

UNDERACTIVE BLADDER IN OBESE-PRONE RATS FED A HIGH FAT DIET

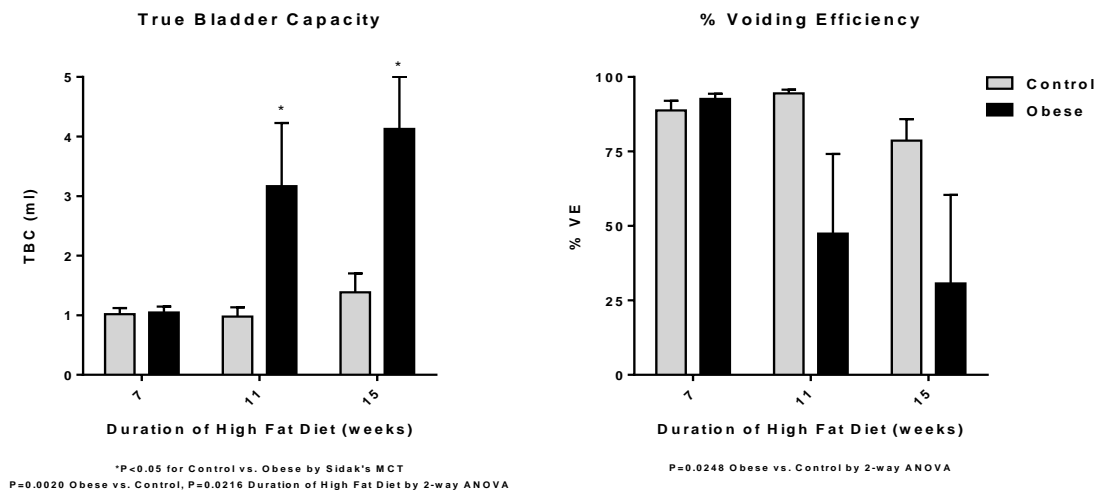
Hypothesis / aims of study

Changes in systemic metabolism lead to a spectrum of alterations in bladder physiology. For example, diabetic and non-diabetic adults with insulin resistance have a high prevalence of overactive bladder (OAB).[1] In those with diabetes, a form of underactive bladder, characterized by impaired bladder sensation and decreased detrusor contractility, can also occur.[2] High fat diets (HFD) have been implicated in the development of obesity and insulin resistance, and thus might be associated with changing bladder symptoms. However, few preclinical studies have examined these associations. The Obese Prone (OP-CD) and Obese Resistant (OR-CD) rats (Charles River, Boston, MA) are unique animal models used in obesity-associated research.[3] These rats have normal leptin function and the same parent genetic background, but have been selectively bred such that on the same HFD, OP-CD rats become obese while OR-CD rats stay lean. We hypothesized that, as a result of obesity-related insulin resistance, OP-CD rats on HFD would develop cystometric correlates of overactive bladder (high amplitude non-voiding contractions and reduced bladder capacities), while OR-CD rats would maintain normal bladder function. To test this hypothesis, we characterized the bladder function in both of these rat strains during chronic HFD feeding.

Study design, materials and methods

This preliminary experiment examined cystometric changes in female OP-CD and OR-CD rats chronically given HFD over a 15 wk time period. Briefly, 4 OP-CD and 4 OR-CD rats were purchased at 9 wk of age; all animals were immediately placed on the same HFD. From 9 to 21 wk of age this was 30% fat/50% carbohydrate (Research Diets, D12266B). From 21 to 24 wk of age this was 60% fat/20% carbohydrate (Research Diets, D12492). Chronic bladder catheters were surgically implanted at 15 wk of age; all catheters were tunnelled subcutaneously to the midscapular region for future cystometric evaluations. Conscious restrained cystometry was performed at 16, 20, and 24 wk of age (corresponding to 7, 11 and 15 wk HFD). Prior to each cystometry, animals were weighed, body length was measured (nose to rump), and body mass index was calculated (BMI = cm/g²). After 15 wk HFD, serum glucose was also measured.

For all cystometric evaluations, animals were initially anesthetized with isoflurane, the catheters were exposed, and the animals were mounted in Ballman restraint cages. Following recovery from anaesthesia, normal saline was infused via chronic bladder catheters at 0.1 ml/min. After a 60 min control period, bladders were emptied and single fill cystometrograms were performed to determine true bladder capacity (TBC), followed by ~60 min of continuous cystometry to determine functional bladder capacity (FBC), measured as the volume infused during each intermicturition interval. Voiding efficiency (%VE) was calculated as follows: %VE = [average FBC/TBC]*100. For statistical calculations, animals who developed overflow incontinence were *a priori* assigned a large TBC of 5 ml and a voiding efficiency of 1%. Cystometric and BMI data were analysed by 2-way analysis of variance, +/- repeated measures as appropriate, with Sidak's multiple comparison post-test. Mean blood glucose was compared between groups using the t-test.



Results

BMI measurements were taken from the 4 OP-CD and 4 OR-CD rats at each time interval. Mean BMIs were significantly higher after 7, 11, and 15 wk of HFD in OP-CD compared to OR-CD rats (0.64 vs. 0.46 at 7wk, 0.72 vs. 0.52 at 11 wk, 0.74 vs. 0.52 at 15 wk, respectively; p = 0.002). At 15 wk HFD, serum glucose ranged from 82 – 115 mg/dL across the 8 animals. There were no significant differences in mean blood glucose at 15 wk HFD between OP-CD and OR-CD groups (102 +/-14 vs. 97 +/- 11, respectively; p=0.58).

Cystometry was performed after 7, 11, and 15 wk of HFD. TBC and %VE were compared across all time points and results are shown in the figure below. After 7 wk HFD, all rats exhibited normal voiding patterns, without high amplitude non-voiding contractions, and there were no significant differences in bladder capacity measurements between groups (mean values = 1.05 and 1.02 ml for OP-CD and OR-CD, respectively). After 11 wk HFD, 2/4 (50%) of OP-CD rats exhibited overflow incontinence while the remaining 2/4 OP-CD and all 4 OR-CD rats exhibited normal voiding patterns. After 15 wk HFD, the chronic catheter in one OP-CD rat who demonstrated overflow incontinence at 11 wk HFD was not functioning, and thus cystometry was not performed on this animal. Of the remaining OP-CD rats, 2/3 (66%) exhibited overflow incontinence while all OR-CD rats showed normal voiding patterns. Mean %VE decreased significantly in the OP-CD group by 2-way ANOVA, although post-test analysis did not reveal significant differences at individual time points.

Interpretation of results

As expected, on high fat diets consisting of 30-60% fat, the obese prone (OP-CD) rats became obese and the obese resistant (OR-CD) rats stayed lean. In obese animals, bladder capacity increased, voiding efficiency decreased, and by the end of the study period the majority of obese animals exhibited overflow incontinence. Notably, the rat with a malfunctioning catheter at 15 wk HFD was one of the animals showing overflow incontinence at 20 wk and would have presumably continued to show overflow at the later time point. Thus, in this particular model, rats who become obese on a high fat diet develop bladder *underactivity* compared to non-obese rats on the same diet. Importantly, after 15 wk HFD, none of the animals were classified as diabetic based on blood glucose measurements.

Concluding message

Somewhat surprisingly, although obese non-diabetic animals exhibited significant changes in bladder function, we did not identify any cystometric correlates of OAB in this animal model. Rather, OP-CD rats given a chronic HFD may serve as a promising new model to study underactive bladder. Further studies are required to understand the mechanisms involved.

References

1. Brown JS, Vittinghoff E, Lin F, et al. Prevalence and risk factors for urinary incontinence in women with type 2 diabetes and impaired fasting glucose: findings from the National Health and Nutrition Examination Survey (NHANES) 2001-2002. *Diabetes Care*. Jun 2006;29(6):1307-1312
2. Kaplan SA & Blaivas JG. Diabetic cystopathy. *J Diabet Complications*. Jul-Sep 1988; 2(3): 133-9
3. Swithers SE, Sample CH, Davidson TL. The effects of high-intensity sweeteners on energy intake and weight control in male and obesity-prone female rats. *Behavioral Neuroscience* 2013; 127(2):262-274.

Disclosures

Funding: 1) National Institutes of Health/NIDDK 1K12 DK100024-01; 2) Duke University Charles B. Hammond Research Fund
Clinical Trial: No **Subjects:** ANIMAL **Species:** Rat **Ethics Committee:** Duke University IACUC (Institutional Animal Care and Use Committee) & Durham VA Medical Center IACUC; Durham, North Carolina, USA