = 0.9835$), or middle urethra (5.3 ± 2.7 vs. 9.5 ± 3.1 mN, $p = 0.9835$), and all force measurements were normalized to KCl evoked forces.

3.3.1 | Bladder

OP rats exhibited decreased contraction strength when exposed to carbachol at concentrations greater than $10^{-5.5}$ M ($p < 0.001$; Figure 3A). There were no differences in contraction strength to EFS between OP and OR rats ($p = 0.9618$; Figure 3B). Purinergic and cholinergic mechanisms were also assessed during EFS. Purinergic-resistant ($p \geq 0.7257$) and cholinergic-resistant ($p \geq 0.0883$) neurogenic contractions were not different between OP and OR rats from 1 to 32 Hz; however, OP rats demonstrated decreased contraction to ABMA ($p = 0.0004$; Figure 3C–E).

3.3.2 | Proximal urethra

OP rats exhibited decreased contraction strength when exposed to higher concentrations of carbachol ($10^{-5.5}$ M: $p = 0.0083$, 10^{-5} M: $p = 0.0136$, $10^{-4.5}$ M: $p = 0.0004$; Figure 4A). OP rats also had a 0.23 mN/KCl increase in strength when exposed to EFS at 32 Hz ($p = 0.0054$; Figure 4B). Sustained muscle contraction to caffeine was not changed between OP and OR rats ($p = 0.0958$; Figure 4C).

3.3.3 | Middle urethra

OP rats had a 0.16 mN/KCl increase in contraction strength when exposed to carbachol at 10^{-6} M ($p = 0.0014$; Figure 5A). OP rats also had a 1 mN/KCl increase in contraction strength with EFS at 32 Hz ($p = 0.0289$; Figure 5B). Sustained muscle contraction to caffeine was increased by 899 mN*s/KCl in OP compared to OR rats ($p = 0.0031$; Figure 5C).

4 | DISCUSSION

OP rats exhibited a constellation of changes consistent with DU and impaired cholinergic- and purinergic-dependent bladder contractions. We observed decreased urine flow rate in uroflowmetry, incomplete emptying in cystometry, and bladder contractions of reduced strength in myography. We also observed increased urethral activity during bladder filling in cystometry and increased middle urethral contractility in myography. Collectively, these findings suggest that alterations in both bladder and urethral function contribute to poor bladder emptying in OP rats.

4.1 | Uroflowmetry

Patients with UAB report symptoms of storage (nocturia, increased daytime frequency, and urgency), voiding (slow stream, hesitancy, and straining), and post micturition dysfunction (feeling of incomplete emptying).[16] In addition, patients with DU undergoing pressure-flow studies exhibit decreased urinary stream, decreased voided volume, decreased sensation, and straining.[5] After 12 weeks of DIO, OP rats exhibited increased urinary frequency compared to OR rats, as well as decreased voided volume and urine flow rate after 13 or 15 weeks, respectively. These results suggest that OP rats display both storage and voiding signs and symptoms that reflect those in patients with DU.

The management of UAB and DU is limited to improving detrusor contractility (cholinergic activation), reducing urethral outlet resistance (alpha-blockers, botulinum toxin A), sacral neuromodulation, or clean intermittent catheterization.[17] Randomized controlled

FIGURE 1 Voiding function during awake uroflowmetry. Obese prone (OP) rats exhibited increased urinary frequency (A, $p = 0.0031$), decreased voided volume (B, $p = 0.0093$), and decreased urine flow rate (C, $p = 0.0064$) compared to obese resistant (OR) rats when adjusted for water consumption. Bethanechol did not alter voiding function parameters. Water consumption was not different between strains (D, $p = 0.0743$) but OP rats weighed more than OR rats (E, $p = 0.0029$). $n = 4$/strain, linear mixed model. Point estimates and 95% confidence intervals derived from the statistical model adjusted for water consumption (A–C) and bethanechol (A–E) are shown in graphs with black coloring equating to $p < 0.05$

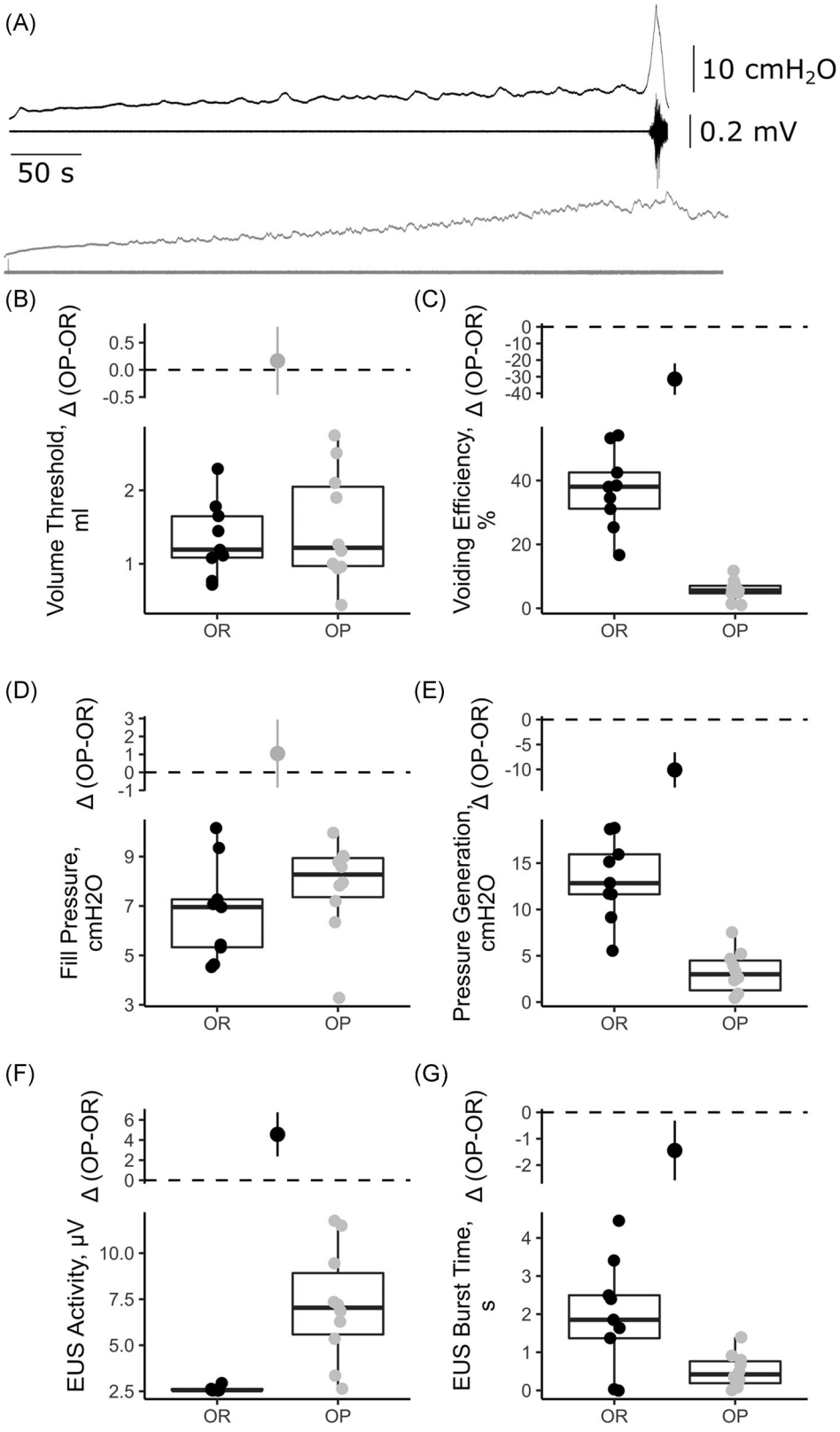

FIGURE 2 Voiding function during urethane-anesthetized cystometry. Representative traces of bladder pressure and EUS EMG in OR and OP rats (A). OP rats exhibited decreased voiding efficiency (C, $p < 0.0001$), decreased intravesical pressure to generate a void (E, $p < 0.0001$), increased EUS EMG activity during filling (F, $p = 0.0011$), and decreased EUS bursting time (G, $p = 0.0175$) compared to OR rats. $n = 9$–10/strain, linear model using generalized least squares. Point estimates and 95% confidence intervals derived from the statistical model are shown in graphs with black coloring equating to $p < 0.05$. EMG, electromyogram; EUS, external urethral sphincter; OP, obese prone; OS, obese resistant

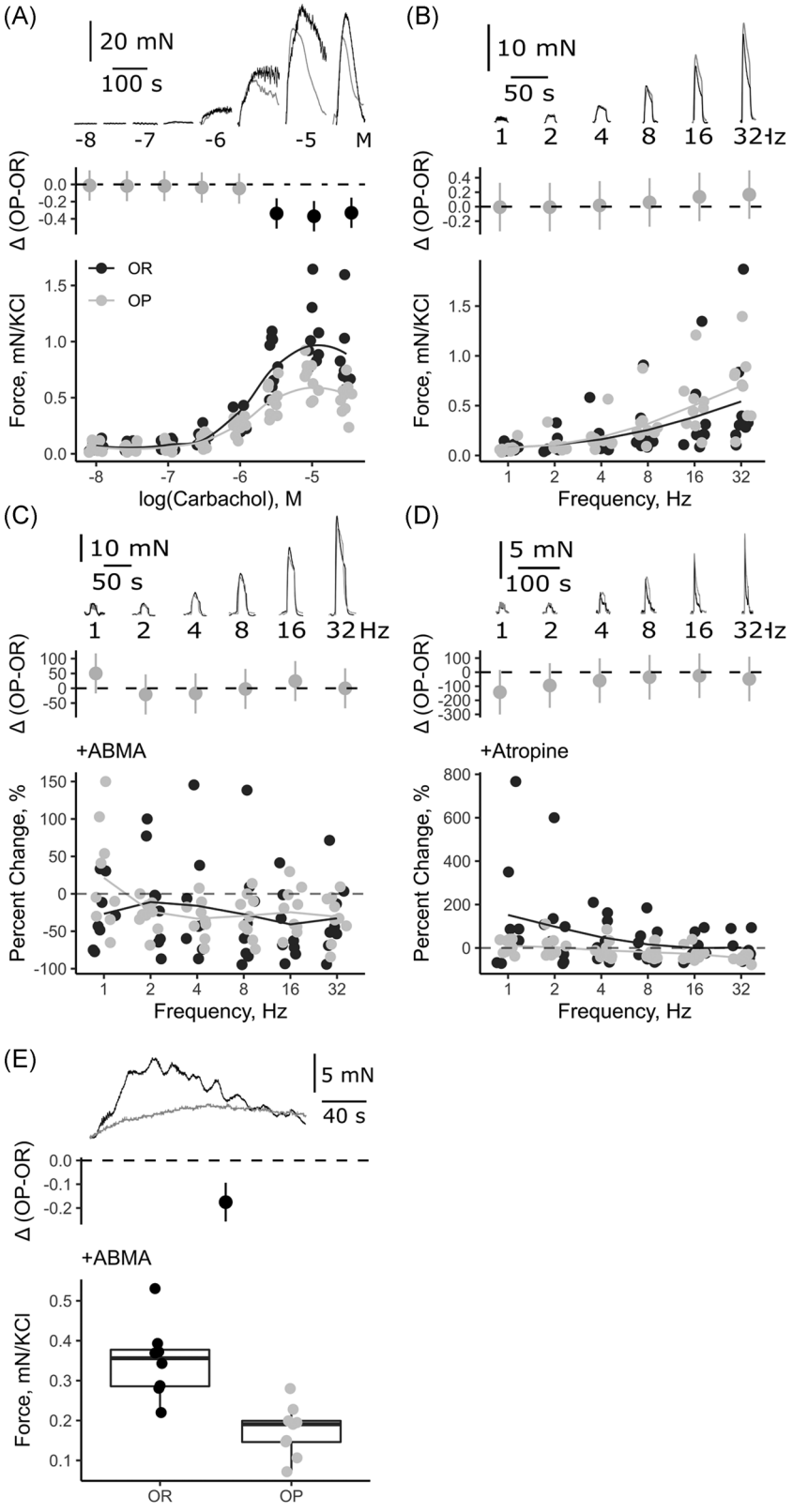

FIGURE 3 Bladder contraction strength to carbachol, electric field stimulation (EFS), and ATP. OP rats exhibited decreased contraction strength when exposed to carbachol at $10^{-5.5}$ ($p = 0.0002$), 10^{-5} ($p = 0.0001$), and $10^{-4.5}$ M ($p = 0.0003$) (A). There were no differences between strains in contraction strength during EFS (B) or during EFS with alpha, beta-methylene ATP (ABMA, C), or atropine (D). OP rats also exhibited decreased contraction strength when exposed to ABMA alone ($p = 0.0004$) (E). $n = 8$–9/strain, linear mixed model. Point estimates and 95% confidence intervals derived from the statistical model are shown in graphs with black coloring equating to $p < 0.05$. OP, obese prone

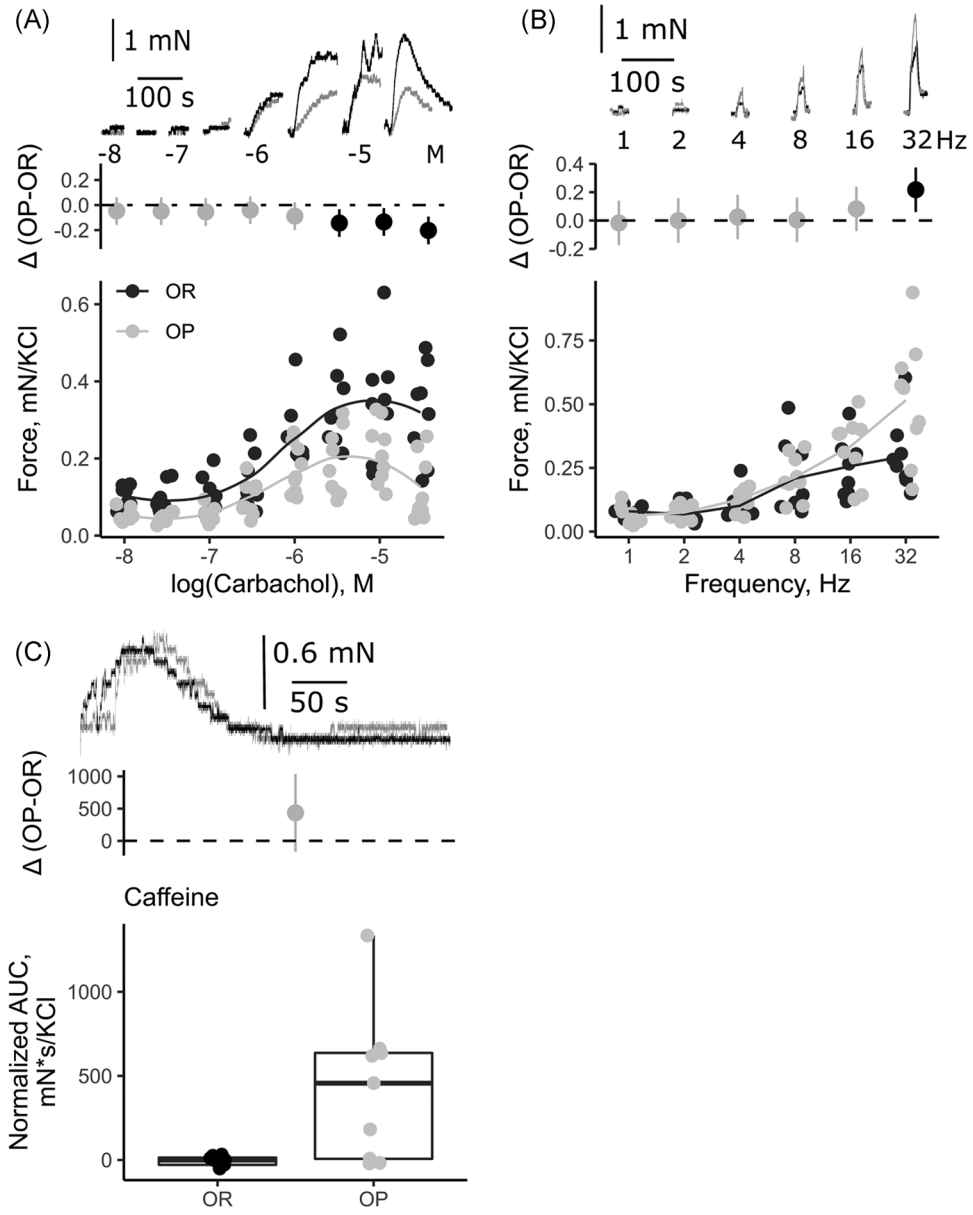

FIGURE 4 Proximal urethra contraction strength to carbachol, electric field stimulation (EFS), and caffeine. OP rats exhibited decreased contraction strength when exposed to carbachol at $10^{-5.5}$ ($p = 0.0083$), 10^{-5} ($p = 0.0136$), and $10^{-4.5}$ M ($p = 0.0004$) (A). Contraction strength to EFS at 32 Hz was increased in OP rats (B, $p = 0.0054$). There were no differences between strains in contraction strength during EFS with caffeine (C, $p = 0.0958$). $n = 8$–9/strain, linear mixed model. Point estimates and 95% confidence intervals derived from the statistical model are shown in graphs with black coloring equating to $p < 0.05$. OP, obese prone

trials currently show minimal benefit in the use of parasympathomimetics in preventing or treating underactivity.[18] Our studies support the ineffectiveness of parasympathomimetics on recovering underactive voiding function. Over the course of 3 weeks, bethanechol had no effect on frequency, volume, or flow rate. This suggests that the OP rat UAB may be less responsive to cholinergic activation, which was later confirmed in myography.

4.2 | Cystometry

Invasive cystometry under urethane anesthesia was conducted to determine whether incomplete emptying underlay the changes observed in uroflowmetry. OP rats exhibited increased postvoid residual and decreased voided volume, resulting in decreased voiding efficiency. This suggests that incomplete emptying may be contributing to the increased urinary frequency in

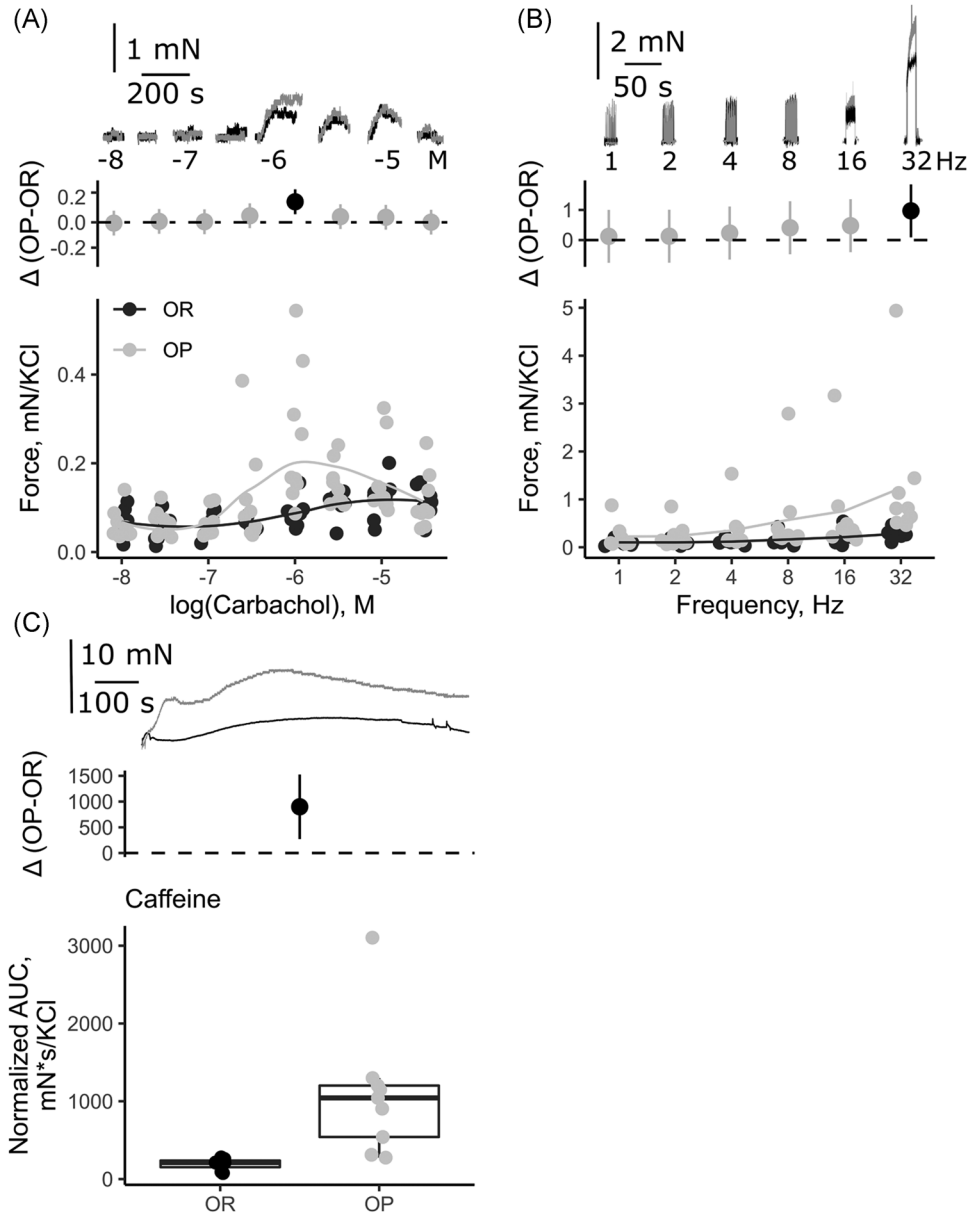

FIGURE 5 Middle urethra contraction strength to carbachol, electric field stimulation (EFS), and caffeine. OP rats exhibited increased contraction strength when exposed to carbachol at 10^{-6} M (A, $p = 0.0014$) and EFS at 32 Hz (B, $p = 0.0289$). Sustained contraction strength to caffeine was also increased in OP rats (C, $p = 0.0031$). $n = 7$–9/strain, linear mixed model. Point estimates and 95% confidence intervals derived from the statistical model are shown in graphs with black coloring equating to $p < 0.05$. OP, obese prone

uroflowmetry. It should be noted that OR rats also exhibit lower voiding efficiency compared to control, urethane-anesthetized rats[19] that may be due to high-fat feeding, intrinsic functional differences, or methodological variation.[10] Further, OP rats were unable to generate sufficient intravesical pressure to expel urine. These results align with a contraction of reduced strength that is used currently to define DU.[4] In addition to deficits in bladder function, we observed alterations in EUS EMG activity in OP rats including increased EUS activity during bladder filling and decreased EUS bursting time. Increased EUS activity with reduced bladder contractility and urine flow suggests OP rats may exhibit components of the clinical features of Fowler's syndrome.[20] Further studies with urethral pressure profilometry may help to clarify urethral dysfunction in OP rats.

Increased volume threshold, decreased voiding efficiency, decreased micturition pressure, and decreased EUS bursting time were demonstrated previously in OP rats.[10] We corroborate these results except for capacity, where we detected no change between OP and OR rats. This minor difference when comparing cystometric

measures between studies may be due to age (24 vs. 40–56 weeks) or animal source (Charles River vs. Duke Breeding Core). Interestingly, female patients with DU have decreased cystometric capacity, whereas male patients have increased capacity compared to controls.[5,21] This suggests capacity may not be a uniform feature of DU despite an association between increased capacity and decreased sensation.[21]

4.3 | Myography

Bladder and urethral muscle force were measured in vitro to determine the mechanisms underlying the impaired cystometric activity. Contractility of the OP rat bladder was decreased when exposed to carbachol and ATP. This suggests an impairment in post-junctional cholinergic and purinergic receptors within the detrusor smooth muscle. These deficits also support the lack of effect of bethanechol in uroflowmetry and indicate that targeting pathways downstream of the cholinergic receptor may be more effective. We also examined the purinergic and cholinergic contributions of neurogenic contractions of the bladder. We did not see a difference in purinergic-resistant or cholinergic-resistant force response to EFS between OP and OR rats. The conflicting results between EFS and carbachol and ATP response may be attributable to indiscriminate activation of excitatory and inhibitory nerves during EFS masking effects or non-cholinergic/non-purinergic contributions during EFS.

The urethra is comprised of inner longitudinal smooth muscle, outer circumferential smooth muscle, and striated muscle. The muscle force measured in our study from the proximal urethral ring is likely from the outer circumferential smooth muscle, whereas the middle urethral ring would contain a mixture of both smooth and striated muscle.[22] The proximal urethra in OP rats exhibited decreased contractility in response to carbachol but increased contractility in response to EFS. Carbachol was used to assess cholinergic smooth muscle contraction in the urethra that may have been influenced by bethanechol in uroflowmetry. These results suggest a deficit in cholinergic signaling with a concurrent increase in neurogenic activation. The neurogenic response may result from an increase in excitatory adrenergic pathways or a decrease in relaxation pathways (nitric oxide, peptides, purines, etc.).[23] Targeted pharmacological studies are necessary to determine which pathways contribute to this EFS response in the proximal urethra, and our current studies are limited by not assessing urethral contractility to adrenergic.

Unlike the proximal urethra, the middle urethra of OP rats exhibited increased contractility in response to both carbachol and EFS. This is consistent with the increased EUS EMG activity observed during bladder filling with cystometry. Contraction of smooth and striated muscle is also mediated by calcium-dependent mechanisms, and caffeine was used to probe calcium release through ryanodine receptors.[24] The middle, but not proximal, urethra of OP rats had a sustained increase in force to caffeine. This suggests that the striated muscle of the OP rat middle urethra may have increased sensitivity to calcium signaling. The combined outcomes from carbachol, EFS, and caffeine suggest a hyperexcitable state of the middle urethra that may reflect a pathological increase in outlet resistance that contributes to incomplete emptying.

5 | CONCLUSIONS

Impaired cholinergic and purinergic signaling in the bladder may contribute to increased urinary frequency, poor bladder emptying, and insensitivity to bethanechol in OP rats. In addition, the middle urethra of OP rats exhibited increased activity during bladder filling and increased responsiveness to pharmacological and electrical stimulation. This increase in urethral excitability may engage a guarding reflex to augment continence and exacerbate incomplete emptying.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

ETHICS STATEMENT

Duke University IACUC approval of protocol registry number A009-19-01.

AUTHOR CONTRIBUTIONS

Conceived and designed the research: Eric J. Gonzalez, Johanna L. Hannan, and Warren M. Grill. *Collected the data*: Eric J. Gonzalez, Michael R. Odom, and Johanna L. Hannan. *Performed the analysis*: Eric J. Gonzalez. *Interpreted the analysis*: Eric J. Gonzalez, Michael R. Odom, Johanna L. Hannan, and Warren M. Grill. *Prepared the*

figures: Eric J. Gonzalez. *Drafted the manuscript*: Eric J. Gonzalez. *Approved the final manuscript*: Eric J. Gonzalez, Michael R. Odom, Johanna L. Hannan, and Warren M. Grill.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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