

The Effect of Diabetes mellitus on Urethral Neuromuscular Function

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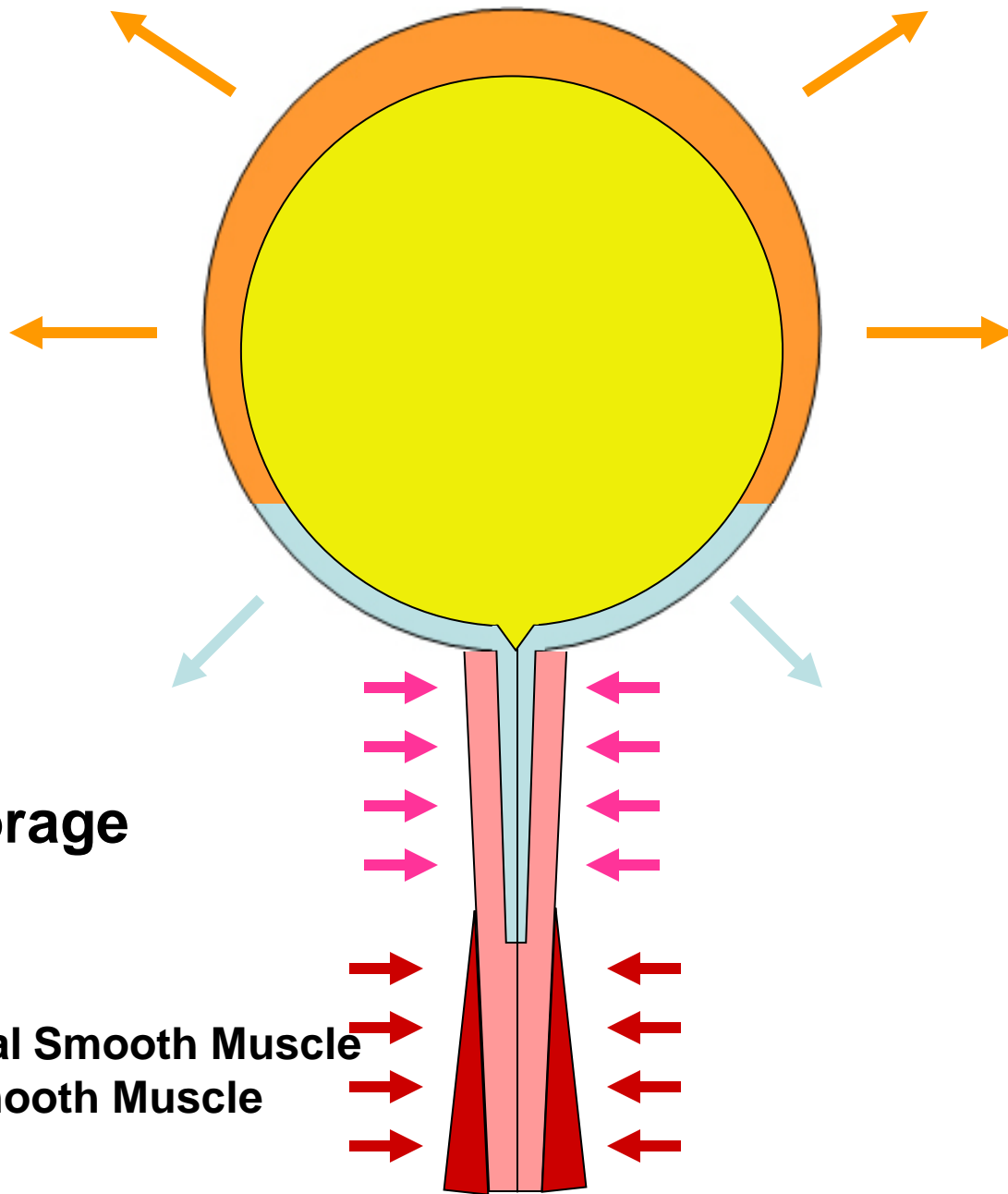
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AMDCC - Pilot and Feasibility Program

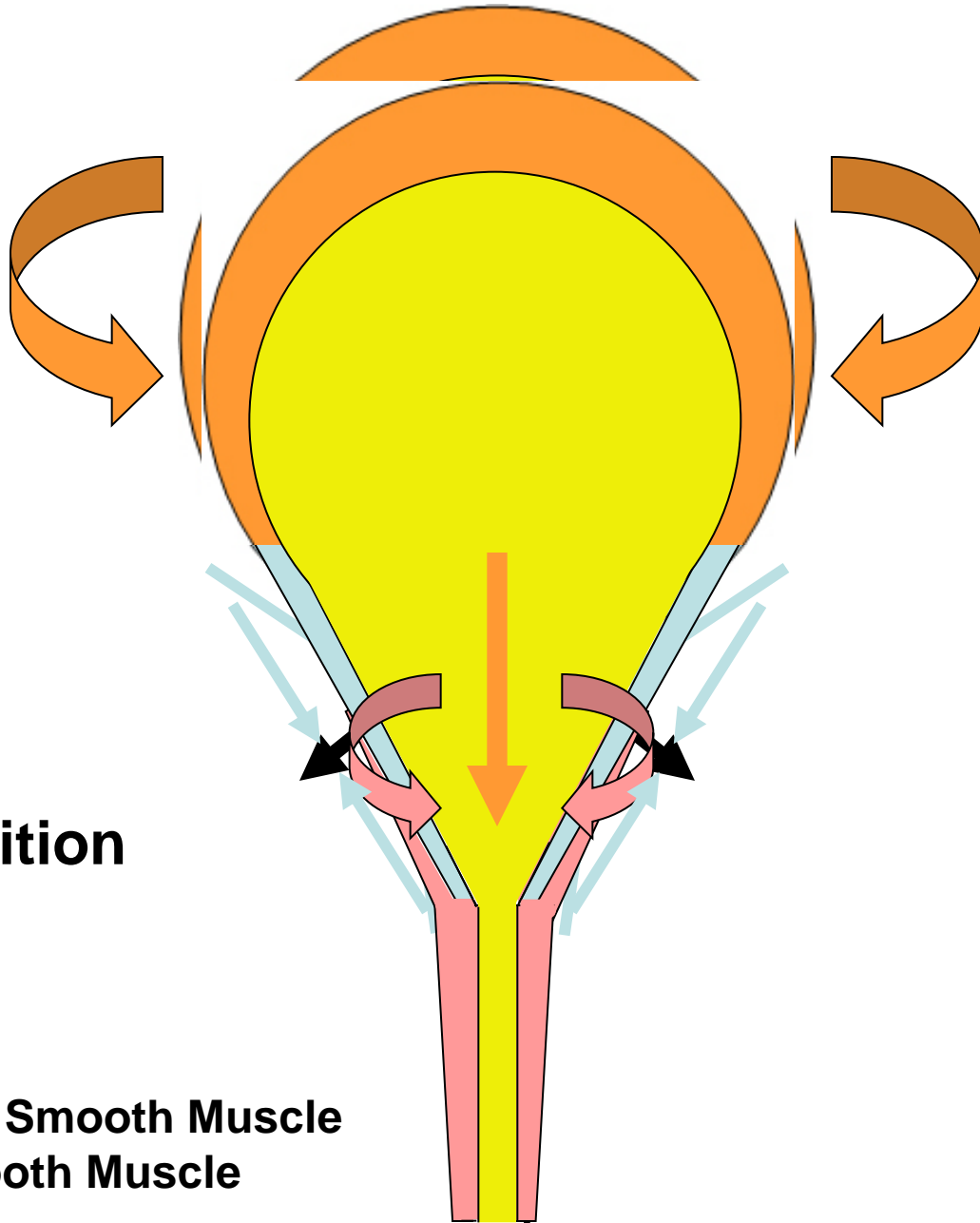
Annual Meeting

091022



Storage

- Detrusor**
- Longitudinal Smooth Muscle**
- Circular Smooth Muscle**
- EUS**



Micturition

- Detrusor
- Longitudinal Smooth Muscle
- Circular Smooth Muscle

LUT Smooth Muscles

	Bladder Circular	Urethral Circular	LUT Longitudinal
Storage	—	+	—
Micturition	+	—	+

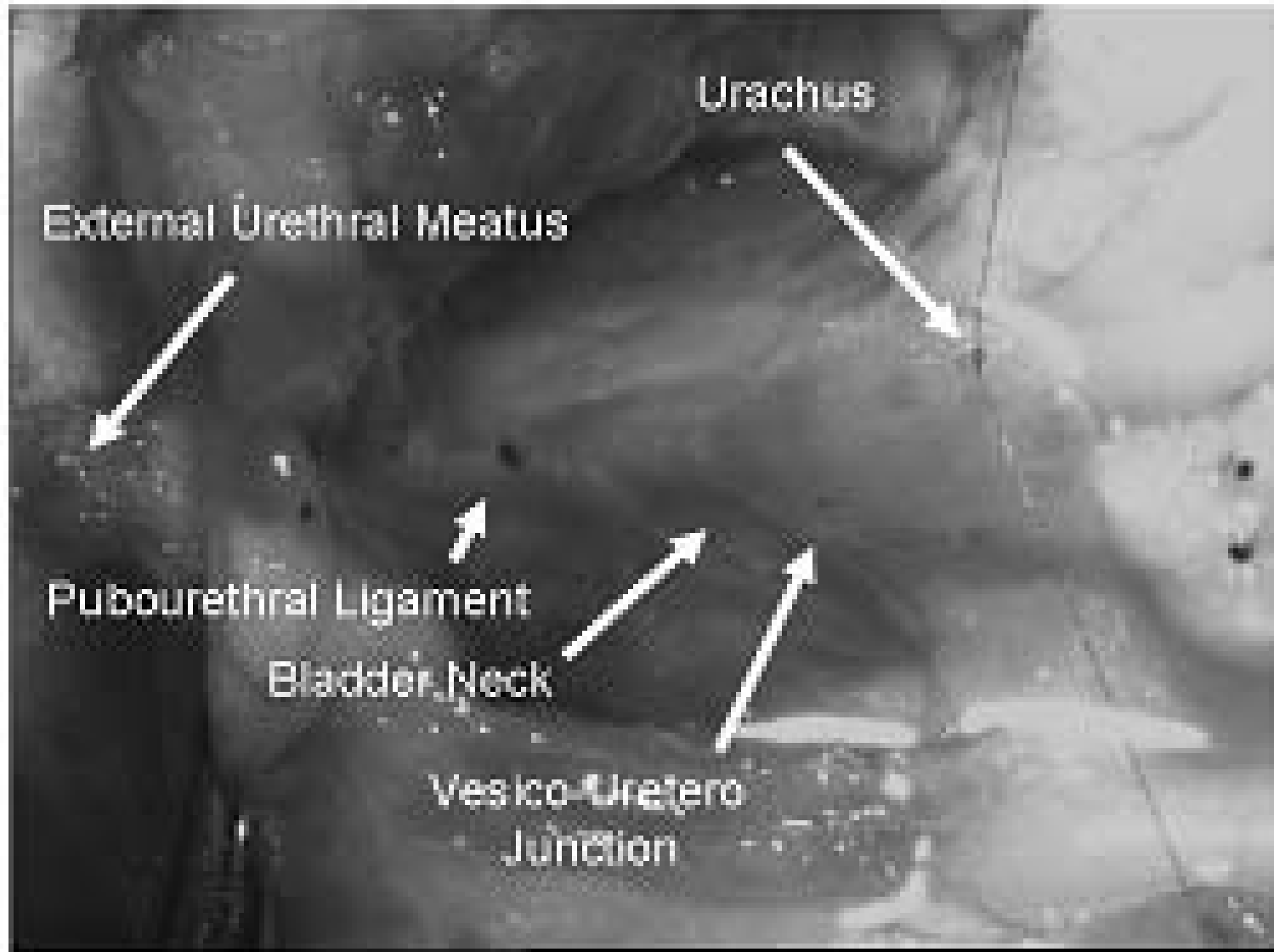
Specific Aims

- **Specific Aim 1:** In Vivo Physiology of LUT Smooth Muscles in 5, 10 and 20 week Vehicle treated and STZ-induced DM animals
- **Specific Aim 2:** In Vitro Pharmacology of LUT Smooth Muscles in 5, 10 and 20 week Vehicle treated and STZ-induced DM animals
- **Specific Aim 3:** In Vitro Passive Biomechanics of LUT Smooth Muscles in 5, 10 and 20 week Vehicle treated and STZ-induced DM animals
- **Specific Aim 4:** Microscopic Anatomical Studies of LUT Smooth Muscles in 5, 10 and 20 week Vehicle treated and STZ-induced DM animals

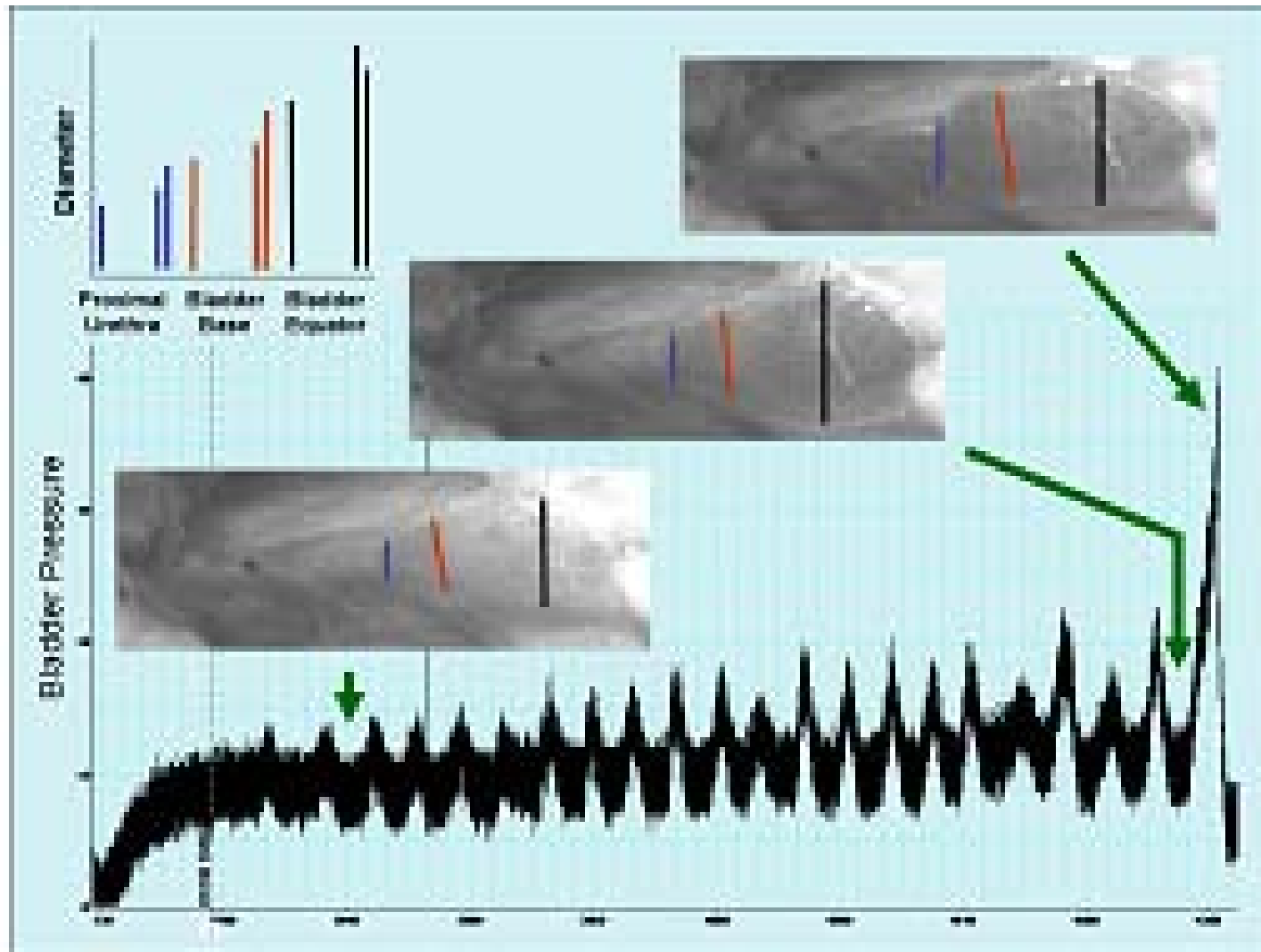
Specific Aim 1

- **Specific Aim 1: In Vivo Physiology of LUT Smooth Muscles in 5, 10 and 20 week Vehicle treated and STZ-induced DM animals**
 - Video urodynamics in urethane anesthetized (1.2 g/kg) control or DM rats with pubic symphysis removal for urethral visualization
 - Punctate Evans Blue dye injections or other anatomical landmarks at bladder neck, mid urethra and distal urethra will allow for movement tracking of ULSM, while the lateral edges of the urethra will allow for UCSM activity measurements
 - Video imaging capture system developed by Dr. Dolber to quantify in vivo activity of ULSM and UCSM during repetitive voiding cycles

Specific Aim 1

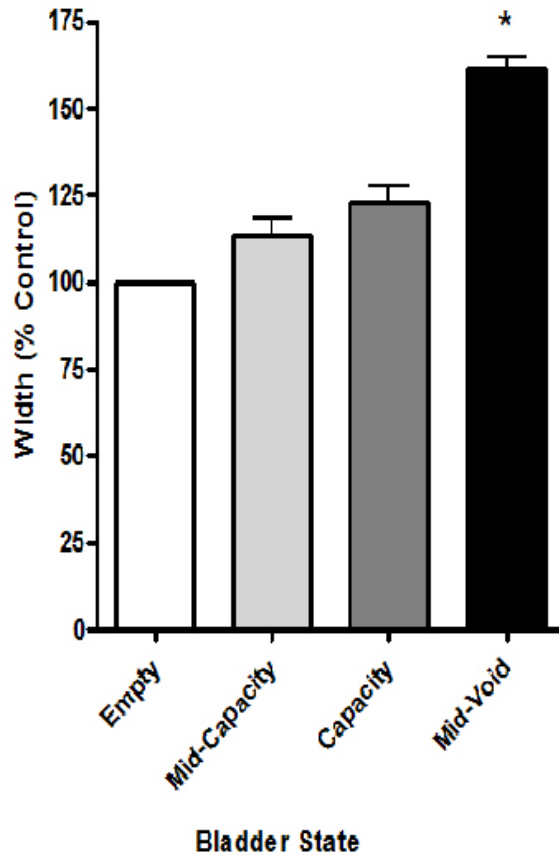


Specific Aim 1



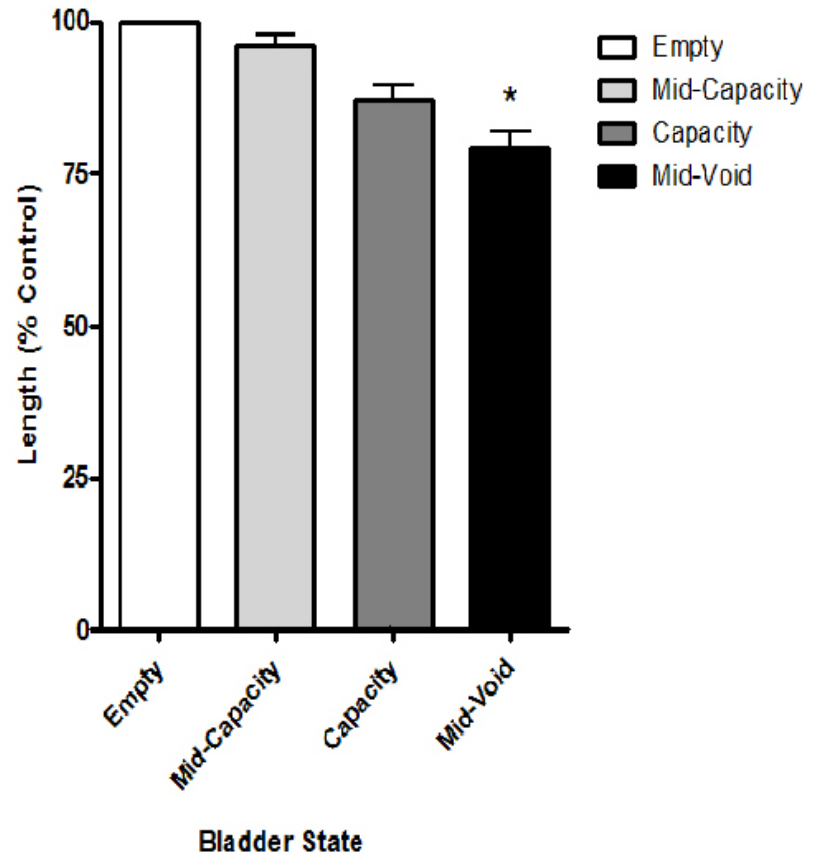
Specific Aim 1

Vesico-urethral Junction Width during Voiding Cycle in Rats (n=3)



P<0.05 vs. Empty by Dunn's MCT
P=0.0017 by Friedman Test from Empty

Proximal Urethral Length during Voiding Cycle in Rats (n=3)



P<0.05 vs. Empty by Dunn's MCT
P=0.0017 by Friedman Test from Empty

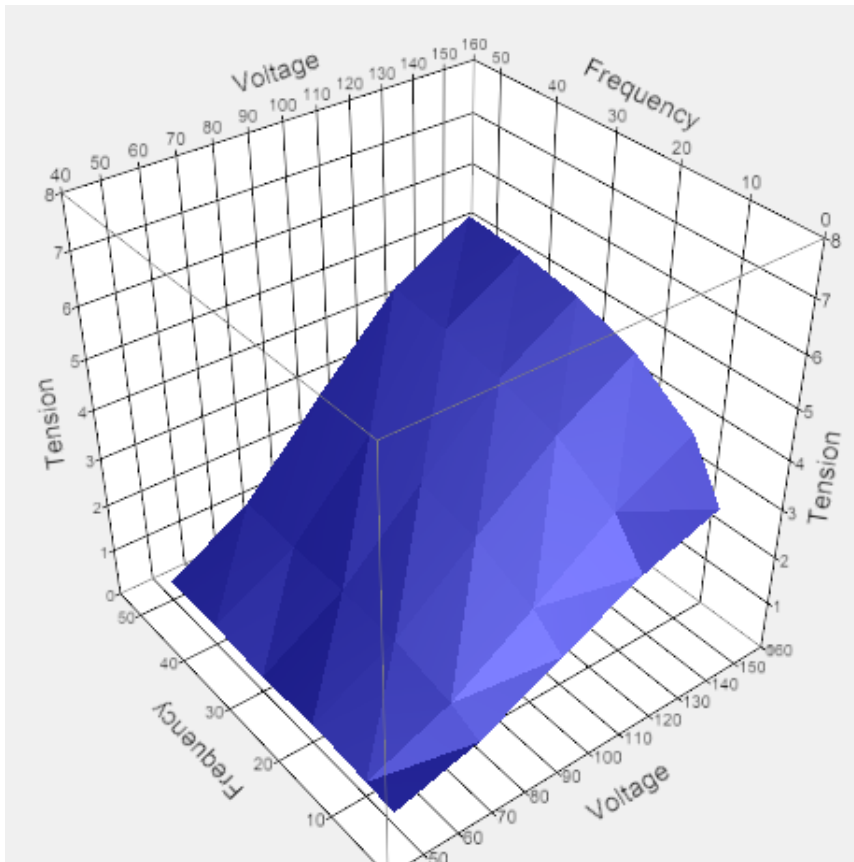
Specific Aim 2

- **Specific Aim 2: In Vitro Pharmacology of LUT Smooth Muscles, and Bladder in 5, 10 and 20 week Vehicle treated and STZ-induced DM animals**
 - Urethral strips, both longitudinal (proximal urethra) and circular (proximal and mid-urethra) and bladder strips, both circumferential base and detrusor and longitudinal (whole bladder dorsal and ventral) will be harvested from the animals used in SA1
 - Frequency-voltage-tension relationships will be performed for each
 - Following EFS stimulus-response determination, the responses to best representative candidate contractors (carbachol, α,β -methylene-ATP and phenylephrine) and relaxers (isoproterenol and sodium nitroprusside) will be tested in 7 point dose-response relationships

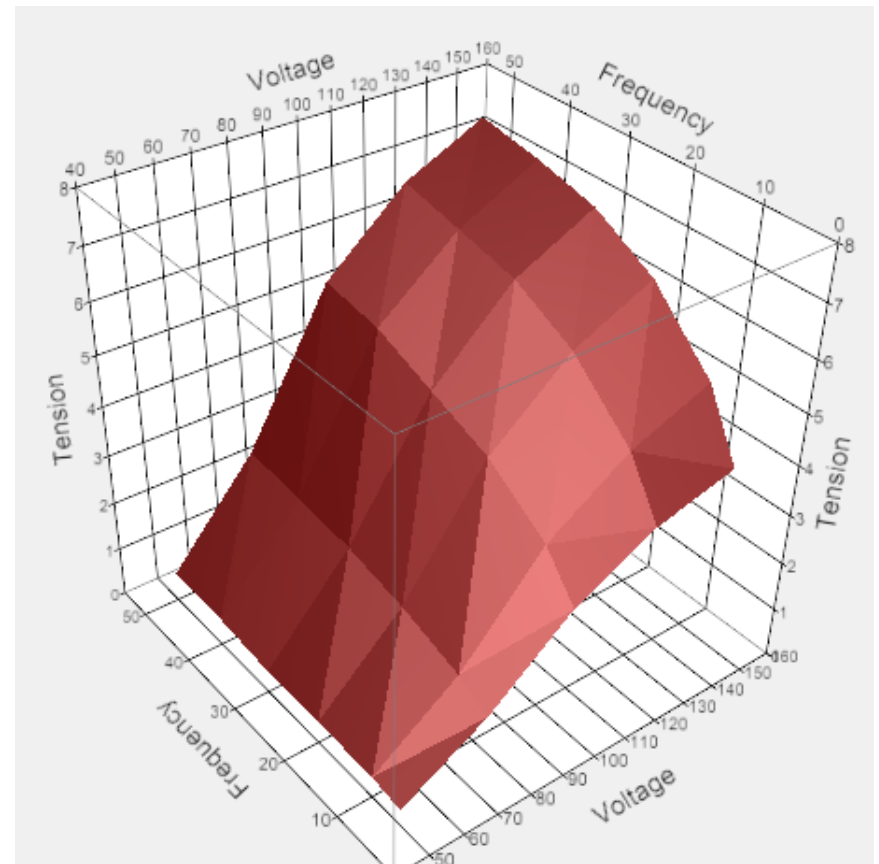
Specific Aim 2

Bladder Strips

Control



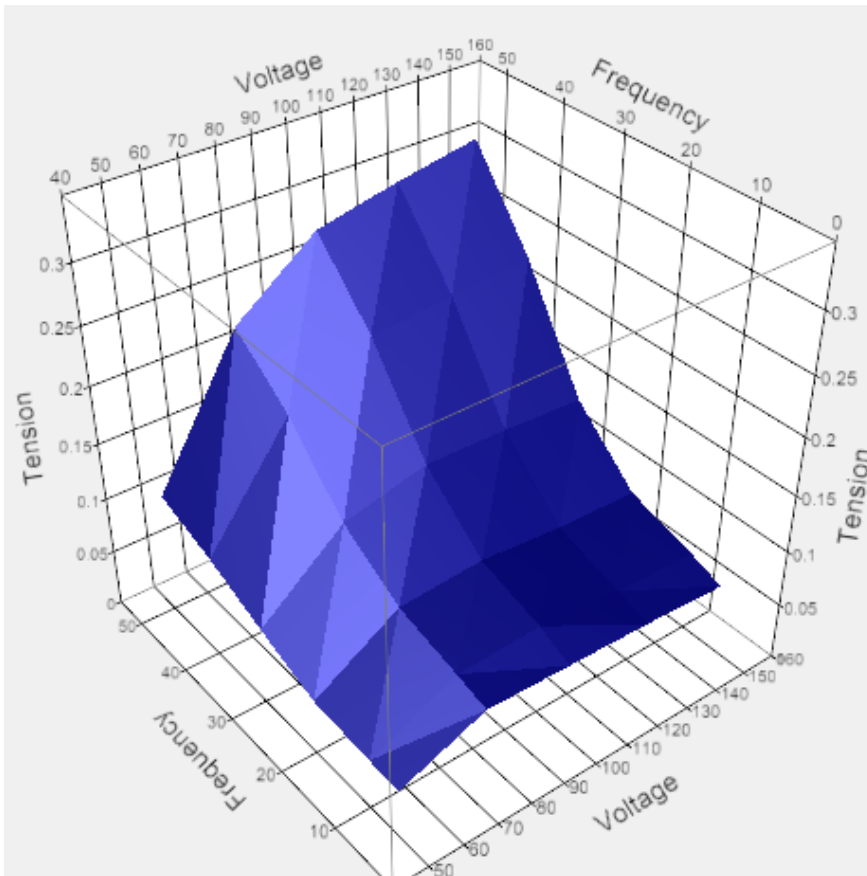
DM



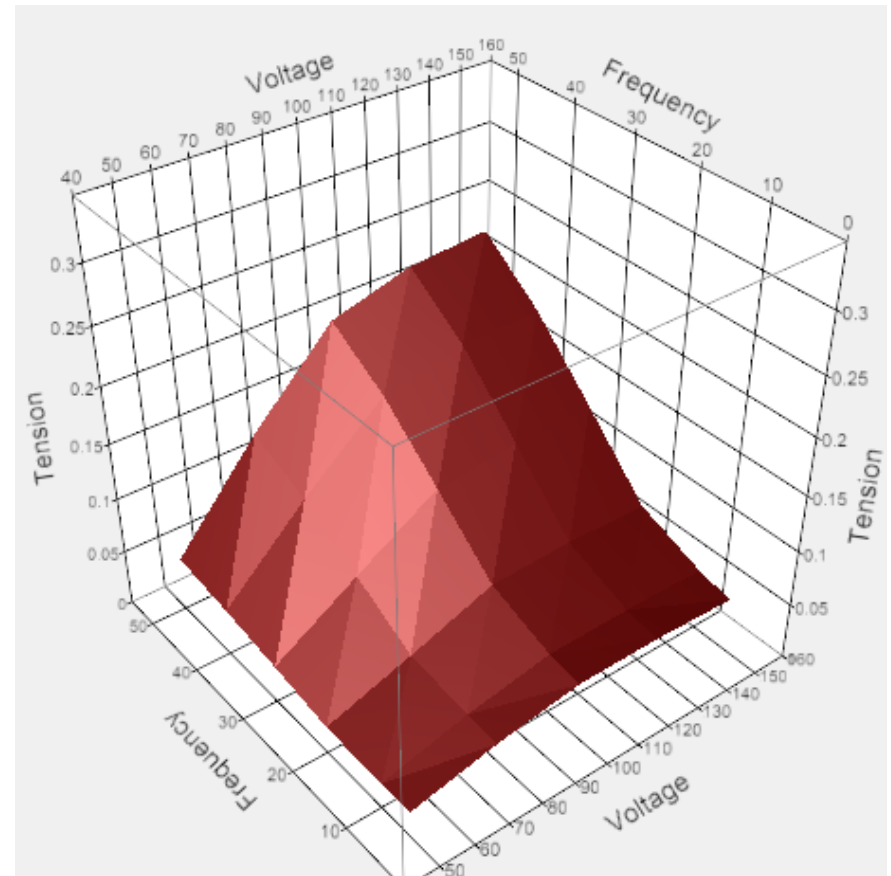
Specific Aim 2

Urethral Strips

Control

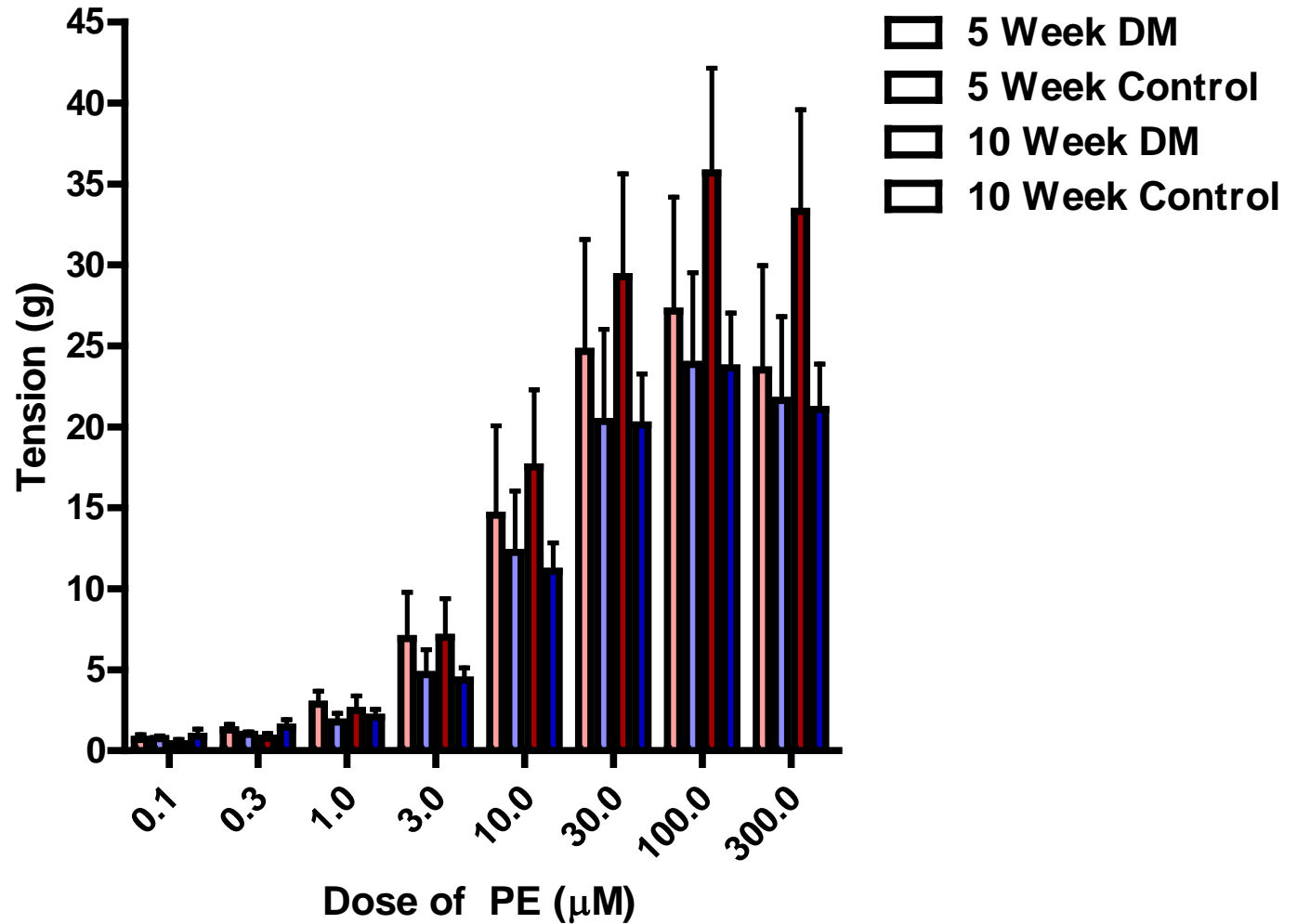


DM



Specific Aim 2

PE Dose-Response

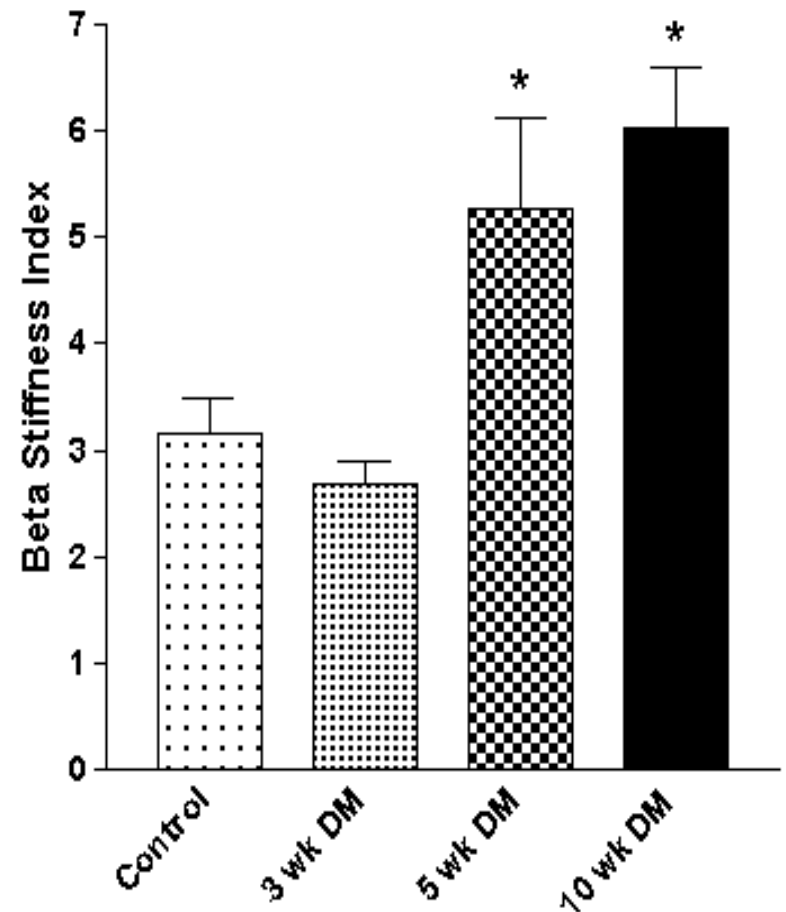
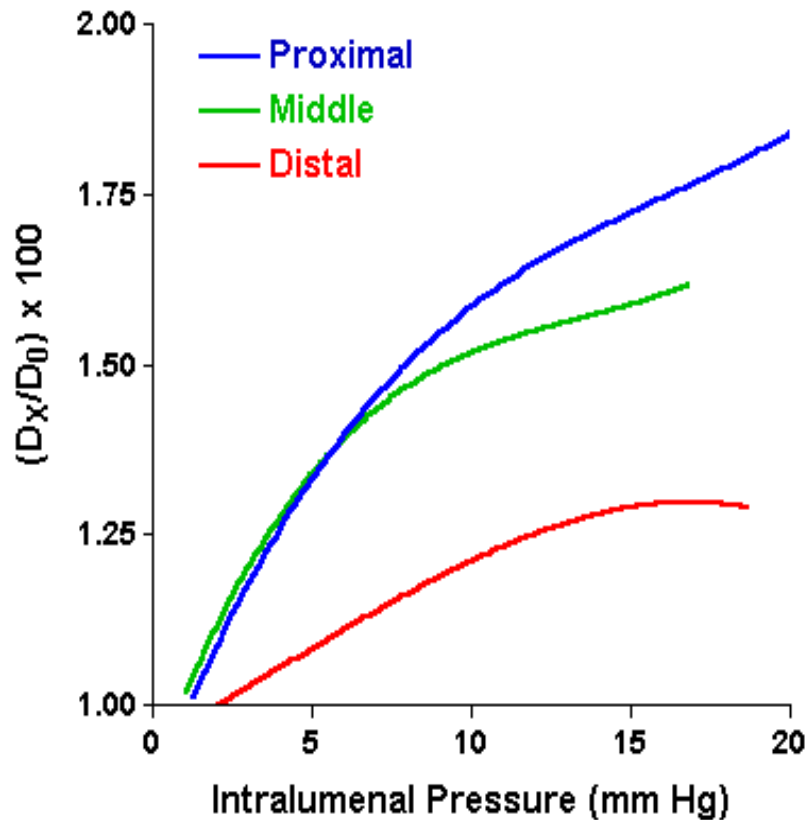


Specific Aim 3

- **Specific Aim 3: In Vitro Passive Biomechanics of LUT Smooth Muscles in 5, 10 and 20 week Vehicle treated and STZ-induced DM animals**
 - Utilizing the same strips used in SA2, we will construct length-tension relationships in EDTA treated strips in order to determine passive tissue compliance of these muscles

Specific Aim 3

Whole Mount Urethra – Circumferential Diameter Measurement

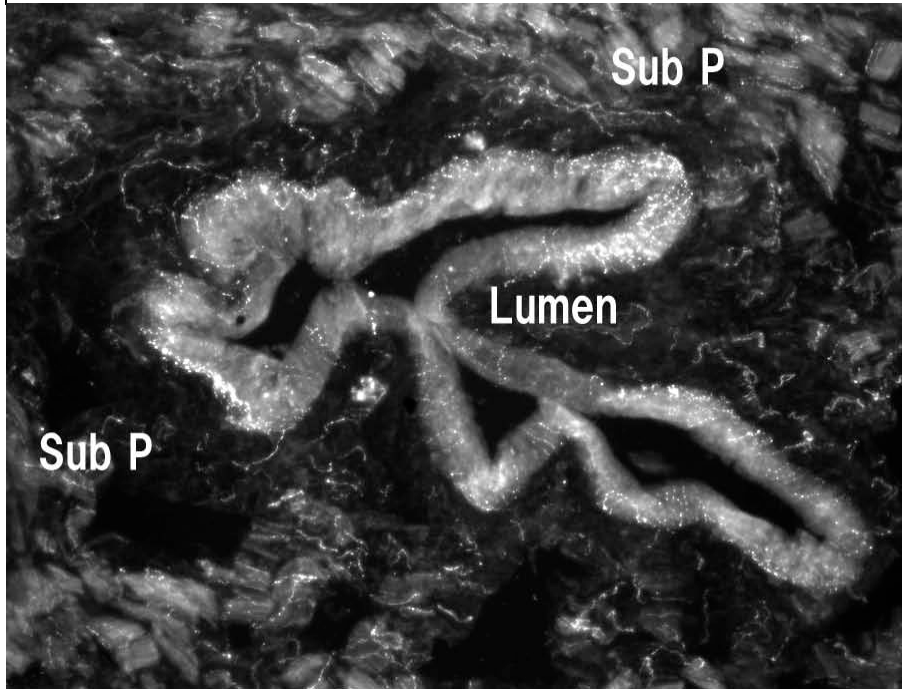


Specific Aim 4

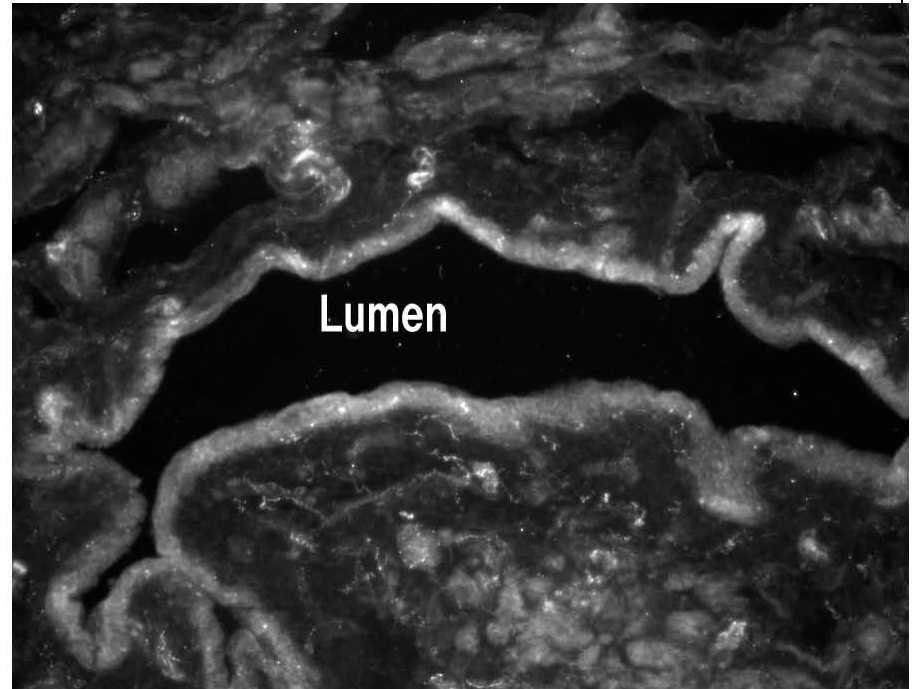
- **Specific Aim 4: Microscopic Anatomical Studies of LUT Smooth Muscles in 5, 10 and 20 week Vehicle treated and STZ-induced DM animals**
 - LUT tissues will be drop fixed in formalin fixative, sucrosed and frozen in OCT media, and sectioned across longitudinal and circumferential axes.
 - Alternate slides will be stained for H&E, collagen, elastin, AGEs, general neuronal (PGP-9.5), efferent markers (TH, ChAT), and afferent markers (Substance P and Neurofilament 200)

Specific Aim 4

Control Sub P



DM Sub P



Goals

- **We hope to understand the role of LUT smooth muscles, including the understudied longitudinal system, in storage and release of urine, and how DM affects this role**
- **We will have in vitro studies using EFS and pharmacological studies to determine function innervation over the course of DM**
- **We will examine the biomechanical properties of urethral and bladder longitudinal and circumferential smooth muscle systems**
- **We will characterize the histological correlates of our functional results**