ADJUSTABLE PRELOAD AND DYNAMIC COMPLIANCE IN THE HUMAN DETRUSOR

Hypothesis / aims of study
Acute changes in detrusor tension may be important in both overactive bladder syndrome (OAB) and detrusor underactivity (DU). Total detrusor tension can be analyzed according to its component parts: the active tension (T_a) generated by detrusor smooth muscle (DSM) contraction and the preload (T_p). T_p can be further subdivided into passive tension (T_pas) derived from non-regulatable structural components (i.e. collagen and elastin) and an adjustable preload (T_ap) that has been identified in rabbit DSM (rDSM) [1] and other smooth muscle types. Alterations of T_ap are observed through the process of reversible strain softening [1]. Our published data using rDSM supports the hypothesis that T_ap is adjusted by breakage and reformation of slowly cycling actomyosin crossbridges, permitting reduced muscle stiffness (increases compliance) at increased muscle lengths [2]. Thus, we propose that T_ap is a mechanism by which bladder compliance is adjusted acutely during filling (dynamic compliance). In biologic tissues, strain softening is defined as progressive loss of stiffness with repeat stretches due to cross-link breakage. Unlike the irreversible strain softening that occurs when a latex balloon is stretched and released prior to its initial inflation, T_ap can be actively restored after contraction at short muscle lengths [1]. Recent pre-clinical studies have established that repeated passive fills of the bladder can acutely increase compliance. Moreover, acute compliance changes can be reversed by active contraction, a process we term "dynamic compliance."

The present study was designed to examine whether human DSM (hDSM) demonstrates T_ap in vitro via manipulation of hDSM tissue samples and the macroscopic correlate of T_ap, dynamic compliance, during in vivo human urodynamics. Improved understanding of the biomechanical mechanisms that can acutely regulate detrusor tension may therefore lead to novel treatments for OAB and DU and are the focus of the current investigation.

Study design, materials and methods
Tissue strip study: After IRB approval, hDSM tissue was obtained from cystectomy specimens. A validated stair-step length-tension protocol [3] was applied to each hDSM strip to determine the reference active tension and length (L_ref). To quantify T_ap in hDSM, tissues were released to 60% L_ref (slack tension) and actively contracted twice to re-form cycling actomyosin crossbridges and restore T_ap to baseline. Tissues were then subjected to three sequential load-unload (strain softening) cycles to 130% L_ref designed to break established crossbridges and quantify the reduction in T_ap. After cycle 2, tissues were actively contracted twice at 60% L_ref to reform crossbridges. The difference in T_p at L_ref between cycle 1 (T_p,1: before strain softening) and cycle 2 (T_p,2: after strain softening) estimates T_ap. T_p in cycle 3 (T_p,3: after active contraction) was expected to approximate T_p,1 due to re-establishment of actomyosin crossbridges lost during strain softening. All T_p values obtained in the T_ap protocol were normalized to T_p,1 measured at L_ref at the beginning of cycle 1 and reported as means ± SEM. A repeated-measures one-way ANOVA was performed with Holm-Sidak post hoc analysis to test for statistical significance.

Dynamic compliance study: Individuals with OAB defined as ICI-q-OAB question 5a ≥3 were enrolled in an IRB-approved extended urodynamics protocol (Figure 1). An initial urodynamic study was performed per best practice guidelines for clinical purposes and to determine maximum cystometric capacity (C_cap). Four repeat fills were then initiated at a rate 10% C_cap/min as follows: 1) fill to 30% C_cap and passively empty via syringe aspiration, 2) fill to 60% C_cap and passively empty, 3) fill to C_cap and void (voluntary or involuntary) and 4) fill to C_cap and void. Repeat Fills 1-3 were performed to strain soften the bladder with incrementally increasing preload. Dynamic compliance was evidenced by decreases in intravesical pressure (p_ves) with passive filling. The void after Fill 3 was performed to demonstrate the reversibility of dynamic compliance with active bladder contraction. Fill 4 was expected to demonstrate p_ves similar to that seen in Fill 1. Extracted p_ves values at 75mL infused for Fills 1-4 were normalized to p_ves at 75mL in Fill 1 and significance was determined via repeated-measures one-way ANOVA with Holm-Sidak post hoc analysis.

Results
Tissue strip study: Urinary bladder tissue specimens from a total of six patients were used for the T_ap protocol (n=6). Following the T_ap protocol, T_p decreased after strain softening (Figure 2). Normalized T_p,2 = 0.46 ± 0.11 which was a significant loss of T_p due to strain softening when compared to T_p,1 (p = 0.01). Normalized T_p,3 = 1.2 ± 0.30 was similar to T_p,1 (p = 0.50) demonstrating the presence of T_ap in hDSM (T_p lost to strain softening was restored by active contraction). Two patients in the T_ap cohort had neurogenic bladder, however, there were no differences in normalized T_p values between neurogenic and non-neurogenic tissues (t-test, p=NS).

Dynamic compliance study: Four patients completed the study and had results available for analysis (n=4). In comparison to repeat Fill 1, dynamic compliance was evident in subsequent fills (Fills 2 and 3) as seen by decreased p_ves values. This was likely due to strain softening during filling not reversed by passive emptying. Voiding occurred at the end of Fill 3 and p_ves during Fill 4 approached p_ves during Fill 1, demonstrating the reversibility of dynamic compliance caused by strain softening (representative data, Figure 3). Average normalized p_ves at 75 ml infused volume demonstrated dynamic compliance after passive emptying (Fill 3: normalized p_ves = 0.69±0.10 vs. Fill 1: 1.0, p<0.05) that was reversed after active voiding (Fill 4: normalized p_ves = 0.92±0.04 vs. Fill 1: 1.0, p=NS) (Figure 4).

Interpretation of results
This study identified and quantified the in vitro biomechanical property of T_ap in hDSM tissue strips and its in vivo correlate, dynamic compliance, seen during an extended repeat filling cystometry protocol. T_ap can be decreased acutely by strain softening and restored by active contraction of hDSM. Dynamic compliance is the macroscopic manifestation of T_ap: repeat fills strain soften
the bladder, similar to repeat stretches in hDSM tissue strips, and the reversibility of strain softening with voiding, similar to contraction of hDSM tissue strips. It is tempting to speculate that a derangement in the active process regulating $T_{ap}$ could contribute to the pathophysiology of an OAB-subtype – an increase in $T_{ap}$ causing an increase in urinary bladder stiffness during filling would result in increased afferent nerve activity and thus increased urgency at lower bladder volumes. Conversely, a decrease in $T_{ap}$ may cause impaired contractility as seen in DU due to a decreased bladder preload.

Concluding message
In this investigation, we revealed that the biomechanical process of $T_{ap}$, previously shown in rDSM exists in hDSM tissue, and that $T_{ap}$ can be seen during in vivo human urodynamics as dynamic compliance. Future research designed to explore the regulatory mechanism of this biomechanical process may lead to improved understanding and treatment of OAB and DU.

References

Disclosures
Funding: Virginia Commonwealth University Presidential Research Quest Fund Clinical Trial: No Subjects: HUMAN Ethics Committee: Virginia Commonwealth University Institutional Review Board Helsinki: Yes Informed Consent: Yes