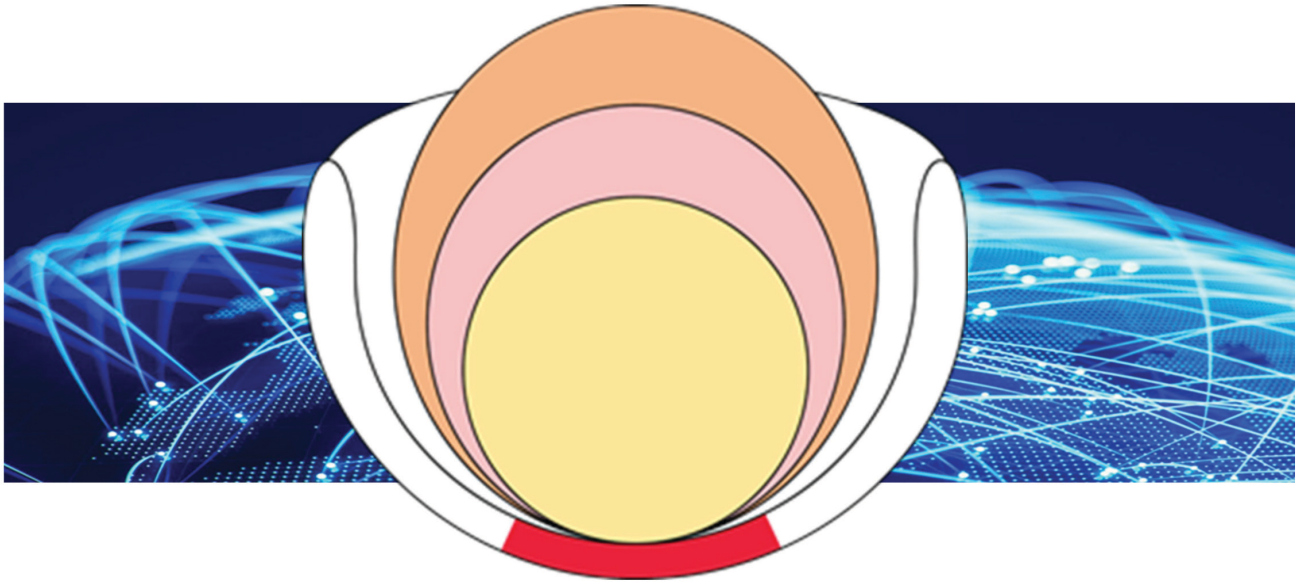
THE SOCIETY FOR PELVIC RESEARCH

Eighth Annual Meeting



Meeting Program

December 7-9, 2023
Savannah, GA

#SPR2023

A scientific society founded by and for career basic and translational scientists interested in normal function and benign disease states of the pelvic viscera and pelvic floor

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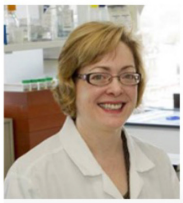
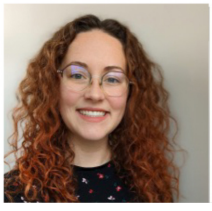


Member at Large
Matthew O. Fraser, PhD
Research Scientist
Durham VA Medical Center

Member at Large
Dr. Alvaro Munoz, PhD
Professor
University of Guadalajara

Trainee Affairs Chair
Cara Hardy, PhD
Post-Doctoral Research Fellow
Jackson Laboratory



Thanks to:

NIH R13 AG084283-01
2023 Annual Meeting of Society for Pelvic Research

International Continence Society

Collaborating for the Advancement of Interdisciplinary Research in Benign Urology

SPR Code of Conduct

Statement of commitment to provide a safe environment

The Society of Pelvic Research (SPR) is dedicated to providing a harassment-free conference experience for everyone, regardless of gender, gender identity and expression, age, sexual orientation, disability, physical appearance, body size, race, ethnicity, religion (or lack thereof), or technology choices.

Expectations of behavior

•Our aim is to maintain the highest standards of professional and ethical conduct.

•We will not tolerate harassment of conference participants in any form, with specific emphasis on harassment related to gender, gender identity and expression, age, sexual orientation, disability, physical appearance, body size, race, ethnicity, religion (or lack thereof), or technology choices.

•In-person and/or virtual attendees agree and acknowledge that they are undertaking participation in the meeting code of conduct, with unacceptable behavior defined as harassment, intimidation, or discrimination in any form, verbal abuse of any attendee, speaker, volunteer, sponsor, staff member, service provider, or other meeting guests.

•Sexual language and imagery will not be permitted at the conference venue, including talks, workshops, parties, Twitter and other online media.

Filing a complaint

Individuals who have questions, concerns or complaints related to harassment are encouraged to contact the conference organizers (Society for Pelvic Research, kelvin.davies@einsteinmed.edu or msullivan@rics.bwh.harvard.edu) or the HHS Office for Civil Rights (OCR) at https://www.hhs.gov/civil-rights/filing-a-complaint/index.html). Filing a complaint with the conference organizer (Society of Pelvic Research) is not required before filing a complaint of discrimination with HHS OCR, and that seeking assistance from the conference organizer in no way prohibits filing complaints with HHS OCR. Individuals can also notify NIH about concerns of harassment, including sexual harassment, discrimination, and other forms of inappropriate conduct at NIH-supported conferences at https://grants.nih.gov/grants/policy/harassment/find-help.htm

Program-at-a-glance: Thursday December 7, Friday December 8

Thursday, December 7, 2023		
Trainee Affairs Workshop (Lafayette Suite)	6:00 PM - 7:00 PM	Leader - Cara Hardy, PhD
	7:00 PM - 8:00 PM	Leader - Cara Hardy, PhD
Trainee Social & Networking	8:00 PM	
Friday, December 8, 2023		
Breakfast & Networking (Pulaski Room)	8:00 AM - 9:00 AM	
Opening Remarks - Day 1(Pulaski Room)	9:00 AM - 9:05 AM	Kelvin Davies, PhD
Session 1: Keynote Presentation	**Moderators:** F - Kelvin Davies, PhD and T - Janhvi Parsai	
	9:05 AM - 9:55 AM	**Indira Mysorekar, PhD**
	9:55 AM - 10:05 AM	Q&A
Bio Break	10:05 AM - 10:20 AM	
Session 1 Abstracts: Aging, IC/BPS and nerve injury	**Moderators:** F - Johanna Hannan, PhD and T - Fenix de Araujo, MSc	
(7 min presentations, 3 min Q&A)	10:20 AM - 10:30 AM	Cara Hardy, PhD (T)
	10:30 AM - 10:40 AM	Catherine Brownstein, PhD
	10:40 AM - 10:50 AM	David Klumpp, PhD
	10:50 AM - 11:00 AM	Eleanora Melkonian (T) (pre-Doc)
	11:00 AM - 11:10 AM	Soumya Kamath, (T) (pre-Doc)
	11.10 AM - 11-20 AM	Shaimaa Maher, PhD (T)
	11:20 AM - 11:30 AM	Nagat Frara, PhD
Session 1: NIH presentation	11:30 AM - 12:00 AM	**Kristy Nicks, PhD**
Lunch & Networking	12:00 AM - 1:00 PM	Ossabaw Room
Session 2: Keynote Presentation (Pulaski Room)	**Moderators**: F - Vivian Cristofaro, PhD and T - Anissa Cervantes	
	1:00 PM - 1:50 PM	**Zachary Danziger, PhD**
	1:50 PM - 2:00 PM	Q&A
Session 2: Best Abstracts from Sociedad Mexicana de Ciencias Urogenitales	Moderators: F - Alvaro Munoz PhD, T - Anissa Cervantes	
	2:00 PM - 2:10 PM	Carlos F. Montes de Oca (pre-Doc)
	2:10 PM - 2:20 PM	Sonia G. Perez-Aguirre (pre-Doc)
	2:20 PM - 2:30 PM	Sergio Marin de Jesus (pre-Doc)
	2:30 PM - 2:40 PM	Andres Baca-Hernandez (pre-Doc)
	2:40 PM - 2:50 PM	Pabeli S. Becerra-Romero (pre-Doc)
Coffee Break & Networking	2:50 PM -3:10 PM	
Session 3: Keynote Presentation (Pulaski Room)	**Moderators:** F - Monty Hughes, Ph.D. and T - Soumya Kamath	
	3:10 PM - 4:00 PM	**John DeLancey, MD**
	4:00 PM - 4:10 PM	Q&A
Session 3 Abstracts: Inflammation, urinary obstruction and diabetes	**Moderators:** F - Michael DiSanto, PhD and T - Shaimaa Maher, PhD	
(8 min presentations, 2 min Q&A)	4:10 PM - 4:20 PM	Anissa Cervantes (T) (pre-MD)
	4:20 PM - 4:30 PM	Monty Huges, PhD
	4:30 PM - 4:40 PM	Vivian Cristofaro, PhD
	4:40 PM - 4:50 PM	Jonathan Beckel, PhD
	4:50 PM - 5:00 PM	Michael Odom, PhD (T)
	5:00 PM - 5:10 PM	Warren Hill, PhD
	5:10 PM - 5:20 PM	Michael Odom, PhD (T)
	5:20 PM - 5:30 PM	Janhvi Parsai, (T) (pre-Doc)
	5:30 PM - 5:40 PM	Monty Hughes, PhD
Close Day 1	5:40 PM -5:50 PM	Vivian Cristofaro, PhD
Cheese and Wine Reception & Networking	6:15 PM -7:15 PM	Harborview

Locations

Trainee Affairs Workshop: Lafayette Suite; **Sessions 1-3:** Pulaski

Breakfast: Pulaski; Lunch: Ossabaw Room; **Networking Reception:** Harborview

Program-at-a-glance: Saturday December 8

Saturday, December 9, 2023		
Breakfast & Networking (Pulaski Room)	8:00 AM - 9:00 AM	
Opening Remarks - Day 2 (Pulaski Room)	9:00 AM - 9:05 AM	Maryrose Sullivan, PhD
Session 4: Keynote Presentation	**Moderators:** F - Maryrose Sullivan, PhD and T - Cintia Vieira dos Sant	
	9:05 AM - 9:55 AM	**William Ricke, PhD**
	9:55 AM - 10:05 AM	Q&A
Bio Break	10:05 AM -10:20 AM	
Session 4 Abstracts: Male and females sexual (dys)function	**Moderators:** F - Carol Podlasek, PhD and T - Eleanora Melkonian	
(8 min presentations, 2 min Q&A)	10:20 AM - 10:30 AM	Molly Alexander (T) (pre-Doc)
	10:30 AM - 10:40 AM	Rayna Birnbaum, PhD (T)
	10:40 AM - 10:50 AM	Carol Podlasek, PhD
	10:50 AM - 11:00 AM	Carol Podlasek, PhD
	11:00 AM - 11:10 AM	Fernanda Priviero, PhD
	11.10 AM - 11-20 AM	Rinaldo Rodrigues dos Passos Jr, MSc (T)
	11:20 AM - 11:30 AM	Cintia Vieira dos Santos, MSc (T)
	11:30 AM - 11:40 AM	Moses Tar, MD
	11:40 AM - 11:50 AM	Fenix de Araujo, MSc (T)
	11:50 AM - 12:00 AM	Stephanie Wilczynski (T) (pre-Doc)
Lunch & Networking	12:00 AM - 1:00 PM	Madison Ballroom
Session 5: Pelvic anatomy and bladder (dys)function	**Moderators:** F - Mary Barbe, PhD and T - Stephanie Wilczynski	
(8 min presentations, 2 min Q&A)	1:00 PM - 1:10 PM	Daniel Medina Aguinaga, MD, PhD (T)
	1:10 PM - 1:20 PM	Zuha Yousuf (T) (pre-Doc)
	1:20 PM - 1:30 PM	Mohamed Elazab (T) (pre-Doc)
	1:30 PM - 1:40 PM	Adam Doelman (T)
	1:40 PM - 1:50 PM	Duncan Morhardt, MD
	1:50 PM - 2:00 PM	Farial Rahman, PhD
	2:00 PM - 2:10 PM	Violeta Mutafova-Yambolieva, PhD
	2:10 PM - 2:20 PM	Britney Hudson (T) (pre-Doc)
	2:20 PM - 2:30 PM	Emely Munoz (T) (pre-Doc)
	2:30 PM - 2:40 PM	Emily Ricke, MS
	2:40 PM - 2:50 PM	Hannah Ruetten, PhD (T)
Coffee Break & Networking	2:50 PM - 3:10 PM	
Session 6: Dr. Phillip P. Smith Memorial Lecture	3:10 PM - 3:15 PM	Cara Hardy, PhD
	3:15 PM - 4.05 PM	**George Kuchel, MD**
	4.05 PM - 4:15 PM	Q&A
Trainee Awards	4:15 PM - 4:35 PM	Mary Barbe, PhD and Monty Hughes, PhD
Closing Ceremony	4:35 PM - 4:45 PM	Kelvin Davies, PhD and Maryrose Sullivan, PhD
Gala Dinner	6:00 PM	Harborview

Locations

Sessions 4-5: Pulaski

Breakfast: Pulaski; **Lunch:** Madison Ballroom

Saturday Gala Dinner: Harborview

Invited Speakers:

Indira Mysorekar, PhD, Professor of Medicine, Chief of Basic and Translational Research Baylor College of Medicine, will discuss the effects of Aging of the bladder in a Keynote Address.

Dr. Myosrekar was born in India where she lived for the first 8 years of her life, before moving with her family to East Africa. Indira obtained a combined BS/MS degree in Molecular Biology from the University of Lund in Lund, Sweden in 1994, and received her Ph.D. degree in Developmental Biology from Washington University in 2002. She developed and characterized mouse models to study adult urothelial stem cell niche activation responses to injury and identified molecular markers of stem cell activation. She also established systems for inducible urothelial-specific generation of loss of function mutants in key genes important for epithelial turnover in the bladder. Dr. Mysorekar is continuing her work on mechanisms of epithelial renewal in the normal and diseased urinary bladder. Her research work encompasses studies of acute and recurrent UTIs to fundamental mechanisms of urinary bladder development and homeostasis.
School of Medicine.

Zachary Danziger, PhD, Assistant Professor, Department of Biomedical Engineering, Florida International University, Miami, FL, will present data on Effects of aging on neuroregulation of the lower urinary tract" in a State of the Art Address.

Dr. Danziger received his Ph.D. from Northwestern University in the area of human motor learning and computational neuroscience, and his postdoctoral studies at Duke University were in electrophysiology and neurourology. Dr. Danziger's primary research interests lie at the intersection of these areas of neuroscience theory and application. His approach is to focus first on understanding the underlying behavior of the neural system, and second, to exploit that understanding to optimize the design of neural interfaces. His lab is currently developing tools to 1) understanding brain activity in motor cortex, with the goal of improving performance of brain-computer interfaces and 2) understanding nerve activity in the urinary tract, with the goal of improving efficiency of stimulation technology designed to restore bladder function.

William Ricke, PhD. Professor of Urologic Research, Director of the George M. O'Brien Center, University of Wisconsin, will present information on Age associated changes in prostatic mitochondrial function and development of LUTS and BPH in a Keynote Address.

Dr. Ricke's laboratory is a translational research laboratory focused on the tumor microenviroment as it relates to hormone action and stromal-epithelial interactions in prostate diseases including benign prostatic hyperplasia (BPH) and prostate cancer. Dr. Ricke discovered that steroid hormone action is critical in the stroma rather than epithelia for malignant transformation and metastasis in prostate cancer, and, that targeting stromal receptors with therapies are the primary mode of therapy. The Ricke lab recently discovered that stromal cells are critical to prostate cancer progression, metastasis, and castrate resistant prostate cancer. Additionally, paracrine interactions are necessary for the development of key genetic, epigenetic, biochemical, and gene-fusion events in aggressive prostate cancer. This understanding provides the framework for elucidating key molecular and cell biological events through research as well as providing therapeutic targets which can be tested via clinically relevant and experimental drugs.

Invited Speakers:

John O. DeLancey, MD, Professor, Department of Obstetrics and Gynecology, University of Michigan, Ann Arbor, Michigan, will discuss the interaction of Aging and other Life Events on Pelvic Floor Function in a Keynote Address.

John O. L. DeLancey, MD is the Norman F. Miller Professor of Gynecology at the University of Michigan Medical School and Director of Pelvic Floor Research in the Department of Obstetrics and Gynecology. He graduated from Oberlin College with Honors in Biology and the University of Michigan Medical School where he also did his residency training. Dr. DeLancey has won many awards for his clinical expertise and research contributions. He was elected to the National Academy of Medicine and induced as an Honorary Member of the Royal College of Obstetrics and Gynecology ad eundem for pioneering discoveries about the fundamental biomechanics of pelvic organ prolapse, birth-related pelvic floor injury, and the mechanisms of urinary incontinence. He has been President of the American Urogynecologic Society and the Society of Gynecologic Surgeons having also received its Distinguished Surgeon Award.

George A. Kuchel, MD. Professor and Chair Department of Medicine, University of Connecticut will present the Dr. Phillip P. Smith Memorial Lecture on the topic of age and its effect on pelvic function. George A. Kuchel, MD, is Professor of Medicine, Travelers Chair in Geriatrics and Gerontology, Director, UConn Center on Aging, University of Connecticut. Dr. Kuchel's research interests include multidisciplinary approaches to the study of aging emphasizing inter-individual variability and resilience mechanisms pertaining to mobility, voiding, cognition and host defense. Design and validation of geroscience-guided interventions into aging and common chronic diseases of aging. Personalized and mechanism-guided approaches to the diagnosis and management of common geriatric syndromes – "Precision Geroscience"

Kristy Nicks, PhD, Program Director for the Bone Biology, Metabolic Bone Disorders, and Osteoporosis portfolio at National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), NIH, Bethesda will discuss "NIH Initiatives to Promote Diversity in Science". She is a co-chair the NIAMS Diversity Supplement Scholars Program work group.

Congratulations!

Travel Award Recipients:

Shaimaa Maher, Cleveland Clinic

Rayna Birnbaum, Albert Einstein College of Medicine

Rinaldo Rodrigues dos Passos, University of South Carolina School of Medicine

Mohamed Elazab, Cleveland State University, Cleveland Clinic

Britney Hudson, Clemson University

Anissa Cervantes, Duke University Medical Center

Top-10 Abstracts

Afrida Rahman-Enyart et al., "Microglia Mediate Symptoms of Chronic Pelvic Pain".

Jillian Sullivan et al., "Inhibition of cyclophosphamide-induced bladder inflammation and hyperactivity through knockdown of the endogenous cholinergic toxin-like signaling peptide SLURP-2".

Adam Doelman et al., "Wireless catheter-free pressure sensors to assess neurogenic lower urinary tract dysfunction in a porcine model of spinal cord injury".

Shelby Harper et al., "Strict glucose control and elimination of NLRP3-induced inflammation prevents diabetic bladder dysfunction in the female Akita mouse model".

Michael Odom et al., "Testosterone Contributes More to the Development of Diabetic Bladder Dysfunction in Type 1 Diabetic Male Mice Than Hyperglycemia".

Armand Allkanjari et al., "Male Akita Mice Develop Bladder Underactivity Independent of NLRP3 as a Result of a Decrease in Neurotransmitter Release from Efferent Neurons".

Rayna Birnbaum et al., "Targeting FL2 after cavernous nerve injury promotes nerve regeneration and erectile function through modulating microtubule dynamics in multiple cell types".

Soumaya Kamath et al., "Characterization of female bladder and vaginal function in a preclinical model of Ehlers-Danlos Syndrome.

Rinaldo Rodrigues dos Passos, Jr. et al., "Erectile dysfunction in young adult rats might be associated with obesity in early life".

Nagat Frara et al., "Upregulation of brain-derived nerve factor (BDNF) expression and enhanced reactive oxygen species production in lower motor neuron-lesioned dog bladder mucosa following somatic-motor nerve transfer".

SPR2023 Annual meeting – In Depth Schedule

Thursday, December 7, 2023

Trainee Affairs Workshop (Lafayette Suite)

6:00 PM	The Art of Scientific Writing: Crafting the Perfect Specific Aims Page.	Leader - Cara Hardy, PhD
7:00 PM	Career Exploration: Knowing your options and preparing for your next step.	Leader - Cara Hardy, PhD
8:00 PM	Trainee Social	TBD

Friday December 8, 2023 Day 1

8:00-9:00	Breakfast and Networking (Pulaski Room)	
9:00-9:05	Opening Remarks (Pulaski Room)	Kelvin Davies, PhD
Session 1: Keynote presentation (Pulaski Room)		Moderators: F- Kelvin Davies, PhD T- Jahnvi Parsai (Undergrad)
9:05-9:55	Aging of the bladder	Indira Mysorekar, PhD
9:55-10:05	Q & A	
10:05-10:20	Bio break	
Session 1: Aging, IC/BPS and nerve injury - Abstracts (Pulaski Room)		Moderators: F - Johanna Hannan, PhD T - Fenix de Araujo, MSc
10:20-10:30	Investigating kidney and bladder functional declines associated with aging.	Cara Hardy, PhD (T)
10:30-10:40	Sentiments of interstitial cystitis/bladder pain syndrome patients towards Elmiron	Catherine Brownstein, PhD
10:40-10:50	Microglia Mediate Symptoms of Chronic Pelvic Pain	David Klumpp, PhD
10:50-11:00	Racial disparities in treatment of interstitial cystitis/bladder pain syndrome	Eleanora Melkonian (T) (pre-doc)
11:00-11:10	Characterization of female bladder and vaginal function in a preclinical model of Ehlers-Danlos Syndrome	Soumya Kamath (T) (pre-doc)
11:10-11:20	Pudendal Nerve Injury and its Contribution to Stress Urinary Incontinence in a Male Rat Model	Shaimaa Maher, PhD (T)
11:20-11:30	Upregulation of brain-derived nerve factor (BDNF) expression and enhanced reactive oxygen species production in lower motor neuron-lesioned dog bladder mucosa following somatic-motor nerve transfer	Nagat Frara, PhD
Session 1: NIH Presentation (Pulaski Room)		
11:30-12:00	NIH Initiatives to Promote Diversity in Science	Kristy Nicks
12:00-1:00	**Networking Lunch (Ossabaw Room)**	

Friday, December 8, 2023

Session 2: Keynote Presentation (Pulaski Room)		Moderators: F – Vivian Cristofaro, PhD T – Anissa Cervantes
1:00-1:50	Effects of ageing on neuroregulation of the lower urinary tract	Zachary Danziger, PhD
1:50-2:00	Q&A	
Session 2: Best abstracts from the Sociedad Mexicana de Ciencias Urogenitales (Mexican Society of urogenital Sciences (Pulaski Room) 2023 annual meeting		Moderators: F – Alvaro Munoz, PhD T – Anissa Cervantes (pre-doc)
2:00-2:10	Analysis of molecular iodine effects in TRAMP mice prostate tumors	Carlos F. Montes de Oca (T) (pre-doc)
2:10-2:20	The antioxidant effect of epigallocatechin-3-gallate on testicular function of puberty and adulthood rat induced by exposure to cadmium.	Sonia G. Pérez-Aguirre (T) (pre-doc)
2:20-2:30	Zinc and its role on testicular and epididymal antioxidant protection in rats exposed to cadmium	Sergio Marín de Jesús (T) (pre-doc)
2:30-2:40	Therapeutic effects of iodine in patients with benign prostatic hyperplasia medicated or not with Tamsulosin	Andrés Baca-Hernandez (T) (pre-doc)
2:40-2:50	Analysis of immune infiltrate, serum cytokine profile and histology in the denervated prostate	Pabeli S. Becerra-Romero (T) (pre-doc)
2:50-3:10	Coffee Break & networking	
Session 3: Keynote Presentation (Pulaski Room)		Moderators: F – Monty Hughes, PhD T – Soumya Kamath
3:10-4:00	Interaction of ageing and other life events on pelvic floor function	John O. Delancey, MD
4:00-4:10	Q &A	
Session 3: Inflammation, urinary obstruction and diabetes - abstracts (Pulaski Room)		Moderators: F – Michael DiSanto, PhD T – Shaimaa Maher, PhD
4:10-4:20	Resolvin E1, a specialized pro-resolving mediator, has a transient beneficial effect on diabetes-associated bladder inflammation in a mouse model	Anissa Cervantes (T) (pre-doc)
4:20-4:30	Strict glucose control and elimination of NLRP3-induced inflammation prevents diabetic bladder dysfunction in the female Akita mouse model	Monty Hughes, PhD
4:30-4:40	High Glucose Increases Bladder Excitability by Altering Caveolae-Mediated Inhibition of Muscarinic M2-Receptor Activation	Vivian Cristofaro, PhD
4:40-4:50	Inhibition of cyclophosphamide-induced bladder inflammation and hyperactivity through knockdown of the endogenous cholinergic toxin-like signaling peptide SLURP-2	Jonathan Beckel, PhD
4:50-5:00	Testosterone Contributes More to the Development of Diabetic Bladder Dysfunction in Type 1 Diabetic Male Mice Than Hyperglycemia	Michael Odom, PhD (T)
5:00-5:10	Bladder Smooth Muscle Integrins are Dysregulated in Mouse Models of Acute Urinary Retention and Partial Bladder Outlet Obstruction	Warren Hill, PhD

Friday, December 8, 2023

5:10-5:20	Male Akita Mice Develop Bladder Underactivity Independent of NLRP3 as a Result of a Decrease in Neurotransmitter Release from Efferent Neurons	Michael Odom, PhD (T)
5:20-5:30	Higher Levels of p-MLKL Suggest that Necroptosis Associated with Bladder Nerves is a Possible Driver of Diabetic Bladder Dysfunction	Jahnvi Parsai (T) (Undergrad)
5:30-5:40	Blocking Necroptosis Partially Prevents Bladder Dysfunction in Diabetic Male Akita Mice	Monty Hughes, PhD
5:40-5:50	Close day 1	Vivian Cristofaro, PhD
6:15-7:15	Wine and cheese reception and networking (Harborview)	

Saturday, December 9, 2023 Day 2

8:00-9:00	Breakfast and Networking (Pulaski Room)	
9:00-0:05	Opening Remarks (Pulaski Room)	Maryrose Sullivan, PhD

Session 4: Keynote Address (Pulaski Room) Moderators: F – Maryrose Sullivan, PhD
T – Cintia V. dos Santos

9:05-9:55	Age associated changes in prostatic mitochondrial function and development of LUTS and BPH	William Ricke, PhD
9:55-10:05	Q&A	
10:05-10:20	Bio break	

Session 4: Male and female sexual (dys)function – Abstracts (Pulaski Room) Moderators: F – Carol Podlasek, PhD
T – Eleanora Melkonian

10:20-10:30	Sub-chronic perfluorooctane sulfonate (PFOS) or perfluorooctanic acid (PFOA) exposure has no impact on erectile function in Sprague-Dawley rats	Molly Alexander (T) (pre-doc)
10:30-10:40	Targeting FL2 after cavernous nerve injury promotes nerve regeneration and erectile function through modulating microtubule dynamics in multiple cell types.	Rayna Birnbaum, PhD (T)
10:40-10:50	BMP4 and GREM1 are targets of SHH signaling and downstream regulators of collagen in the penis	Carol Podlasek, PhD
10:50-11:00	SHH regulates penile morphology and smooth muscle through a mechanism involving BMP4 and GREM1	Carol Podlasek, PhD
11:00-11:10	Erectile dysfunction in young adult rats might be associated with obesity in early life	Fernanda Priviero, PhD
11:10-11:20	The role of NLRP3 (nucleotide-binding domain, leucine-richâ€“containing family, pyrin domainâ€“containing-3) in chronic stress-induced vascular and erectile dysfunction	Rinaldo Rodrigues dos Passos Jr., MSc (T)
11:20-11:30	Leucine Rich Repeat Containing 8 channels modulate superoxide anion production and hypercontractility of pudendal arteries and corpus cavernosum in diabetic vasculogenic erectile dysfunction	Cintia Vieira dos Santos (T)
11:30-11:40	A wide range of PDE5 inhibitors act synergistically with a topical nitric oxide delivery system to elicit an erectile response in an animal model of radical prostatectomy.	Moses Tar, MD

Saturday, December 9, 2023

11:40-11:50	More than a feeling: Unraveling the sexual difference in TRPM8 (Transient Receptor Potential Melastatin 8) channel vascular function.	Fenix de Araujo, MS (T)
11:50-12:00	The Role of Leucine Rich Repeat Containing 8A Channels in Female Sexual Dysfunction and Vaginal Smooth Muscle	Stephanie Wilczynski (T) (pre-doc)
12:00-1:00	**Lunch and networking (Madison ballroom)**	

Session 5: pelvic floor anatomy and bladder (dys)function (Pulaski Room)

Moderators: F – Mary Barb, PhD
T – Stephanie Wilczynski

1:00-1:10	A surgical and functional approach to the pelvic gross neuroanatomy of the female Yucatan minipig	Daniel Medina Aguinaga MD, PhD (T)
1:10-1:20	Innervation of the ovine pelvic floor muscles by levator ani and perineal nerves	Zuha Yousuf (T) (pre-doc)
1:20-1:30	Bladder Volume Estimation Using a Catheter-free Wireless Pressure-Volume Sensor in Minipigs	Mohamed Elazab (T) (pre-Doc)
1:30-1:40	Wireless catheter-free pressure sensors to assess neurogenic lower urinary tract dysfunction in a porcine model of spinal cord injury	Adam Doelman (T)
1:40-1:50	Transcriptomic and functional assessment of the developing zebrafish bladder	Duncan Morhardt, PhD
1:50-2:00	Sub-chronic Neuromodulation of Bulbospongeosus Nerve Improves deficits associated with Stress Urinary Incontinence in Mature Multiparous Female Rabbits	Farial S. Rahman (pre-Doc)
2:00-2:10	Prostaglandins Facilitate the ATP Degradation by Soluble Ectonucleotidases in the Urinary Bladder Lamina Propria	Violeta Mutafova-Yambolieva, PhD
2:10-2:20	Effects of elevated pressure cycling on urothelial exosomes in vitro	Britney Hudson (T) (pre-Doc)
2:20-2:30	Short terms effects on the stereotyped voiding behavior of female rats induced by the consumption of sweetened water	Emely Munoz (T) (pre-Doc)
2:30-2:40	Use of anti-fibrotics in the treatment of Lower Urinary Tract Dysfunction in mice	Emily Ricke, MS
2:40-2:50	Local tissue response to a CXCL12 chemokine therapy for fecal incontinence in a rabbit model.	Hannah Ruetten, PhD (T)
2:50-3:10	Coffee Break & Networking	

Session 6: Dr. Phillip P. Smith Memorial lecture (Pulaski)

3:10-3:15	Introduction	Cara Hardy, PhD
3:15-4:05	Age and its effect on pelvic function	George Kuchel, MD
4:05-4:15	Q&A	
4:15-4:35	**Trainee awards**	Monty Hughes, PhD Mary Barb, PhD
4:35-4:45	**Closing ceremony**	Kelvin Davies, PhD Maryrose Sullivan, PhD
6:00-8:00	**Gala dinner (Harborview)**	

SUBMITTED ABSTRACTS

Title: *Sub-chronic perfluorooctane sulfonate (PFOS) or perfluorooctanic acid (PFOA) exposure has no impact on erectile function in Sprague-Dawley rats*

Authors: Alexander, Molly K.[1], Hou Anjalee[1], DeWitt, Jamie C.[2], Hannan, Johanna L.[1]

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Introduction/Objectives: Per and polyfluoroalkyl substances (PFAS) are commonly used in industrial and consumer products and are environmental and health contaminants. The most common PFAS chemicals, perfluorooctane sulfonate (PFOS) and perfluorooctanic acid (PFOA), are used in firefighting foam, nonstick bakeware, water-resistant fabrics, grease-resistant paper and fire-resistant materials. PFAS is a known endocrine disruptor and can reduce testosterone and lower semen quality. The impact of PFAS exposure on erectile physiology is unknown. Our study will assess the effect of PFOS and PFOA on erectile function in Sprague-Dawley rats. We hypothesize that PFOS and PFOA will decrease erectile function.

Methods: Adult male Sprague-Dawley rats were divided into three groups (n=10/group): 1) Control; 2) PFOS (10mg/kg/day); and 3) PFOA (10mg/kg/day). PFOS/PFOA was dissolved in 0.05% Tween 20 and administered in drinking water for 28 days. Controls received 0.05% Tween 20 in drinking water. The study was carried out in two different cohorts of rats (n=4 and n=6/group). Following 28 days of PFOS/PFOA exposure, rats were placed on regular drinking water for 2 weeks. Body weights were collected weekly and terminal organ weights of liver, testes, spleen, heart, lungs, brain, seminal vesicles, kidneys, bladder, and thymus were recorded. Erectile function was assessed prior to PFAS exposure and every 2 weeks thereafter via apomorphine-induced behavioral erections and yawns. Nerve-stimulated intracavernosal pressure and mean arterial pressure (ICP/MAP) was measured 2 weeks after exposure stopped.

Results: Four weeks of PFOS exposure significantly decreased body weight while PFOA exposure did not impact body weight (Con: 588±63.5g; PFOS: 453±16.3g; PFOA: 535±29.9g, $p<0.01$). The PFOS dose in the second cohort was overtly toxic with 5/6 rats dying following 3-4 weeks of exposure. Two weeks following PFOS/PFOA exposure, livers were markedly enlarged (Con: 19.4±2.85g; PFOS: 25.4±0.16g; PFOA: 20.2±3.77g, $p<0.01$), spleens were smaller (Con: 0.93±0.12g; PFOS: 0.65±0.03g; PFOA: 0.88±0.12g, $p<0.05$), and testes were larger in PFOS rats (Con: 1.8±0.07g; PFOS: 2.0±0.06g; PFOA: 1.8±0.07). No other organ weights were different across exposure groups. PFOS/PFOA exposed animals had similar numbers of apomorphine-induced erections throughout the 6 week period. Two weeks post-exposure, ICP/MAP was unchanged between all groups. Ongoing studies will assess testosterone levels.

Conclusions: A sub-chronic PFOS exposure markedly decreased body weight, increased liver size, decreased spleen weight, and increased testes weight. PFOA exposure did not impact body or organ weights. Following PFOS or PFOA exposure, there was no change in apomorphine-induced behavioral erections or ICP/MAP. Additionally, the dose of PFOS assessed was quite toxic to the second cohort of animals. Future studies will assess if a more chronic exposure will impact erectile function.

Funding Source(s): Sexual Medicine Society of North America

Male Akita Mice Develop Bladder Underactivity Independent of NLRP3 as a Result of a Decrease in Neurotransmitter Release from Efferent Neurons

Authors: Armand Allkanjari, Michael R. Odom*, Francis M. Hughes, Jr.*, Jack E. Mulcrone, Huixia Jin and J. Todd Purves

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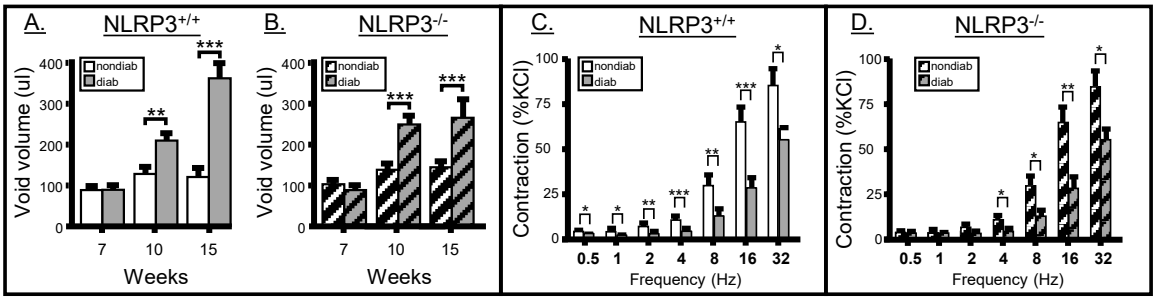
Introduction: Diabetic Bladder Dysfunction is a prevalent complication that is recalcitrant to glucose control and without a targeted therapy. Using the Akita diabetes model (type 1) crossed with an NLRP3 knockout, we previously found that females (glucose 200-300 mg/dl), develop overactivity at 15 weeks which progresses to an underactive bladder. Both are the result of NLRP3-induced inflammation. In this study we examine DBD in the males which develop a stronger hyperglycemia (500-600 mg/dl).

Methods: Akita were bred to be NLRP3$^{+/+}$ or NLRP3$^{-/-}$. Blood glucose was assessed by glucometer, bladder inflammation by Evans blue dye extravasation, bladder function by urodynamics, nerve densities by PGP9.5, NF200 or vesicular acetylcholine transporter (VACHt) staining and bladder strip contractility by myography.

Results: NLRP3 expression did not affect blood glucose. Bladder inflammation was present in diabetics by 10 weeks and dependent on NLRP3. Urodynamics demonstrated only diabetic underactivity with no overactive stage (A.) or dependence on NLRP3 (B.). There was a decrease in overall nerve density (PGP9.5^{+}) and one class of afferent nerves (NF200^{+} Aδ-fibers) in diabetic bladders. However, this loss was dependent on NLRP3 and so deemed unlikely to explain the NLRP3-independent underactivity. To determine if changes in the bladder smooth muscle (BSM) was responsible we analyzed contractility with KCl (depolarizing agent), carbachol (muscarinic agonist) and α,β-methylene-ATP (purinergic agonist). The contractions were equivalent in control and diabetics, suggesting no changes in BSM. Consequently we proposed underactivity may be due to decreases in neurotransmitter release from efferent nerves. Indeed, electrical field stimulation revealed a decrease in contractility in diabetic mice (C.) that was not blocked in the NLRP3$^{-/-}$ strain (D). Quantitation of VACHt nerve terminals marked a decrease in the number of these efferent terminals in bladder cross section.

Conclusions: Diabetes in male Akita mice causes bladder underactivity after 15 weeks through a decrease in efferent neurotransmitter release.

Funding: NIH-RO1 DK117890, NIH-K12 DK100024



Title: More than a feeling: Unraveling the sexual difference in TRPM8 (Transient Receptor Potential Melastatin 8) channel vascular function.

Authors: Fênix A. de Araujo[1,2]*, Raiana Moraes[1], Rinaldo R. dos Passos[2], Camilla F. Wenceslau[2,3], R. Clinton Webb[2,3], Fernanda Priviero[2,3], Cameron G. McCarthy[2,3], Darizy F. Silva[1,4]

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Introduction/Objectives: Sexual dysfunction is a complication of many diseases and compromises the quality of life of both men and women. Menthol, a TRPM8 agonist, is widely used in preparations to improve gland sensation and vaginal lubrication during sexual intercourse, however no data are reported for TRPM8 vascular function in the pelvic region from both sexes. Therefore, we hypothesized that TRPM8 participates in classical agonists pathways in different manner in male and female mice.

Methods: Adult (3 to 6 months) male and female C57Bl/6 mice and -/-TRPM8 (KO) mice were used, and internal pudendal arteries (IPA) were dissected and mounted on DMT wire myographs for isometric tension measurements. Concentration response-curves were performed for acetylcholine (ACh), norepinephrine (NE), histamine (HIST) and serotonin (5HT), with or without TRPM8 agonist (menthol 30μM) or antagonist (M8B 1μM). Results are expressed as mean±SEM, n=5. Data analyzed using unpaired or paired Student's t-test, $P<0.05$.

Results: In both sexes the incubation with menthol did not change NE contraction, whereas M8B increased NE potency (male: pD2 NE_{before}:7.12±0.11, NE_{M8B}: 7.57±0.08; female: pD2 NE_{before}:6.49±0.11, NE_{M8B}: 6.64±0.10). In males, 5HT-induced contraction was not affected by TRPM8 activation or inhibition, however in females, TRPM8 inhibition reduced 5HT contraction (pD2 5-HT_{before}: 6.47±0.12, 5-HT_{M8B}: 6.26±0.09) and the opposite occurred during TRPM8 activation (pD2 5-HT_{before}: 6.60±0.08, 5-$HT_{Menthol}$: 6.69±0.06). M8B incubation increased ACh relaxation in male IPA (pD2 ACh_{before}: 6.75±0.17, ACh_{M8B}: 7.02±0.11) and menthol had no effect. The HIST-induced relaxation was not affected by M8B or menthol incubation in male IPA. In female IPA from KO mice the M8B had the same effect in NE contraction as in IPA from female control, with no changes in ACh-induced relaxation. This suggests that the pharmacological tools used to activate or block TRPM8 could have some off target effect or that the truncated TRPM8 expression in KO still have functional effectiveness in the arteries.

Conclusions: TRPM8 channels have different roles in vascular function in male and female IPA. TRPM8 influences in classical agonists responses and could be a receptor or pathway related. Further studies are needed to better understand the sex difference in TRPM8 involvement in arterial responses to contraction and relaxation pathways.

Funding Source(s): Fiocruz's Internationalization Project (Capes PrInt-Fiocruz); National Council for Scientific and Technological Development (CNPq); Federal University of Bahia (UFBA); The Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB); The Rede Baiana de Bioprospecção de Fármacos (BIOPROFAR-BA). National Institutes of Health (R00HL151889).

Title: Targeting FL2 after cavernous nerve injury promotes nerve regeneration and erectile function through modulating microtubule dynamics in multiple cell types.

Authors: Rayna Birnbaum[1], Moses T. Tar [2], Adam H. Kramer [1#], Lisa A. Baker[1#], David Sharp[1], Kelvin P. Davies [1,2].

Affiliations: [1]Department of Molecular Pharmacology, [2]Dept. of Urology Dept., Albert Einstein College of Medicine, Bronx, NY, United States. [#]Current affiliation: MicroCures, Inc., Bronx, NY, United States.

Introduction/Objectives: We have identified Fidgetin-like 2 (FL2) as a novel regulator of microtubule dynamics, with an inhibitory effect on both cell motility and axonal growth *in vitro*. For axons to successfully regrow after peripheral nerve transection, the nerve bridge must first be re-vascularized: endothelial cells must traverse the nerve bridge forming new blood vessels, and Schwann cells form parallel cords that guide regenerating axons back to their target tissue. The cavernous nerves (CNs) are parasympathetic nerves that regulate blood flow to the penis and are commonly damaged during the surgical procedures of radical prostatectomy (RP), resulting in erectile dysfunction (ED). We hypothesize that based on the inhibitory effects of FL2 on axonal growth and cell motility, depletion of FL2 after CN injury promotes regeneration and recovery of erectile response. We further hypothesize the pro-regenerative effects of FL2 depletion are due to knockdown in multiple cell types, including endothelial cells and Schwann cells. Here, we tested the effects of FL2 depletion after CN injury on recovery of erectile response in a rodent CN transection model and conducted *in vitro* studies to test if FL2 depletion promotes angiogenesis.

Methods: Both CNs of rats were transected, after which a gelatin wafer containing FL2 siRNA liposomes or control siRNA liposomes was placed onto the severed nerves (n=15 per treatment group). Three weeks later, rats were anesthetized and the CNs re-exposed and subjected to cavernosometry. To test whether FL2 depletion enhances angiogenesis *in vitro,* we used an endothelial tube formation assay, in which endothelial tubes formed by human umbilical vein endothelial cells (HUVECs) transfected with control or FL2 siRNA over a 12-hour period were characterized using the ImageJ plugin Angiogenesis Analyzer.

Results: Visual inspection revealed regeneration of at least one of the two CNs in 13 out of 15 rats, whereas 0 of 15 control rats had regenerated CNs. In rats with regenerated nerves, erectile response was assessed by cavernosometry. ICP/BP scores showed partial recovery of erectile response (mean ICP/BP = 0.4 with 2 mA stimulation), indicating reinnervation of the target tissue (control animals ICP/BP could not be assessed due to retraction of the proximal nerve segment preventing electrical stimulation). HUVECs treated with FL2 siRNA formed meshwork at a faster rate than control treated cells, with 27% more nodes, 41% more meshes and 32% more segments.

Conclusions: FL2 depletion after CN transection is effective in inducing nerve repair and partial recovery of erectile response. Our *in vitro* studies in HUVECs indicate FL2 depletion can enhance angiogenesis and support the hypothesis that endothelial cells may be one of the cell types through which FL2 depletion promotes CN repair.

Funding Source(s): NIH/NIDDK 2 R42 DK117684-02; NIH RO1 GM109909; Sexual Medicine Society of North America.

Title: Bladder Smooth Muscle Integrins are Dysregulated in Mouse Models of Acute Urinary Retention and Partial Bladder Outlet Obstruction

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Affiliations: [1]Renal-Electrolyte Division, University of Pittsburgh School of Medicine, Pittsburgh, PA, U.S.A; [2]Laboratory of Voiding Dysfunction, Division of Nephrology, Beth Israel Deaconess Medical Center & Harvard Medical School, Boston, MA, U.S.A.

Introduction/Objectives: The bladder experiences mechanical stress in a number of pathologies including outlet obstruction, overactivity and diabetes-related polyuria. Here, we tested the hypothesis that a key event in the mechanically stressed bladder is altered binding interactions by mechanosensory integrins to extracellular matrix (ECM) and actin cytoskeleton, resulting in physiological changes. Accordingly, we examined expression of integrins and related molecules in mouse models of acute urinary retention (AUR) and partial bladder outlet obstruction (pBOO).

Methods: AUR Model: To mimic the stress the bladder wall receives during AUR, we applied 50 and 80 cm water pressure to the bladder lumen of female C57BL/6J mice via urethra to simulate different AUR conditions. Pressures were kept constant for 30 min then the catheter removed**. pBOO Model**: We used a nerve-sparing mid-urethral obstruction (NeMO) model for pBOO as described in Sidler *et al.* (J Vis Exp; 2017; 122:55288). Voiding spot assay and cystometrogram were as described (Function;2022;3(5):zqac042), JCI Insight;2021;6(19): e152984).

Results: Following AUR, mice exhibited incontinence and overactivity with diminished voiding pressure at 6 and 24 h. Bladder smooth muscle (BSM) from pressure-treated mice had significantly diminished expression of muscarinic M3 receptor as well as elevated expression of β1-integrin and downstream effectors including integrin-linked kinase, focal adhesion kinase (FAK), talin-1, and paxillin, suggesting an acute remodeling. pBOO mice after 1 month of obstruction exhibited enlarged bladders without increased ECM. Mice exhibited dysfunctional voiding by RT-VSA with increased spot number and reduced volumes/void. Using x-ray video cystometry on awake mice, micturition took significantly longer than sham operated controls, urine flow rates were slower, and residual volumes larger. Western blotting of pBOO bladders revealed significant reductions in integrins β1-, β3-, and α1-, and FAK; as well as reduced M3 receptor expression.

Conclusions: We propose that integrin mechanosensors may be universal participants in reduced detrusor contractility and remodeling following biomechanical stress in bladder diseases.

Funding Source(s): NIDDK RO1DK134431 and RO1 DK119183

Title: Resolvin E1, a specialized pro-resolving mediator, has a transient beneficial effect on diabetes-associated bladder inflammation in a mouse model

Authors: Anissa Cervantes[1], Francis M Hughes Jr[1], Huixia Jin[1], J Todd Purves[1]

Affiliations: [1]Department of Urology, Duke University Medical Center, Durham, NC, USA

Introduction/Objectives: Diabetes is a chronic inflammatory condition with many complications including diabetic bladder dysfunction (DBD), which affects over 50% of patients with diabetes. Inflammation consists of two stages: initiation, mediated by inflammasomes, and resolution, mediated by specialized pro-resolving mediators (SPMs). SPMs, in particular Resolvin E1 (RvE1), have previously been shown to be effective in decreasing inflammation in other sterile inflammatory bladder models including cyclophosphamide-mediated damage and surgical obstruction. The Akita mouse model was selected as the males show extremely elevated blood glucose levels and significant bladder inflammation and DBD at 15 weeks. We hypothesize that RvE1 treatment will decrease bladder inflammation and improve DBD in Akita male mice.

Methods: Beginning at 15 weeks of age, Akita male mice were treated daily with RvE1 (Diab+RvE1) for one or two weeks and compared with the following methods to age-matched wild type (WT) and untreated Akita mice (Diab). Blood glucose, body weight, and bladder weights were examined to evaluate for structural changes. Evans Blue extravasation evaluated level of inflammation. Finally, cystometry was used to investigate any functional changes.

Results: Blood glucose and bladder weight were increased in diabetic mice compared to WT mice while body weight was decreased. RvE1 did not affect bladder or body weight. However, blood glucose was decreased after two weeks of treatment relative to the untreated Akita mice. Evans Blue extravasation was increased in the Diab group versus the WT group. Excitingly, one week alone of RvE1 decreased extravasation back to WT levels. However, two weeks of treatment saw a rise of inflammation levels closer to levels of untreated diabetics. Cystometry results displayed a similar pattern. Void volumes, intercontraction interval, and bladder capacity were increased in the diabetic group relative to the WT group. One week of RvE1 treatment significantly decreased these measurements to WT levels. However, after two weeks of treatment these parameters were back to values nearer to diabetic levels.

Conclusions: RvE1 treatment transiently decreases diabetes-associated bladder inflammation.

Funding Source(s): NIDDK (grant no.R01DK117890)

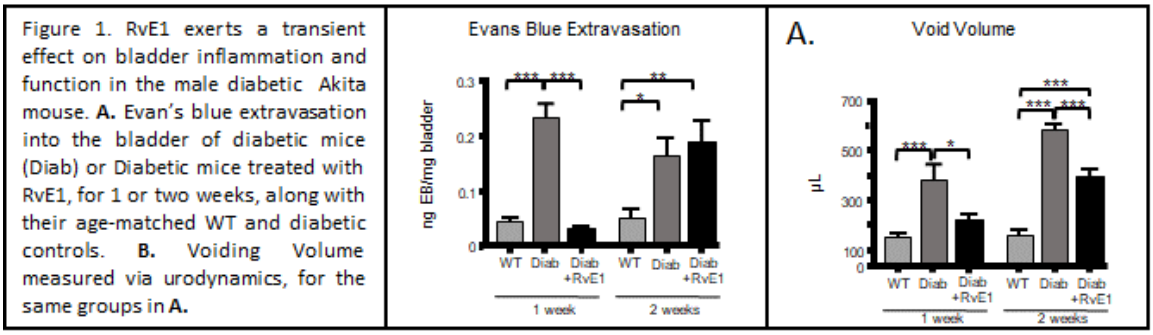

Figure 1. RvE1 exerts a transient effect on bladder inflammation and function in the male diabetic Akita mouse. **A.** Evan's blue extravasation into the bladder of diabetic mice (Diab) or Diabetic mice treated with RvE1, for 1 or two weeks, along with their age-matched WT and diabetic controls. **B.** Voiding Volume measured via urodynamics, for the same groups in **A.**

Title: High Glucose Increases Bladder Excitability by Altering Caveolae-Mediated Inhibition of Muscarinic M_2-Receptor Activation

Authors: Vivian Cristofaro*[1,2], Josephine A. Carew[1,2], Maryrose P. Sullivan[1,2]

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Introduction/Objectives: Caveolae are cholesterol-enriched membrane microdomains which serve as a platform for the differential modulation of several downstream signaling events. In the bladder, the loss of caveolae or the altered expression of caveolins (Cav, the constitutive proteins of caveolae) is associated with either reduction or augmentation of specific receptor-activated bladder smooth muscle (BSM) responses. With diabetes, several organs including the bladder, display alterations in caveolar elements and the early phase of diabetic bladder dysfunction (DBD) is characterized by detrusor hyperreactivity. However, since it is unknown whether hyperglycemia affects the caveolae-mediated regulation of BSM contractility, the aim of this study was to investigates the effects of caveolar depletion on muscarinic-mediated BSM responses and determine whether these responses were altered by hyperglycemia.

Methods: BSM tissue was procured from C57 mice. Caveolin protein localization and interaction with muscarinic receptors (MRs) were determined by western blotting and immunoprecipitation. BSM contractile responses induced by carbachol (CCh, 1μM) were repeated before and after the depletion of caveolae (achieved by treatment with methyl-β-cyclodextrin, mβCD, 10mM, 1hr), in the presence of either 4-DAMP (10nM) or AFDX (0.1μM), M_3 and M_2 receptor antagonists respectively. Baseline CCh contractions were measured under normal glucose (NG 11.5mM) and then repeated after tissue exposure to high glucose (HG, 23mM, 2hrs). CCh responses generated under HG were also evaluated after mβCD or in the presence of AFDX. The effect of HG on actin polymerization in BSM tissue was analyzed by F/G actin assay.

Results: M_3R and M_2R interact with caveolin proteins. At baseline under NG, CCh-induced contractile responses were unaffected by caveolae depletion. In contrast, when the M_2R-mediated component of CCh contraction was isolated by the presence of 4-DAMP, the subsequent depletion of caveolae enhanced CCh responses significantly. Exposure to HG caused a significant increase in CCh responses, as well as an augmented BSM F/G actin ratio. Moreover, HG exposure induced the translocation of caveolins from membrane to cytosol, along with the dissociation of the Cav-3/M_2R complex. Finally, the HG-mediated increases in CCh contractions and actin polymerization were both prevented in the presence of M_2R antagonist AFDX.

Conclusions: The enhanced contractile response to CCh in BSM tissue after mβCD in the presence of M_3R antagonist indicates that the M_2R component of cholinergic contractions in the bladder is negatively regulated by caveolae. The increased BSM actin polymerization and the enhanced CCh responses observed after exposure of tissue to HG suggest that hyperglycemia contributes to the development of BSM hypercontractility during the compensatory phase of DBD. The dissociation of Cav-3 from M_2R after stimulation in HG reveals that hyperglycemia alters the reciprocal interactions between these proteins. These findings together with the effect of AFDX in preventing the HG-induced increases in both CCh responses and F/G actin suggest that hyperglycemia enhances cholinergic excitability by disrupting the inhibitory regulation of M_2R.

Funding Source: Department of Veterans Affairs, Biomedical Laboratory R&D, Washington DC.

Title: SHH regulates penile morphology and smooth muscle through a mechanism involving BMP4 and GREM1

Authors: Jiangping Deng[1], MD, Sarah Martin[1], Tim Searl, PhD[1], Samuel Ohlander, MD[1], Daniel Harrington, PhD[2], Kevin McVary, MD[3], Podlasek CA, PhD[1*]

Affiliations: [1]University of Illinois at Chicago; [2]UTHealth, [3]Loyola University Medical Center

Introduction/Objectives: The cavernous nerve (CN) is frequently damaged in prostatectomy and diabetic patients with ED, initiating changes in penile morphology including an acute and intense phase of apoptosis in penile smooth muscle and increased collagen, which alter penile architecture, make corpora cavernosa smooth muscle less able to relax in response to neurotransmitters, and ED results. Sonic hedgehog (SHH) is a critical regulator of penile smooth muscle, and SHH treatment suppresses penile remodeling after CN injury through an unknown mechanism; we examine if part of the mechanism of how SHH preserves smooth muscle after CN injury involves bone morphogenetic protein 4 (BMP4) and GREMLIN (GREM1).

Methods: Primary cultures of smooth muscle cells were established from prostatectomy, diabetic, hypertension and Peyronie's (control, n=18) patients. Cultures were characterized by ACTA2, CD31, P4HB and nNOS immunohistochemical analysis. Patient smooth muscle cell growth was quantified in response to BMP4 and GREM1 treatment. Adult Sprague Dawley rats underwent 1 of 3 surgeries: 1) Uninjured or CN injured rats were treated with BMP4, GREM1 or MSA (control) proteins via Affi-Gel beads (n=16) or peptide amphiphile (PA, n=26) for 3 and 14 days, and trichrome stain was performed. 2) Rats underwent sham (n=3), CN injury (n=9), or CN injury and SHH PA treatment for 1, 2 and 4 days (n=9) and western for BMP4 and GREM1 was performed. 3) Rats were treated with 5E1 SHH inhibitor (n=6) or IgG (control, n=6) for 2 and 4 days, and BMP4 and GREM1 localization was examined. Statistics were performed by ANOVA with Scheffe's posthoc test.

Results: BMP-4 increased patient smooth muscle cell growth, and GREM1 decreased growth. In uninjured and CN injured rats, BMP4 treatment via Aff-Gel beads and PA increased smooth muscle at 3 and 14 days of treatment. GREM1 treatment caused increased collagen and smooth muscle at 3 days, which switched to primarily collagen at 14 days. CN injury increased BMP4 and GREM1 while SHH PA altered western band size suggesting alternative cleavage and range of BMP4 and GREM1 signaling. SHH inhibition in rats increased BMP4 and GREM1 in fibroblasts.

Conclusions: SHH treatment alters BMP4 and GREM1 localization and range of signaling, which can affect penile morphology. Part of the mechanism of how SHH regulates corpora cavernosa smooth muscle, involves BMP4 and GREM1. Understanding how SHH PA preserves and regenerates penile morphology after CN injury will aid development of ED therapies.

Funding Source(s): NIH/NIDDK DK101536

Title: BMP4 and GREM1 are targets of SHH signaling and downstream regulators of collagen in the penis

Authors: Jiangping Deng[1], MD, Tim Searl, PhD[1], Samuel Ohlander, MD[1], Daniel Harrington, PhD[2], Kevin McVary, MD[3], Podlasek CA, PhD[1*]

Affiliations: [1]University of Illinois at Chicago; [2]UTHealth, [3]Loyola University Medical Center

Introduction/Objectives: Cavernous nerve (CN) injury caused by prostatectomy and diabetes, initiates a remodeling process (smooth muscle apoptosis and increased collagen) in the corpora cavernosa of the penis of patients and animal models that is an underlying cause of erectile dysfunction (ED). The Sonic hedgehog (SHH) pathway plays an essential role in the response of the penis to denervation; collagen increases with SHH inhibition and decreases with SHH treatment. We examined if part of the mechanism of how SHH prevents penile remodeling and increased collagen with CN injury, involves bone morphogenetic protein four (BMP4) and GREMLIN (GREM1). The relationship between SHH, BMP4, GREM1 and collagen was examined in the penis of ED patients and rat models of CN injury, SHH inhibition, and SHH, BMP4 and GREM1 treatment.

Methods: Corpora cavernosa of Peyronie's (control), prostatectomy and diabetic ED patients was obtained (n=30). Adult Sprague Dawley rats (n=90) underwent either: **1**. CN crush (1-7 days) or sham surgery, **2**. CN injury and BMP4, GREM1 or MSA (control) protein treatment via Affi-Gel beads or peptide amphiphile (PA) for 14 days, **3**. 5E1 SHH inhibitor, IgG or PBS (control) treatment for 2-4 days. Immunohistochemical and western analysis for BMP-4 and GREM1, and collagen analysis by hydroxyproline and trichrome stain were performed

Results: BMP4 and GREM1 proteins were identified in corpora cavernosa smooth muscle of prostatectomy, diabetic and Peyronie's patients, and in rat smooth muscle, sympathetic nerve fibers, perineurium, blood vessels, and urethra. Hydroxyproline assay showed 25.4% decreased collagen in rats with CN injury and BMP4 treatment ($p=0.02$) and 61.3% increased collagen with CN injury and GREM1 treatment ($p=0.005$). Trichrome stain showed increased collagen in rats treated with GREM1. Western analysis identified increased BMP4 and GREM1 in corpora cavernosa of prostatectomy and diabetic patients, and after CN injury (1-2 days) in our rat model. Localization of BMP4 and GREM1 changed with SHH inhibition. SHH treatment increased the monomer form of BMP4 and GREM1, altering their range of signaling.

Conclusions: BMP4 and GREM1 are downstream targets of SHH that impact collagen and may be useful in collaboration with SHH to prevent penile remodeling and ED. A better understanding of penile remodeling and how fibrosis occurs with loss of innervation is essential for development of novel ED therapies.

Funding Source(s): NIH/NIDDK DK101536

Title: Wireless catheter-free pressure sensors to assess neurogenic lower urinary tract dysfunction in a porcine model of spinal cord injury

Authors: Adam W. Doelman[1], Jay Ethridge[1], Audrey Warner[1], Ashnoor Arora[1], Megan Webster[1], Kitty So[1], Neda Tearle[1], Avril Billingsley[1], Alex Munro[1], Femke Streijger[1], Steve J.A. Majerus[2,3], Margot S. Damaser[3,4], and Brian K. Kwon[1,5].

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Introduction/Objectives: Neurogenic lower urinary tract dysfunction remains a leading cause of morbidity after spinal cord injury (SCI). Urodynamics (UDS) is presently the clinical standard for the assessment of bladder dysfunction after neurological injury. However, this method presents many well-recognized limitations which may interfere with bladder function and its subsequent interpretation. To address this, we tested a wireless intravesical pressure sensor (the "UroMonitor"), as well as an implanted transmural pressure sensor to permit continuous bladder pressure recording without the use of catheters. The aim of the presented study was to compare the UroMonitor and transmural telemetric systems to conventional catheter-based pressure measurement and to characterize neurogenic bladder dysfunction during UDS and ambulatory urodynamic monitoring in a large animal model of SCI.

Methods: Yucatan minipigs (n=9) were used under IACUC and veterinary oversight. SCI was induced via contusion-compression impact at the 10th thoracic level. A transmural telemetric sensor was implanted 4-weeks prior to SCI. UDS experiments were performed before and 4-, 8-, and 11-weeks after SCI. UroMonitor was inserted transurethrally before each UDS experiment under general anesthesia.

Results: We demonstrated that the UroMonitor and transmural sensors reliably identified >90% of voiding and non-voiding contractions during UDS assessment before and after SCI. UroMonitor and transmural pressure recordings showed strong, statistically significant correlation to conventional UDS catheters. The amplitude of bladder contractions measured from wireless sensors were within ~4 cmH2O of the present clinical standard. During ambulatory monitoring, voided volumes were found to be significantly reduced relative to those collected during UDS in pre-SCI animals. Further, bladder contraction amplitudes were found to be greater during ambulation relative to UDS assessment in SCI pigs.

Conclusions: Wireless, catheter-free devices may offer an alternative to traditional bladder assessment techniques permitting more natural, comfortable observation of lower urinary tract dysfunction after neurological injury in pre-clinical animals and humans. The present study tested two devices that can reliably identify and quantify bladder contractions before and after SCI in a large animal model with a high degree of accuracy.

Funding Source(s): DoD Translational Research Award – Spinal Cord Injury Research Program.

Title: The role of NLRP3 (nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3) in chronic stress-induced vascular and erectile dysfunction.

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Introduction/Objectives: Stress is known to cause vascular and erectile dysfunction where inflammation has been considered a major contributing factor. However, the precise mechanisms through which chronic stress impacts the vasculature and contributes to vasculogenic erectile dysfunction remain unclear. Here, we hypothesize that chronic stress exacerbates NLRP3 inflammasome activation leading to vascular and erectile dysfunction in male mice.

Methods: Male C57Bl/6 mice were submitted to chronic unpredictable stress (CUS) via daily exposure to different types of stressors for 28 days, while control animals were submitted to handling (non-CUS). Anxiety-like behavior, body weight, and systolic blood pressure (SBP) were assessed. Mesenteric resistance arteries (MRA), pudendal arteries (PA), and corpus cavernosum (cc) were removed and mounted in myographs to evaluate reactivity. Concentration-response curves to acetylcholine (ACh; 1 nM – 30 uM) and phenylephrine (PE; 1 nM – 30 uM) were performed. Using non-linear regression, we obtained the maximal response (Emax) and potency (pEC50) of ACh and PE. The expression of NLRP3 and caspase-1 was assessed by Western blot. Student t test was performed for comparasion between groups. Data are presented as mean ± S.E.M and significance was set at $p<0.05$.

Results: Stress increased SBP by 12.1±4.9%, decreased body weight gain by 53% ($p=0.04$), and evoked anxiety-like behavior ($p=0.01$) in mice exposed to CUS when compared to non-CUS. In the vasculature, CUS led to endothelial dysfunction observed by reduced potency of ACh in the MRA (pEC50; CUS: 6.17±0.09 vs non-CUS: 6.81±0.11; $p=0.003$) and PA (pEC50; CUS: 6.31 ±0.07 vs non-CUS: 6.65±0.09; $p=0.008$). Moreover, CUS led to a significant decrease in the maximal relaxation induced by ACh in the CC (CUS: 75 ± 4% vs. Non-CUS: 88 ± 4%) as well as an impaired contraction to PE (CUS: 1.07 ± 0.15% vs. non-CUS: 1.72 ± 0.17%). Finally, we observed increased expression of NLRP3 (CUS: 1.5 ± 0.6 vs non-CUS: 0.7 ± 0.2) as well as caspase-1 (CUS: 2.5 ± 0.5 vs non-CUS: 0.3 ± 0.2) in the MRA from male mice exposed to CUS when compared to non-CUS.

Conclusions: These data suggest that CUS led to an activation of the NLRP3 inflammasome pathway, and impaired the relaxation by a mechanism that might be associated with decreased nitric oxide bioavailability (endothelium-dependent relaxation), whereas impaired contraction might be associated with desensitization of alpha-adrenergic receptors in response to stress.

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Title - Erectile dysfunction in young adult rats might be associated with obesity in early life**.**

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Introduction/Objectives – Erectile dysfunction (ED) is largely associated with obesity, however, the consequences of early life obesity for ED in young adulthood are unknown. We hypothesize that obesity induced by preweaning overfeeding impairs vascular and erectile function and affects blood pressure in young adult male rats (5-month-old).

Methods – At postnatal day (PND) 1, Wistar rat were divided into a normal litter (NL, dams were kept with 5 female and 5 male pups) or small litter (SL, dams were kept with 2 male and 1 female pup) during lactation. After weaning, pups from the SL group presented with increased body weight. Next, rats from both groups returned to normal size colonies (4-5 rats per cage) and were fed standard chow. At PND160, we evaluated body weight and fat pad deposition, mean arterial pressure (MAP) by tail-cuff and vascular (pudendal artery) and cavernosal reactivity. Concentration-response curves to phenylephrine (PE) or acetylcholine (ACh) were performed in both preparations. In the corpus cavernosum, contraction and relaxation to electrical field stimulation (EFS) were obtained. Concentration-response curves were analyzed using non-linear regression analysis. Data was analyzed by Student's t-test and presented as mean ± S.E.M. Statistical significance was set at $p<0.05$.

Results – At PND160 no differences in body weight were observed in SL (594±14g) compared to NL (559±13g), however, there was an increase in fat deposition of the SL group, observed by increased retroperitoneal adipose tissue (NL 7.47±0.69g vs SL 11.25±1.23g, $p<0.05$), and perigonadal adipose tissue (NL 10.05±0.55g vs SL 13.24±1.16g, $p<0.05$). SL rats presented increased MAP (NL 114.9±0.58 vs SL 120.5±1.20 mmHg). In the pudendal artery, it was observed decreased acetylcholine-induced relaxation in the SL group (Emax: 28±6%) compared to NL group (Emax: 68±5.5%). No changes in the contraction to PE were observed. In the corpus cavernosum, the relaxation induced by EFS was significantly decreased for all frequencies of stimulation in SL rats compared to NL rats (1 Hz: NL 0.77 ± 0.18% vs SL 0.27 ± 0.09%; 4 Hz: NL 2.48 ± 0.26% vs SL 1.36 ± 0.41%; 16 Hz: NL 3.06 ± 0.28% vs SL 2.39 ± 0.53%).

Conclusions – Our data suggests that early life obesity can cause long-lasting vascular and cavernous damage to males that might be associated with erectile dysfunction in young adulthood.

Funding Source(s) – NIDDK 132948 and Institute on Cardiovascular Disease Research, University of South Carolina.

Title: Bladder Volume Estimation Using a Catheter-free Wireless Pressure-Volume Sensor in Minipigs

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Introduction/Objectives: Multi-channel urodynamics is a clinical exam used to diagnose storage and voiding dysfunctions associated with the bladder, urethra and pelvic floor. Although the clinical gold standard, urodynamics only provides a short snapshot of bladder function in an artificial, unfamiliar setting. Catheters are inserted into the bladder and rectum to measure intra-vesical and intra-abdominal pressures, while the bladder retrogradely fills with saline at a supraphysiological rate. In animal research, urodynamics is even less physiological, as it requires anesthetics which interact with normal lower urinary tract function. The non-physiological nature of the exam makes it difficult to translate urodynamic findings from animal studies to clinical use. As a result, there is a need for a catheter-free sensor of bladder function during natural filling. We have developed the Urological Monitor of Conscious Activity (UroMOCA), a surgically inserted wireless bladder pressure and volume monitoring device. Here, we describe the *in-vivo* performance of the UroMOCA for continuous bladder volume sensing in large animals.

Methods: The UroMOCA was surgically implanted in eight female Yucatan miniature pigs under anesthesia via laparotomy and cystotomy on an approved IACUC protocol. The device was implanted for 1 week in five animals and for 4 weeks in three animals. In the 1 week experiments, the device was evaluated in conscious free-moving animals with an anesthetized follow-up on Day 7. In the 4 week experiments, UroMOCA accuracy was evaluated in conscious animals and on Days 14 and 28 under anesthesia as follows. Filling cystometry was performed to compare UroMOCA pressure to a dual-lumen reference transurethral catheter while filling the bladder at 20-25 mL/min. Bolus filling of the bladder with 0.9% and 4% NaCl solutions was performed to evaluate the volume sensitivity of the UroMOCA up to bladder capacity. Normal bladder function was recorded by the UroMOCA in awake untethered animals. Events such as voids, bowel movements and motion artifacts were manually marked. The UroMOCA was surgically explanted following the Day 7 or Day 28 bolus fill testing, and the animals were euthanized.

Results: Ambulatory data recording captured over 100 hours of catheter-free bladder function across all animals. UroMOCA volume measurements increased non-linearly with bolus fills of 0.9% and 4.0% NaCl solutions. Bladder emptying events were associated with sharp decreases in the UroMOCA volume measurements concomitant with rises in bladder pressure. Variability in volume measurements was noticed between free-moving and anesthetized animals and is likely due to the positional sensitivity of the UroMOCA within the bladder.

Conclusions: The UroMOCA can be used for monitoring bladder pressure and volume in chronic experiments of awake, behaving animals. A look-up table will be developed to convert UroMOCA measurements to units of volume.

Funding Source(s): NIH 3OT2 OD023873

Title: Upregulation of brain-derived nerve factor (BDNF) expression and enhanced reactive oxygen species production in lower motor neuron-lesioned dog bladder mucosa following somatic-motor nerve transfer

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Introduction and Objective: Loss of motor and sensory function due to spinal cord injury has been linked to bladder pathologies and increased susceptibility to urinary tract infections (UTIs). Neurotrophins, including brain-derived nerve factor (BDNF) and associated reactive oxygen species (ROS), play roles in modulating neuronal plasticity peripherally and centrally. We used an established dog model of lower motor neuron-lesioned bladder in which somatic nerve transfer to the vesical branch of the pelvic nerve is used to promote bladder reinnervation to evaluate BDNF expression and nicotinamide adenine dinucleotide phosphate (NADPH)-dependent ROS production after long-term bladder decentralization, with or without reinnervation procedures. Levels of the pro-inflammatory cytokine, tumor necrosis factor alpha (TNFα), and the sensory nerve marker, calcitonin gene related peptide (CGRP), were also evaluated.

Methods: Three groups of female mongrel hound dogs: 1) Decentralized (n=9), achieved by bilateral transection of coccygeal and sacral spinal roots and dorsal roots of lumbar 7, and a 6–21 mo recovery; 2) ObNT-Reinn (n=9), which were animals that underwent similar decentralization and 9–13 mo recovery, then reinnervation by bilateral transfer of obturator nerves to vesical branches of pelvic nerves and another 8–12-mo recovery; and 3) Controls (n=9; age-matched sham operated and unoperated animals). Urination postures and frequency of UTIs were monitored. At study end, animals were anesthetized, and bladders harvested. Detrusor wall mucosa and smooth muscle tissues were dissected, homogenized, and used for biochemical assays. The urothelial marker, uroplakin III, was assessed in bladder mucosa homogenates using Western blot. Mucosa and smooth muscle levels of total BDNF, TNFα, and CGRP were measured using ELISA, and superoxide production measured using lucigenin-enhanced chemiluminescence.

Results: All Decentralized and ObNT-Reinn animals had multiple instances of culture-confirmed bacteriuria. Several Decentralized animals showed micturition-like postures during periods when UTIs were confirmed; these postures disappeared with antibiotic clearance of UTIs. At study end (~ 21 mo after initial decentralization), uroplakin III protein levels in the mucosa were 2.7-fold lower in Decentralized vs Control bladders, a decline associated with urothelial sloughing in Decentralized bladders. ObNT-Reinn animals showed the highest levels of BDNF in both their mucosa and smooth muscle (> 3-fold each), and highest levels of ROS in their mucosa (1.5- to 2-fold), compared to the other two groups. In contrast, ObNT-Reinn as well as Decentralized animals showed lower CGRP (mucosa and muscle layers), lower TNFalpha (> 2-fold in the mucosa only), and lower ROS in smooth muscle layers, compared to Controls.

Conclusions: Decentralized animals showed increased UTIs, impaired urothelial structure and function through loss of cellular stress response as shown by the lower mucosa levels of uroplakin III and ROS, compared to Controls. The enhanced BDNF and ROS levels in ObNT-Reinn bladders could be due to the new obturator to bladder axonal pathway, perhaps as compensatory mechanisms to recover bladder function. Since CGRP is mostly expressed in sensory nerves as a primary afferent excitatory neurotransmitter, it is reasonable to propose that the observed decreased CGRP levels in Decentralized and ObNT-Reinn bladder layers occurred because of bladder deafferentation that remained even after bladder reinnervation in the latter group. The decreased TNFα levels in Decentralized and ObNT-Reinn mucosal layers might be the consequence of the prolonged antibiotic treatments needed to control the recurrent UTIs.

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Title: Prostaglandins Facilitate the ATP Degradation by Soluble Ectonucleotidases in the Urinary Bladder Lamina Propria

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Introduction/Objectives: Prostaglandins are essential signaling molecules in the regulation of bladder excitability and are thought to play a particular role in the sensory arm of the micturition reflex. These mediators, as well as adenosine 5'-triphosphate (ATP), alter bladder excitability during filling and may contribute to increased bladder activity during interstitial cystitis/bladder pain syndrome. Soluble ecto-nucleotidases (s-ENTDs) that hydrolyze ATP to ADP, AMP, and adenosine are released in the suburothelium/lamina propria (LP) in response to bladder distention and are key to determining the effective concentrations of extracellular purines at receptor sites of various cell types in the LP at rest and during bladder filling. It is currently unknown whether prostaglandins regulate the release of s-ENTDs, thus affecting the levels of extracellular purines in the LP and subsequently modulating bladder excitability.

Methods: We evaluated changes in the concentration of 1N^6-etheno-ATP substrate (eATP, 2 μM) and the formation of its metabolites eADP, eAMP, and e-adenosine (eADO) in LP of murine bladder using highly sensitive HPLC techniques with fluorescence detection. The hydrolysis of eATP by s-ENTDs was measured during one hour of reaction between substrate and s-ENTDs in concentrated extraluminal solutions (cELS) that had been in contact with distended and nondistended LP of detrusor-free murine bladders, in the absence of tissue, and in the presence or absence of prostaglandin E2 (PgE2), PgF2α, PgI2, and PgD2.

Results: In cELS from nondistended preparations at 60 minutes of reaction, eATP levels diminished significantly from 92.13±0.47 to 56.67±2.37 % of total purines in vehicle (DMSO 0.2%, n=6) to 38.68±7.45% in PgE2 (n=4, P=0.0015), 46.18±3.1% in PgF2α (n=4, P=0.0029), 47.9±6.54% in PgI2 (n=4, P=0.2092), and 42.19±6.39 % in PgD2 (n=4, P=0.0013); ea. 10 μM. Therefore, the spontaneous release of s-ENTDs in LP was enhanced by PgE2, PgF2α, and PgD2, but not by PgI2. In cELS from distended bladders, however, the decrease of eATP remained unchecked in the presence of PgF2α, PgI2, and PgD2 and was slightly enhanced by PgE2. In the presence of all prostaglandins tested, the increasing effect on eATP degradation in cELS from distended preparations was more apparent in the formation of eADO than in the eATP decrease.

Conclusions: Our results suggest novel mechanisms of regulation of bladder excitability by prostaglandins by controlling the effective concentrations of purine mediators at receptor sites. While PgI2 did not seem to play a significant role in the extracellular ATP metabolism by s-ENTDs, PgF2α, PgD2, and particularly PgE2 accelerated the degradation of ATP and formation of ADO by increasing the release of s-ENTDs. The effects of prostaglandins were greater on the spontaneous than on the distention-induced release of s-ENTDs in the LP. Further studies are warranted to understand the interdependence of prostanoid and purinergic signaling mechanisms in the bladder LP in health and disease.

Funding Source: NIH R01 grant DK 119482

Title: Investigating kidney and bladder functional declines associated with aging**.**

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Affiliations: The Jackson Laboratory, Bar Harbor, ME, USA

Introduction/Objectives: Aging is the greatest risk factor for many conditions, including kidney and bladder dysfunction. Despite the known declines in both kidney function and bladder function observed in aging systems, it is unknown whether kidney functional declines are negatively associated with bladder performance, or vice versa. We hypothesize that kidney function will directly correlate with bladder function, emphasizing the need for systems-based approaches of investigating urinary tract aging.

Methods: Glomerular filtration rate (GFR) and cystometry, or GFR and strip studies were performed on aged 24 month old C57BL/6J mice (n=8-10/sex). This study seeks to determine if correlations exist between GFR and cystometric outcomes, or GFR and pharmacologic responses.

Results: Cystometric results revealed an increased number of male aged mice that failed to respond to cystometry, however female non-responder rates were analogous to previous reports. Strip studies revealed decreased responsiveness to pharmacologic agonists compared to mature animals. Preliminary results indicate that GFR decreases with age correlate with decreased bladder function.

Conclusions: To date, there is little known about how physiological changes in one urinary tract organ influence the other. These data support a systemic-functional model in which upper and lower urinary tract function are predictive of one another.

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Strict glucose control and elimination of NLRP3-induced inflammation prevents diabetic bladder dysfunction in the female Akita mouse model

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Introduction/Objective: Diabetic bladder dysfunction (DBD) is a prevalent diabetic complication with no targeted therapy. While strict blood glucose control is logically thought to reverse complications, the Epidemiology of Diabetes Interventions and Complications study found no beneficial effects on lower urinary tract symptoms. However, it is possible that strict glucose control can prevent DBD, if administered early. Unfortunately, ≈50% of diabetics are classified as poorly controlled. In this study, we examine the effect of early glucose control (strict and poor) on development of DBD in the female Akita mouse, a Type 1 diabetic model with mild hyperglycemia. In addition, we have shown that NLRP3-induced inflammation was critical to DBD in this model. Therefore, we have expanded this study to test the potential of inhibiting/deleting NLRP3 as adjunct therapy to glucose control, particularly in poorly controlled diabetes.

Methods: Akita were bred NLRP3$^{+/+}$ or NLRP3$^{-/-}$. Only females were used. At week 6, some diabetics were given insulin pellets (s.c., Linshin, Toronto) that poorly (0.05 U/day) or strictly (0.125 U/day) controlled blood glucose. At week 15, blood glucose (glucometer), HbA1c levels (ELISA), bladder inflammation (Evans blue) and bladder function (urodynamics) was assessed.

Results: Blood glucose (A) averaged 113±5 mg/dl (nondiabetic), 244±20 mg/dl (uncontrolled diabetic), 186±13 mg/dl (poorly controlled) and 131±16 mg/dl (strictly controlled). These levels were not affected by deletion of NLRP3. HbA1c levels reflected glucose levels. Bladder inflammation (B) was also directly correlated to glucose control and eliminated by deletion of NLRP3 in all groups. Examining bladder function (C) we found signs of overactivity (decreased void volume, etc.) in the uncontrolled group and improved urodynamics in the poorly controlled group. Strict control eliminated the dysfunction, showing that effective control, initiated early, can prevent DBD. Moreover, when NLRP3 was deleted, no bladder dysfunction developed, suggesting that elimination of NLRP3-induced inflammation will prevent DBD.

Conclusions: Both early-initiated strict glycemic control and NLRP3 elimination can effectively prevent DBD, suggesting hyperglycemia acts through NLRP3-induced inflammation to trigger DBD.

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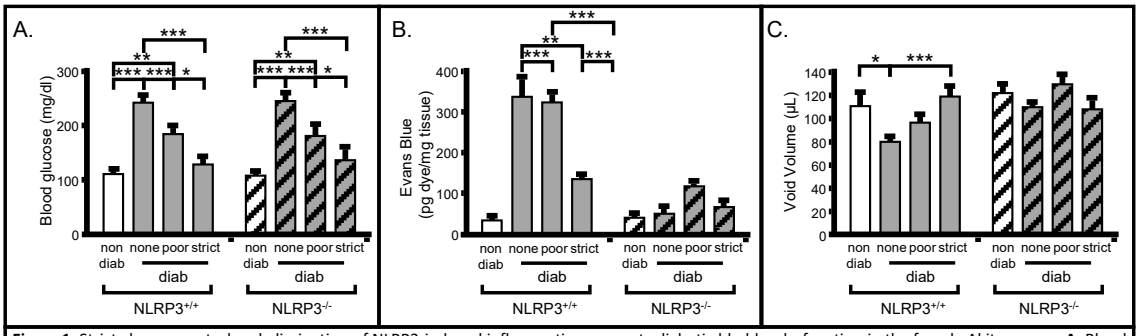

Figure 1. Strict glucose control and elimination of NLRP3-induced inflammation prevents diabetic bladder dysfunction in the female Akita mouse. **A.** Blood glucose levels, **B.** Evan's blue inflammation and **C**. Voiding volume of the indicated groups at 15 weeks of age. Glucose control was initiated in week 6

Title:

Sentiments of interstitial cystitis/bladder pain syndrome patients towards Elmiron

Authors:

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Introduction/Objectives: Interstitial cystitis/bladder pain syndrome (IC/BPS) is a multifactorial, chronic syndrome involving urinary frequency, urinary urgency, and bladder discomfort that affects an estimated 10 million individuals in the United States. The only FDA-approved medication to treat IC/BPS is Pentosan polysulfate sodium, PPS (US trade name Elmiron). A recent study identified a unique pattern of ocular pigmentary maculopathy in six patients out of 38 (16%) with PPS exposure due to IC/BPS treatment. It is not known what individuals with IC/BPS think of PPS and if the discovery of ocular pigmentary maculopathy impacts their thoughts on PPS treatment.

Methods: Data were collected from Inspire.com–an online health community. Data were limited to patients who were members of the IC/BPS community and mentioned PPS in their discussion posts. Sentiment analysis was conducted using VADER (Valence Aware Dictionary for Sentiment Reasoning) to identify compound scores and percentages of negative, positive, and neutral sentiment for PPS discussions and for each topic. Topic modeling was conducted using Latent Dirichlet Allocation (LDA). To categorize topics, words with the highest probability were ranked for each topic, and a manual investigation of patient discussions was examined and labeled by authors.

Results: Topic modeling with LDA revealed 5 topic categories: "ineffectiveness or discontinuing use", "alternative treatments", "personal treatments suggestions based on experience", "severe side effects", and "risk of long-term use". The overall compound patients' sentiment of PPS was -0.083, 32.48% negative, 22.03% positive, and 45.48% neutral.

Conclusions: Patients' discussions surrounding Elmiron included its effectiveness at treating IC/BPS in view of its severe side effects e.g., blindness. Patient overall compound sentiment score was neutral, indicating that patients are still considering PPS usage despite understanding the risks because of the severe symptoms of IC/BPS. Health forums are useful for patients with complex disorders to gain insights into how to manage their condition when there is uncertainty around existing medical treatments.

Funding Source(s):

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Title: Effects of elevated pressure cycling on urothelial exosomes in vitro

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Introduction/Objectives: Bladder outlet obstruction (BOO) leads to elevated storage and voiding pressures, which lead to inflammation, epithelial-mesenchymal transition (EMT), and eventually fibrosis.[1,2] Patients do not seek treatment until the condition is bothersome and the chance to detect EMT in the bladder cells may be missed. Thus, there is a need for a non-invasive method to detect these alterations in the early stages of BOO. Extracellular vehicles (EVs), such as exosomes, in urine can carry stable RNAs and proteins for cell-to-cell communication. Studies have shown that stressors, such as pressure, can cause changes in the cargo within exosomes.[3,4] Therefore, we hypothesize that signaling molecules such as small RNAs related to EMT, and fibrosis may be contained in exosomes released by the urothelium and could potentially be used as an early diagnostic maker for BOO. To date, however, the effects of elevated pressure on urothelial exosomes and their contents are unknown. In the present study, exosomes released by rat urothelial cells (MYP3) or human bladder epithelial cells (BDeC) exposed to elevated pressure were collected and examined.

Methods: MYP3 and BDeC cells were either maintained under atmospheric pressure or exposed to elevated pressure cycles (15 cmH_2O for 175 min and 75 cmH_20 for 5 min) for 48 or 72 hours. At the end of the experiment, supernatant culture media were collected, and exosomes were harvested using either classical ultracentrifugation or a novel method involving capillary-channeled polymer (C-CP) fiber spin down tips. Size distribution of the EVs was characterized using dynamic light scattering (DLS).[5,6] Protein content was quantified using Bradford assay (Fisher). To verify that the EVs were exosomes, CD81 was quantified via a dot blot. Total RNA was isolated using a Zymo kit and RNA was sequenced using Oxford Nanopore technology.

Results: When EVs were isolated from supernatant media of MYP3 cells via ultracentrifugation, the yield was very low and results of CD81 detection was inconsistent. However, when EVs were isolated from MYP3 and BDeC cells using C-CP, the yield was higher, and all were within exosome size range (30-150 nm). Moreover, compared to the control group, the EVs obtained from cells exposed to high pressure cycles contained higher amounts of protein and RNA.

Conclusions: The results of the present study demonstrated that the C-CP method allowed for higher yields of EVs collected from the supernatant media of urothelial cells. In addition, exposure of urothelial cells to high pressure cycling that simulated BOO led to an increase in EV release. Further research, however, is needed to characterize the small RNAs from exosomes.

Funding Source(s): Clemson University TGER Grant and NSF GRFP (BNH)
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Blocking Necroptosis Partially Prevents Bladder Dysfunction in Diabetic Male Akita Mice

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Introduction: Diabetes is reaching epidemic levels worldwide and the most common complication of this disease is uropathy, otherwise known as diabetic bladder dysfunction (DBD). DBD can present as overactivity or underactivity and can seriously erode quality of life. Our lab has been characterizing DBD in the Akita mouse model of Type 1 diabetes which occurs as a result of a natural mutation in the INS2 gene and results in an increase in blood glucose starting around week 5 of life. By 15 weeks females develop overactivity dependent on NLRP3-induced inflammation while males develop underactivity independent of NLRP3. Exploring the males further, we have surmised the underactivity arises from a decrease in neurotransmitters released from efferent nerves and this is due, at least in part, to a decrease in the density of acetylcholinergic nerve terminals. Retraction, or "dying back" of nerve terminals (often with preservation of cell bodies) is a well-known event in diabetic neuropathy, which is mostly worked out in studies of peripheral sensation. Exciting new studies suggest this retraction is caused by a programmed form of necrosis, known as necroptosis, occurring in Schwann cells. Loss of Schwann cells leads to reduced signaling and axonal transport and eventually retraction of the axons. Thus, we hypothesize that necroptosis of Schwann cells in the bladder leads to retraction of acetylcholinergic nerve terminals and bladder dysfunction in male Akita mice. To address this hypothesis, we have given mice an inhibitor of necroptosis (Necrostatin) daily from week 6-15 of life and assessed bladder function by urodynamics

Methods: Type 1 diabetic Akita male mice and nondiabetic male mice were used in this study. Beginning at 6 weeks of age one group of diabetic male mice were given Necrostatin-1 (1.65 mg/kg/day, ThermoFisher). On week 14 suprapubic tubes were implanted and one week later urodynamics performed. Blood glucose was assessed by glucometer at the terminal time point.

Results: Necrostatin-1 treatment did not affect blood glucose levels. As expected, male Akita mice showed signs of underactivity by cystometry, including increased void volume and decreased frequency, along with an increase in compensated capacity. In contrast, daily Necrostatin attenuated the severity of the bladder dysfunction. For example, voiding volumes were 220 ul compared to 370 ul for diabetics and frequencies were 4.8 micturitions/hr compared to 2.82. However, levels were not entirely reduced back to control levels (void volume = 108 ul, frequency = 6.97 micturitions/hr).

Conclusions: Diabetic bladder dysfunction in male Akita mice can be attributed, in part, to necroptosis, presumably occurring in the Schwann Cells.

Funding: NIH-RO1 DK117890, NIH-K12 DK100024

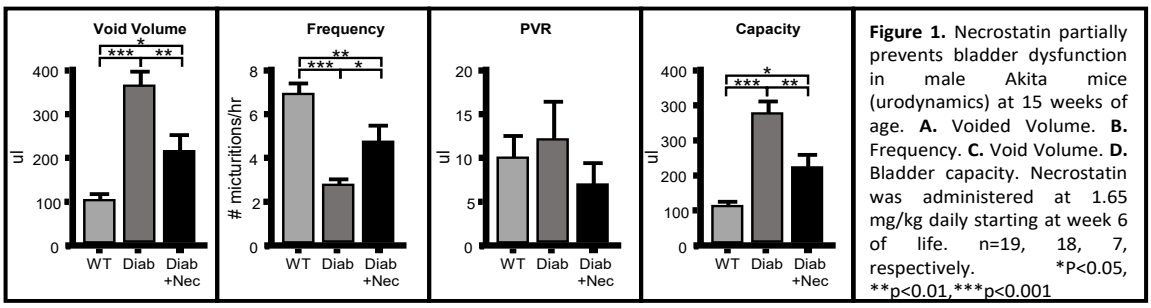

Figure 1. Necrostatin partially prevents bladder dysfunction in male Akita mice (urodynamics) at 15 weeks of age. **A.** Voided Volume. **B.** Frequency. **C.** Void Volume. **D.** Bladder capacity. Necrostatin was administered at 1.65 mg/kg daily starting at week 6 of life. n=19, 18, 7, respectively. *P<0.05, **p<0.01,***p<0.001

Title: Characterization of female bladder and vaginal function in a preclinical model of Ehlers-Danlos Syndrome

Authors: Soumya Kamath, Andrew Readyoff, Nick Williamson, Everett Minchew, Espen E. Spangenburg, Johanna L. Hannan

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Introduction/Objectives: Ehlers-Danlos syndrome (EDS) is an inheritable connective tissue disorder characterized by collagen abnormalities, leading to tissue fragility. Individuals with EDS often experience urinary incontinence, pelvic pain, and sexual dysfunction. While EDS affects both genders, it's more prevalent in women (73-89%). Classic EDS (type 1) results from mutations in collagen type V (COL5a1) gene, which plays a crucial role in regulating fibrillogenesis. Using a murine EDS model with Col5a1 gene mutation that mimics classic EDS symptoms, we aimed to characterize smooth muscle function in the vaginal and bladder tissues of female mice. **We hypothesized that lower levels of collagen V in the EDS mice would lead to decreased bladder contraction, increased vaginal contraction, and more frequent voiding**.

Methods: We utilized 16-week wild type (WT; Col5a1 +/+) and heterozygous (EDS; Col5a1 +/-) mice (n=13-14). To assess bladder function, void spot assays were performed. Tissue bath experiments were conducted on both bladder and vaginal strips. KCl contractions assessed tissue viability, followed by concentration response curves to carbachol and norepinephrine in bladder and vagina, respectively. In bladders, electric field stimulated (EFS) contractions with or without atropine (cholinergic antagonist) and PPADS (purinergic antagonist) were measured. Additionally, relaxation curves to DEA NONOate and EFS were assessed in the vagina.

Results: At 16 weeks of age, the body and bladder weights between the two genotypes were unchanged. Young female EDS mice exhibited reduced voiding frequency (WT: 2.59±1.0, EDS: 2.00±0.8; $p=0.0536$). No significance was observed in total void area or primary void size. At lower concentrations of carbachol, EDS mice contracted higher than WT mice ($p<0.05$) and significantly increased EC50 (WT: -6.8±0.4, EDS: -7.3±0.6; $p<0.05$). Overall EFS mediated contractions were no different between groups, but the EDS mice had a greater cholinergic inhibition to atropine. Maximum KCl contractions, norepinephrine, and EFS relaxation in vaginal strips were no different between groups. EDS mice displayed increased sensitivity to DEA NONOate relaxation with lower EC50s (WT: -5.9±0.7, HET: -6.9±0.04; $p>0.05$).

Conclusions: Young female EDS mice demonstrated less frequent voiding, and increased bladder sensitivity to muscarinic stimulation. Overall, the vaginal tissue was unchanged between genotypes. Ongoing studies will assess morphology of the bladder and vagina as well as morphology and function of the pelvic floor muscle. We will also assess sexual receptivity and pain studies. We believe that this model can be used to help further understand the mechanism urinary incontinence in EDS.

Funding Source(s): International Society of the Study of Women's Sexual Health (ISSWSH)

Title: Pudendal Nerve Injury and its Contribution to Stress Urinary Incontinence in a Male Rat Model

Authors: Shaimaa Maher[1*], Daniel Gerber[2], Teja Malakalapalli[1], Brett Hanzlicek[1,3], Mei Kuang[1], Lan Wang[1] and Margot Damaser[1,2,3]

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Introduction/Objectives: Maintaining urinary continence is vital following prostatectomy, as the surgery can disrupt essential anatomical structures responsible for continence. This study aimed to assess how pudendal nerve injury affects male continence and establish a rat model for exploring innovative neuro-regenerative treatments to improve post-prostatectomy continence.

Methods: Six male rats were subjected to acute leak point pressure (LPP) and external urethral sphincter electromyography (EMG) testing with an: intact urethra, exposed urethra (UE), exposed pudendal nerve (NE), and after pudendal nerve transection (PNT). Additionally, 67 male rats underwent sham pudendal nerve injury (sham), pudendal nerve crush (PNC), or PNT and were tested for LPP and EMG 4 days, 3 weeks, or 6 weeks after injury. Urethras were assessed histologically after dissection. The Kruskal-Wallis test followed by Dunn's multiple comparisons test was used to compare LPP, EUS EMG amplitude, and EUS EMG firing rate for the acute study. For the chronic study, an unpaired two-tailed t-test was used to compare outcomes between the sham and PNC groups 4 days and 3 weeks after injury. Ordinary one-way analysis of variance (ANOVA) followed by the Tukey's multiple comparisons test was used to compare outcomes among the sham, PNC, and PNT groups 6 weeks after injury.

Results: Acute PNT led to a significant reduction in LPP, EMG amplitude, and EMG firing rate compared to the intact, UE, and NE groups. In the chronic study, PNC resulted in significant LPP and EMG amplitude/firing rate reductions 4 days and 6 weeks after injury. Three weeks after injury, PNC significantly diminished LPP and EMG firing rate. Sham-injured animals had compact, innervated neuromuscular junctions (NMJ) compared to the PNC and PNT groups at all 3 timepoints. Conversely, in the PNC and PNT groups at all timepoints, NMJs were less organized and less innervated with axons. Collagen infiltration was markedly increased in both PNC and PNT groups compared to the sham group.

Conclusions: The pudendal nerve exerts a significant influence on male rat urinary continence. The established rat model holds promise for preclinical evaluation of neuro-regenerative therapies aimed at enhancing post-prostatectomy continence.

Funding Source(s): Research Project is supported by a grant from The Cleveland Clinic Research Program Committee (RPC IF110222)

Title - A surgical and functional approach to the pelvic gross neuroanatomy of the female Yucatan minipig.

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Introduction/Objectives – larger animal models offer additional advantages for investigations of the pelvic floor and lower urinary tract (LUT), included translational potential. Yucatan minipigs have been considered ideal for preclinical model for the study of LUT. However, the information regarding the pelvic organs and innervation is nonspecific and contradictory between studies. The aim of this study was to explore anatomically the organs and nerves relevant for micturition and sexual functions and describe a surgical approach for the physiological study of the pelvic organs.

Methods – Seven postmortem and perfused with 4% paraformaldehyde female Yucatan miniature pigs' specimen were obtained from swine models of T10 contusion spinal cord injury at UofL. Using a surgical stereoscope, the lumbo-sacral plexus was dissected using 3 different approaches: infra-pelvic, abdomino-pelvic and dorsal. Laminectomies of the last 3 lumbar vertebras and right unilateral laminectomies of S1-S3 were performed to expose the nerve roots of the lumbo-sacral plexus. The partial S1-S3 laminectomy was made to spare the sacral foramens as anatomical reference. Additionally, the sacro-tuberous ligaments were also disinserted to ease the dissection of the perineal branches of pudendal nerve.

Results – The hypogastric, pelvic, and pudendal nerves were fully identified and tracked. Sacral roots (S1-S3) provide branches to form the pudendal and pelvic nerve. The pudendal nerve is formed for 2 different and fully distinguishable branches, the first one formed for fiber coming from S1-S2 who leaves the pelvis through the greater foramen with the sciatica nerve and a major branch formed for fivers from S2-S3 who runs intrapelvic on the pararectal space and leaves the pelvis through the pelvic facia behind the pubococcygeus muscle. Both branches mixed and reenter the pelvis through the minor sciatic foramen diverging into the perineal, dorsal nerve of clitoris (DNC) and suprapubic branches. Some branches going to the urethra-vaginal compressor and external urethral sphincter muscles were identified. The pelvic nerve is formed by S2 branches but is possible that S2 and S3 also contribute by anastomotic fivers. The pelvic nerve mixes with the hypogastric and form the pelvic plexus (terminology suggested by Dr Majewski). Vesical and urethral nerves emerge from the plexus as well as the cavernous nerve which travels caudally to the perineum. By the infra-pubic approach, the DNC and suprapubic branches were identified, as well as, the U-VC, ischiocavernosus and external anal sphincter muscles and clitoris. The posterior approach looks like is the best way to reach the pudendal nerves and the abdominal approach for pelvic and hypogastric nerves.

Conclusions – The results show the anatomical references of the major structures involved in the urogenital systems paving the way for further surgical and physiological investigations.

Funding Source(s) – This study was supported by NIH R21 (Grant no.1R21NS114982-01A1) and Ole A., Mabel Wise & Wilma Wise Nelson Endowment.

Title: Racial disparities in treatment of interstitial cystitis/bladder pain syndrome

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Introduction/Objectives: Interstitial cystitis/bladder pain syndrome (IC/BPS) is a multifactorial, chronic syndrome involving urinary frequency, urinary urgency, and bladder pain. Diagnosing IC/BPS focuses on the exclusion of other diseases, and receiving a diagnosis typically involves multiple healthcare providers and usually takes several years. We hypothesized that significant discrepancies in treatment exist for racial Minority groups with IC/BPS. Therefore, we compared the experiences of different races and ethnicities for their symptoms, treatment status, and treatment methods.

Methods: A web survey of IC/BPS symptomatology and pain experiences was administered between June and August 2022. The survey included the Interstitial Cystitis Problem Index (ICPI) and The O'Leary-Sant Interstitial Cystitis Symptom Index (ICSI) in addition to questions about demographics, comorbidities, treatments, and pain. T-tests were used to compare between racial groups.

Results: 1,631 individuals responded to the survey. 89.09% reported their race as White or Caucasian, 6.93% as Black or African American, and 6.39% reported another race or did not answer. 7.2% of respondents reported Hispanic ethnicity.

Although there was no significant difference between racial and ethnic groups in either ICPI or ISCI, treatment levels for Non-White participants were significantly lower when compared to White participants ($p<0.01$). 22.16% of Non-White patients had never received treatment for IC/BPS. Conversely, only 7.37% of White patients had never been treated for their IC/BPS. The non-treatment rate for Hispanic respondents was 22.2% and the non-treatment rate for non-Hispanic respondents was 7.9%.

Significant disparities were observed across almost all treatment categories. A distinct gap was found in the utilization of non-narcotic pain medications, with 35.79% of White respondents receiving this treatment compared to only 14.62% among Non-White respondents. Narcotic pain medication was slightly more equitable among the two groups; however, White participants were still more likely to receive opioids than Non-White participants at 26.8% and 19.2% treatment rates respectively. Similar trends were evident for antidepressants (51.93% White and 25.38% Non-White), and antihistamines (45.39% White and 28.46% Non-White). Pentosan polysulfate sodium, the only oral drug approved by the FDA specifically for interstitial cystitis, was also more likely to be prescribed to White participants (36.76% White and 20.77% Non-White). All results are considered significant at $p < 0.01$.

Conclusions: While there were no observed differences in symptomology by race or ethnicity, participants of color were significantly less likely to have ever received treatment for IC/BPS or to have access to essential medications to manage their condition including narcotic medication, non-narcotic medication, antidepressants, antihistamines, and Pentosan polysulfate.

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Transcriptomic and functional assessment of the developing zebrafish bladder

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BACKGROUND: Our knowledge of development has benefited from direct visualization of early ontogenic events. While great strides in understanding bladder and lower urinary tract development have been made using mammalian models, as *in utero* amniotes, mammals develop internally and thus present issues with visualization. Zebrafish offer unique insights because they are transparent during development, are amenable to a host of genetic tools, and allow for excellent assessment of molecular and morphologic changes that result from pharmacological or toxicant exposure. Based largely on larval studies, zebrafish have long been thought to possess only a common cloaca, without separately terminating urologic, genital, anal structures, and a distinct urinary bladder has been assumed absent in zebrafish. These assumptions limited the utility of zebrafish as a model for urinary tract development. We recently rigorously confirmed the presence of a contractile urinary bladder in adult zebrafish, allowing us to now exploit this finding to characterize morphologic and molecular characteristics of bladder development in zebrafish.

METHODS: We employed the Tg(cdh17:mCherry) zebrafish line, which has a fluorescently labelled urinary system, to characterize morphologic and molecular signatures of distal urinary structures in developing and adult zebrafish. Distal urinary structures were imaged in zebrafish from 7 to 30 days post fertilization (dpf) and analyzed for morphology. Distal urinary structures from larval zebrafish at multiple developmental timepoints were dissected and processed for bulk RNA-seq. Nearby distal gastrointestinal tissue was used for differential expression comparison. Preliminary expression timelines were assessed. Dextran-conjugated Alexa dye was injected into the pericardium to trace distal urinary structures and excretion. We defined the bladder as a discrete distal urinary segment that increases fluorescence with filling and decreases with a urinary stream.

RESULTS: Pericardial injection of fluorescent dye revealed bladder filling and emptying with periodic contractions coordinated with voiding as early as 40 dpf. RNA-seq of dissected adult zebrafish bladder revealed enrichment of orthologs to mammalian bladder-specific genes (including uroplakin 3b and aquaporin 3a). RNA-seq in juvenile zebrafish (14-27dpf) revealed increased differential GU expression of smooth muscle and contractile protein genes (e.g., desmin and smooth muscle myosin heavy chain), relatively similar expression of synaptic and neuronal genes (e.g., synaptophysin-like 1, NMDAR, and VGLUT), and the expression of mGluR1-5, alpha- and beta- adrenergic receptors, and purinergic receptors.

CONCLUSIONS: Zebrafish have a bladder with compelling transcriptomic similarities to mammalian counterparts. Before a formal bladder, distal urinary structures undergo morphologic changes that parallel differential increase in contractile protein genes as well as expression of neuronal genes and bladder-relevant pharmacological targets. These findings support the emerging utility of zebrafish to examine the influence of bladder development on its function and disease.

Title: Short terms effects on the stereotyped voiding behavior of female rats induced by the consumption of sweetened water.

Authors: Emely V. Munoz[1,2], Vianca M. Gandara[1], Alvaro Munoz[1].

Affiliations: [1]Northern Region University Center and [2]University Center for Biological and Agricultural Sciences, University of Guadalajara, Mexico.

Introduction/Objectives: Excessive consumption of sugary beverages has been associated with conditions such as obesity, stress, metabolic syndrome, and type 2 diabetes. However, there are few pre-clinical studies that assess the effects on voiding behavior (VB) when providing beverages sweetened with natural-honey or refined-sugar. The aim of this study was to determine the short-term effects of these beverages on VB in young female rats.

Methods: Twelve female rats (Wistar, 220-240 g) were used to assess the VB combined with an absorbent paper evaluation. On the first day, the VB was evaluated with the presence of water; on the second day, a 5% sucrose-sweetened solution was provided (Suc); on the third day, a 5% honey-sweetened solution was provided (Hon), and on the fourth day, plain water was given (Suc-Hon group, N=6). In the second group, the exposure to honey and sucrose was reversed (Hon-Suc group, N=6). After assessing VB (2 h), we determined body weight, blood glucose, fluid intake, post-VB urinary bladder volume (UBV), number of voidings and urine-spot areas. Voids and fluid intake were correlated to determine Pearson coefficients (PC). Data were analyzed utilizing one-way ANOVA and student's t-tests.

Results: No differences were found in weight or glucose levels. In the Suc-Hon group, rats had an increase in the number of voids, especially when drinking Hon, with a larger positive PC ($r=0.1425$ for Suc, and $r=0.6431$ for Hon). In the Hon-Suc group the number of voids were increased on days 2-4, with a negative PC for Suc ($r=0.5249$ for Hon, and $r=-0.2517$ for Suc). In both groups, the intake of sweetened liquids was significantly increased (ANOVA). The post-VB UBV in the Suc-Hon group was significantly higher when compared to the Hon-Suc ($P<0.05$, t-test), while the average for the top 5 urine-spot areas was significantly lower in the Suc-Hon ($p<0.05$, t-test). The total number of voids on the first day were similar in both groups, while they were higher in the Hon-Suc vs Suc-Hon on day 2 ($p=0.05$, t-test), with no differences on the third day. Surprisingly, the total number of voids on the fourth day, when drinking water, were significantly increased in the Hon-Suc group in comparison with the Suc-Hon group ($p<0.05$, t-test).

Conclusions: The current approach is useful to determine short-term effects of beverages on voiding behavior. Overall, our results suggest a preference of rats for honey-sweetened drinks, however, sucrose-sweetened drinks seem to induce an acute but significant increase in the frequency of voidings even when drinking regular water. Additional studies are necessary to determine how the exposure to sweetened beverages, either natural or artificial, could affect the physiology of the urinary tract on the long term.

Funding Source(s): PRO-SNI-2022-23, University of Guadalajara, Mexico.

Testosterone Contributes More to the Development of Diabetic Bladder Dysfunction in Type 1 Diabetic Male Mice Than Hyperglycemia

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Introduction: Diabetic bladder dysfunction (DBD) affects over half of all patients with diabetes. Their symptoms manifest as a diverse range of presentation from overactive bladder (OAB) to underactive bladder (UAB) and the cause of such variable presentation is unknown. Emerging evidence suggests sex hormones influence the development of DBD symptoms. In addition to clinical data, this theory is supported by data from preclinical models such as the Type 1 diabetic Akita mouse in which by 15 weeks of age, females develop OAB while, for unknown reasons, males develop UAB. It has been proposed the more severe hyperglycemia in males is responsible for UAB development rather than the OAB observed in females; however, we hypothesize the high levels of endogenous testosterone in males facilitates the development of diabetic UAB. Here, we test this hypothesis in the Akita mouse model by surgically castrating males before UAB symptoms develop and measuring blood glucose weekly before urodynamic assessment of bladder function at 15 weeks, when UAB is evident in gonadally intact diabetic males.

Methods: Type 1 diabetic Akita males and nondiabetic males were surgically castrated (-T) at 8 weeks of age. Blood glucose was measured weekly from 8 weeks of age to 15 weeks of age in diabetic Akita females (n=10) and four groups of gonadally intact and castrated males: diabetic (n=10), non-diabetic (n=10), diabetic -T (n=15), and nondiabetic -T (n=5). At 15 weeks, awake-restrained cystometry was performed in all four groups of male mice (respective n=9,11,11,3). Voiding volume and voiding frequency were the primary endpoints for urodynamic assessment.

Results: Female diabetic mice exhibit moderate hyperglycemia of 300 mg/dL from weeks 8 to 15 while male diabetics exhibit consistent and severe hyperglycemia of 550 mg/dL. After castrating diabetic males at 8 weeks of age, blood glucose gradually declines ($p<0.05$) to 300 mg/dL by week 15 – identical to female diabetics. Castration does not impact blood glucose of nondiabetic males as both nondiabetic and nondiabetic -T groups maintain blood glucose levels of 150 mg/dL. As a consequence of diabetes, urodynamic assessment reveals diabetic males develop signs of UAB by 15 weeks as indicated by a significant increase in void volume and decrease in voiding frequency ($p<0.05$). Interestingly, given the similar blood glucose levels of female diabetic mice and castrated male diabetic mice, we anticipated diabetic -T mice would develop a similar OAB phenotype as females. However, eliminating the production of testosterone by castrating male diabetic mice prevents the development of DBD altogether. Diabetic -T mice demonstrate near identical voiding volumes and frequencies as nondiabetic male mice, which are both significantly different ($p<0.05$) from gonadally intact diabetic males. Castration has no impact on the voiding volumes and frequencies of nondiabetic males.

Conclusions: Testosterone has a greater impact on the development of DBD in diabetic males than the severity of hyperglycemia. Hormonal therapies which eliminate testosterone production may be a much needed therapeutic option to prevent UAB in patients with diabetes – a condition for which there is currently no pharmacologic treatment option available. Given the efficacy and common use of FDA-approved hormonal therapies for other benign urological disorders, this presents a novel therapeutic target with potentially swift translational value.

Funding: NIH-K12 DK100024; NIH-RO1 DK117890

Higher Levels of p-MLKL Suggest that Necroptosis Associated with Bladder Nerves is a Possible Driver of Diabetic Bladder Dysfunction

Authors: Janhvi Parsai, Michael R. Odom, Francis M. Hughes, Jr., A. Ata Gultekin, Huixia Jin, J. Todd Purves

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Introduction/Objectives: Diabetes is a growing problem globally with >643 million people predicted to be affected by 2030. ≈80-93% of patients experience bladder dysfunction with limited treatments available. Diabetic bladder dysfunction has long been linked to diabetic neuropathy which causes the denervation of afferent and efferent nerves in the bladder. New research shows that necroptosis, a form of programmed cell death, of Schwann cells may drive diabetic neuropathy by slowing neuronal signaling and/or causing axonal retraction. To assess a possible role of necroptosis-driven diabetic neuropathy in bladder dysfunction, we characterized the expression of MLKL, a critical protein in the necroptosis signaling pathway, in male wildtype and Akita Type 1 diabetic mice. Diabetic male akita mice exhibit underactive bladder pathology by 15 weeks of life which we have shown is a result of a decrease in acetylcholine signaling at nerve terminals. In this study we aim to explore the possibility that this decrease is a result of an increase in necroptosis associated with bladder nerves.

Methods: Two groups of male mice (wildtype- C57BL/6J and diabetic- C57BL/6-*Ins2*^{Akita}*/J)* were evaluated at 15 weeks of age. Mice bladder was fixed, sectioned (5 um) and stained with antibodies for phosphorylated Mixed Lineage Kinase-Domain-Like Pseudokinase (p-MLKL), a biomarker for active necroptosis. P-MLKL was imaged and quantified. In addition, proteins were isolated from detrusor muscle and assessed by western blotting for total MLKL. Additional sections were co-stained for total MLKL and myelin.

Results: At 15 weeks of age, diabetic male Akita mice demonstrated an increase in the density of neuron bundles expressing necroptosis-inducing p-MLKL compared to wildtype (Fig. 1A). Western blot data also shows an increase in overall MLKL expression in diabetic mice (Figure 1-B). Wildtype bladders stained for myelin fibers (a marker of Schwann cells) with a small portion co-staining with MLKL. Co-staining was dramatically increased in the diabetic bladders. In summary, diabetic mice had a higher density of p-MLKL^{+} nerve bundles, an increased in overall MLKL expression and increased colocalization of MLKL with Schwann cells compared to wildtype mice.

Conclusions: The higher levels of necroptosis-inducing p-MLKL in diabetic Akita male mice, suggests that necroptosis may be responsible for the decreased neuronal signaling exhibited in these animals. Further research aims to assess if necroptosis inhibition is a possible therapeutic target for diabetic bladder dysfunction and other neuropathy-related complications of diabetes.

Funding Source(s): NIDDK (R01DK117890)

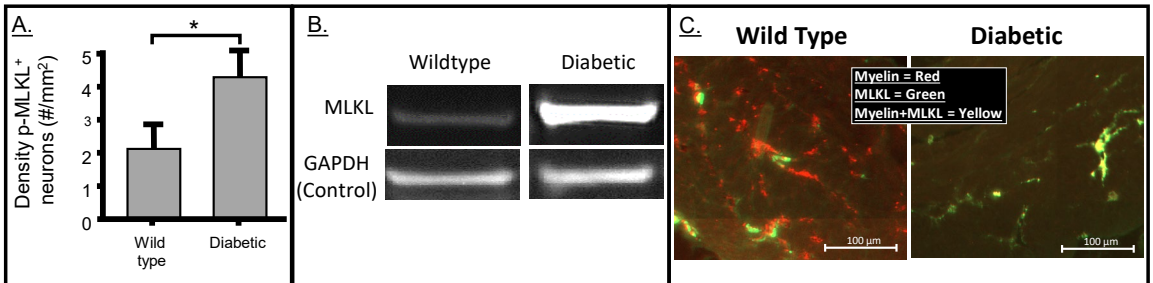

Figure 1: A) Density of p-MLKL^{+} neuronal bundles (n=4/4) and B) Total MLKL and GAPDH expression in wildtype and diabetic Akita mice

Title: Microglia Mediate Symptoms of Chronic Pelvic Pain

Authors: Rahman-Enyart, A., Yaggie, R.E., Winter, D.A., Schaeffer, A.J., and Klumpp, D.J.

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Introduction/Objectives: Patients with urologic chronic pelvic pain syndrome (UCPPS) suffer chronic pelvic pain and often also voiding and/or cognitive dysfunctions. Neuro-immune mechanisms underlying chronic pelvic pain are not fully understood, but microglia are increasingly understood to mediate and modulate normal neuronal functions and dysfunction. Here, we examine the role of microglia in a clinically relevant UCPPS model of interstitial cystitis/bladder pain syndrome (IC/BPS).

Methods: Microglia were examined in AOAH-deficient mice that spontaneously exhibit chronic pelvic pain symptoms and cognitive dysfunction. Microglia were phenotyped by quantitative morphometry, immunofluorescence, and RNA-seq. To functionally evaluate microglia, mice were treated by oral gavage of the CSF-R1 antagonist PLX5622 that transiently ablates microglia. Mice were evaluated for pelvic pain and associated cognitive deficits by mechanical allodynia and by novel-object recognition (NORT) and object location (OLT) tests for learning and memory.

Results: Morphometry revealed altered morphology of AOAH-deficient microglia in brain regions associated with pain (prefrontal cortex) and memory (hippocampus). PLX5622 treatment significantly reduced pelvic allodynia and deficits in learning and memory. Microbiota manipulation similarly reduced pain and cognitive dysfunction and reduced microglial activation. RNA-seq analyses of purified microglia revealed a neuro-inflammatory phenotype in CD11b+ AOAH-deficient microglia characterized by induction of known microglial inflammatory markers (e.g., Irf7, Tspo, Ccr2, IL1b) and consistent with TLR4 activation.

Conclusions: Microglia exhibit altered morphologies in distinct brain regions in a model of IC/BPS consistent with activation and exhibit a neuro-inflammatory transcriptome phenotype characteristic of TLR4 signaling. Microglial also mediate pain and cognitive dysfunction and respond to manipulation of microbiota, suggesting that microglia are transducers of dysbiosis that represent therapeutic targets for symptoms of UCPPS.

Funding Source(s): U01 DK082342 and R01 DK134817

Title: Sub-chronic Neuromodulation of Bulbospongeosus Nerve Improves deficits associated with Stress Urinary Incontinence in Mature Multiparous Female Rabbits

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Introduction/Objectives: Mature multiparous (MM) rabbits have reduced urethral pressure and lower voided volume congruent with SUI symptoms in women. We previously demonstrated that acute electrical stimulation (ES) of the bulbospongiosus nerve (BsN) improve both metrics. Hypothesis: Daily ES for 30 days of the BsN will provide further improvements of SUI-related functional deficits in this animal model.

Methods: MM female New Zealand White rabbits were randomly divided into two groups: 1) Experimental treatment and 2) sham control group (n=8 each). During a 2-week baseline period we use a void spot assay to determine involuntary leaking (as percentage of leak events per day). The animals underwent a unilateral wireless electrode implantation onto the BsN, followed by a 2-week recovery and 4-week treatment period. Efficacy was also evaluated by measuring daily micturition mass, voiding frequency, and daily water intake. A cystometry was done at the end of the study. Statistical analysis was conducted using two-tailed Welch's t-test for normal data sets and two-tailed Mann-Whitney test for non-normal data sets when comparing between groups, and paired t-test when comparing within group (baseline vs treatment).

Results: 4-week stimulation of the BsN in MM rabbits induced on average a 40.0 ± 4.46% decrease in the number of voiding events, from 69.4 ± 8.15% during baseline to 29.4 ± 5.24% average leak frequency during the treatment period ($p<0.0001$). This is a significant improvement compared to the negative control group, which showed no significant changes in leak event frequency between baseline and treatment period (-1.83 ± 2.96%). This improvement in the experimental group was congruent with the observed significant increase between baseline and treatment in the daily micturition mass (33.1 ± 8.04 g; $p<0.01$) and average micturition mass per void (14.3 ± 2.36, $p<0.001$) compared to sham controls (-14.3 ± 13.5 g; 5.13 ± 0.588 g, respectively). No difference were observed in voiding frequency and daily water intake between groups. Cystometry analysis results also showed experimental animals having significantly improved functional parameters compared to negative control animals, including higher average vesical pressures (P_{ves}) during storage phase (4.13 ± 0.46 mmHg vs 1.02 ± 0.14 mmHg, $p<0.005$), higher maximum P_{ves} during storage (9.723 ± 1.002 mmHg vs 5.127 ± 0.591 mmHg, $p<0.005$), higher leak point P_{ves} (8.023 ± 0.8992 mmHg vs 4.364 ± 0.6343 mmHg, $p<0.05$), and higher voiding efficiency (8.441 ± 1.382% vs 2.300 ± 0.3618%, $p<0.005$).

Conclusions: Sub-chronic neuromodulation treatment of BsN is improving SUI-related deficits in behavioral and functional evaluations in the female rabbit model.

Funding Source(s): NIH 1 R01 DK120307-01

Title: Use of anti-fibrotics in the treatment of Lower Urinary Tract Dysfunction in mice

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Introduction/Objectives: Lower urinary tract dysfunction (LUTD) is commonly found in aged men and women. Benign prostatic hyperplasia (BPH) affects nearly all aged men. BPH is commonly accompanied by lower urinary tract symptoms (LUTS) which consists of increased urinary frequency, urgency, and nocturia, as well as decreased urinary flow. World-wide, the incidence of BPH is on the rise and the rate and incidence of BPH is higher than other urological diseases including prostate and bladder cancer. Common processes of BPH/LUTS include hyperplasia and smooth muscle contractility which are therapeutically targeted with 5-alpha reductase inhibitors and alpha-blockers (respectively). Recently, our group and others have implicated prostatic fibrosis as a mediator of BPH/LUTS and its treatment failure. Currently there are no therapies targeting fibrosis in the treatment of BPH/LUTS. We hypothesize that treatment of age associated prostate fibrosis and LUTS with the anti-fibrotics, such as pirfenidone, would decrease prostate fibrosis and improve urinary frequency in an aging mouse model of BPH/LUTS.

Methods: We utilized 24 month old male C57Bl6/J mice exhibiting increased urinary frequency (relative to younger male mice) to determine the effects of pirfenidone on prostate fibrosis and LUTD. We established baseline urinary frequency in control young (2-month old) and aged (24-month old) male mice using void spot assays (VSA) and they were analyzed using Void Whizzard software. Mice were subsequently assessed on a weekly basis for up to 4 weeks. Aged mice were treated per os (i.e. fed) 300mg/kg/day pirfenidone mixed in peanut butter for 4 weeks. Changes in prostate fibrosis were assessed using picrosirius red (polarized light) and histological analyses and were analyzed.

Results: Aged 24 month old mice with LUTD treated with pirfenidone, showed a decrease in collagen content within the anterior prostate compared to untreated controls. Furthermore, voiding frequency was significantly ($P<0.05$) decreased in voiding frequency by week 1, which remained through 4 weeks. The urinary frequency was similar to those of young mice.

Conclusions: Our study demonstrates that prostate fibrosis, associated with LUTD in mice and men, is therapeutically targetable with FDA approved anti-fibrotic agents such as pirfenidone. Reduction of prostate fibrosis by using anti-fibrotic agents appears to improve urinary frequency and may be suitable for treatment in men suffering from BPH/LUTS or possibly other LUTS independent of prostate function.

Funding Source: NIH U54DK104310

Title: Local tissue response to a CXCL12 chemokine therapy for fecal incontinence in a rabbit model.

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Introduction/Objectives: Fecal incontinence is a common complication of childbirth. Trauma to the anal sphincter results in a combination of muscle and nerve damage that leads to fibrosis and loss of anal sphincter muscle tone. CXCL12 is a chemokine that is known to recruit stem/progenitor cells to sites of injury. We hypothesized that a CXCL12 chemokine therapy would 1) reduce sphincter fibrosis, 2) restore sphincter muscle content, vascularization, and innervation, and 3) recruit stem cells to the injury site in a rabbit model of fecal incontinence.

Methods: Adult female rabbits were assigned to 3 groups Uninjured/No treatment, Injured/Treated, and Injured/No Treatment (n=4 each). Injured groups were anesthetized and a 5x10mm section of external anal sphincter was removed at the 9 o'clock position. Treated rabbits were injected with 200mg of human recombinant CXCL12 six weeks after injury. Humane euthanasia and necropsy was performed six weeks post treatment. The external anal sphincter was removed, fixed, embedded in paraffin, sectioned, and mounted to slides for histologic analysis. Collagen was assessed using Picrosirus red, muscle with Fast Myosin Skeletal Heavy Chain immunohistochemistry (IHC; RRID:AB_2297993), innervation with PGP9.5 IHC (RRID:AB_306343), and vascularization with CD31 IHC (RRID:AB_2942096). We are currently troubleshooting IHC for stem/progenitor cells in rabbit tissue. Comparisons between groups were made with a regular ANOVA for normally distributed data and with a Kruskal-Wallis test for data that could not be transformed to a normal distribution. Either Tukey's post test or Dunn's post test were performed if initial comparison was significant ($p<0.05$).

Results: A single 10X image of Fast Myosin Skeletal Heavy Chain IHC stain was taken at the 9 o'clock position of the anal sphincter and study group was masked during image assessment. Images were assessed for presence of distinct muscle layering and organization of fibers within the layer. The Injured/No Treatment group had indistinct or absent muscle layering and the circumferential and inner longitudinal layers were disorganized compared to controls ($p<0.05$). A single 10X image of Picrosirius red stain was taken at the 9 o'clock position of the anal sphincter and collagen fiber characteristics assessed using CT Fire. Collagen fiber density was increased in the Injured/No treatment group compared to the other groups ($p<0.05$). Staining for PGP9.5 and CD31 is complete and analysis is in progress.

Conclusions: Local injection of CXCL12 reduces post-injury fibrosis and results in restoration of muscle content. Further analysis and studies are needed for this promising new treatment for post-parturient anal incontinence.

Funding Source(s): Study funded by a grant from The Veteran's Association Merit Award. Ruetten supported by NIH T32 OD010957.

Title: Leucine Rich Repeat Containing 8 channels modulate superoxide anion production and hypercontractility of pudendal arteries and corpus cavernosum in diabetic vasculogenic erectile dysfunction

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Introduction/Objectives: Erectile dysfunction (ED) is associated with hypercontractility of the corpus cavernosum (CC) and pudendal arteries (PA) in consequence of increased reactive oxygen species (ROS), which favors the flaccid state of the penis. Leucine Rich Repeat Containing 8 (LRRC8A) Volume Regulated Anion Channels (VRACs) modulates the influx of extracellular superoxide anion ($O_2^{\bullet-}$) produced by Nox1, contributing to the creation of an oxidized domain. Downregulation of LRRC8A is associated with inhibition of Nox1 and decreased production of $O_2^{\bullet-}$ and we have demonstrated improved relaxation and reduced contraction of the pudendal artery in LRRC8A knockout mice. We hypothesized that downregulation of Nox1 or LRRC8A decreases contractile response to phenylephrine (PE)-induced and electrical field stimulation (EFS) in the PA and CC of diabetic mice.

Methods: the CC and PA of diabetic (*dbdb*) mice were mounted in myographs, and contraction elicited by PE was performed in the presence of apocynin (non-selective NOX inhibitor), GKT137831 (NOX1/4 inhibitor) and montelukast (LRRC8A inhibitor). In LRRC8A KO *dbdb* mice, contractions to PE and EFS were obtained in the absence or in the presence of GKT137831.

Results: In the PA, the potency of PE was increased in *dbdb* mice, and apocynin (1 mM) restored the contraction to normal levels. In the CC, the presence of 3 mM of apocynin markedly diminished the contractile response in both control and *dbdb* mice. The LRRC8A inhibitor, montelukast, significantly reduced PE-induced contraction of the CC reaching maximal contraction of 69 $\pm$ 7% and 65 $\pm$ 10% in the control and *dbdb* group, respectively, compared to the contraction in the absence of the inhibitor. In the presence of the Nox1/Nox4 inhibitor, GKT137831, the contraction elicited by PE was reduced only in the diabetic group (Emax: 68 $\pm$ 9%), whereas in the control group this reduction was not significant (Emax: 82 $\pm$ 14%). In the LRRC8A KO *dbdb* mice, the contraction to PE in the PA was reduced by 30% compared to the *dbdb*. In the CC, contraction to EFS was similar in both groups, however, GKT137831 reduced the response with lesser magnitude of inhibition in the LRRC8A KO *dbdb* mice (35% of inhibition) compared to *dbdb* mice (50% of inhibition), suggesting that the participation of $O_2^{\bullet-}$ in the contractile response may be reduced by the lack of functional LRRC8A.

Conclusions: LRRC8A modulates the contractile response by managing the influx of ROS in smooth muscle cells, which plays a role in the development of ED. Inhibition of LRRC8A may be a therapeutic target to prevent hypercontractility of arteries and CC in conditions where there are marked increases in ROS production, such as diabetes mellitus.

Funding Source(s): NIH – R01 132948; Institute on Cardiovascular Disease Research, University of South Carolina

Title: Inhibition of cyclophosphamide-induced bladder inflammation and hyperactivity through knockdown of the endogenous cholinergic toxin-like signaling peptide SLURP-2.

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Introduction/Objectives: Previously published research has shown that urothelial nicotinic acetylcholine receptors (nAChRs) can modulate bladder reflexes by controlling urothelial ATP release, with α7 nAChRs being inhibitory and α3 nAChRs being excitatory. This biphasic control of bladder function makes urothelial nAChRs attractive targets for the treatment of bladder pathology. Recently, a family of signaling peptides with homology to snake venoms has been shown to modulate nAChR signaling, raising the possibility that these peptides, known as Secreted Ly-6/uPAR-Related Proteins (SLURPs) could play a role in modulating urothelial nAChR signaling. Additionally, there is some evidence that mutations in these proteins can lead to epithelial pathology. The goal of the current project, then, is to examine the effect one of these peptides, SLURP-2, has on nAChR-mediated urothelial ATP release and if SLURP-2 could play a role in bladder pathology.

Methods: ATP release was measured in TRT-HU1 cells grown in 96-well plates using a luciferin-luciferase assay kit. PCR was performed on RNA extracted from rat urothelial tissue surgically separated from the detrusor muscle or from cultured TRT-HU1 cells using commercially available reverse transcriptase and PCR kits and primers for SLURP-2 designed in house. To knockdown SLURP-2 *in vivo*, three separate siRNAs against SLURP-2 were encapsulated in liposomes and instilled into the urinary bladder of Sprague Dawley rats (200-225g) using a transurethral catheter. The procedure was repeated 3 days later, and then, on the 4th day, bladder inflammation was induced using an intraperitoneal injection of cyclophosphamide (CYP, 150mg/kg) 24 hours before urethane anesthetized bladder cystometry was performed. Bladder edema was measured using plasma extravasation of Evans Blue dye after completion of the CMG.

Results: PCR analysis confirmed that SLURP-2 was expressed in both rat urothelial tissue and the human urothelial cell line TRT-HU1. Stimulation of TRT-HU1 cells with recombinant SLURP-2 (1μg/ml) greatly increased ATP release in response to the α3 nAChR cytisine (100uM) 3.5-fold without stimulating ATP release by itself. SLURP-2 application also prevented the inhibition of basal ATP release induced by the α7 nAChR agonist PNU282987 (10μM). qPCR analysis showed increased SLURP-2 mRNA in the urothelium of both 8-day CYP treated rats (3-fold) and 10-day water avoidance stress (WAS) (2-fold) rats. siRNA knockdown of urothelial SLURP-2 in the rat fully reversed the increase in voiding frequency and increase in bladder plasma extravasation following CYP administration.

Conclusions: Our results suggest that SLURP-2 plays a significant role in CYP-induced bladder hyperactivity and inflammation, possibly by modulating nAChR-mediated urothelial ATP release.

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Title: A wide range of PDE5 inhibitors act synergistically with a topical nitric oxide delivery system to elicit an erectile response in an animal model of radical prostatectomy.

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Introduction/Objectives: Our recently published studies on a rat model of radical prostatectomy (RP) demonstrate that a topically applied nitric oxide microparticle delivery formulation (NO-MP) elicits a stronger erectile response ($P<0.05$ for both time-to-first-erection and erections per time period) when applied in combination with orally administered sildenafil (a PDE5 inhibitor, PDE5i) than when NO-MP is used alone. However, there are several FDA-approved PDE5i treatments for ED; these treatments act through common mechanisms (i.e. inhibiting PDE5), but exhibit several characteristics that may impact the clinical efficacy and commercial impact of combination therapy. Therefore, the goal of the present studies was to determine if other commercially FDA-approved PDE5i act cooperatively when used in combination with NO-MP to elicit an erectile response.

Methods: A rat model of RP was used whereby male Sprague-Dawley rats underwent bilateral cavernous nerve transection. One-week later, the rats were administered via oral gavage sildenafil, tadalafil, vardenafil, and avanafil at 0.05, 0.005, 0.01 and 0.1 mg mg/kg, respectively (approximating $1/10^{th}$ the lowest available human-equivalent dose for each drug); they were then anaesthetized and canulae inserted to determine the intracorporal pressure (ICP) and systemic blood pressure (BP). After a stable trace was recorded, topical application of 100 mg of NO-MP (or empty-MP for the control group) was investigated for an effect on the erectile response.

Results: In the ED model of RP, when oral sildenafil was administered in combination with topical application of NO-MP to the penile shaft, there was a more rapid onset of the first erectile response (11 compared to 22 min; t-test, p value = 0.041) and an increase in spontaneous erections per hour (4.6 compared to 2 erections per hour, t-test; p value = 0.043), compared to NO-MP applied alone. However, the maximal ICP/BP recorded was not significantly different between the combination therapy and monotherapy. Results from combination therapy using other PDE5i (avanafil, vardenafil, and tadalafil) were equally effective (i.e., not significantly different) in improving time-to-first-erection and increasing the number of spontaneous erections per hour; similarly, combination therapy with these other PDE5i did not affect the maximal ICP/BP versus NO-MP as monotherapy.

Conclusions: Our results suggest that oral administration of any one of four PDE5 inhibitors, when used in combination with topically applied NO-MP, could be a potential novel therapeutic approach to treat men with ED resulting from RP.

Funding Source(s): NIH/NIDDK R44 DK121587, R41 DK121587.

Title: The Role of Leucine Rich Repeat Containing 8A Channels in Female Sexual Dysfunction and Vaginal Smooth Muscle

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Introduction/Objectives: Vaginal smooth muscle (VaSM) is critical to sexual function, desire, and arousal. In cases of sexual dysfunction, many patients may experience lessened or inadequate lubrication or experience pain with intercourse. A loss of function in the vascular, vaginal smooth muscle, and endothelium function could contribute to many symptoms present in individuals with female sexual dysfunction (FSD). In women who are diabetic, there is a correlation with increased risk of vaginal fibrosis, which can lead to other pelvic conditions such as pelvic organ prolapse. This study aims to bridge the gap to treat diabetic individuals' sexual dysfunction and increase blood flow to the VaSM and clitoral tissue to improve overall pelvic organ health. We hypothesize that the inhibition of leucine rich repeat containing 8A (LRRC8A) volume regulated anion channels (VRACs) will decrease the contraction in vaginal smooth muscle of lean, diabetic (db/db), db/db vascular smooth muscle LRRC8A knockout mice and improve the relaxation of the tissues by lowering the flux of extracellular superoxide into the vascular and vaginal smooth muscle.

Methods: Female lean and db/db (12 wks), and db/db vascular smooth muscle (VSM) LRRC8A knockout mice (26-34 weeks old) underwent distal vaginal dissection. The VaSM was then mounted on a strip myograph for measurement of isometric force development. Contraction to carbachol (CCh) was performed in the absence and presence of the LRRC8A inhibitor, montelukast (1uM) (MONT) and volume regulated anion channel inhibitor, carbenoxolone (CBX), after a 30-minute incubation and relaxation to isoproterenol (ISO) or carbenoxolone (CBX) was performed.

Results: The weight/length of VaSM (mg/mm) was used to normalize the maximum contraction (mN). Measures of weight/length in db/db mice VaSM tissue were less than that in lean mice ($p=0.0112$). Relaxation is significantly different in Emax of control db/db mice to (ISO 7.004 ± 1.727 vs. CBX 2.787 ± 1.064, $p = 0.0315$) and in Emax relaxation of db/db VSM-LRRC8A knockout mice to (ISO 7.993 ± .8538 vs. CBX 3.251 ± .9659, $p = 0.0353$).

Conclusions: Treatment with an inhibitor of LRRC8A offers a novel approach to treat FSD and should be further researched to observe its overall impact. LRRC8A may play an important physiological role in the maintenance of the contractile response by managing the flux of $O^{\bullet -}$ in the VaSM, making it an important potential target for individuals suffering from sexual dysfunction.

Funding Source(s): University of South Carolina Start-up Fund, NIH- R01 80005644, SMSNA Scholars in Sexuality Research Grant.

Title: Innervation of the ovine pelvic floor muscles by levator ani and perineal nerves

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Introduction/Objectives: Stress Urinary Incontinence (SUI) affects approximately 35% of the adult female population in the US and is characterized by uncontrolled urine leakage during increased intra-abdominal pressure. It is associated with pelvic floor neuro-muscular injuries during childbirth, and exacerbated by aging or menopause. Pelvic floor muscle training (PFMT) is an effective therapy but is limited by low patient compliance; surgical sling implants are effective but suffer from deleterious adverse effects. Pelvic floor neuromodulation (PFNM) has been proposed as a novel treatment for SUI and preliminary data has been reported in the rabbit model. However, for clinical translatability a larger animal model with similar pelvic and reproductive anatomy as women is needed. Sheep are a suitable preclinical model for overactive bladder (OAB) and neuromodulation testing. However, this animal model has not been tested for SUI and there is a lack of anatomical and functional studies on the pelvic floor innervation in these animals. The goal of this study is to map and characterize the anatomy of ovine pelvic floor innervation.

Methods: Four adult female yearling sheep were used to determine the gross anatomy of the pelvic neuromuscular system. The pudendal nerve was visualized using a high-resolution ultrasound system. Post-euthanasia, the pelvic area was dissected, and the innervation pattern and morphometry of the perineal and levator ani nerves was described.

Results: The pelvic floor muscles are innervated by pudendal-perineal branches and levator ani nerve (LaN) branches. The pudendal nerve (1.28±0.27 mm OD) arose from spinal levels S2-S4. After passing through the Alcock canal, it branched into medial perineal (~0.5 mm OD) and lateral (~0.8 mm OD) divisions, where the lateral nerve superficially innervated the skin over the perineal region. The medial perineal nerve branched into the dorsal clitoralis nerve (DcN) (1.04±0.34 mm OD) and the bulbospongiosus nerve (BsN) (0.74±0.20 mm OD), innervating the clitoris and bulbospongiosus muscle (BsM) respectively. LaN (1.25±0.25 mm OD) arose from spinal levels S4-S5 and innervated the levator ani muscle (LAM) prior to innervating the perineal musculature surrounding the clitoris, vagina and rectum. The BsM is a paired muscle around the urethra and vagina and was innervated by the BsN. The pubococcygeus muscle (PcM) was innervated by LaN branches.

Conclusions: This study described the anatomical arrangement of the ovine levator ani and pelvic floor nerves, and quantitatively defined the average nerve diameters. Further studies are needed to confirm the functional innervation of the ovine pelvic floor as well as histologically characterize nerve microanatomy. These will serve as a basis for future neuromodulation testing.

Funding Source(s): This study was conducted with the support of the Institute for Translational Sciences at the University of Texas Medical Branch, supported in part by a Clinical and Translational Science Award (2UL1TR001439-06) from the National Center for Advancing Translational Sciences.

Top 5- SMCU ABSTRACTS

Analysis of molecular iodine effects in TRAMP mice prostate tumors

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Introduction/objective: Advanced prostate cancer therapy includes androgen deprivation; however, epithelial cancer cells can develop androgen-independence in the long-term and acquire an aggressive neuroendocrine (NE)-like phenotype. In prostate cancer cell lines, molecular iodine (I_2) potentiates the antiproliferative actions of androgen receptor (AR) antagonists, and in breast cancer, it promotes the formation of an iodolipid (halogenation reaction) capable of activating PPARG receptors. I_2 and PPARG induce antitumor effects in breast cancer, whereas in prostate cancer, PPARG is considered to promote tumor progression. Thus, the aim of this study was to analyze the effects of I_2 on the acquisition of an NE-like phenotype induced by castration in prostates of TRAMP mice (transgenic adenocarcinoma mouse prostate). To analyze the effects of I_2 in the histopathology of tumoral prostate (TRAMP) in non-castrated and castrated mice.

Methods: Eighteen weeks-old TRAMP mice were subjected to sham surgery or castration, and were provided or not with I_2 (0.025%, drinking water) for four weeks. Prostates were processed for histopathological analysis (H&E and Masson's trichrome) and immunohistochemistry (AR, synaptophysin [SYP, NE protein], and PPARG). Scoring systems were employed to grade the severity of high proliferative intraepithelial lesions (HPIN), and the extent of acini stomal hypertrophy (desmoplasia).

Results: In sham TRAMP mice, I_2 increased the number of mice with high-grade epithelial lesions, selectively reduced the immunodetection of AR protein within the nucleus and had not any effect in PPARG. As expected, the androgen deficiency in castrated TRAMP mice reduced the proportion of mice with high-grade epithelial lesions, induced epithelial atrophy, and desmoplasia. In addition, castration decreased the total AR levels, as well as PPARG nuclear levels, yet contrary to what was expected, the number of NE (SYP-positive) cells did not change in this condition. The addition of I_2 to castrated TRAMP mice did not modify the score of epithelial lesions, but exacerbated the desmoplasia, and increased the presence of NE cells within prostate acini.

Conclusions: Our results suggest that I_2 actions depend on both the cell type compartment, and the androgenic status; being the epithelial compartment more sensitive in normal androgenic conditions, and the stromal compartment more sensitive under androgen deficiency. Castration alone does not seem to mediate the acquisition of a NE-like phenotype, but a complex molecular interaction between castration and I_2 was evidenced. Additional studies are required to understand the NE mechanisms of I_2 in carcinogenesis.

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The antioxidant effect of epigallocatechin-3-gallate on testicular function of puberty and adulthood rat induced by exposure to cadmium.

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Introduction/objective: Exposure to cadmium (Cd) in environmental and occupational environments is a major public health problem. This exposure has been associated with the production of free radicals and reactive oxygen species. The main exposure to Cd is through contaminated food and cigarette smoke, causing severe damage to various organs such as the male reproductive system, with the testicle being the main organ affected. In the testicle, the synthesis of testosterone (T) and production of spermatozoa takes place; however, the exposure and accumulation of Cd in tis organ can affect the antioxidant enzymatic mechanisms that protect these processes of male fertility in this sense, it has been proposed that the imbalance in oxidative stress is involved in alterations of testicular functionality. Therefore, it is important to study the effect of Cd exposure on the testis and the relationship with the activity of antioxidant enzymes, the evaluation of damage due to oxidative stress using Malondialdehyde (MDA), which is the final product of lipoperoxidation. In order to counteract all these damages, studies have been carried out on supplementary antioxidants such as vitamins and catechins, among others. Green tea has been studied, which contains antioxidants such as catechins (Wang, et al., 2020). Among these compounds it contains Epigallocatechin-3-gallate (EGCG), which is an antioxidant that acts as a free radical scavenger and activates antioxidant enzymes.

Methods: Two experimental groups were formed, treated from days 21 to 49 of life, each group (1 and 2) were made up of a subgroup (A) which was a control administered with saline solution and another subgroup (B) treated with 1 mg/Kg/day of CdCl2, another third subgroup (C) treated with 1mg/Kg of CdCl2/10 mg/Kg/day of EGCG intraperitoneally (IP), euthanasia was performed to the first group at 49 days of age; group 2 was euthanized at 90 days of age. The testes and serum were obtained from both groups, with which tests carried out for histological analysis of semi-thin sections, quantification of MDA and T concentrations.

Results: It was observed that Cd increased the MDA quantification figures in both groups (49 and 90 days of life), as well as a decrease in concentrations of T, in the histological analysis we observed various alterations in the testicular epithelium; the groups administered with Cd/EGCG showed a decrease in the quantification of MDA. Regarding the concentration of T, an increase was observed; in histology, we made possible the recovery of the sleeping epithelium in both groups of 49 and 90 of life.

Conclusion: Our results suggest that Cd administered at an early age has a direct impact on testicular function and remains during adulthood, but when EGCG is administered, a protective effect is observed, restoring T concentrations and protecting the testicle against oxidative damage that is induced by Cd.

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Zinc and its role on testicular and epididymal antioxidant protection in rats exposed to cadmium.

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Introduction/objectives: Cadmium (Cd) is a heavy metal that affects male fertility by causing harmful effects on the testicle and epididymis, which is why the use of supplements that are capable of preventing or reducing the damage caused by Cd have been considered, among this zinc, which is an essential trace element for livings; its biological functions include benefical effects on the reproductive system, testosterone synthesis, spermatogenesis and sperm maturation. **Objectives:** Analyze the effects of Zn on the histology and antioxidant activity of the testis and epididymis of the rat with exposure to Cd.

Methods: Four groups of one-day-old male Wistar rats were required.

1) Saline group (50ul)
2) Cd (0.5 mg/kg body weight (b.w.) of CdCl2
3) Zn (1mg/kg b.w. of ZnCl2)
4) Zn + Cd, all administered intraperitoneally.

Zn was administered from 1 to 56 days and Cd from 35 to 56 days. Euthanasia was performed at 90 days of age, blood was recovered to determine T concentrations, testes and epididymies were obtained from histological processing, análisis of antioxidant activity and evaluation of sperm parameters.

Results: Cd group presented a decrease in T concentrations, histological damage at the testicle and epididymis, as well as altered antioxidant activity. The Zn + Cd group maintained T concentrations, there was no alteration in the antioxidant activity and histology of testicular and epididymal epithelium, nor even in the sperm parameters. One-way ANOVA´s followed by Tukey were performed, a significance of $p < 0.05$ was considered.

Conclusions: Pretreatment with Zn protects and reduces damage to the seminiferous and epididymal epithelium, its antioxidant activity and sperm parameters in rats exposed to Cd.

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Therapeutic effects of iodine in patients with benign prostatic hyperplasia medicated or not with Tamsulosin.

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Introduction/Objectives: Benign prostatic hyperplasia (BPH) is a chronic pathology, with a high incidence in men over 50 years. When there is not risk of obstruction, the main therapeutic options include watchful waiting and pharmacological treatment with α1 adrenergic antagonists and 5-alpha-reductase among others. Preclinical studies show that molecular iodine (I_2) prevents hyperplasia, oxidative stress, and prostate inflammation. The purpose of this work was to analyze potential therapeutic effects if I_2 in BPH subjects treated or not with Tamsulosin (Tams, α1 adrenergic antagonist).

Methods: Study conducted in collaboration with the Hospital General de Querétaro. Protocol approved by Bioethics and Research Committees (555/INB-UNAM 29-04-10/CEI-SESEQ; 001/INB-UNAM 25-05-10/CB-SESEQ; 015 INB-UNAM). 83 patients with HBP diagnosis were recruited to evaluate the effects of the I_2 oral supply (0.05%), in the clinical symptoms (IPSS), prostate volume (PV), post-micturition residual (PVR) urine, bladder wall thickness (BWT), prostate antigen levels (PSA) and thyroid profile. Groups: placebo, I_2, placebo + Tams, I_2 + Tams. Patients with Tams had at least one year of treatment. I_2 was administered for 8 months and a subgroup of patients (I_2 + Tams) were followed up for 4 years. The results for each parameter were analyzed using a nonparametric-paired T (baseline vs 8monts) or nonparametric repeated measures ANOVA.

Results: All treatments (including placebos) reduced the score of IPSS and BWT. I_2 alone decreased PSA levels and PV in those subjects with PSA levels greater than 4 ng/ml at the beginning of the study and PV greater than 40 cc. Unexpectedly, the combination of placebo + Tams reduced PV. The I_2 + Tams combination did not reduced the PV, but reduced PSA and the PVR urine. I_2 transiently increased TSH and reduced T4 levels to subclinical levels in 12% of patients.

Conclusions: This study confirms the well-known placebo effect on clinical symptoms. I_2 alone had a protective effect against prostate growth, and combined with Tams enhanced the urinary voiding. The I_2 supply for long term maintained the prostatic parameters and did nor modify the thyroid function. The adjuvant effects of the I_2 and antiandrogen will be analyzed in subsequent studies.

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Analysis of immune infiltrate, serum cytokine profile, and histology in the denervated prostate.

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Introduction and Objectives: The prostate, an accessory sexual gland in male reproduction, is regulated by both the endocrine system and the autonomic nervous system. Preganglionic fibers from the viscerocutaneous branch of the pelvic nerve and the hypogastric nerve converge in the major pelvic ganglion, from which postganglionic innervation to the prostate arises. Recent studies have shown the presence of inflammatory infiltrates in this gland in response to preganglionic axotomy. Therefore, the fundamental purpose of this research was to characterize the type of infiltrate present in the denervated prostate, the types of cytokines present, and their correlation with prostatic histology.

Methods: For this, male rats weighing 300 grams were subjected to preganglionic axotomy and left for a period of 15 days. The weight of each prostatic lobe was measured, immune populations were characterized through flow cytometry, proinflammatory and anti-inflammatory cytokines were quantified by ELISA, and the prostatic tissue was stained with Hematoxylin-Eosin for histological analysis.

Results: The results showed a decrease in the weight of both prostatic lobes because of preganglionic injury to the pelvic and hypogastric nerves. The presence of CD3+ T lymphocytes, CD8+ cytotoxic lymphocytes, B lymphocytes, and macrophages was identified in the infiltrate, along with an increase in interleukins IL-1β, IL-6, IL-10, and IFN-γ. Additionally, the presence of hyperplasia and metaplasia in response to the axotomy of these nerves was evidenced, and axotomy of the hypogastric nerve also induced an increase in the infiltrate of neutrophils.

Conclusions: Taken together, these results indicate that the loss of nervous control leads to an inflammatory process, along with histological changes and the loss of prostatic cells. This suggests that prostatic lesions are not exclusively the result of hormonal alterations, but that the nervous and immune components also play a crucial role in this process. These findings provide a new perspective for approaching prostatic diseases.

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LATE BREAKING ABSTRACT

Transcriptomic and functional assessment of the developing zebrafish bladder

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BACKGROUND: Our knowledge of development has benefited from direct visualization of early ontogenic events. While great strides in understanding bladder and lower urinary tract development have been made using mammalian models, as *in utero* amniotes, mammals develop internally and thus present issues with visualization. Zebrafish offer unique insights because they are transparent during development, are amenable to a host of genetic tools, and allow for excellent assessment of molecular and morphologic changes that result from pharmacological or toxicant exposure. Based largely on larval studies, zebrafish have long been thought to possess only a common cloaca, without separately terminating urologic, genital, anal structures, and a distinct urinary bladder has been assumed absent in zebrafish. These assumptions limited the utility of zebrafish as a model for urinary tract development. We recently rigorously confirmed the presence of a contractile urinary bladder in adult zebrafish, allowing us to now exploit this finding to characterize morphologic and molecular characteristics of bladder development in zebrafish.

METHODS: We employed the Tg(cdh17:mCherry) zebrafish line, which has a fluorescently labelled urinary system, to characterize morphologic and molecular signatures of distal urinary structures in developing and adult zebrafish. Distal urinary structures were imaged in zebrafish from 7 to 30 days post fertilization (dpf) and analyzed for morphology. Distal urinary structures from larval zebrafish at multiple developmental timepoints were dissected and processed for bulk RNA-seq. Nearby distal gastrointestinal tissue was used for differential expression comparison. Preliminary expression timelines were assessed. Dextran-conjugated Alexa dye was injected into the pericardium to trace distal urinary structures and excretion. We defined the bladder as a discrete distal urinary segment that increases fluorescence with filling and decreases with a urinary stream.

RESULTS: Pericardial injection of fluorescent dye revealed bladder filling and emptying with periodic contractions coordinated with voiding as early as 40 dpf. RNA-seq of dissected adult zebrafish bladder revealed enrichment of orthologs to mammalian bladder-specific genes (including uroplakin 3b and aquaporin 3a). RNA-seq in juvenile zebrafish (14-27dpf) revealed increased differential GU expression of smooth muscle and contractile protein genes (e.g., desmin and smooth muscle myosin heavy chain), relatively similar expression of synaptic and neuronal genes (e.g., synaptophysin-like 1, NMDAR, and VGLUT), and the expression of mGluR1-5, alpha- and beta- adrenergic receptors, and purinergic receptors.

CONCLUSIONS: Zebrafish have a bladder with compelling transcriptomic similarities to mammalian counterparts. Before a formal bladder, distal urinary structures undergo morphologic changes that parallel differential increase in contractile protein genes as well as expression of neuronal genes and bladder-relevant pharmacological targets. These findings support the emerging utility of zebrafish to examine the influence of bladder development on its function and disease.

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