

THE EFFECT OF VAGINAL DISTENSION ON BLADDER AND URETHRAL FUNCTION IN NULLIPAROUS LYSYL OXIDASE LIKE-1 KNOCKOUT AND C57BL6 MICE.

Hypothesis / aims of study:

Female pelvic floor dysfunction (FPFD) is a complex and often debilitating group of conditions which include urinary incontinence, voiding dysfunction, pelvic organ prolapse, and anal incontinence. These disorders affect up to 75% of women, and require surgery in 11% of women, yet, little is known about their pathophysiology. Pregnancy and childbirth are well documented risk factors for FPFD, with urinary incontinence being particularly associated with a prolonged second stage of labor. Animal models of female pelvic floor dysfunction are limited. In rats, prolonged vaginal distension, using an inflated Foley catheter inserted into the vagina, leads to tissue hypoxia and reduced leak point pressures [1]. These findings suggest that urethral/vaginal trauma associated with ischemia during a prolonged second stage of labor may play a role in the pathophysiology of FPFD.

A new model of FPFD may help to elucidate the pathophysiology of FPFD associated with birth trauma. In 2004, Liu et al. noted that mice lacking the protein, lysyl oxidase-like 1 (LOXL1) routinely develop pelvic organ prolapse [2]. LOXL1 is required to synthesize elastin polymers from tropoelastin monomers in adult tissues. LOXL1 knockout (LOXL1 KO) mice develop pelvic organ prolapse after pregnancy and delivery or with aging, presumably due to an inability to maintain homeostasis of mature elastic fibers. In 2006, Liu et al. further characterized the FPFD seen in these mice [3]. LOXL1 KO mice develop overt prolapse 1-3 days post partum (Acute Stage), which retracts over 1-2 weeks, leaving a persistent perineal bulge with internal pelvic organ descent (Stable Stage). The vaginal wall was noted to be markedly distended with uterine descent into the upper vagina. Liu et al. also demonstrated that absence of LOXL1 leads to voiding dysfunction and possibly urinary incontinence in these mice. Parous prolapsed LOXL1 KO mice voided more frequently with smaller volumes than parous non-prolapsed wild type controls. The role of elastic fiber homeostasis in response to tissue trauma related to vaginal childbirth remains unclear. The objective of the study was to determine the effect of vaginal distension on voiding function and abdominal leak point pressures in Lysyl Oxidase Like 1 deficient (LOXL1 KO) and C57Bl6 mice.

Study design, materials and methods:

Nulliparous, virginal LOXL1 KO mice and C57Bl6 mice, aged 3-6 months, were assigned to either vaginal distension or no vaginal distension (control) groups. Mice in the vaginal distension group were anesthetized and the vagina was serially dilated to accommodate a 12 Fr vaginal dilator. A modified pediatric Foley catheter was placed in the vagina and secured with silk suture. The balloon was distended with 0.5 ml of saline for four hours. After four hours of vaginal distension, the catheter was removed and the mice were allowed to awaken from anesthesia. Two days later, a suprapubic bladder catheter was placed under ketamine anesthesia. Two days after catheter placement, the mice underwent awake cystometrograms (CMG). Mice were then anesthetized with urethane and underwent abdominal leak point pressure testing (LPP). Control mice underwent catheter placement, followed by CMG and LPP testing two days later, as outlined above but did not undergo the vaginal distension procedure. The mean peak bladder pressures, baseline bladder pressures, fill time, fill volume, void volume, void pressure, voiding frequency, frequency of nonvoid contractions, and overall bladder contraction frequency were calculated from CMG data, and the mean peak bladder pressures, baseline bladder pressures, fill volume and abdominal leak point pressures were calculated from LPP data.

Univariate testing was performed using Student t-tests for normative data and Wilcoxon Rank Sum tests for non-normative data. Differences between group mean values were compared using two way ANOVA testing. Pairwise multiple comparisons were performed using the Student-Neuman-Keuls method. A P-value less than 0.05 was considered statistically significant.

Results:

Nine LOXL1 KO mice and 9 C57Bl6 mice were assigned to the vaginal distension group and 7 LOXL1 KO mice and 15 C57Bl6 mice were assigned to the control group. LOXL1 KO mice had a significantly increased frequency of non-void bladder contractions after vaginal distension than LOXL1 KO mice in the control group (24.95 ± 5.58 contractions/hour vs. 5.39 ± 6.32 contractions/hour, respectively. $P=0.028$). C57Bl6 mice also demonstrated an increase in non-void contraction frequency after vaginal distension compared with controls, although the results were not statistically significant (13.58 ± 6.32 contractions/hour vs. 3.82 ± 5.29 contractions/hour, respectively. $P=0.25$). No differences in fill volume, fill time, void pressure, void volume, or void frequency between the vaginal distension or control groups were identified on CMG testing. No differences were identified between LOXL1 and C57Bl6 mice within the vaginal distension or control groups. Abdominal leak point pressure testing revealed no differences within intervention groups in either mouse strain, however LOXL1 KO mice in the control group demonstrated a significantly greater abdominal leak point pressure than C57Bl6 controls (11.74 ± 2.58 vs. 4.47 ± 1.76 cm water, respectively. $P=0.026$). No mice developed acute pelvic organ prolapse up to four days after vaginal distension.

Interpretation of results:

LOXL1 KO mice demonstrate increased frequency of non-void bladder contractions after vaginal distension when compared with LOXL1 KO controls. These findings suggest that elastic fiber homeostasis after pelvic floor injury may play a role in the pathophysiology of detrusor overactivity. These findings are consistent with human studies which have shown decreased elastin gene expression in non-compliant human bladder tissue.

Our findings contrast with published studies of vaginal distension in rats, where reduction in LPP is observed after vaginal distension. Neither Lox1 KO nor C57Bl/6 mice demonstrate decreased LPP 4 days after vaginal distension. It is possible that in mice the bladder is more susceptible to injury from VD than the urethra, resulting in detrusor overactivity as opposed to stress urinary incontinence. Recovery rates may also differ between rats and mice. Longer term studies are needed to clarify this issue. Vaginal distension does not appear to induce acute pelvic organ prolapse in Lox1-deficient mice. No mice demonstrated pelvic organ prolapse four days after vaginal distension. This may be explained by either insufficient pelvic floor injury or inadequate length of follow up to demonstrate this effect. Alternatively, POP may result from a multifactorial etiology, and may not be caused by mechanical injury alone.

This study also suggests that different mouse strains may demonstrate significant differences in bladder and urethral function. Baseline differences in LPP were demonstrated between Lox1 KO controls and C57Bl/6 controls, suggesting a difference in urethral function between strains without intervention. Future studies are needed to determine if the higher LPP seen in Lox1 KO mice are due to altered elastic fiber homeostasis or other genetic differences.

Concluding message:

We conclude that vaginal distension in LOXL1 KO mice may lead to an increase in the frequency of nonvoid contractions, suggesting that altered elastic fiber homeostasis may play a significant role in the pathophysiology of detrusor overactivity.

References

1. [BJU International](#). 2002 Sep;90(4):403-7.
2. [Nature Genetics](#). 2004 Feb;36(2):178-82.
3. [American Journal of Pathology](#). 2006 Feb;168(2):519-28.

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ANIMAL SUBJECTS: This study followed the guidelines for care and use of laboratory animals and was approved by Louis Stokes Cleveland DVA Medical Center Institutional Animal Care and Use Committee