aps® Conference Program and Abstracts

2018 Cardiovascular, Renal and Metabolic Diseases: Sex-Specific Implications for Physiology

Knoxville, Tennessee
September 30–October 3, 2018
the-aps.org/sexgender

#SexGender18
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“APS meetings—including the big EB meeting and other smaller affiliated meetings—are the best way to keep up with what is going on in my field.”

—Eleanor Lederer, APS member
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2018 APS Conference
Cardiovascular, Renal and Metabolic Diseases:
Sex-Specific Implications for Physiology
September 30 – October 3, 2018
Knoxville, TN

APS Council
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Acknowledgements
The Conference Organizers and The American Physiological Society gratefully recognize the generous financial support from the following:

National Institute Of Diabetes and Digestive and Kidney Diseases (NIDDK)
National Heart, Lung, and Blood Institute (NHLBI)
American Heart Association Councils on Kidney in CV Disease, Basic CV Sciences, and Hypertension
Journal of Clinical Science

*Funding for this conference was made possible (in part) by 1R13HL143981-01 from the National Heart, Lung and Blood Institute. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the NIH; nor does mention by trade names, commercial practices, or organizations imply endorsement by the U.S. Government.
LOCATION:
The 2018 APS Cardiovascular, Renal and Metabolic Diseases: Sex-Specific Implication for Physiology Meeting is held at the Crowne Plaza Knoxville, 401 W Summit Hill Drive, Knoxville, Tennessee 37902, Phone: 865-522-2600

MEETING REGISTRATION HOURS:
Sun., September 20 ......................... 3:00 PM – 8:30 PM
Mon. October 1 ......................... 7:00 AM – 6:00 PM
Tue., October 2 ......................... 7:00 AM – 6:00 PM
Wed., October 3 ......................... 7:00 AM – 12:30 PM

STUDENT REGISTRATION:
Any student member or regularly matriculated student working toward a degree in one of the biomedical sciences is eligible to register at the student fee. Nonmember postdoctoral fellows, hospital residents and interns, and laboratory technicians do not qualify as students. Non-member students who register onsite must provide a valid university student ID card. APS student members should present their current APS membership card indicating their student category status.

POSTDOCTORAL REGISTRATION:
Any person who has received a Ph.D. degree in physiology or related field, within five years of the meeting start date, as attested to by the department head is eligible to register at the postdoctoral fee. A statement signed by the department head must accompany the registration form and remittance when registering.

INCLUDED IN YOUR REGISTRATION:
Your registration to this meeting includes entry into all oral and poster scientific sessions, breakfasts, AM and PM breaks, the awards banquet, and a program book. Registration is nontransferable. You must pay the entire fee regardless of the number of sessions/events you attend. Guests of attendees are not permitted in the scientific sessions, opening reception or meeting breaks and social events.

PRESS REGISTRATION:
Press badges will be issued at the Meeting Registration Desk to members of the working press and freelance writers bearing a letter of assignment from an editor. Representatives of allied fields (public relations, public affairs, etc.) must register as nonmembers.

PHOTOGRAPH/VIDEO RECORDING:
Photo or video capture of any scientific presentation in whole or part is expressly prohibited. Recording or taking photography of another person without their explicit permission is prohibited.

Individuals observed photographing or videotaping any presentation, in whole or part, will be asked to leave the meeting immediately, forfeiting the registration fee.

CODE OF CONDUCT:
APS is committed to providing a safe, productive and welcoming environment for all meeting participants and staff. All participants including, but not limited to, attendees, speakers, volunteers, APS staff, hotel staff, service providers and others are expected to abide by the APS Meeting Code of Conduct which maintains that all individuals should: be treated with respect and consideration, valuing a diversity of views and opinions; be considerate, respectful and collaborative; communicate openly and with respect, critiquing ideas rather than individuals; avoid personal attacks; be mindful of your surroundings and fellow participants; and, be respectful of the rules and the meeting venue. Contact the APS staff at the Meeting Registration Desk if you notice a dangerous situation, someone in distress, or in violation of this Code of Conduct.

PROGRAM OBJECTIVE:
The focus of the meeting will be to educate, explore, and expand your understanding of the unique considerations for the study of cardiovascular physiology of males vs. females. The program has been be designed to provide a broad understanding of the impact of biological sex on disease mechanisms and outcomes as well as highlight recent advances in the field.

The meeting will build on the success of previous APS meetings by expanding the clinical and translational component of the meeting and to bring together basic scientists and clinician scientists to discuss sex differences and sex-specific pathologies related to cardiovascular, renal, and metabolic disorders.

Attendees should expect to gain both greater appreciation and understanding of how the unique physiology of the female vs. the male impacts cardiovascular and renal health in the settings of both normal physiology and in cardiovascular, renal, and metabolic disorders.
### APS CONFERENCE
**CARDIOVASCULAR, RENAL AND METABOLIC DISEASES**  
**SEX-SPECIFIC IMPLICATIONS FOR PHYSIOLOGY**  
**SEPTEMBER 30 – OCTOBER 3, 2018**  
**KNOXVILLE, TENNESSEE**

<table>
<thead>
<tr>
<th>TIME</th>
<th>SUNDAY, SEPTEMBER 30</th>
<th>MONDAY, OCTOBER 1</th>
<th>TUESDAY, OCTOBER 2</th>
<th>WEDNESDAY, OCTOBER 3</th>
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<td>7:50AM – 8:00PM</td>
<td>Welcome and Introduction</td>
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<tr>
<td>8:00AM – 9:45AM</td>
<td>SESSION 1: Sex and Gender Differences in Physiology and Function: The Brain and Nervous System</td>
<td>SESSION 4: Physiology and Gender: Obesity and Metabolism</td>
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<td>8:00AM – 10:10AM</td>
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<td>SESSION 7: Impact of Sex on Vascular Function in Cardiovascular and Metabolic Disorders</td>
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<tr>
<td>9:45AM – 11:00AM</td>
<td>Coffee Break and Poster Session 1</td>
<td>Coffee Break and Poster Session 2</td>
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<td>10:40AM – 12:00PM</td>
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<td>SESSION 2: Physiology and Gender: Aging and Senescence</td>
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<td>11:00AM – 12:45PM</td>
<td>SESSION 8: Male-Specific Cardiovascular, Renal and Metabolic Complications</td>
<td>SESSION 5: Sex and Gender Differences in Physiology and Function: The Kidney</td>
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<td>12:00PM – 12:30PM</td>
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<td>Highlights and Closing Remarks</td>
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<td>12:45PM – 3:00PM</td>
<td>Lunch on your own</td>
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<td>1:00PM – 3:00PM</td>
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<td>Lunch on your own</td>
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<td>3:00PM – 3:45PM</td>
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<td>PLENARY LECTURE: National Initiatives in Sex and Gender-Based Medicine (SGBM)</td>
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<tr>
<td>TIME</td>
<td>SUNDAY, SEPTEMBER 30</td>
<td>MONDAY, OCTOBER 1</td>
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<td>3:00PM – 4:45PM</td>
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<td><strong>SESSION 3:</strong> Sex and Gender Differences in Physiology and Function: The Heart</td>
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<td>4:00PM – 6:00PM</td>
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<td><strong>SESSION 6:</strong> Female-Specific Cardiovascular, Renal and Metabolic Complications</td>
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<td>5:00PM – 6:00PM</td>
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<td>Career Development and Trainee Network Session</td>
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<td>3:00PM – 8:30PM</td>
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<tr>
<td>6:30PM – 8:30PM</td>
<td>Opening Reception</td>
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<td>7:00PM – 9:00PM</td>
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<td><strong>BANQUET AND AWARDS CEREMONY</strong></td>
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Thank you for attending!
2018 APS CONFERENCE
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Cardiovascular, Renal and Metabolic Diseases: Sex-Specific Implications for Physiology
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Intersociety Meeting, Comparative Physiology: Complexity and Integration
Society for Integrative and Comparative Biology
Society for Experimental Biology
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Comparative Biochemistry and Physiology
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Journal of Experimental Biology
Loligo Systems
American Journal of Physiology—Regulatory, Integrative and Comparative Physiology

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1.0 Welcome and Introduction from Conference Chairs  
Mon., 7:50-8:00 AM, Summit I

Jennifer Sullivan, Augusta Univ.  
Michael Ryan, Univ. of Mississippi Med. Center

2.0 SESSION 1: SEX AND GENDER DIFFERENCES IN PHYSIOLOGY AND FUNCTION: THE BRAIN AND NERVOUS SYSTEM  
Mon., 8:00-9:45 AM, Summit I

Chairs: Gina Yosten, St. Louis Univ. School of Med.  
Taylor Schlotman, US Army Institute of Surgical Research

8:00 AM 2.1 Adipokines and the Central Regulation of Cardiovascular Function  
Gina Yosten St. Louis Univ. School of Med.

8:25 AM 2.2 Abstract 15: Comparing Time to Presyncope during Simulated Hemorrhage Across Menstrual Cycle  
Taylor Schlotman US Army Institute of Surgical Research

8:35 AM 2.3 Abstract 48: Estrogen Receptor Alpha Contributes to the Inhibitory Action of Central Cytochrome P4501B1 Generated 17β Estradiol Metabolite 2-Methoxyestradiol to Angiotensin II-Induced Hypertension  
Purnima Singh Univ. of Tennessee Health Science Center

8:45 AM 2.4 The Role of Sensitization and Neuroplasticity in Sex Differences in Blood Pressure and Hypertension  
A. Kim Johnson Univ. of Iowa

9:10 AM 2.5 Abstract 89: Central Acetylcholinesterase Inhibitor, Galantamine, Prevents Lipid-Induced Oxidative Stress in African American Women  
Cyndya Shibao Vanderbilt Univ. Med. Center

9:20 AM 2.6 Brain Blood Flow Control: Sex Differences and Sex-Specific Conditions  
Jill Barnes Univ. of Wisconsin-Madison

3.0 POSTER SESSION I  
Mon., 9:45-11:00 AM, Tennessee Ballroom

Board #

1 3.1 Diabetes Promotes a Sexual Dimorphic Expansion of Circulating and Cerebrovascular Th17 Cells in Female Rats  
LaDonya Jackson, Weiguo Li, Yasir Abdul, Guangkuo Dong, Babak Baban, Susan C. Fagan, Adviye Ergul. Medical College of Georgia

2 3.2 Sexual Dimorphism in Central Modulation of Baroreceptor Afferent Input in Hypertension  
Ibrahim M. Salman, Omar Z. Ameer, Sheridan McMurray, Arun Sridhar, Stephen J. Lewis, Yee-Hsee Hsieh. Case Western Reserve Univ.; Galvani Bioelectronics
| 3 | 3.3 | Central Leptin Receptor Antagonism Attenuates the Development of Menopausal-Induced Hypertension in the Rat  
**Maria Barnes, Sarah Clayton. Des Moines Univ.** |
| 4 | 3.4 | Time Course Assessment of Acute and Chronic Hypotonic Brain Edema by Dynamic NMR: Topographical and Gender Differences  
**Marta Tejedor, Alberto Lázaro, Alejandro Rojo, Lorena Cussó, Marian González-Nicolás, Jorge J García Seoane, Meritxell López, Alberto Tejedor. Universidad Complutense Madrid;Hospital General Gregorio Marañón** |
| 5 | 3.5 | Estrogen Receptor Alpha Contributes to the Inhibitory Action of Central Cytochrome P4501B1 Generated 17β Estradiol Metabolite 2-Methoxyestradiol to Angiotensin II-Induced Hypertension  
**Purnima Singh, Chi Young Song, Shubha Ranjan Dutta, Scott A. Heldt, Kafait U. Malik. Univ. of Tennessee Health Science Center** |
| 6 | 3.6 | Sex-Dependent Effects of Prediabetes in Mouse Models of Alzheimer's Disease and Mixed Dementia  
**Olivia Gannon, Lisa Robison, Abigail Salinero, Melissa Thomas, Alya Tyson, Kristen Zuloaga. Albany Medical College** |
| 7 | 3.7 | DOCA-Salt Hypertension Leads to Neurocognitive Deficiencies in Female SD Rats  
**Kasey Belanger, Ellen Gillis, Jennifer Sullivan. Augusta Univ.** |
| 8 | 3.8 | Sex-Specific Hormones Produced During Proestrous and Estrous Exert a Regulatory Effect on the Nefatin-1 mRNA Levels and Autonomic Function in Female Cycling Rats  
**Alicia Pate, Gina Yosten. St. Louis College of Pharmacy; Saint Louis Univ.** |
| 9 | 3.9 | Central Acetylcholinesterase Inhibitor, Galantamine, Prevents Lipid-Induced Oxidative Stress in African American Women  
**Cyndya Shibao, Jorge Celedonio, Shahram E, Sachin Paranjape, Andre Diedrich. Vanderbilt Univ. Medical Center** |
| 10 | 3.10 | Angiotensin Type 2 Receptor Stimulation With Compound 21 Improves Stroke Outcome in Female Rats: Possible Role For Peroxisome Proliferator-activated Receptor Gamma  
**Wael Eldahshan, Bindu Pillai, Mohammed Sayed, Abdulrahman Alwhaibi, Abdelrahman Fouda, Tauheed Ishrat, Adiye Ergul , Susan Fagan. Univ. of Georgia;Augusta Univ.;The Univ. of Tennessee Health Science Center** |
| 11 | 3.11 | Ovariectomy induces anxiety-like behavior and short-term recognition memory impairment  
**Glenda Campos, Aline De Souza, Crystal West, Hong Ji, Rodrigo De Menezes, Kathryn Sandberg. Georgetown Univ.;Federal Univ. of Ouro Preto** |
| 12 | 3.12 | Sex Differences in Healthy Human Heart Revealed by Cap Analysis Gene Expression (CAGE)  
**Anna Gams, Ndeye Rokhaya Faye, Ruslan Deviatiiarov, Aaron C. Koppel, Igor R. Efimov. The George Washington Univ.;Kazan Federal Univ.** |
| 13 | 3.13 | Comparison of Measures of Compensatory Reserve in Differentiating Tolerance to Simulated Hemorrhage in Males versus Females  
**Taylor Schlotman, Victor Convertino. US Army Institute of Surgical Research** |
| 14 | 3.14 | Comparing Time to Presyncope during Simulated Hemorrhage Across Menstrual Cycle  
**Winfred Stacey, Carmen Hinojosa-Laborde, Taylor Schlotman, Victor Convertino. US Army Institute of Surgical Research** |
<table>
<thead>
<tr>
<th>Time</th>
<th>Title</th>
<th>Authors</th>
<th>Institutions</th>
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<tr>
<td>3.15</td>
<td>Stress Hormone inhibition of Estrogen Transcriptional Regulation of the Serotonin Signaling in Cardiomyocytes is deleterious for the Female Heart in Myocardial Infarction</td>
<td>Natalie Burford, Lilly Kamberov, Wayne Orr, John Cidlowski, Diana Cruz-Topete</td>
<td>LSU Health Sciences Center Shreveport; National Institute of Environmental Health Sciences</td>
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<tr>
<td>3.16</td>
<td>Heart Failure with Preserved Ejection Fraction (HFpEF) is Augmented in Obese Mice with an XX Sex Chromosome Complement</td>
<td>Sean Thatcher, Yasir AlSiraj, Lisa Cassis.</td>
<td>Univ. of Kentucky</td>
</tr>
<tr>
<td>3.17</td>
<td>AMP-activated Protein Kinase and Estrogen-Dependent Mechanisms Underlying Increased Susceptibility to Cardiovascular Disease During Menopause</td>
<td>Marissa Pier, John Konhilas.</td>
<td>Univ. of Arizona</td>
</tr>
<tr>
<td>3.18</td>
<td>Beta-Carotene Metabolism in the Maternal Heart During Pregnancy</td>
<td>Chelsee Holloway, Youn-Kyung Kim, Loredana Quadro.</td>
<td>Rutgers Univ.</td>
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<tr>
<td>3.19</td>
<td>Responses in Cardiac Stroke Volume Index and Cardiac Index Do Not Differ Between Genders During Moderate Central Hypovolemia</td>
<td>Nathalie Linn A. Holme, Martin Andreas Lehre, Signe Søvik, Maja Elstad, Maria Skytioti.</td>
<td>Univ. of Oslo</td>
</tr>
<tr>
<td>3.21</td>
<td>Interaction between GLP-1 Receptor Agonists and Renin Angiotensin System in the Metabolic Syndrome in a Model of Postmenopausal PCOS</td>
<td>Edgar Torres Fernandez, Damian Romero, Licy Yanes Cardozo.</td>
<td>Univ. of Mississippi Med. Center</td>
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<tr>
<td>3.22</td>
<td>Angiotensin II Induces a Pro-Inflammatory Shift in the Splenic CD4+ T Cell Proteome in Menopausal Mice</td>
<td>Dennis Pollow Jr, Nathaniel Husband, Jill Romero-Aleshire, Joshua Uhlorn, Caitlin Moffett, Jennifer Uhrlaub, Janko Nikolich-Zugich, Heddwen Brooks.</td>
<td>Univ. of Arizona</td>
</tr>
<tr>
<td>3.23</td>
<td>Sex-Specific Regulation of Sirtuin-3 Mediates Differences in Ischemia-Reperfusion Kidney Injury</td>
<td>Jenny Pan, Vincent Yu, Qingtian Li, David Sheikh-Hamad.</td>
<td>Michael E. DeBokey VAMC; Baylor College of Medicine</td>
</tr>
<tr>
<td>3.24</td>
<td>Group IV Cytosolic Phospholipase A2α is Required for 6β-Hydroxytestosterone Mediated Angiotensin II Induced Hypertension in Male Mice</td>
<td>Ajeeth Pingili, Purnima Singh, Chi Young Song, Ji Soo Shin, Joseph Bonventre, Kafiat Malik.</td>
<td>Univ. of Tennessee HSC; Brigham and Women’s Hospital, Harvard Medical School</td>
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<tr>
<td>3.26</td>
<td>Sex Differences in the Diurnal Natriuretic Response to Benzamil in Sprague Dawley Rats</td>
<td>Reham Soliman, Jermaine Johnston, Eman Gohar, David Pollock.</td>
<td>Univ. of Alabama at Birmingham</td>
</tr>
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</table>
27 3.27 Increased Severity of Renal Ischemia Reperfusion Injury in Male vs. Female Mice is Associated with Greater Expression of Choline Acetyltransferase in Tubules
Shannon Allen, Jacob Zalewski, Jacqui Potter, Conor Miles, Donald Hoover, Aaron Polichnowski. East Tennessee State Univ.

28 3.28 Gender Differences in Human Skin Na⁺ and Monocyte Salt-Sensitivity
Annet Kirabo, Natalia Barbaro, Jason Foss, Fernando Elijovich, Justin Van Beusecum, Cheryl Laffer, Mingfang Ao, Aseel Alsouqi, Alp Ikizler, David Harrison, Annet Kirabo. Vanderbilt Univ. Medical Center

29 3.29 Greater Aortic Inflammation in Male SHR Corresponds to Vascular Dysfunction
Lindsey Ramirez, Jacqueline Musall, Jennifer Sullivan. Augusta Univ.

30 3.30 Pretreatment with Low Dose Lipopolysaccharide Attenuates Medullary Congestion in Male WKY Following Acute Kidney Injury
Sarah Ray, G. Ryan Crislip, Riyaz Mohamed, Bansari Patel, Katie Wilson, Jinging Sun, Paul O’Connor. Augusta Univ.

31 3.31 Immune Cell Influx in Cisplatin-Induced Acute Kidney Injury: Sex Differences
Lisa M. Curtis, Chunlan Fan, Ravindra Boddu. Univ. of Alabama at Birmingham

32 3.32 Sex Differences in Renal Ammonia Metabolism
Autumn N. Harris, Hyun-Wook Lee, Gunnar Osis, Kierstin L. Webster, Jill W. Verlander, I. David Weiner. Univ. of Florida

LB32A 3.33 Sex Differences in the Metabolic and Physiological Effects of a High Fat Diet in the 3xTg-AD Mouse Model of Alzheimer's Disease

LB32B 3.34 Sex Differences in the Cerebral Vascular Function and K Channel Role in Adult Sprague Dawley (SD) Rats
Sumit Sontakke. Univ. of Mississippi Med. Center

LB32C 3.35 Cerebrovascular Dysfunction in the Dahl S Rat Model of Superimposed Preeclampsia
Kenji Maeda, Daniel McClung, Junie Paula Warrington, Michael Garrett, Michael Ryan, Jennifer Sass. Univ. of Mississippi Med. Center

4.0 SESSION 2: PHYSIOLOGY AND GENDER: AGING AND SENESCENCE

Mon., 11:00 AM-12:45 PM, Summit I

Chairs: Christopher DeSouza, Univ. of Colorado
        Jennifer DuPont, Tufts Med. Center

11:00 AM 4.1 Sex Differences, Aging and Vascular Function
Christopher DeSouza Univ. of Colorado

11:25 AM 4.2 Abstract 6: Sex Differences in the Effects of Prediabetes on Vascular Contributions to Dementia
Kristen Zuloaga Albany Med. Coll.

11:35 AM 4.3 Abstract 9: Sex Differences in the Role of the Smooth Muscle Cell Mineralocorticoid Receptor in Cardiovascular Aging
Jennifer DuPont Tufts Med. Center

11:45 AM 4.4 Maternal Aging and Cardiovascular Dysfunction
Sandra Davidge Univ. of Alberta
## DAILY SCHEDULE

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<th>Time</th>
<th>Session</th>
<th>Title</th>
<th>Speakers</th>
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<tbody>
<tr>
<td>12:10 PM</td>
<td>4.5</td>
<td>Abstract 32: Central Leptin Receptor Antagonism Attenuates the Development of Menopausal-Induced Hypertension in the Rat</td>
<td>Maria Barnes <em>Des Moines Univ.</em></td>
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<tr>
<td>12:20 PM</td>
<td>4.6</td>
<td>Bioenergetic and Metabolic Consequences of the Loss of Ovarian Function in Women</td>
<td>Wendy Kohrt <em>Univ. of Colorado</em></td>
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<tr>
<td><strong>5.0</strong></td>
<td><strong>SESSION 3: SEX AND GENDER DIFFERENCES IN PHYSIOLOGY AND FUNCTION: THE HEART</strong></td>
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<tr>
<td>Mon., 3:00-4:45 PM, Summit I</td>
<td>Chairs: Zdenka Pausova, <em>Hospital for Sick Children</em></td>
<td>Eli Louwagie, <em>Univ. of South Dakota-Sanford School of Med.</em></td>
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<tr>
<td>3:00 PM</td>
<td>5.1</td>
<td>Sex and Gender Differences in Cardiac Function</td>
<td>Zdenka Pausova <em>Hospital for Sick Children</em></td>
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<tr>
<td>3:25 PM</td>
<td>5.2</td>
<td>Abstract 7: Sex Differences in Healthy Human Heart Revealed by Cap Analysis Gene Expression (CAGE)</td>
<td>Anna Gams <em>The George Washington Univ.</em></td>
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<tr>
<td>3:35 PM</td>
<td>5.3</td>
<td>Abstract 39: Heart Failure with Preserved Ejection Fraction (Hfpef) is Augmented in Obese Mice with an XX Sex Chromosome Complement</td>
<td>Sean Thatcher <em>Univ. of Kentucky</em></td>
</tr>
<tr>
<td>3:45 PM</td>
<td>5.4</td>
<td>Estrogen Modulation of the Cardiac RAS in Diastolic Dysfunction</td>
<td>Leanne Groban <em>Wake Forest School of Med.</em></td>
</tr>
<tr>
<td>4:10 PM</td>
<td>5.5</td>
<td>Abstract 71: Prenatal Diabetes and High-Fat Diet Exposure Impair Mitochondrial Function in Adult Rat Cardiomyocytes</td>
<td>Eli Louwagie <em>Univ. of South Dakota-Sanford School of Med.</em></td>
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<tr>
<td>4:20 PM</td>
<td>5.6</td>
<td>Understanding the Unique Considerations for the Treatment of Heart Disease in Women vs. Men</td>
<td>Martha Gulati <em>Ohio State Univ. Wexner Med. Ctr.</em></td>
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<td><strong>6.0</strong></td>
<td><strong>CAREER DEVELOPMENT AND TRAINEE NETWORK SESSION</strong></td>
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<td>Mon., 5:00-6:00 PM, Summit I</td>
<td>5:00 PM 6.1</td>
<td>Get the Job You Want: Tips for Interviewing and Negotiating</td>
<td>Kelly Hyndman <em>Univ. of Alabama at Birmingham</em></td>
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<td><strong>7.0</strong></td>
<td><strong>SESSION 4: PHYSIOLOGY AND GENDER: OBESITY AND METABOLISM</strong></td>
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<td>8:00 AM</td>
<td>7.1</td>
<td>Sex and Sex Steroids Regulate of Metabolic Dysfunction</td>
<td>Franck Mauvais-Jarvis <em>Tulane Univ.</em></td>
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</table>
8:25 AM  7.2  Abstract 29: Progesterone Upregulates Endothelial Mineralocorticoid Receptor Expression which Predisposes Female Mice to Obesity-induced Endothelial Dysfunction
Jessica Faulkner Med. Coll. of Georgia at Augusta Univ.

8:35 AM  7.3  Abstract 55: Identification of Sex-Specific miRNA and mRNA Expression Patterns in Type 1 Diabetes
Preethi Krishnan Indiana Univ.

8:45 AM  7.4  Sex Differences in Kidney Injury in a Rat Model Of Prepubertal Obesity
Jan Michael Williams Univ. of Mississippi Med. Ctr.

9:10 AM  7.5  Abstract 67: Female Rats Offered Free Access to Lard, Sucrose, and Chow Developed Features of Metabolic Syndrome and Periuterine Adipose Tissue Expansion
Hijab Ahmed Univ. of North Texas Health Science Center

9:20 AM  7.6  X Marks the Spot: Sex Chromosomes Regulate Hypercholesterolemia and Atherosclerosis
Lisa Cassis Univ. of Kentucky

8.0  POSTER SESSION 2
Tue., 9:45-11:00 AM, Tennessee Ballroom

Board #

33  8.1  Long Term Consequences of Food Restriction on Body Composition and Angiotensin System
Aline Souza, Crystal West, Glenda Campos, Amrita Pai, Hong Ji. Georgetown Univ.; Universidade Federal de Ouro Preto

34  8.2  Progesterone Upregulates Endothelial Mineralocorticoid Receptor Expression which Predisposes Female Mice to Obesity-induced Endothelial Dysfunction
Jessica Faulkner, Simone Kennard, Galina Antonova, Zsolt Bagi, Iris Jaffe, Vijay Patel, Eric Belin de Chantemele. Medical College of Georgia at Augusta Univ.; Tufts Medical Center

35  8.3  CD8 T-cells Isolated from Female Mice have Increased in vitro Cell Activation in Response to Nutrient Deprivation
Merry Lindsey, Joshua Clayton, Elizabeth Flynn, Donald Menick, Kristine DeLeon-Pennell. Univ. of Mississippi Medical Center; Medical Univ. of South Carolina

36  8.4  Arginase 2 is Involved in Diet-Induced Obesity and Metabolic Dysregulation in Male and Female Mice

37  8.5  Identification of Sex-Specific miRNA and mRNA Expression Patterns in Type 1 Diabetes

38  8.6  Sex Specific Upregulation of Hepatic FGF21 Expression in Metabolic States Contrast by Nutrient Availability
Nadezhda Bazhan, Tatyana Yakovleva, Natalia Sitnikova, Elena Makarova. Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia

39  8.7  Female Rats Offered Free Access to Lard, Sucrose, and Chow Developed Features of Metabolic Syndrome and Periuterine Adipose Tissue Expansion
Hijab Ahmed, Johanna Hannan, John Apolzan, Styliani Gouloupoulou. Univ. of North Texas Health Science Center; Brody School of Medicine; Pennington Biomedical Research Center, Louisiana State Univ. System
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<th>Time</th>
<th>Poster</th>
<th>Title and Authors</th>
<th>Institution(s)</th>
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<tr>
<td>8.8</td>
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<td><strong>Prenatal Diabetes and High-Fat Diet Exposure Impair Mitochondrial Function in Adult Rat Cardiomyocytes</strong>&lt;br&gt;<em>Eli Louwagie, Tricia Larsen, Michelle Baack.</em> Sanford School of Medicine - Univ. of South Dakota; Sanford Research</td>
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<td>8.9</td>
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<td>8.10</td>
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<td><strong>His and Her Computational Models of Long-term Blood Pressure Regulation</strong>&lt;br&gt;<em>Jessica Leete, Anita Layton.</em> Duke Univ.</td>
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<td>8.11</td>
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<td><strong>Sex Differences in the Role of the Smooth Muscle Cell Mineralocorticoid Receptor in Cardiovascular Aging</strong>&lt;br&gt;<em>Jennifer DuPont, Seung Kim, Qing Lu, Rachel Kenney, M. Elizabeth Moss, Zhe Sun, Mark Aronovitz, Gregory Martin, Wendy Baur, Gerald Meineger, Michael Hill, Iris Jaffe.</em> Tufts Medical Center; Univ. of Missouri</td>
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<td>8.12</td>
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<td><strong>Gender and Ageing Influence on Vascular Responses in a Pulmonary Hypertensive Rat Animal Model</strong>&lt;br&gt;<em>Jesus Prieto-Lloret, Elena Olea, Angela Gomez-Niño, Ana Obeso, Asuncion Rocher.</em> Univ. of Valladolid. IBGM. CIBERES ISCiii</td>
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<td>8.13</td>
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<td><strong>The Role of Sex in Oxidative Stress and Implications on Vascular Function and Blood Pressures</strong>&lt;br&gt;<em>Rebecca Kappus, Anna Ruth Carmichael, Caroline Blackman, Jessica Yomano.</em> Appalachian State Univ.</td>
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<td>8.14</td>
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<td><strong>Estrogen Determines the Sex-Differences in Adrenergic Vessel Tone Regulation</strong>&lt;br&gt;<em>Kristin Riedel, Irakli Kopaliani, Zatscherl Birgit, Müller Bianca, Carmen Friebel, Andreas Deussen.</em> Technische Universität Dresden, Med. Faculty Carl Gustav Carus</td>
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<td>8.15</td>
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<td><strong>Inhibition of Neprilysin Attenuates Angii-Induced Abdominal Aortic Aneurysms (AAAs) and Atherosclerosis in Hypercholesterolemic Male Mice</strong>&lt;br&gt;<em>Yasir Alsiraj, Sean Thatcher, Mark Ensor, Lisa Cassis.</em> Univ. of Kentucky</td>
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<td>8.16</td>
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<td>8.18</td>
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<td><strong>Sex Differences in Vascular Reactivity and Biomarkers of Inflammation in Offspring of Dams Exposed to Perinatal High Salt Diet</strong>&lt;br&gt;<em>Ahmed Oloyo, Santan Olley, Esther Ohihoin, Abdulahi Adejare, Khadijat Ismail-Badmus, Olusoga Sofola.</em> Coll. of Med., Univ. of Lagos</td>
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<td>8.19</td>
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<td><strong>Nitric Oxide Helps Maintain the Buffering Capacity of Perivascular Adipose Tissue in Female Dahl SS in response to a High Fat Diet despite Increases in Blood Pressure and Vascular Inflammation</strong>&lt;br&gt;<em>Lia Taylor, Babak Baban, Jennifer Sullivan.</em> Augusta Univ.</td>
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<td>8.21</td>
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<td><strong>Sex-Specific Differences in Primary Neonatal Lung Fibroblasts and Microvascular Endothelial Cells Exposed to Hyperoxia in Vitro: Implications for Bronchopulmonary Dysplasia (BPD)</strong>&lt;br&gt;<em>Xiaoyu Dong, Yuhao Zhang, Jason Gleichorn, Swati Balaji, Krithika Lingappan.</em> Baylor College of Medicine; Univ. of Delaware</td>
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<td>8.22</td>
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<td><strong>Serelaxin Infusion Does Not Attenuate the Development of Hypertension in a Mouse Model of Systemic Lupus Erythematosus</strong>&lt;br&gt;<em>Victoria Wolf, Jennifer Sasser, Michael Ryan.</em> Univ. of Mississippi Med. Center</td>
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Afferent Arteriolar Responsiveness to Endothelin Receptor Activation: Does Sex Matter?
Eman Y. Gohar, Anthony K. Cook, Edward W. Inscho, David M. Pollock. *Univ. of Alabama at Birmingham*

Purinoceptor-dependent Regulation of Sodium Excretion is Sexually Dimorphic
Eman Y. Gohar, Malgorzata Kasztan, Shali Zhang, Edward W. Inscho, David M. Pollock. *Univ. of Alabama at Birmingham*

Differential Protein Expression of Renal Dopamine Receptors but Similar AT1R Activity in Salt-Sensitive Male and Female C57Bl/6J Mice
Xiaoyan Wang, Laureano Asico, Xiaobo Ma, Pedro Jose. *George Washington Univ.*

Renal Ischemia Reperfusion Injury in a Pig Model Reveals Gender Specific Expressed Genes as Potential New Biomarkers of Renal Injury/Regeneration Processes Driving to Chronic Kidney Disease
Stéphane Nemours, Luis Castro, Didac Ribatallada, Miguel Aranda, Marina Ferrer, Juan Morote, Anna Meseguer. *Vall d’Hebron Institut de Recerca (VHIR)*

Female Rats with Preexisting CKD Exhibit Impaired Recovery From AKI and the Subsequent Development of Proteinuria

Oral L-Arginine Treatment Significantly Increases Renal Tregs in Female DOCA Salt Hypertensive Rats

Necrox-5 Abolished Maturation Induced Sex Differences in Blood Pressure (BP) in Spontaneously Hypertensive Rats (SHR)

Androgen Influence on Renal Fibrosis Associated with Pyelonephritis

Tissue-Specific Estrogen Receptor Profiling Using Droplet Digital PCR

A Study on the Incidence and Types of Twinning in the South Indian Population
Shakthi Kumaran Ramasamy. *Aarupadai Veedu Med. Coll., Puducherry*

T Cell Specific Knockdown of Estrogen Receptor-α Does Not Eliminate Premenopausal Protection from Angiotensin II-Induced Hypertension, but Does Impact Renal T Cell Expression of CD28 and CTLA-4

Sex Shapes Cancer Cachexia and the Response to Therapeutic Blocking of ACVR2B Ligands in the Genetically Engineered KPC Mouse Model of Pancreatic Ductal Adenocarcinoma
Xiaoling Zhong, Jianguo Liu, Ashok Narasimhan, Leonidas Koniaris, Teresa Zimmers. *Indiana Univ.*
DAILY SCHEDULE

| LB67  | 8.35 | Effect of Oleanolic Acid on Lipid Metabolism in Neonatal Rats with Metabolic Syndrome  
Molefhi Moirapula Abotseng, Emmanuel Mukwevho, Ademola Ayeleso, Trevor Nyakudya.  
North West Univ.; Adeleke Univ., Ede.; Univ. of Johannesburg |
| LB68  | 8.36 | Effects of Tender Coconut Water on the Coronary Artery of Male Diabetic Wistar Rats  
Churchill Inneh, Oghenakhogie Momodu, Vanessa Oigbochie, Eseosa Adaniwmowan.  
Univ. of Benin |
| LB69  | 8.37 | Therapeutic Role of Intrapartum PDE-5 Inhibition on Blood Pressure and Renal Injury in Offspring  
of Preeclamptic Rats  
Hannah Turbeville, Sean Didion, Michael Garrett, Jennifer Sasser.  
Univ. of Mississippi Med. Center |
| LB70  | 8.38 | Effects of Prenatal Sildenafil Treatment On Long-term Cardiovascular Function in Offspring From  
Dahl Salt-Sensitive Rats  
Fieke Terstappen, Frank Spradley, Sinead Clarke, Ying Ge, Courtney Ross, Michael Garrett, Jaap Joles, Titia Lely, Jennifer Sasser.  
Univ. Medical Centre Utrecht; Univ. of Mississippi Medical Center |
| LB71  | 8.39 | Testosterone Supplementation in Postmenopausal Hypertensive Rats  
Rodrigo Maranon, Jane Reckelhoff.  
UMMC |
| LB72  | 8.40 | Hypogonadal Hypertension in Male Sprague-Dawley Rats is Reversed by Testosterone Replacement Therapy, Which Down-Regulates Renin-Angiotensin System Message Expression  
Andrea Hanson, Nikolas Garcia, Joshua Mckenna, Mercedes Perusquia, John Stallone.  
Texas A&M Univ.; Universidad Nacional Autonoma de Mexico |

9.0 SESSION 5: SEX AND GENDER DIFFERENCES IN PHYSIOLOGY AND FUNCTION: THE KIDNEY

Tue., 11:00 AM-1:05 PM, Summit I

Chairs:  
David Pollock, Univ. of Alabama at Birmingham  
Ellen Gillis, Augusta Univ.  

11:00 AM 9.1 Sex Differences in Renal Function: Lessons From the ET-1 System  
David Pollock Univ. of Alabama at Birmingham  

11:25 AM 9.2 Abstract 5: Sex-Specific Regulation of Sirtuin-3 Mediates Differences in Ischemia-Reperfusion Kidney Injury  
Jenny Pan Michael E. DeBakey VAMC  

11:35 AM 9.3 (Patho)Physiological Consequences of Sex Differences in Renal Sodium Transporters  
Alicia McDonough Univ. of Southern California  

12:00 PM 9.4 Abstract 68: Oral L-Arginine Treatment Significantly Increases Renal Tregs in Female DOCA Salt Hypertensive Rats  
Ellen Gillis Augusta Univ.  

12:10 PM 9.5 Sex Differences in Renal Ischemia-Reperfusion Injury  
Attila Szabó Univ. of Budapest  

12:30 PM 9.6 Abstract : 92Sex Differences in Renal Ammonia Metabolism  
Autumn Harris Univ. of Florida
12:40 PM 9.7  Sex Differences in the Regulation of Blood Pressure by the Circadian Clock Proteins PER1 and BMAL1 in C57Bl/6J Mice
Michelle Gumz  Univ. of Florida

10.0  PLENARY LECTURE

Tue., 3:00-3:45 PM, Summit I

3:00 PM  National Initiatives In Sex And Gender-Based Medicine (SGBM)
Marjorie Jenkins  Texas Tech Univ. Health Sci. Ctr.

11.0  SESSION 6: FEMALE-SPECIFIC CARDIOVASCULAR, RENAL AND METABOLIC COMPLICATIONS

Tue., 4:00-6:10 PM, Summit I

Chairs:  Jennifer Sasser, Univ. of Mississippi Med. Center
         Dennis Pollow Jr., Univ. of Arizona

4:00 PM 11.1  Clinical and Basic Science Considerations of Cardiovascular Health in Pregnancy
Jennifer Sasser  Univ. of Mississippi Med. Center

4:25 PM 11.2  Abstract 59: Beta-Carotene Metabolism in the Maternal Heart During Pregnancy
Chelsee Holloway  Rutgers Univ.

4:35 PM 11.3  Vascular Changes in the Postmenopausal Female
Sarah Lindsey  Tulane Univ.

5:00 PM 11.4  Abstract 64: Female Rats with Preexisting CKD Exhibit Impaired Recovery From AKI and the Subsequent Development of Proteinuria
Jacqui Potter  East Tennessee State Univ.

5:10 PM 11.5  Long-term Sequelae of Preeclampsia: A Clinical Perspective
Michelle Hladunewich  Stonybrook Health Sci.s Centre, Toronto

5:35 PM 11.6  Abstract 93: Angiotensin II Induces a Pro-Inflammatory Shift in the Splenic CD4+ T Cell Proteome in Menopausal Mice
Dennis Pollow Jr.  Univ. of Arizona

5:40 PM 11.7  Leptin Accelerates Disease Progression and the Development of Hypertension in an Experimental Model of Autoimmune Disease
Erin Taylor  Univ. of Mississippi Med. Ctr.

WEDNESDAY, OCTOBER 3, 2018

12.0  SESSION 7: SEX AND GENDER DIFFERENCES IN PHYSIOLOGY AND FUNCTION: THE VASCULATURE

Wed., 8:00-10:10 AM, Summit I

Chairs:  Eric Belin de Chantemele, Augusta Univ.
         Eman Y. Gohar, Univ. of Alabama at Birmingham

8:00 AM 12.1  Impact of Sex on Vascular Function in Cardiovascular and Metabolic Disorders
Eric Belin de Chantemele  Augusta Univ.
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<thead>
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<th>Time</th>
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<th>Title</th>
<th>Speaker</th>
<th>Institution</th>
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<tr>
<td>8:25 AM</td>
<td>12.2</td>
<td>Abstract 28: Estrogen Determines the Sex-Differences in Adrenergic Vessel Tone Regulation</td>
<td>Kristin Riedel</td>
<td>Technische Universität Dresden, Med. Faculty Carl Gustav Carus</td>
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<td>8:35 AM</td>
<td>12.3</td>
<td>Sex Differences in Endothelial Cells of the Microvasculature</td>
<td>Virginia Huxley</td>
<td>Univ. of Missouri</td>
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<td>9:00 AM</td>
<td>12.4</td>
<td>Abstract 78: Nitric Oxide Helps Maintain the Buffering Capacity of Perivascular Adipose Tissue in Female Dahl SS in response to a High Fat Diet despite Increases in Blood Pressure and Vascular Inflammation</td>
<td>Lia Taylor</td>
<td>Augusta Univ.</td>
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<tr>
<td>9:10 AM</td>
<td>12.5</td>
<td>Using Mathematical Modeling to Understand the Basis for Sex Differences in Vascular Function</td>
<td>Anita Layton</td>
<td>Duke Univ.</td>
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<tr>
<td>9:45 AM</td>
<td>12.7</td>
<td>The Impact of Sex and Diabetes on Vascular Function in Diabetes</td>
<td>Adviye Ergul</td>
<td>Augusta Univ.</td>
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<td>10:40 AM</td>
<td>13.0</td>
<td>SESSION 8: MALE-SPECIFIC CARDIOVASCULAR, RENAL AND METABOLIC COMPLICATIONS</td>
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<td>10:40 AM</td>
<td>13.1</td>
<td>Androgens in Cardiovascular Health and Disease</td>
<td>Jane Reckelhoff</td>
<td>Univ. of Mississippi Med. Ctr.</td>
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<tr>
<td>11:05 AM</td>
<td>13.2</td>
<td>Abstract 12: Group IV Cytosolic Phospholipase A2 is Required for 6B-Hydroxytestosterone Mediated Angiotensin II Induced Hypertension in Male Mice</td>
<td>Ajeeth Pingili</td>
<td>Univ. of Tennessee HSC</td>
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<td>11:15 AM</td>
<td>13.3</td>
<td>Abstract 79: Pretreatment with Low Dose Lipopolysaccharide Attenuates Medullary Congestion in Male WKY Following Acute Kidney Injury</td>
<td>Sarah Ray</td>
<td>Augusta Univ.</td>
</tr>
<tr>
<td>11:25 AM</td>
<td>13.4</td>
<td>Abstract 82: Androgen Influence on Renal Fibrosis Associated with Pyelonephritis</td>
<td>Teri Hreha</td>
<td>Washington Univ.</td>
</tr>
<tr>
<td>11:35 AM</td>
<td>13.5</td>
<td>Testosterone Deficiency in the Aging Male</td>
<td>Licy Yanes Cardozo</td>
<td>Univ. of North Carolina</td>
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<tr>
<td>12:00 PM</td>
<td>14.0</td>
<td>HIGHLIGHTS &amp; CLOSING REMARKS</td>
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**Chair:** Jane Reckelhoff, Univ. of Mississippi Med. Ctr.
Teri Hreha, Washington Univ.
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5.0  Session 3: Sex and Gender Differences in Physiology and Function:
The Heart .................................................................................................................. Page 47

7.0  Session 4: Physiology and Gender: Obesity and Metabolism .................................... Page 50

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9.0  Session 5: Sex and Gender Differences in Physiology and Function: The Kidney ........ Page 75

11.0 Session 6: Female-Specific Cardiovascular, Renal and Metabolic Complications ....... Page 80

12.0 Session 7: Sex and Gender Differences in Physiology and Function:
The Vasculature ........................................................................................................ Page 83

13.0 Session 8: Male-Specific Cardiovascular, Renal and Metabolic Complications .......... Page 87

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**2: SESSION 1: SEX AND GENDER DIFFERENCES IN PHYSIOLOGY AND FUNCTION: THE BRAIN AND NERVOUS SYSTEM**

2.1 ADIPOKINES AND THE CENTRAL REGULATION OF CARDIOVASCULAR FUNCTION

Gina Yosten1

1Pharmacology and Physiology, Saint Louis University

Adipokines such as leptin and nesfatin-1 act as important metabolic signals that inhibit food intake in the presence of nutrient excess. These hormones also act in brain to modulate cardiovascular function by stimulating sympathetic nervous system activity and increasing blood pressure. Our recent studies suggest that female sex hormones protect against nesfatin-1-induced elevations in blood pressure, and that expression of nesfatin-1 is altered across the rat estrous cycle. We hypothesize that nesfatin-1 may play an important role in etiology of hypertension, particularly in postmenopausal women, who have lost the protective effect of ovarian steroid hormones against nesfatin-1 action. Future studies will investigate the function and expression of nesfatin-1 in aging females, particularly in the setting of obesity.

2.2 COMPARING TIME TO PRESYNCOPE DURING SIMULATED HEMORRHAGE ACROSS MENSTRUAL CYCLE

Winfred Stacey1, Carmen Hinojosa-Laborde1, Taylor Schlotman1, Victor Convertino1

1Battlefield Health & Trauma Center for Human Integrative Physiology, US Army Institute of Surgical Research

**Background:** Several studies indicate that females have lower orthostatic tolerance compared to males. The mechanisms that contribute to the evident sex differences are not well understood. Multiple characteristics including sex hormones appear to contribute to low tolerance. Menstrual cycle and concurrent hormonal fluctuations are particularly relevant to understanding why females have lower tolerance to central hypovolemia. A number of studies designed to investigate contributions of ovarian hormones (particularly estradiol) to cardiovascular function have generated conflicting findings. Furthermore, there is paucity in the literature on contribution of the anterior pituitary hormones, luteinizing (LH) and follicle-stimulating hormones (FSH), which are elevated during the ovulatory phase of the menstrual cycle.

**Objective:** In our current study, we induced central hypovolemia similar to hemorrhage using progressively stepwise lower body negative pressure (LBNP) in women during various phases of the menstrual cycle. We tested the hypothesis that less time would be required to manifest presyncope in women during the follicular and luteal phases of the menstrual cycle when estradiol is at its highest levels compared to the ovulatory phase.

**Methods:** In this cross-sectional experimental design, LBNP application was induced on healthy volunteer female subjects (n=22, mean age 26.8 ± 6 years; mean weight 63.9 ± 9 kg; mean BMI 23.7 ± 2.7; mean height 164.3± 8.2 cm). All subjects were not taking oral contraceptives. Time to presyncope was calculated from start of baseline to termination of LBNP at three menstrual cycle phases; early follicular (Days 1 – 7; n=10), ovulatory (Days 12 – 16, n=5) and mid luteal (Days 20-26, n=7). Data are presented as mean ± SEM. The probability that any differences in LBNP tolerance across menstrual cycle did not exist by greater than chance were determined by ANOVA and expressed as exact ‘p’ values.

**Results:** The average time to presyncope was lower for females in the ovulatory phase (1254.2 ± 92 seconds) compared to those in early follicular (1613 ± 48 seconds; p = 0.013) and mid luteal (1496 ± 80 seconds; p = 0.099) phases. Time to presyncope for females in early follicular and mid luteal phases were marginally similar.

**Conclusion:** A major finding in this study is the lower tolerance to LBNP in the ovulatory phase of the menstrual cycle. Contrary to our hypothesis, the ovulatory phase of the menstrual cycle that is characterized by elevated LH and FSH with reduced levels of estradiol and progesterone is associated with lower tolerance to central hypovolemia. As such, our findings indicate that estradiol may not contribute to compromised tolerance to central hypovolemia in women given that its highest levels are before the ovulatory phase and during the luteal phase of the menstrual cycle. Such a hypothesis may be tested by subsequent cross-sectional comparisons of females with and without use of contraceptives.

**Funding:** Funding was provided by an appointment to the Post-doctoral research fellowship program, administered by the Oak Ridge Institute for Science and Education and a grant from the US Army Combat Casualty Care Research Program (D-009-2014-USAISR).
2.3

ESTROGEN RECEPTOR ALPHA CONTRIBUTES TO THE INHIBITORY ACTION OF CENTRAL CYTOCHROME P4501B1 GENERATED 17B-ESTRADIOL METABOLITE 2-METHOXYESTRADIOL TO ANGIOTENSIN II-INDUCED HYPERTENSION

Purnima Singh1, Chi Young Song3, Shubha Ranjan Dutta1,2, Scott A. Heldt1, Kafait U. Malik1

1Pharmacology, University of Tennessee Health Science Center, 2Anatomy and Neurobiology, University of Tennessee Health Science Center

Hypertension in postmenopausal females is attributed to diminished levels of 17β-estradiol (E2). It has been documented that in various experimental models of hypertension including angiotensin (Ang) II, the protection against hypertension is lost in females following ovariectomy (OVX). Ang II produces hypertension by its action in the brain which is minimized by E2 through its action on estrogen receptor alpha (ERα). Cytochrome P4501B1 (CYP1B1) and catechol-O-methyltransferase (COMT) that sequentially metabolize E2 to 2-methoxyestradiol (2-ME) are expressed in the brain. This study was performed to test the hypothesis that 2-ME mediates the inhibitory effect of E2 on Ang II-induced hypertension via ERα in the brain of female mice. Intracerebroventricularly (ICV) administered E2 (1.5µg/2µL/every 2nd day) in OVX wild-type (Cyp1b1+/+) mice attenuated the Ang II (700 ng/kg/min, 14 days)-induced increase in mean arterial pressure (MAP) measured by radiotelemetry, but not in the mice injected with COMT siRNA (ICV, 0.4 nmol) (108±2 vs. 144±3, n=5-6, P<0.05). ICV injections of 2-ME (1.5 µg/2µL/every 2nd day) but not E2 attenuated the increase in MAP by Ang II in OVX Cyp1b1+/+ mice (112±1 vs 142±5, n=5-6, P<0.05); this effect was minimized in the mice by ICV injected ERα siRNA (0.4 nmol; 137±9). Power spectral analysis of the data on day 12 showed that Ang II-infusion increased the low to high frequency ratio of heart rate variability, index of sympathetic outflow modulation in ICV E2 injected OVX Cyp1b1+/+ compared to OVX Cyp1b1−/− mice (2.6±0.1 vs 1.6±0.1) or ICV 2-ME injected OVX Cyp1b1+/+ mice (1.6±0.1); these effects were blunted by ICV injected COMT siRNA (3.0±0.4) or ERα siRNA (2.6±0.4). Administration of ganglionic blocker hexamethonium (30 mg/Kg, IP) on day 14 of Ang II infusion resulted in greater reduction in MAP in OVX Cyp1b1−/− than OVX Cyp1b1+/+ mice injected ICV with E2 (Δ89±7 vs Δ64±3, mmHg) or 2-ME in OVX Cyp1b1−/− mice (Δ60±3 mmHg); these effects were attenuated in mice by ICV injected COMT siRNA (Δ84±7 mmHg) or ERα siRNA (Δ84±9, mmHg). Furthermore, in the intact Cyp1b1+/+ female mice, adenovirus (Ad)-CYP1B1 shRNA but not Ad-scrambled (scr) siRNA (ICV, 2µL, 1.3X1010 particles/mL), potentiated Ang II-induced increase in systolic blood pressure (SBP, mm Hg) measured by tail-cuff method (170±4 vs 141±3, n=8-9, P<0.05). In the intact Cyp1b1−/−, but not OVX Cyp1b1−/− mice, reconstitution of CYP1B1 in the brain by transduction with Ad-CYP1B1-DNA (ICV, 2µL, 1.0 X 1011 particles/mL) reduced Ang II-induced increase in SBP (135±1 vs 167±6, n=7-10, P<0.05). These data provide the first evidence that 1) central effect of E2 to attenuate Ang II-induced hypertension is dependent on brain CYP1B1 and is most likely mediated via generation of 2-ME, and 2) 2-ME protects against Ang II induced hypertension by acting through ERα in the brain by reducing the sympathetic outflow. Also, deoxycorticosterone acetate (DOCA, 50 mg/kg) salt (1% NaCl in drinking water) in uni-nephrectomized Cyp1b1−/− female mice at 4 weeks caused greater increase in SBP than in Cyp1b1−/− mice (165±6 vs. 133±4, n=4, P<0.05). In the intact Cyp1b1−/− female mice, reconstitution of CYP1B1 in the brain by transduction with Ad-CYP1B1-DNA but not its control Ad-GFP-DNA reduced DOCA salt-induced increase in SBP (133±3 vs 169±7, n=5, P<0.05). These data suggest that the brain CYP1B1 also protects against DOCA salt-induced hypertension in female mice. Whether this protection is mediated via DOCA-salt effect on brain Ang II, metabolism of E2 or ERα remains to be determined. The significance of this study is that the hormone replacement therapy in the USA have failed to lower BP or decrease cardiovascular disease in postmenopausal females. Therefore, 2-ME could be useful in treating hypertension in postmenopausal and hypoestrogenemic premenopausal females or in those with menstrual irregularities because of ovarian failure. This study was supported by NIH-HLBI Grant 19134-43.

2.4

THE ROLE OF SENSITIZATION AND NEUROPLASTICITY IN SEX DIFFERENCES IN BLOOD PRESSURE AND HYPERTENSION

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1Psychological and Brain Sciences, Pharmacology, Health and Human Physiology, and the François M. Abboud Cardiovascular Center, University of Iowa, 2Psychological and Brain Sciences, University of Iowa

The nervous system has the capacity to not only control reflex responses, but also can be modified by experience to alter the magnitude of the responses of systems it controls and then maintain these changes over long periods of time. These types of adaptive changes in the control of systemic responses are characteristic of processes involving memory and implicate a mediating brain neuroplasticity. In recent years we have demonstrated under several experimental conditions that the hypertensive response can be sensitized in response to challenges (stressors) presented earlier in the lifetime of an animal (rat or mouse). Sensitization is a simple form of non-associative learning. To
demonstrate Hypertensive Response Sensitization (HTRS), we employ an Induction-Delay-Expression (IND-DEL-EXP) experimental paradigm. Adult males readily show HTRS even when IND of sensitization is conducted as early as during the perinatal period. Intact females do not show HTRS. This talk will focus on the mechanisms responsible for induction of HTRS, why it occurs only in males and why males and females display such a sexual dimorphism.

Funding Sources: NIH HL14388, HL098207, HL073986, HL84027

2.5
CENTRAL ACETYLCHOLINESTerase INHIBITOR, GALANTAMINE, PREVENTS LIPID-INDUCED OXIDATIVE STRESS IN AFRICAN AMERICAN WOMEN

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1Clinical Pharmacology, Vanderbilt University Medical Center

African American women (AAW) have one of the highest prevalence of hypertension in the US. Obese AAW have decreased parasympathetic (PNS) activity compared to whites. Continuous lipid infusion that causes cardiovascular autonomic imbalance (decrease in PNS and increase in sympathetic activity) induces a greater increase in oxidative stress in AA compared to whites. Considering that PNS protects against oxidation and that central acetylcholinesterase inhibitors have been shown to suppress oxidative stress in animal models. We tested the hypothesis that the central acetylcholinesterase inhibitor, galantamine attenuates oxidation in response to lipid infusion in obese AAW compared to white women. We randomized 14 healthy obese AAW (39.5±10.7 yo, BMI 38.8±3.4) and 10 (35.9±8.3 yo, BMI 36.3±2.1) white women. All subjects underwent 4-h infusions of Intralipids and heparin. On separate days subjects received either 16 mg galantamine or placebo in a crossover fashion. Lipid-induced oxidative stress and inflammation were assessed with plasma F2 isoprostanes and cytokines at baseline, 2 and 4 h post-intralipid infusion. In AA, 16 mg of galantamine significantly suppressed the increase in lipid-induced oxidative stress (10±18 vs. -3.0±12 pg/mL with galantamine, P=0.014). No effect was noted in whites. Galantamine tended to increase IL10 (4.8±7.58 vs. 17.3±20.7 pg/mL with galantamine, P=0.06). We did not observe any effect on blood pressure or heart rate. Conclusion: Increased parasympathetic tone with central acetylcholinesterase inhibitor, galantamine, suppressed lipid-induced oxidative stress in African American women.

2.6
BRAIN BLOOD FLOW CONTROL: SEX DIFFERENCES AND SEX-SPECIFIC CONDITIONS

Jill Barnes1

1Kinesiology, University of Wisconsin-Madison

Despite sexual dimorphism present in risk of stroke and cognitive decline, sex differences in brain blood flow regulation are understudied. Premenopausal women demonstrate higher basal brain blood flow and middle cerebral artery blood velocity (MCAv) compared with age-matched men. However, the brain blood flow response to a stimulus (such as hypercapnia), termed cerebrovascular reactivity, may be more relevant for determining future risk of cerebrovascular disease. Sex differences in cerebrovascular reactivity will be discussed using several different methodological approaches. In addition, conditions unique to women, such as menopause and pregnancy, may further affect brain blood flow regulation. We will discuss the effect of age and menopause in women, the influence of previous use of menopausal hormones, and impact of pregnancy history in postmenopausal women on cerebrovascular reactivity. Collectively this work suggests there are sex-differences in cerebrovascular reactivity and sex-specific conditions that may place postmenopausal women at greater risk of stroke or cognitive decline.

Support: NIH HL118154, Alzheimer’s Association

3: POSTER SESSION I

3.1
DIABETES PROMOTES A SEXUAL DIMORPHIC EXPANSION OF CIRCULATING AND CEREBROVASCULAR TH17 CELLS IN FEMALE RATS

LaDonya Jackson1,2, Weiguo Li3, Yasir Abdul3, Guangkoo Dong1, Babak Baban3, Susan C. Fagan1,2, Adviye Ergul1,2,3

1Physiology, Medical College of Georgia, 2Clinical and Experimental Therapeutics, University of Georgia, 3Charlie Norwood Veterans Affairs, Medical Center

Diabetes is a sexually dimorphic disease. Women not only experience more severe diabetes but also suffer from a higher rate of diabetic complications including stroke and cognitive impairment. Yet, experimental data on sex-differences in cerebrovascular complications of diabetes is limited. We recently showed that diabetes negates the cerebrovascular protection typically seen in adult female rats. It has been reported that a high salt diet promotes expansion of IL17-producing T cells (Th17) in the gut microbiome and contributes to cerebrovascular dysfunction and cognitive impairment in male mice. Based on these grounds, we postulated that 1) circulating and/or cerebrovascular IL17 are elevated in
high fat diet (HFD)-induced diabetic female rats, and 2) diabetes promotes Th17 expansion in the gut.

**Methods:** Diabetes was induced in male and female Wistar rats by a HFD and low dose streptozotocin combination. After 8-11 weeks of diabetes, cell suspensions prepared from freshly harvested blood, brain and small intestine specimens were analyzed by flow cytometry using antibodies against cell surface markers (CD3, CD4, γδTCR) and intracellular markers (IL4, IL10, IL17, IFNγ and FOXP3) of T cells. Plasma IL17 concentration was measured through ELISA.

**Results:** (Table) Although HFD did not alter Th17 cells within the gut, it differentially increased both circulating (p<0.05) and cerebral Th17 cells (p<0.05), as well as plasma IL17 concentrations in females, but not males. Non-diabetic females also had significantly more circulating and cerebral inflammatory cells than their males counterparts (Th17 blood and brain p<0.05,0.01, Th1 blood and brain, p<0.05,0.05, δTCR brain p<0.05). This separation is further expanded in the presence of diabetes (Th17 blood and brain p<0.001,0.001, Th1 blood p<0.001, δTCR blood p<0.001).

**Conclusion:** Expansion of Th17 cells may contribute to the sexual dimorphic differences that exists in diabetes-related complications. Whether diabetes-mediated expansion of Th17 cells and IL17 concentrations contributes to cerebrovascular and cognitive dysfunction in females remain to be determined.

### Table 1: Protein Concentration in Blood and Brain

<table>
<thead>
<tr>
<th>Protein</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Th17</strong></td>
<td>5.2±1.5</td>
<td>7.0±1.5</td>
</tr>
<tr>
<td><strong>γδTCR</strong></td>
<td>9.3±4.3</td>
<td>5.7±4.0</td>
</tr>
<tr>
<td><strong>Th1</strong></td>
<td>5.8±2.6</td>
<td>5.4±0.7</td>
</tr>
<tr>
<td><strong>TREG</strong></td>
<td>4.3±0.7</td>
<td>5.7±0.8</td>
</tr>
</tbody>
</table>

**3.2 SEXUAL DIMORPHISM IN CENTRAL MODULATION OF BARORECEPTOR AFFERENT INPUT IN HYPERTENSION**

Ibrahim M. Salman, Omar Z. Ameer, Stephen J. Lewis, Yee-Hsee Hsieh

1. Department of Pediatric, Case Western Reserve University, 2. Pediatric of Department, Case Western Reserve University, 3. Department of Disease Biology, Galvani Bioelectronics, 4. Department of Pulmonary, Critical Care, and Sleep Medicine, Case Western Reserve University

Baroreflex activation therapy (BAT) reduces blood pressure in patients with resistant hypertension; however, it remains unknown if hemodynamic responses to this novel approach are influenced by sex. Electrical activation of the baroreceptor afferents within the aortic depressor nerve (ADN) of spontaneously hypertensive rats (SHRs) may bring new insights into the mechanisms underlying BAT in males and females. Accordingly, the differences in cardiovascular responses triggered by stimulation of the left and right ADN were studied in male versus female SHRs. Pentobarbital-anesthetized SHRs of either sex (25-29 weeks, n=5-9) were instrumented for left and right ADN stimulation (1-40 Hz, 0.2 ms, 0.4 mA for 20s) and recording of mean arterial pressure (MAP), heart rate (HR) and mesenteric (MVR) and femoral (FVR) vascular resistance. Female rats were also matched for the diestrus phase of the estrus cycle. Male SHRs had greater resting MAP (177±7 vs. 141±6 mmHg, P<0.01) but comparable resting HR (357±7 vs. 333±11 bpm), FVR (207±32 vs. 225±19 mmHg.min.ml⁻¹) and MVR (35±3 vs. 30±4 mmHg.min.ml⁻¹) compared to females. Irrespective of sex, both left and right ADN stimulation evoked frequency-dependent drops (P<0.01) in MAP, HR, FVR and MVR. Left ADN stimulation mediated greater reflex reductions in MAP, HR and FVR but not in MVR in male SHRs relative to females. Stimulation of the right ADN resulted in similar drops in MAP, HR and FVR but greater drops in MVR in female versus male SHRs. Thus, left ADN stimulation in males evokes a greater depressor response than stimulation of the right ADN, whereas in females the depressor response mediated by left and right ADN stimulation is comparable. Our data shows a differential central modulation of left but not right baroreceptor afferent neurotransmission in male versus female SHRs. Enhanced hypotensive responses to left ADN stimulation in male SHRs are likely driven by more potent baroreflex-mediated reductions in HR and FVR relative to females. The minor reductions in MVR in females in response to right ADN stimulation do not seem to contribute major differences in overall hemodynamic control in either sex. Collectively, this indicates that sexual dimorphism in baroreceptor afferent control of cardiovascular function...
should be considered when developing novel therapeutic strategies targeting hypertension in both sexes.

3.3 CENTRAL LEPTIN RECEPTOR ANTAGONISM ATTENUATES THE DEVELOPMENT OF MENOPAUSAL-INDUCED HYPERTENSION IN THE RAT
Maria Barnes, Sarah Clayton
Biochemistry and Nutrition, Des Moines University, Physiology and Pharmacology, Des Moines University

The fact that estrogen plays a protective role against hypertension in pre-menopausal women is well established. Additionally, the rapid increase in the rate of hypertension among post-menopausal female provides additional support for beneficial effects of estrogen. However, the events that occur in the absence of estrogen to augment the development of hypertension in post-menopausal women have not been fully elucidated. One event that occurs in menopausal women that may contribute to the development of hypertension is the significant increase in body weight in the form of adipose tissue. Increased adiposity is associated with an increase in the levels of circulating adipokines, substances released from adipose cells. One such adipokine is leptin, which is known to increase with increasing adiposity and play a role in the regulation of cardiovascular function, energy homeostasis and autonomic tone. Therefore, in the current study, using a post-menopausal rat model, we hypothesize that leptin, acting through central leptin receptors, augments menopausal-induced hypertension. Bilateral ovariectomized (OVX) female rats were used in these experiments. On the day the ovaries were removed, rats were instrumented with a telemetry probe for continuous monitoring of blood pressure and heart rate and an osmotic pump to deliver the leptin antagonist (LAN-6) (3µg/day, n=6) or vehicle (saline, n=6) into the lateral ventricle for four weeks. Sham animals were subjected to the same surgeries as OVX animals; however, the ovaries were not excised after opening the abdominal cavity. These animals received only vehicle infusion (SHAM-saline, n=4). Four weeks after the surgery, OVX-saline and OVX-antagonist daily food intake (58 grams) and average weight gain after 4 weeks (74 grams) were significantly greater than SHAM-saline (23 and 21 grams respectively; p<0.05). Four (4) weeks following the OVX surgery, the blood pressure of OVX-saline rats was significantly higher than SHAM-saline (106±2 mmHg vs. 96±2 mmHg respectively, P=0.0017), confirming the menopausal-induced hypertension model. The blood pressure of OVX-antagonist rats was similar to SHAM-saline (102±1 mmHg, P>0.05). To determine if the change in blood pressure in this model was due to differential activation of the sympathetic nervous system, we tested the blood pressure response to an acute injection of hexamethonium (30µg/kg, IP). The drop in blood pressure was significantly greater in OVX-saline animals, as compared to OVX-antagonist (-41±2 mmHg vs. -30±3 mmHg, P=0.021), which suggests an enhanced basal sympathetic tone in menopausal-induced hypertension due to leptin receptor activation. Taken together, these data suggest leptin, acting on its receptors in the central nervous system, modulates sympathetic nervous system activity and augments blood pressure in menopausal-induced hypertension.

The Iowa Osteopathic Educational Research Foundation supported this work.

3.4 TIME COURSE ASSESSMENT OF ACUTE AND CHRONIC HYPTONIC BRAIN EDEMA BY DYNAMIC NMR: TOPOGRAPHICAL AND GENDER DIFFERENCES
Marta Tejedor, Alberto Lázaro, Alejandro Rojo, Lorena Cussó, Marian González-Nicolás, Jorge J García Seoane, Meritxell López, Alberto Tejedor

Introduction and Aims: Hypotonic brain edema has not been studied in depth with imaging techniques such as dynamic NMR. Aims: to assess spatial, temporal and gender differences in brain responses to hypotonicity following acute and chronic hyponatremia induction.

Methods: Chronic hyponatremia was induced in Wistar rats and BALBc mice by intraperitoneal (ip) injection of desmopressin (0.4 µg/kg/d), hyposodic liquid diet and free access to water for 7 days (G1). A group of control normonatremic animals was fed with pellet based diet (G2). Brain edema was studied in real time with NMR at baseline and after ip injection of either 10% of body weight in water (both G1 and G2) or 2 mL of NaCl3% for every 100 grams of body weight (G1) over 120 minutes. ADC (apparent diffusion coefficient) assessed the degree of intracellular water content in different regions of interest: cortex, hypothalamus, nervous fibers, extrapyramidal system.

Results: Baseline [Na] were 136.75±1.59mmol/L (G2) and 136±0.94mmol/L (G1); after acute water load: 112±5mmol/L and 119±5mmol/L respectively. Baseline ADC values were lower in G1, indicating relevant brain edema. After acute water load, a further drop in the ADC levels was observed in both control and SIADH group. Such ADC reduction started earlier in females. A transient period of cellular defense where water was actively pumped outside the cells was observed, being more efficient in G1, becoming the ADC levels similar in both

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groups at 60 min, but worsening again at 90 and 120 min. This defense was less intense in females and the final ADC value was lower than that observed in males. Lateral hypothalamus was the first region to become edematous, followed by the cortex, and then, at different time points, by the extra-pyramidal system and myelinated fibers. Response to edema appeared with different time delays from the maximum degree of edema, but it seemed to follow a structural order: hypothalamus, cortex, extrapyramidal system and fibers. Treatment of G1 with hypertonic saline (NaCl3%) induced a correction of the edema that was three times faster than the spontaneous one, and extremely delayed in neural fiber regions. Sodium concentrations went from 136±0.94mmol/L to 140±7mmol/L.

Conclusions: Brain response to hypotonicity is not homogeneous, and edema develops at different time points in different regions. The time course of the response to brain edema is not homogeneous either. Different speed in the response to brain edema in adjacent areas suggests that damage leading to central pontine myelinolysis could be earlier than observed in clinical practice. Male and female response to brain edema is significantly different and should be considered when assessing this condition in the clinical practice.

3.5 ESTROGEN RECEPTOR ALPHA CONTRIBUTES TO THE INHIBITORY ACTION OF CENTRAL CYTOCHROME P4501B1 GENERATED 17β ESTRADIOL METABOLITE 2-METHOXYESTRADIOL TO ANGIOTENSIN II-INDUCED HYPERTENSION

Purnima Singh1, Chi Young Song1, Shubha Ranjan Dutta1,2, Scott A. Heldt3, Kafait U. Malik1
1Pharmacology, University of Tennessee Health Science Center, 2Anatomy and Neurobiology, University of Tennessee Health Science Center

Hypertension in postmenopausal females is attributed to diminished levels of 17β-estradiol (E2). It has been documented that in various experimental models of hypertension including angiotensin (Ang) II, the protection against hypertension is lost in females following ovariectomy (OVX). Ang II produces hypertension by its action on estrogen receptor alpha (ERα). Cytochrome P4501B1 (CYP1B1) and catechol-O-methyltransferase (COMT) that sequentially metabolize E2 to 2-methoxyestradiol (2-ME) are expressed in the brain. This study was performed to test the hypothesis that 2-ME mediates the inhibitory effect of E2 on Ang II-induced hypertension via ERα in the brain of female mice. Intracerebroventricularly (ICV) administered E2 (1.5µg/2µL/every 2nd day) in OVX wild-type (Cyp1b1+/+) mice attenuated the Ang II (700 ng/kg/min, 14 days)-induced increase in mean arterial pressure (MAP) measured by radiotelemetry, but not in the mice injected with COMT siRNA (ICV, 0.4 nmol) (108±2 vs. 144±3, n=5-6, P<0.05). ICV injections of 2-ME (1.5 µg/2µL/every 2nd day) but not E2 attenuated the increase in MAP by Ang II in OVX Cyp1b1−/− mice (112±1 vs 142±5, n=5-6, P<0.05); this effect was minimized in the mice by ICV injected ERα siRNA (0.4 nmol; 137±9). Power spectral analysis of the data on day 12 showed that Ang II-infusion increased the low to high frequency ratio of heart rate variability, index of sympathetic outflow modulation in ICV E2 injected OVX Cyp1b1−/− compared to OVX Cyp1b1+/− mice (2.6±0.1 vs 1.6±0.1) or ICV 2-ME injected OVX Cyp1b1−/− mice (1.6±0.1); these effects were blunted by ICV injected COMT siRNA (3.0±0.4) or ERα siRNA (2.6±0.4).

Administration of ganglionic blocker hexamethonium (30 mg/Kg, IP) on day 14 of Ang II infusion resulted in greater reduction in MAP in OVX Cyp1b1−/− than OVX Cyp1b1+/− mice injected ICV with E2 (Δ89±7 vs Δ64±3, mmHg) or 2-ME in OVX Cyp1b1−/− mice (Δ60±3 mmHg); these effects were attenuated in mice by ICV injected COMT siRNA (Δ84±7 mmHg) or ERα siRNA (Δ84±9, mmHg). Furthermore, in the intact Cyp1b1+/− female mice, adenovirus (Ad)-CYP1B1 shRNA but not Ad-scrambled (scr) shRNA (ICV, 2µL, 1.3X1013 particles/mL) potentiated Ang II-induced increase in systolic blood pressure (SBP, mm Hg) measured by tail-cuff method (170±4 vs 141±3, n=8-9, P<0.05). In the intact Cyp1b1−/−, but not OVX Cyp1b1−/− mice, reconstitution of CYP1B1 in the brain by transduction with Ad-CYP1B1-DNA (ICV, 2µL, 1.0 X 1013 particles/mL) reduced Ang II-induced increase in SBP (135±1 vs 167±6, n=7-10, P<0.05). These data provide the first evidence that 1) central effect of E2 to attenuate Ang II-induced hypertension is dependent on brain CYP1B1 and is most likely mediated via generation of 2-ME, and 2) 2-ME protects against Ang II induced hypertension by acting through ERα in the brain by reducing sympathetic outflow. Also, deoxycorticosterone acetate (DOCA, 50 mg/kg) salt (1% NaCl in drinking water) in uni-nephrectomized Cyp1b1−/− female mice at 4 weeks caused greater increase in SBP than in Cyp1b1−/− mice (165±6 vs. 133±4, n=4, P<0.05). In the intact Cyp1b1−/− female mice, reconstitution of CYP1B1 in the brain by transduction with Ad-CYP1B1-DNA but not its control Ad-GFP-DNA reduced DOCA salt-induced increase in SBP (133±3 vs 169±7, n=5, P<0.05). These data suggest that the brain CYP1B1 also protects against DOCA salt-induced hypertension in female mice. Whether this protection is mediated via DOCA-salt effect on brain Ang II, metabolism of E2 or ERα remains to be determined. The significance of this study is that the hormone replacement therapy in the USA have failed to lower BP or decrease cardiovascular disease in postmenopausal females. Therefore, 2-ME could be useful in treating hypertension in postmenopausal and hypoestrogenic premenopausal females or in those with menstrual
because of ovarian failure. This study was supported by NIH- HLBI Grant 19134-43.

3.6 SEX-DEPENDENT EFFECTS OF PREDIABETES IN MOUSE MODELS OF ALZHEIMER’S DISEASE AND MIXED DEMENTIA

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1Neuroscience & Experimental Therapeutics, Albany Medical College

Alzheimer’s Disease (AD) and vascular dementia are the two most common forms of dementia, and it has been estimated that 60% of individuals with AD have underlying cerebrovascular pathology/mixed dementia (MxD). Diabetes increases the risk for both vascular and non-vascular dementia (including AD). Less is known about the effects of prediabetes, insulin resistance and glucose intolerance in the absence of hyperglycemia. This is a critical gap in knowledge, as prediabetes is 3x more common than diabetes and affects 38% of Americans. Prediabetes has been linked to early indicators of dementia, including hippocampal atrophy and memory deficits. Despite strikingly high prevalence and rates of comorbidity, much less is known about the effects of prediabetes on vascular and AD pathology and symptomology, particularly when these pathologies co-occur. In addition, since females have higher rates of dementia and faster rates of decline in cognition, sex differences must be explored. Therefore, the aim of the current study was to determine whether sex differences in the effects of prediabetes (modeled by chronic high fat diet) exist in mouse models of AD and MD. Male and female 3xTg-AD mice received either sham (AD model) or right unilateral common carotid artery occlusion surgery (MxD model) at ~2.5 months of age; these were compared to wild-type (WT)/sham surgery (control) mice of both sexes. These metabolic deficits appear to be greater in females compared to males, and in 3xTg-AD mice regardless of surgery (AD and MxD models) compared to WT mice. HF diet did not affect novel object recognition performance in WT mice; however, most AD and MxD groups were impaired on this task. HF diet generally impaired spatial memory in the Morris water maze across mouse models, and MxD (regardless of diet) also hindered performance. This study will be critical for understanding sex differences in the effects of prediabetes on Alzheimer’s disease and mixed dementia, and may point to pathways that could be targeted to enhance functional outcomes.

3.7 DOCA-SALT HYPERTENSION LEADS TO NEUROCOGNITIVE DEFICIENCIES IN FEMALE SD RATS

Kasey Belanger 1, Ellen Gillis 1, Jennifer Sullivan 1

1Neurology, Augusta University

Current publications report younger hypertensive women have a greater risk of dementia later in life compared to their hypertensive male counterparts. To investigate the impact of hypertension in cognitive function in females, we measured neurocognitive function in a rat model that mimics the salt-sensitive hypertension observed in humans, the deoxycorticosterone acetate (DOCA)-salt hypertension model. In the current study, we tested the hypothesis that DOCA-salt hypertension results in neurocognitive deficiencies in females. Female Sprague Dawley (SD) rats (10-11 wks of age, n=6) were uni-nephrectomized and a subset of rats (n=3) were subcutaneously implanted with a DOCA pellet (200 mg/rat, 60-d time release) and given 0.9% NaCl to drink ad libitum. Uni-nephrectomized control rats did not receive a DOCA pellet and were given tap water to drink. Blood pressure (BP) was measured weekly via tail cuff plethysmography and cognitive function was assessed by the Y Maze Alternation test, prior to the implanting DOCA pellets as well as following a 4 week DOCA treatment. DOCA-salt treatment resulted in a significant increase in BP from baseline (Baseline: 133 ± 4 mmHg vs Week 4 183 ± 3 mmHg; p= <0.0001). BP did not change in UNX controls (Baseline: 133 ± 2 mmHg vs Week 4: 134 ± 4 mmHg). During the Y Maze Alternation test, rats are exposed to A and B arms for 10 minutes followed by a 15 minute break. Following the break, all rats are then allowed 3 minute access to arms A, B, and the novel arm C. During those 3 minutes, the number of times they consequently enter a different arm, and the total number of arm entries are used to calculate the percent of alternation, a commonly applied measure to quantify cognitive function. Percent of alternation significantly decreased in DOCA-salt rats (Baseline: 50% ± 10 vs Week 3: 30% ± 6; p=.027), but remained unchanged in UNX controls (Baseline: 48% ± 6 vs Week 3: 51% ± 5), suggesting that DOCA-salt hypertension led to cognitive decline. In conclusion, DOCA-salt hypertensive female rats display cognitive deficiencies in relation to the increase in BP. Future
studies will assess the mechanisms by which increases in BP alter cognitive function, including assessment of neuroinflammation, blood brain barrier permeability and the activated brain renin angiotensin system. Exploring sex specific mechanisms mediating hypertension and cognitive function could lead to novel therapy options to reduce the risks of cognitive impairments in early hypertensive populations.

3.8
SEX-SPECIFIC HORMONES PRODUCED DURING PROESTROUS AND ESTROUS EXERT A REGULATORY EFFECT ON THE NESFATIN-1 MRNA LEVELS AND AUTONOMIC FUNCTION IN FEMALE CYCLING RATS.
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Nesfatin-1/ NUCB2 is a protein derived from the nucleobindin-2 precursor originally localized in different appetite controlling areas of the brain, such as the hypothalamic PVN, ARC, SON, LHA and NTS, and is thought to be regulated in a sex-specific manner. It has recently been suggested that nesfatin-1 might play an important metabolic role during pregnancy and fetal development (2) and the expression of nesfatin-1/ NUCB2 is regulated by progesterone and 17β-estradiol in mouse pituitary gland (1). We investigated Nesfatin-1 mRNA expression in female, cycling rats across the estrous cycle and observed a decrease in Nesfatin-1 mRNA during Proestrous, corresponding to a period of increased levels of Estradiol. We therefore hypothesized that the ability of centrally administered Nesfatin-1 to raise mean arterial pressure (MAP) in conscious, cycling, freely moving female rats would no longer be seen during the Proestrous day of the estrous cycle. We monitored MAP changes across estrous cycle with central Nesfatin-1 administration. We found that the ability of Nesfatin-1 to increase MAP is no longer prevalent in female, cycling rats on proestrous and estrous days, suggesting that sex-specific hormones produced during proestrous and estrous exert a regulatory effect on the Nesfatin-1 mRNA levels and autonomic function.


3.9
CENTRAL ACETYLCHOLINESTERASE INHIBITOR, GALANTAMINE, PREVENTS LIPID-INDUCED OXIDATIVE STRESS IN AFRICAN AMERICAN WOMEN
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African American women (AAW) have one of the highest prevalence of hypertension in the US. Obese AAW have decreased parasympathetic (PNS) activity compared to whites. Continuous lipid infusion that causes cardiovascular autonomic imbalance (decrease in PNS and increase in sympathetic activity) induces a greater increase in oxidative stress in AA compared to whites. Considering that PNS protects against oxidation and that central acetylcholinesterase inhibitors have been shown to suppress oxidative stress in animal models. We tested the hypothesis that the central acetylcholinesterase inhibitor, galantamine attenuates oxidation in response to lipid infusion in obese AAW compared to white women. We randomized 14 healthy obese AAW (39.5±10.7 yo, BMI 38.8±3.4) and 10 (35.9±8.3 yo, BMI 36.3±2.1) white women. All subjects underwent 4-h infusions of Intralipids and heparin. On separate days subjects received either 16 mg galantamine or placebo in a crossover fashion. Lipid-induced oxidative stress and inflammation were assessed with plasma F2-isoprostanes and cytokines at baseline, 2 and 4-h post-intralipid infusion. In AA, 16 mg of galantamine significantly suppressed the increase in lipid-induced oxidative stress (10±18 vs. -3.0±12 pg/mL with galantamine, P=0.014). No effect was noted in whites. Galantamine tended to increase IL10 (4.8±7.58 vs. 17.3±20.7 pg/mL with galantamine, P=0.06). We did not observe any effect on blood pressure or heart rate. Conclusion: Increased parasympathetic tone with central acetylcholinesterase inhibitor, galantamine, suppressed lipid-induced oxidative stress in African American women.
3.10
ANGIOTENSIN TYPE 2 RECEPTOR STIMULATION WITH COMPOUND 21 IMPROVES STROKE OUTCOME IN FEMALE RATS: POSSIBLE ROLE FOR PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA
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Introduction: The angiotensin type 2 receptor (AT2R) agonist, compound 21 (C21), has been shown to be neurovascular protective after stroke in male rats. Here we aimed to study the effect of C21 treatment on female rats after stroke.

Methods: Young female intact Wistar rats (10-12 weeks) in the metestrus/diestrus phases of estrus cycle (low endogenous estrogen) were subjected to 3 h middle cerebral artery occlusion (MCAO) using a silicone-coated monofilament and treated at reperfusion with IP C21 0.03 mg/kg. Bederson and paw grasp tests were performed at 24 h and animals were sacrificed for infarct size analysis. Another cohort of ovarioctomized (OVX) and intact female rats were subjected to 1 h of MCAO and treated with IP C21 0.03 mg/kg at reperfusion and daily for 72 h. Bederson test was performed at 24 h and 72 h and animals were sacrificed and whole brain collected for infarct size analysis and western blotting.

Results (mean±SE): At 24 h, C21 treatment in females resulted in significant decrease in infarct size (22±4% vs 34±2%, p value= 0.03) and improvement in Bederson (2.1±0.1 vs 2.6±0.1, p value= 0.03) and Paw grasp (2.7±0.3 vs 1.5±0.3, p value= 0.01) scores. Interestingly, C21 treatment showed a trend toward increased expression of the transcription factor peroxisome proliferator-activated receptor γ (PPARγ) at 72 h in sham and stroked animals suggesting a novel crosstalk between the AT2R and PPARγ after ischemic stroke. In addition, the expression of the AT2R was significantly higher in female ECs compared to male ECs.

Conclusions: Compound 21 improves stroke outcome in female rats probably through increased expression of PPARγ and the AT2R.

Funding: R21 NS083559; VA Merit BX000891; Jowdy Foundation and Distinguished Research Professorship to SCF.

3.11
OVARIECTOMY INDUCES ANXIETY-LIKE BEHAVIOR AND SHORT-TERM RECOGNITION MEMORY IMPAIRMENT
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Introduction: Low levels of ovarian hormones as a result of ovarian follicle failure in menopause decrease the estrogen supply in the brain. Important brain areas involved in the modulation of mood behavior and cognition, including amygdala and hippocampus, express a high density of estrogen receptors, suggesting estrogen deficiency could lead to anxiety development and memory impairments. Taking that into account, the aim of this study was to assess the effect of ovarian hormone loss on anxiety-like behavior, and spatial and recognition short-term memory in ovarioctomized rats.

Methods: Three month old (mo) female Long Evans rats were ovarioctomized (OVX) group or sham-operated (Sham group). Five weeks after surgery, the animals were exposed to a battery of behavioral tests including elevated plus maze (EPM), open field (OF), novel object recognition (NOR) and 12 arms radial maze (RAM) tests. The behavior protocol was performed during 18 days with two days of interval between each test.

Results: Our results found that OVX group exhibited increased anxiety-like behavior showing a lower percentage of time spent in the open arms (p=0.0278, Student t test) and the number of entries in the open arms (p=0.0044, Student t test) of EPM in comparison to Sham group. Additionally, OVX group spent less time exploring the center area of the OF than Sham group (p=0.0044, Student t test), reinforcing the anxiety response in OVX group. Also, OVX group showed reduced time exploring the novel object in the NOR test compared to Sham group (p=0.0131, Student t test), indicating an impairment of short-term recognition memory. In contrast, no difference was found in the short-term spatial memory in the RAM test.

Conclusion: Estrogen deprivation caused by ovarioctomy induces anxiety-like behavior and decreased short-term recognition memory, but not short-term spatial memory.
SEX DIFFERENCES IN HEALTHY HUMAN HEART REVEALED BY CAP ANALYSIS GENE EXPRESSION (CAGE)

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Background: Cardiovascular diseases remain the primary cause of death worldwide. Several epidemiologic and investigative studies have shown the evidence of sex hormones affect in cardiac electrophysiology through genomic effects (ion-channels expression) and non-genomic effects (ion-channels function modulation). However, very little is known about the molecular basis for gender-related discrepancies in cardiac electrophysiology. Due to physiologically distinct functions of atria (electrical impulse initiation) and ventricles (blood pumping), there is a difference in ion-channel expression within a heart that causes different disease susceptibilities between both sexes.

Objective: Compare ion channel gene expression associated with sex differences using CAGE analysis on left atrial (LA) and left ventricular (LV) human donor hearts.

Materials and Methods: Total RNA was extracted from left atria (LA) and left ventricle (LV) of human donor hearts n=4 males (mean age = 53.75) and n=3 females (mean age = 58.7), the cause of death was determined to be non-cardiogenic. Samples were analyzed with CAGE, which is high throughput method for transcriptome analysis that utilizes ‘cap-trapping’. The number of tags gives a frequency of usage that provides information about transcription start sites as well as transcript expression levels. Normalization of raw CAGE tag count was performed as counts per million. The two-sample t-test was used to determine statistical significance.

Results and Discussion: Our results confirmed higher expression of SCN5A in ventricles compared to atria. SCN5A gene encodes Nav1.5 channel α-subunit (I₅Na) which is more abundant in working myocardium compared to nodal cells. Loss of sodium channel function is associated with Brugada phenotype that is more predominant in males. Indeed, males had a lower expression of the gene that might indicate on their higher predisposition to the disease. KCND3 that encodes for outward current potassium voltage-gated channel (IₒK) that is the main contributing current to repolarizing phase 1 of the cardiac action potential had a significantly higher expression in female atria. KCNIP2, voltage-gated potassium channel (IₒK) maintains early repolarization. During heart failure it augments C₅a,1.2 (CACNA1C) and K₅,4,3 (KCND3). Since the hearts were healthy, high expression of KCNIP2 in atria did not affect the expression of KCND3 and CACNA1C. Estrogen is known to up-regulate CACNA1C which was indeed found in female hearts although not statistically significant for our postmenopausal female group. Mutations in potassium-channel genes, KCNH2 (I₆K) and KCNQ1 (I₅K), have been associated with Long QT syndrome type 2 and 1 respectively. Our results for these two ions channels did not show a statistically significant difference in expression across sexes. Interestingly, gene for inward rectifier potassium channel (IₒK), KCNJ3, that plays an important role in heartbeat generation is up-regulated in atria, the region with pacemaker cells. Another inward-rectifier potassium channel (I₅KACH), KCNJ2 is up-regulated in ventricles. That demonstrates ion channel specificity to the anatomical areas of the heart and might carry a unique electrophysiological function.

Conclusion: In the past decade there has been a push towards sex-specific drug development since it has been established that medications affect males and females differently. Our study reveals that there are sex-dependent gene expression differences in cardiac ion channels and that CAGE approach allows high-throughput gene expression profiling which can be beneficial for gender-specific drug development and personalized medicine overall. Supported by NIH 5R01HL114395.

COMPARISON OF MEASURES OF COMPENSATORY RESERVE IN DIFFERENTIATING TOLERANCE TO SIMULATED HEMORRHAGE IN MALES VERSUS FEMALES

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Background: The physiological response to hemorrhage includes significant vasoconstriction to shunt blood to the heart and brain and subsequent cardiovascular collapse (shock). Lower body negative pressure (LBNP) induces central hypovolemia similar to hemorrhage. Healthy humans can either be high tolerant (HT) or low tolerant (LT) to hypovolemia during LBNP. The compensatory reserve (CRM) measures the sum total of all mechanisms that compensate for relative blood volume deficit by analyzing changes in photoplethysmographic (PPG) arterial waveform features. Decreased CRM with progressive reductions in central blood volume can provide a sensitive and specific status of an individual patient. The time to hemodynamic decompensation can be defined as tolerance, where decompensation occurs at 0% CRM. We previously reported that women have less responsiveness during reductions in central blood volume than men and that the female sex was a
Objective: We tested the hypothesis that the higher tolerance to progressive central hypovolemia observed in males can be explained by a slower rate of reduction in CRM compared with females.

Methods: Continuous, noninvasive measures of CRM were collected from 208 healthy volunteer subjects (110 male, 98 female; mean age 27.3 ± 8 years; mean height 171 ± 11 cm; mean weight 73.3 ± 16 kg; mean BMI 24.9 ± 4.4; 143 HT, 65 LT) before and during a progressively stepwise LBNP protocol to the point of presyncope. Tolerance group was defined by those subjects who did (HT) or did not (LT) complete the stepwise protocol to 60 mmHg LBNP. Data were analyzed at equal CRM (60%, 30%, and 0%) using generalized estimating equations (GEE) to account for the repeated measures design.

Results: Comparisons of equal compensatory reserve in male and female subjects revealed mean ± 1 SE times were slower in male subjects compared to female subjects at 60% CRM (13.4 ± 0.4 vs. 13.2 ± 0.3 min; p = 0.660), 30% CRM (21.3 ± 0.4 vs. 19.7 ± 0.4 min; p < 0.01), and 0% [decompensation] (30.1 ± 0.6 vs. 26.4 ± 0.5 min; p < 0.01). HT female subjects (n = 61) had slower times than LT male subjects (n = 28) required to reach 0% CRM [decompensation] (29.0 ± 0.4 vs. 23.6 ± 0.6 min; p < 0.01).

Conclusion: Consistent with our hypothesis, male subjects had higher overall tolerance than female subjects, coincident with a significantly slower rate of reduction in CRM at 30% and 0% CRM. Additionally, HT female subjects required more time to reach 0% CRM (decompensation) compared to LT males, highlighting that the difference in tolerance between these gender cohorts can be explained by the rate of compensatory reserve depletion. While sex differences have been previously reported as a predictor of LBNP tolerance, we report for the first time the comparison of differentiating tolerance in males compared to females based on measures of the capacity to compensate. The results of the current study indicate that differences between males and females in tolerance to central hypovolemia can be explained by a more rapid depletion of the compensatory reserve in women.

Funding: Funding was provided by an appointment to the Post-doctoral research fellowship program administered by the Oak Ridge Institute for Science and Education and a grant from the US Army Combat Casualty Care Research Program (D-009-2014-USAISR).

3.14 COMPARING TIME TO PRESYNCOPE DURING SIMULATED HEMORRHAGE ACROSS MENSTRUAL CYCLE
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Background: Several studies indicate that females have lower orthostatic tolerance compared to males. The mechanisms that contribute to the evident sex differences are not well understood. Multiple characteristics including sex hormones appear to contribute to low tolerance. Menstrual cycle and concurrent hormonal fluctuations are particularly relevant to understanding why females have lower tolerance to central hypovolemia. A number of studies designed to investigate contributions of ovarian hormones (particularly estradiol) to cardiovascular function have generated conflicting findings. Furthermore, there is paucity in the literature on contribution of the anterior pituitary hormones, luteinizing (LH) and follicle-stimulating hormones (FSH), which are elevated during the ovulatory phase of the menstrual cycle.

Objective: In our current study, we induced central hypovolemia similar to hemorrhage using progressively stepwise lower body negative pressure (LBNP) in women during various phases of the menstrual cycle. We tested the hypothesis that less time would be required to manifest presyncope in women during the follicular and luteal phases of the menstrual cycle when estradiol is at its highest levels compared to the ovulatory phase.

Methods: In this cross-sectional experimental design, LBNP application was induced on healthy volunteer female subjects (n=22, mean age 26.8 ± 6 years; mean weight 63.9 ± 9 kg; mean BMI 23.7 ± 2.7; mean height 164.3± 8.2 cm). All subjects were not taking oral contraceptives. Time to presyncope was calculated from start of baseline to termination of LBNP at three menstrual cycle phases; early follicular (Days 1 – 7; n=10), ovulatory (Days 12 – 16, n=5) and mid luteal (Days 20-26, n=7). Data are presented as mean ± SEM. The probability that any differences in LBNP tolerance across menstrual cycle did not exist by greater than chance were determined by ANOVA and expressed as exact ‘p’ values.

Results: The average time to presyncope was lower for females in the ovulatory phase (1254.2 ± 92 seconds) compared to those in early follicular (1613 ± 48 seconds; p = 0.013) and mid luteal (1496 ± 80 seconds; p = 0.099) phases. Time to presyncope for females in early follicular and mid luteal phases were marginally similar.
Conclusion: A major finding in this study is the lower tolerance to LBNP in the ovulatory phase of the menstrual cycle. Contrary to our hypothesis, the ovulatory phase of the menstrual cycle that is characterized by elevated LH and FSH with reduced levels of estradiol and progesterone is associated with lower tolerance to central hypovolemia. As such, our findings indicate that estradiol may not contribute to compromised tolerance to central hypovolemia in women given that its highest levels are before the ovulatory phase and during the luteal phase of the menstrual cycle. Such a hypothesis may be tested by subsequent cross-sectional comparisons of females with and without use of contraceptives.

Funding: Funding was provided by an appointment to the Post-doctoral research fellowship program, administered by the Oak Ridge Institute for Science and Education and a grant from the US Army Combat Casualty Care Research Program (D-009-2014-USAISR).

3.15
STRESS HORMONE INHIBITION OF ESTROGEN TRANSCRIPTIONAL REGULATION OF THE SEROTONIN SIGNALING IN CARDIOMYOCYTES IS DELETERIOUS FOR THE FEMALE HEART IN MYOCARDIAL INFARCTION.

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Exposure to chronic stress is considered a risk factor for myocardial infarction (MI). Despite the fact that estrogen exerts cardioprotective effects, pre-menopausal women (25 to 40 years of age) are more susceptible to stress-induced MI than similarly aged men. These observed gender-specific effects of stress in MI may be partly attributed to the interactions between estrogen and glucocorticoids (the primary stress hormones). The goal of the present study is to investigate the effects of the combined actions of estrogen and glucocorticoids in cardiomyocytes. Our genome-wide studies show that glucocorticoids inhibit estrogen-mediated regulation of genes with established roles in cardiomyocyte homeostasis, including the cardiac serotonin receptor 5-HT2BR. Serotonin signaling via cardiac 5-HT2BR is critical to prevent mitochondrial dysfunction and cardiomyocyte apoptosis in the adult heart. Selective serotonin reuptake inhibitors (SSRIs) block the reabsorption of serotonin in the brain, making more serotonin available to other organs, including the heart. The use of SSRIs is associated with reduced risk of MI; however, the mechanisms underlying this protection are unknown. Immunohistochemistry for human heart slides of patients who died of MI shows higher expression of 5-HT2BR in females than in males. Therefore, we hypothesize that estrogen cardioprotective effects in the heart are mediated in part by serotonin signaling via the cardiomyocyte 5-HT2BR, and this protection is blocked by glucocorticoids. Our data show that treating cardiomyocytes with estrogen significantly up-regulated 5-HT2BR gene expression and protein levels, whereas co-treatment with glucocorticoids inhibited these effects. Supporting these results, treatment with glucocorticoids in vivo repressed estrogen up-regulation of 5-HT2BR expression in hearts from wild-type mice. Using siRNA, gene expression, and chromatin immunoprecipitation (ChIP) assays, we found that 5-HT2BR is a primary target of the glucocorticoid receptor (GR) and the estrogen receptor (ER)-α at the level of transcription. Ligand-bound GR blocks the recruitment of ER-α to the promoter of the 5-HT2BR gene, which may contribute to the adverse effects of stress in the heart of pre-menopausal women. In the context of MI, our data show that glucocorticoid co-treatment with estrogen depressed the expression of 5-HT2BR which lead to a poorer outcome in hearts of female mice challenged with ischemia-reperfusion injury. These findings suggest that increased stress levels in pre-menopausal women promote a negative outcome in MI by blocking estrogen up-regulation of the 5-HT2BR, which results in the inhibition of serotonin signaling cardioprotective effects. Funding for this study was provided by the Research Council and Center for Cardiovascular Diseases and Sciences LSU Health Shreveport.

3.16
HEART FAILURE WITH PRESERVED EJECTION FRACTION (HFpEF) IS AUGMENTED IN OBESE MICE WITH AN XX SEX CHROMOSOME COMPLEMENT

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Objective: Heart failure with preserved ejection fraction (HFpEF) accounts for at least 50% of cardiomyopathies associated with left ventricular dysfunction. HFpEF is associated with diastolic hypertension, atrial fibrillation, obesity, age, and female sex. Alarmingly, given its high prevalence, there are no effective therapies for HFpEF and few experimental models that exhibit features of the human disease. Since female sex is a risk factor for HFpEF, we used a novel murine model (the four core genotypes) that enables dissection of the relative contributions of sex hormones and sex chromosomes. We also incorporated diet-induced obesity and advancing age as risk factors for HFpEF into the experimental design.
Methods and Results: Male (M, XY and XX) and female (F, XX and XY) mice (6 months of age) were fed a 60% high-fat (HF) diet for 6 months. Carotid artery catheters and radiotelemeters were implanted at week 22, mice were allowed to recover for 1 week, and then blood pressure was recorded for 7 days. Echocardiography was performed by ultrasound under anesthesia at study endpoint. There was a significant effect of sex (M>F) and genotype (XX>XY) on systolic (SBP) and diastolic blood pressures (DBP) of HF-fed mice. Within a sex, SBP and DBP were higher in XY than XX males, but a surprising opposite effect was observed in females (XX>XY SBP and DBP). Notably, left ventricular diastolic diameter (XX: M, 3.9 ± 0.1; F, 3.9 ± 0.1 mm; XY: M, 4.1 ± 0.08; F, 4.2 ± 0.06 mm; P<0.05) and volume were significantly decreased in XX compared to XY obese mice regardless of gonadal sex (P<.05). Consistent with indices of HFpEF, ejection fraction and fractional shortening were not different between groups. However, stroke volume was decreased in XX compared to XY obese mice, regardless of gonadal sex (XX: M, 35.3 ± 2; F, 35.9 ± 3 µl; XY: M, 44.7 ± 4.4; F, 39.6 ± 2.8 µl; P<0.05). Assesment of baroreceptor activity from blood pressure records indicated that baroreceptor slope (gain) and activity were impaired in XX mice, regardless of gonadal sex. Measurements of potential HFpEF biomarkers demonstrated increased serum total TGF-β in female mice with an XX sex chromosome complement (XX, 166 ± 20; XY, 116 ± 11 ng/ml; P<0.05). Studies evaluating cardiomyocyte size and assessing cardiac collagen are underway in hearts from XX versus XY mice. Conclusions: Using mice with differing sex chromosome complement, we have generated a murine model of HFpEF that exhibits features of the human disease, including obesity, female (XX) sex, advancing age, hypertension, diastolic stiffness, an impaired baroreceptor response, and higher serum TGF-β concentrations. Future studies will use this model to define potential therapeutic targets for HFpEF. Funding for this project was provided by NIH HL73085 and HL107326 (LAC) and P20RR021954 (COBRE, LAC)

3.17 AMP-ACTIVATED PROTEIN KINASE AND ESTROGEN-DEPENDENT MECHANISMS UNDERLYING INCREASED SUSCEPTIBILITY TO CARDIOVASCULAR DISEASE DURING MENOPAUSE

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Introduction: Premenopausal women are protected against heart and cardiovascular disease (CVD) compared to age-matched men. The cellular and molecular mechanisms underlying the transition from premenopause/perimenopause (CVD-resistance) to postmenopause (CVD-susceptible) in women is unknown and is the focus of this project. The critical barrier impeding translational progress is the lack of appropriate models to study menopause. Most studies have used surgical removal of ovaries as a model of menopause. We overcome this barrier with the 4-vinylcyclohexene diepoxide (VCD) model of menopause, which mirrors progressive ovarian failure and preserve the critical “perimenopause” transitional period. Using this model, we demonstrate that perimenopausal, like cycling (premenopausal) females, are protected from pathological angiotensin II (Ang II)-induced cardiac remodeling, while menopausal females are not. Multiple molecular, genetic and cellular mechanisms have been suggested to underlie protection against CVD in non-cycling females, many of which put estrogen as the key mediator. We discovered and interaction between adenosine monophosphate-activated kinase (AMPK) signaling axis and estrogen that is potentially responsible for this cardioprotection before onset of menopause. We hypothesized that the loss of AMPK signaling is responsible for the exacerbated pathological cardiac remodeling in menopause.

Methods: A gradual transition to menopause was induced by repeated daily injections of VCD (160 mg/kg). Ang II (800ng/kg*min) was infused into perimenopausal and menopausal females for 14 days. A separate cohort of mice received AMPK activator A769662 (30mg/kg) during perimenopause and menopause. We aimed to identify whether AMPK is necessary to mitigate pathological cardiac remodeling in menopausal females. Left ventricle heart tissue was flash frozen and processed for westerns, histology and quantitative proteomics. For proteomics, gene ontology and expression changes were quantified using mus musculus (mouse) genome libraries in Perseus software. DAVID was utilized to discover pathway specific changes across treated groups. To validate proteome across pre-, peri-, and menopausal females, biomarkers from the identified KEGG pathways were validated with immunoblotting of AMPK signaling proteins. Additionally, histological staining validated fibrosis and structure of the heart during menopause.

Results: Quantitative proteomics provided a 5,370 proteome. Using a 3-way-ANOVA and arcine function transformation, 348 proteins revealed unique alterations in expression. A series of heat maps determined cycling and perimenopausal females to have similar expression profiles, while menopausal females had the opposite expression levels. Proteins expressed in menopausal females were further identified in DAVID and revealed that significant (p*<0.05) fold changes in the KEGG pathways “complement coagulation cascade” and “AMPK” were changing. Perimenopausal females also had increased levels of threonine 172 and acetyl-coa-
carboxylase phosphorylation compared to menopausal females, indicating that AMPK activation and signaling is playing a crucial role in mitigating susceptibility to heart disease during menopause. This was further validated by the quantification of fibrosis in the menopausal hearts.

**Conclusions:** Using proteomics and westerns we determined potential cellular and molecular mechanisms in premenopausal and perimenopausal females that prevents associated cardiovascular morbidities; and started to reveal the underlying shift that gives rise to increased susceptibility to CVD in menopausal females. This work was supported by the NIH grant HL098256, the American Heart Association 16GRNT31390006, and a training grant in cardiovascular sciences ST22HL00795515.

### 3.18

**BETA-CAROTENE METABOLISM IN THE MATERNAL HEART DURING PREGNANCY**

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Pregnancy-related deaths associated with cardiovascular diseases have recently increased in the U.S. with a higher occurrence among African American and Hispanic women. Low intake of fruits and vegetables, the main source of vitamin A, is a hallmark of poor nutrition that is manifested in these populations that are mainly affected by gestational cardiac complications. Many signaling pathways have been associated with the physiological hypertrophy (remodeling) of the heart that occurs during pregnancy. However, how these pathways are activated and influenced during pregnancy has yet to be fully understood. Retinoic acid, the active form of vitamin A that functions as a transcriptional regulator, has been implicated in cardiac remodeling in the adult but whether or not retinoids (vitamin A and its derivatives) are essential during the cardiac hypertrophy of pregnancy is still unknown. Preliminary data from our laboratory revealed a small but significant decrease in Dhrs3 (Dehydrogenase reductase 3) and Lrat (Lecithin:retinol acyltransferase) in the heart of pregnant wild-type mice at 14.5 dpc, suggesting that during gestation, heart retinoid metabolism may be shunted towards retinoic acid formation rather than storage. Thus, if cardiac retinoid acid synthesis is favored in wild-type mice during pregnancy, the active vitamin A metabolite may play a potential role in sustaining maternal cardiac hypertrophy (remodeling). Beta-carotene, the most abundant dietary precursor of vitamin A, can be cleaved asymmetrically by the mitochondrial beta-carotene 9',10'-oxygenase (BCO2) to generate beta-apo-10’carotenal, which can serve as a precursor of retinoids, but may also antagonize retinoid acid action per se. Preliminary observations in our lab indicate that cardiac mRNA levels of BCO2, which is the only carotenoid cleavage enzyme expressed in the adult mammalian heart, were elevated in wild-type pregnant mice at mid-gestation. Moreover, in the absence of BCO2 (Bco2⁻/⁻ mice) the maternal heart failed to enlarge during pregnancy. Notably during pregnancy, the heart of Bco2⁻/⁻ mice showed significantly reduced retinyl ester levels compared to wild-type pregnant mice. Based on these premises, we hypothesized that BCO2 may contribute to the physiological hypertrophy of the maternal heart during pregnancy. Current studies aim at further understanding the effects of the lack of murine BCO2 on retinoid metabolism and cardiac function in the maternal heart. Understanding the role of carotenoid and retinoid metabolism in this process will allow us to ultimately design dietary preventative measures to potentially decrease adverse cardiac function during pregnancy.


### 3.19

**RESPONSES IN CARDIAC STROKE VOLUME INDEX AND CARDIAC INDEX DO NOT DIFFER BETWEEN GENDERS DURING MODERATE CENTRAL HYPOVOLEMIA**

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**Introduction:** Gender-specific differences in physiological regulation are of growing interest within both research and clinical treatment. Men have been found to have a greater cardiovascular reserve than women, and female sex has been proposed as a predictor of lower tolerance to hypovolemia (Hinojosa-Labarbe et al. 2015). In this study, we hypothesized that females have a greater relative decrease in cardiac stroke volume index (SVI)
and in cardiac index (CI) during simulated hypovolemia, compared to men.

**Methods:** Seventeen females (median (range) age 21 yrs (21–22)) and Body Surface Area (BSA) (=0.000975482xweight^{0.46}xheight^{1.06}) (Schlich et al. 2010) 1.7 m² (1.5 – 2) and fourteen males (age 23 yrs (21–27) and BSA (=0.000579479xweight^{0.38}xheight^{1.24}) 1.9 m² (1.6 – 2.1) underwent experimental central hypovolemia induced by lower body negative pressure (LBNP) to ~30 mmHg. The study was approved by the regional ethics committee (ref.no: 2012/2251 and 2014/2228) and conforms to the Declaration of Helsinki. Heart rate (HR) was obtained from the duration of the R-R interval from a three-lead ECG signal. Non-invasive finger arterial pressure was recorded continuously from the left middle finger, providing beat-to-beat mean arterial pressure (MAP) and beat-to-beat left cardiac stroke volume (SV) (Finometer, FMS, the Netherlands). Cardiac output (CO) was calculated from HR and SV. SVI and CI were calculated by dividing SV and CO by BSA. Medians and 95% confidence intervals were calculated by Hodges-Lehmann’s estimates. Wilcoxon signed rank sum test for paired samples was used to test differences between conditions. Unpaired Wilcoxon test was used to assess gender differences. P<0.05 was considered significant.

**Results:** In the female group, HR increased by 10% from rest (56 bpm (51, 59)) to LBNP (62 bpm (57, 65)); SVI decreased by 18% from rest (45 ml/m² (40, 48)) to LBNP (37 ml/m² (31, 41)); CI decreased by 9% (p<0.001) from rest (2.5 l/min/m² (2.1, 2.8)) to LBNP (2.3/(min/m²) (1.8, 2.5)); MAP did not change from rest (73 mmHg (68, 75)) to LBNP (74 mmHg (71, 77)). P<0.001.

In the male group, HR increased by 13% from rest (58 bpm (52, 62)) to LBNP (66 bpm (57, 70)); MAP increased by +6% from rest (70 mmHg (66, 73)) to LBNP (75 mmHg (70, 78)); SVI decreased by 19% from rest (52 ml/m² (47, 56)) to LBNP (42 ml/m² (36, 47)); CI decreased by 10 % from rest (3.1 l/(min/m²) (2.8, 3.3)) to LBNP (2.8 l/(min/m²) (2.5, 3)). P<0.001.

Percentage change in HR, MAP, SVI and CI did not differ between female and male groups (P>0.3).

**Discussion and Conclusions:** We could not detect gender differences in the relative cardiovascular responses to moderate hypovolemia in these 31 subjects. Others have reported differences in cardiovascular responses between men and women experiencing decompensated hypovolemia. Thus, other factors (difference in body size, blood volume, hormonal differences) may explain why women reach decompensated hypovolemia sooner than men.

Funding: The University of Oslo and the Research Council of Norway (Grant 230354).


### 3.20

**SEX DIFFERENCES IN THE EFFECTS OF PREDIABETES ON VASCULAR CONTRIBUTIONS TO DEMENTIA**

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**Background:** Diabetes causes endothelial dysfunction and is a major risk factor for vascular contributions to cognitive impairment and dementia (VCID). Diabetic women have increased risk of VCID compared to diabetic men. Data on the effects of prediabetes, which is 3 times more common than type 2 diabetes, are lacking. Prediabetes can be modeled in mice via long-term administration of a high fat (HF) diet, which we have shown causes cognitive deficits. Young females are protected from the metabolic effects of the diet. We hypothesized the middle-aged females would lose their protection, relative to males, from the metabolic and cognitive effects of a HF diet in a mouse model of VCID.

**Methods:** Middle-aged (8.5 month old) male and female C57BL/6J mice were placed on a HF or control diet for 6.5 months (15 months old at the end of the study). After 3 months on the diet, they received either VCID surgery (unilateral carotid artery occlusion) or sham surgery. Body weight and glucose tolerance were monitored. After 6 months on the diet, blood flow through the left carotid was measured via ultrasound and then cognitive function was assessed in the Morris water maze. Finally, blood flow was measured via laser speckle contrast imaging. Brain were also collected and we will be performing histology to examine neuroinflammation, white matter damage, and blood brain barrier damage.

**Results:** HF diet caused greater increases in body weight and glucose intolerance in females than males. Blood flow through the left carotid was reduced in HF-fed mice of both sexes. Cerebral blood flow was reduced in the right hemisphere following VCID surgery, with reductions being more severe in the HF female VCID mice. Spatial memory was impaired in VCID males, regardless of diet; however, in females, either HF diet or VCID (or a combination of the two) impaired spatial memory.

**Conclusions:** The data show that HF-fed/prediabetic middle-aged-females have more severe metabolic and cognitive deficits than prediabetic males. This is in line with the increased risk of VCID in diabetic women.
Polycystic Ovary Syndrome (PCOS), the most common endocrine disorder in women, is characterized by androgen excess and ovarian dysfunction. PCOS is often associated with components of metabolic syndrome (MS) such as obesity, dyslipidemia, insulin resistance (IR) and increased blood pressure (BP). Small clinical trials showed that liraglutide, a glucagon-like peptide-1 receptor agonist (GLP-1RA), caused significant weight loss in young women with PCOS. Additionally, liraglutide decreased IR and BP in reproductive age-PCOS rats.

Postmenopausal women who have had PCOS and chronic androgen excess may be at greater risk for MS and cardiovascular disease than normo-androgenemic postmenopausal women. It was also reported that Liraglutide decreases BP in simple obesity, but does not in obese women with PCOS. We have previously characterized a rat model of postmenopausal PCOS (PM-PCOS) in which chronic androgen excess causes features of MS: increased body weight, fat mass, dyslipidemia, IR, BP and activation of the renin-angiotensin system (RAS). In the present study, we tested the combination of GLP-1RA with RAS blockage as an effective therapeutic tool to treat the MS observed in PM-PCOS.

Methods: Female SD rats, 4 weeks-old, were randomized to chronically receive DHT (dihydrotestosterone, 7.5 mg/90 days) or placebo (n=18/group). At 17 months of age, PM-PCOS and age-matched placebo rats were randomized to consecutively receive: first, liraglutide (0.3 mg/kg/day SC) for 3 weeks, enalapril for 1 week and finally liraglutide+enalapril in combination for 1 additional week. Washout periods of 2 weeks were allowed between treatments. Mean arterial blood pressure (MAP) was recorded by radiotelemetry throughout the experiment. Before and after liraglutide treatment, body composition (by Echo-MRI) and components of the MS were measured.

Results: PM-PCOS rats exhibited significantly higher food intake, body weight, fat mass, total cholesterol, triglycerides, leptin and MAP compared to age-matched placebo rats. After 3 weeks of treatment, liraglutide caused greater reduction in cumulative food intake, body weight (-38.4 ± 4.7 vs -28.8 ± 2.4 g, p<0.05), fat mass/body weight (-7.7 ± 0.6 vs -4.8 ± 0.7, p<0.01), HOMA-IR index (-4.5 ± 0.6 vs -1.4 ± 1.1, p<0.05), leptin, cholesterol, and triglycerides levels in PM-PCOS.

Liraglutide decreased MAP in age-matched placebo rats (108 ± 1 vs 101 ± 1 mmHg, p<0.0001); however, it did not lower MAP in PM-PCOS animals (123 ± 2 vs 126 ± 3 mmHg, p=0.56). Enalapril completely abolished differences in MAP (96 ± 2 vs 99 ± 2 mmHg, p=0.47) between groups. Liraglutide+enalapril in combination further decreased MAP in age-matched placebo rats (96 ± 2 vs 86 ± 3 mmHg, p=0.03), but did not in PM-PCOS animals (102 ± 3 vs 100 ± 3 mmHg, p=0.65).

Summary: Although liraglutide exhibited beneficial effects in several components of the MS, it failed to lower MAP in PM-PCOS model. Enalapril completely abolished the differences in MAP between groups. Enalapril+liraglutide in combination did not further decrease MAP in PM-PCOS, in contrast to age-matched placebo. Our results suggest that RAS blockage in combination with liraglutide could be an effective therapeutic tool to treat all components of MS in PM-PCOS, including hypertension.

Funding: AHA 0830239N (L.L.Y.C.) and 12SDG8980032 (D.G.R.), EFFERG (L.L.Y.C.), and NIH R21 DK-113500 (D.G.R.), and P20 GM-121334 (L.L.Y.C.)

3.22 ANGIOTENSIN II INDUCES A PRO-INFLAMMATORY SHIFT IN THE SPLENIC CD4⁺ T CELL PROTEOME IN MENOPAUSAL MICE

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Premenopausal female mice are protected against Angiotensin II-induced hypertension, however this protection against Ang II-induced hypertension is lost following the onset of menopause. T cells are required for the development of Ang II hypertension in male mice and we have shown that premenopausal females are protected from T cell-mediated hypertension. This protection is also lost in menopausal mice. The purpose of this study was to utilize a bottom-up shotgun proteomics-based approach to examine how CD4⁺ T cell activation and pro-inflammatory signaling is modified in...
Ang II-infused menopausal mice compared to premenopausal counterparts. 10-week-old C57BL/6J female mice received intraperitoneal injections of 4-vinylcyclohexene diepoxyde (VCD; sesame oil vehicle) for 20 consecutive days to induce menopause. Cyclicity was monitored daily to determine the day of onset of menopause. Ang II (800ng/kg/min) was infused for 14 days via osmotic minipump into VCD-treated menopausal (Meno/Ang II) or vehicle-treated premenopausal (Ang II) mice. After 14 days of Ang II infusion, splenic CD4+ T cells were isolated and purified via negative immunomagnetic selection. CD4+ purity was measured via flow cytometry and protein was obtained from these cells. Splenic CD4+ T cell protein samples were fractioned via SDS PAGE prior to trypsin digestion and Zip Cleaning on C18 columns. These peptide samples were analyzed via label-free MS/MS tandem mass spectrometry and were subsequently identified and quantified using Mascot and Progenesis software. 7,123 proteins were identified from the peptide samples. From this protein list, 5,857 proteins were identified by more than one unique peptide sequence and were used for subsequent analysis. 964 proteins were differentially expressed between control, Ang II and Meno/Ang II groups (p < 0.05). Of the 964 differentially expressed proteins, 350 were significantly different between Ang II and Control, while 639 proteins were differentially expressed between Meno/Ang II and Control, and 248 between Meno/Ang II and Ang II. Gene Ontology (GO) enrichment of the 964 differentially expressed proteins was assessed using Perseus software and the DAVID database. Ang II infusion resulted in the overexpression of 220 GO biological pathways (p < 0.05), including positive regulation of cell adhesion (5.1-fold enrichment), negative regulation of interleukin-6 production pathway (4.5-fold enrichment), and negative regulation of cell cycle arrest (4.1-fold enrichment). Overall, expression of proteins positively regulating cell adhesion and negatively regulating interleukin 6 were decreased in the Ang II group versus control and were further decreased in the Meno/Ang II group (see attached table). Proteins associated with negative regulation of cell cycle arrest were equally increased by Ang II in premenopausal and menopausal female mice, suggesting an increase in CD4+ T cell proliferation. These results demonstrate that Ang II induces a significantly greater shift in the splenic CD4+ T cell proteome in female mice after menopause. This shift results in a proteomic profile favoring the proliferation and migration of pro-inflammatory T cells in postmenopausal females.

<table>
<thead>
<tr>
<th>Protein Name</th>
<th>Positive Regulation of Cell Adhesion</th>
<th>Control</th>
<th>Ang II</th>
<th>Meno/Ang II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercellular adhesion molecule 1</td>
<td>1.00 ± 0.04</td>
<td>0.79 ± 0.06*</td>
<td>0.73 ± 0.08*</td>
<td></td>
</tr>
<tr>
<td>Integrin alpha-null</td>
<td>1.00 ± 0.10</td>
<td>0.80 ± 0.08</td>
<td>0.63 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Tyrosine-protein kinase SIK-1</td>
<td>1.00 ± 0.03</td>
<td>0.80 ± 0.08</td>
<td>0.63 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Platelet 12-LOX</td>
<td>1.00 ± 0.04</td>
<td>0.80 ± 0.08</td>
<td>0.63 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Cyclin dependent kinase 6</td>
<td>1.00 ± 0.04</td>
<td>0.80 ± 0.08</td>
<td>0.63 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Apoptosis-inducing factor</td>
<td>1.00 ± 0.04</td>
<td>0.80 ± 0.08</td>
<td>0.63 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Negative Regulation of Interleukin 6 Production Pathway</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta-arrestin-1</td>
<td>1.00 ± 0.04</td>
<td>0.80 ± 0.08</td>
<td>0.63 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Interleukin-1 receptor-associated kinase</td>
<td>1.00 ± 0.04</td>
<td>0.80 ± 0.08</td>
<td>0.63 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Toll-like receptor 9</td>
<td>1.00 ± 0.04</td>
<td>0.80 ± 0.08</td>
<td>0.63 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Zinc finger CCCH-type containing 12A</td>
<td>1.00 ± 0.04</td>
<td>0.80 ± 0.08</td>
<td>0.63 ± 0.03</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05 vs Control; #p<0.05 vs Ang II

3.23 SEX-SPECIFIC REGULATION OF SIRTUIN-3 MEDIATES DIFFERENCES IN ISCHEMIA-REPERFUSION KIDNEY INJURY

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1Nephrology, Michael E. DeBakey VAMC, 2Medicine-Nephrology, Baylor College of Medicine, 3Molecular & Cell Biology, Baylor College of Medicine

Background: While the pathogenesis of ischemic acute kidney injury (AKI) is better defined, the therapeutic options remain limited. Sex influences susceptibility to kidney ischemia-reperfusion injury (IRI), and sex hormones play a crucial role. We have previously shown that a pathway from stanniocalcin-1 (STC1) mediated activation of AMPK to induction of mitochondrial sirtuin-3 (SIRT3) suppresses ROS generation and confers resistance to kidney IRI. Our observations reveal increased baseline kidney expression of STC1, activated AMPK, and SIRT3 in female mice vs. males, and we hypothesize that SIRT3 protects from IRI and mediates the observed sexual dimorphism in response to injury.

Methods: We subjected wild-type (WT) and SIRT3 transgenic (Tg) male or female mice to bilateral kidney IRI (clamping of renal pedicles for 30 minutes). A group of male or female WT mice were treated with testosterone by subcutaneous implantation of a 200 mg (21-day release) testosterone pellet for 2 weeks. Cultured HEK 293T cells were treated with 17β-estradiol, testosterone or vehicle.

Results: We observed higher kidney expression of STC1, mitochondrial SIRT3 and activity of AMPK in WT female mice compared with males. While there was age-dependent decline in kidney SIRT3 and AMPK activity, differential expression in males and females persisted. Aged 6 months-old SIRT3 Tg male mice display less tubular vacuolization vs. similarly aged WT male mice. Compared with WT male mice, SIRT3 Tg male mice
demonstrated resistance to 30-minutes of kidney IRI characterized by: improved survival; preserved creatinine clearance (CrCl); decreased morphological damage and ROS production. SIRT3 Tg male mice tolerated IRI with survival and kidney function impairment similar to WT females. WT or SIRT3 Tg female mice display no measurable change in kidney function with 30-minutes of kidney IRI. In WT female mice, kidney mitochondrial SIRT3 expression correlates with both plasma estradiol and testosterone levels; in WT male mice, kidney mitochondrial SIRT3 expression correlates with only plasma testosterone level. Testosterone administration to aged 6 months-old WT male mice increased plasma testosterone ~4-fold, caused kidney injury (decreased CrCl and increased tubular vacuolization), and decreased kidney mitochondrial SIRT3 expression (with no effect on whole cell SIRT3). Testosterone treatment to WT female mice caused no measurable kidney injury, but increased whole cell and mitochondrial SIRT3 expression; possibly due to an associated increase in plasma estradiol level. In cultured HEK cells, estradiol increased whole cell and mitochondrial SIRT3 protein expression, and SIRT3 mRNA in a dose-dependent manner. Testosterone decreased mitochondrial SIRT3 protein expression in a dose-dependent manner, but had no effect on whole cell SIRT3 protein expression and SIRT3 mRNA. Estradiol also increased estrogen receptor-β and estrogen related receptor-α mRNA expression.

**Conclusion:** The data suggest that: 1) SIRT3 ameliorates kidney IRI, and decreased SIRT3 expression in males mediates the increased susceptibility to ischemic injury; 2) sex steroids regulate mitochondrial SIRT3 expression; estrogen via transcriptional regulation and testosterone via inhibition of mitochondrial targeting.

Funding: This work was supported by Career Development Award #IK2 BX002912 and Merit Award #BX002006 from the United States (U.S.) Department of Veterans Affairs Biomedical Laboratory Research and Development Program, and a generous gift from Dr. and Mrs. Harold Selzman.

### 3.24

**GROUP IV CYTOSOLIC PHOSPHOLIPASE A₂/α IS REQUIRED FOR 6β-HYDROXYTESTOSTERONE MEDIATED ANGIOTENSIN II INDUCED HYPERTENSION IN MALE MICE**

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Previously we showed that cytochrome P450 (CYP) 1B1-testosterone derived metabolite 6β-hydroxytestosterone (6β-OHT), by acting as a permissive factor, contributes to the development of angiotensin II (Ang II)-induced hypertension in male mice. Also, we reported that Ang II-induced hypertension is mediated by group IV cytosolic phospholipase A₂/α (cPLA₂/α) activation, resulting in arachidonic acid (AA) release, and generation predominantly of eicosanoids with pro-hypertensive effects. This study was performed to investigate the interaction of CYP1B1 and cPLA₂/αAA system by testing the hypothesis that 6β-OHT contributes to Ang II-induced hypertension by promoting cPLA₂/α activation and generation of eicosanoids with pro-hypertensive effects. Male intact or castrated (Cas) Cyp1b1⁻/⁻/cPLA₂/α⁻/⁻, Cyp1b1⁺/⁺, and cPLA₂/α⁺/⁺ mice (8 weeks old, n=4-5) were infused with Ang II (700 ng/kg/min) and injected with 6β-OHT (15 μg/g, i.p. every 3rd day), for 2 weeks, and systolic blood pressure (SBP) was measured by tail cuff. In Cyp1b1⁺/⁺/cPLA₂/α⁻/⁻ mice, castration or CYP1B1 gene disruption minimized the Ang II-induced increase in SBP (127±3 and 148±3 vs. 188±3 mmHg, respectively, P<0.05). Ang II infusion in 6β-OHT, but not its vehicle (DMSO, 50ml) treated Cyp1b1⁺/⁺ mice increased SBP (189±5 vs.148±3 mmHg, P<0.05); this increase was minimized by the AA metabolism inhibitor, 5,8,11,14-eicosatetraynoic acid (25 mg/kg, i.p. every 3rd day) (140±4 mmHg, P<0.05). Ang II infusion with 6β-OHT treatment increased SBP in Cas cPLA₂/α⁺/⁻ mice, but not in Cas cPLA₂/α⁻/⁻ mice (176±7 vs. 122±2 mmHg, P<0.05). Treatment with antagonists of prostaglandin (PG) E2 receptors EP1 (SC19220) and EP3 (L-798106) (28 µg/g, s.c. every 2nd day) attenuated the Ang II-induced increase in SBP in 6β-OHT treated Cas cPLA₂/α⁺/⁻ mice (123±4, 123±6 vs. 189±5 mmHg, respectively, P<0.05). These data suggest that 6β-OHT contributes to Ang II-induced increase in SBP via cPLA₂/α activation, the release of AA and generation of eicosanoids, most likely PGE2 that exerts pro-hypertensive effects by stimulating EP1 and EP3 receptors. Therefore, the development of agents that selectively inhibit the cPLA₂/α activity or block EP1 and EP3 receptors could be useful in treating hypertension and its pathogenesis.
Activation of T cell-dependent pro-inflammatory responses are required for Ang II hypertension in male mice. However, females are protected from T cell-mediated hypertension and may suppress hypertension by directly preventing Ang II-induced pro-inflammatory T cell activation. Here we sought to determine whether transferring T cells from hypertensive donor mice eliminates female protection against T cell-mediated hypertension. Splenic CD3+ T cells were transferred from normotensive (NT) or Ang II-hypertensive (HT) C57BL/6J male donors to female Rag-1−/− (NT T cell female-NTF; HT T cell female-HTF) or male Rag-1−/− (HT T cell male-HTM) recipient mice. Blood pressure was monitored (tail cuff) for 5 weeks post-transfer. Ang II (490ng/kg/min) was infused into recipient mice for 14 days during weeks 4 and 5 post-transfer (NTFA; HTFA; HTMA). Ang II significantly increased MAP in donor male mice (NT 114 ± 3 mmHg vs HT 157 ± 3 mmHg, *p<0.05). Transfer of T cells from HT donors did not induce HT in female or male recipients. Similarly, T cell donor environment did not affect Ang II-induced blood pressure in female recipients, which remained protected compared to male recipients (MAP: NTF 83 ± 4 mmHg*, HTF 88 ± 6 mmHg*, NTFA 101 ± 5 mmHg*, HTFA 103 ± 5 mmHg*, HTMA 138 ± 3 mmHg, *p<0.05 vs HTMA). Flow cytometry demonstrated similar splenic T cell frequency across all groups (CD3: NTF 18%, NTFA 16%, HTF 17%, HTFA 14%, HTMA 18%, p>0.05). However, T regulatory cells were significantly reduced in male recipients compared to all female groups (Foxp3: NTF 21.6%*, NTFA 22.2%*, HTF 22.8%*, HTFA 22.6%*, HTMA 15.3%, *p<0.05 vs HTMA). Females had significantly less renal T cell infiltration compared to males and infiltration was not impacted by Ang II infusion or T cell donor status (CD3: NTF 12,083*, NTFA 11,317*, HTF 12,656*, HTFA 8,997*, HTMA 22,405, *p<0.05 vs HTMA; CD4: NTF 6,411*, NTFA 4,702*, HTF 5,831*, HTFA 4,579*, HTMA 9,914, *p<0.05 vs HTMA; CD8: NTF 5,397*, NTFA 6,123*, HTF 6,362*, HTFA 3,792*, HTMA 11,727, *p<0.05 vs HTMA). These results demonstrate that female mice prevent T cell-mediated hypertension and renal T cell infiltration regardless of previous T cell exposure to a hypertensive environment, suggesting a direct preventive mechanism in females against pro-hypertensive T cell responses.
pg/hr in females (n=8) at ZT0. At ZT12, ET-1 increased from 0.09 ± 0.02 to 0.23 ± 0.06 pg/hr in males (n=8) and from 0.14 ± 0.05 to 0.34 ± 0.06 pg/hr in females (n=8). The ET-1 excretory response was significantly different between males and females at ZT0, but not ZT12, demonstrating a sex and time-related difference in ET-1 release.

These results demonstrate that the response to ENaC inhibition is less prominent in females independent of renal ET-1. Since ENaC expression is greater in female rat kidneys, this could be related to having less ENaC activity independent of expression in females or differences in non-ENaC related effects of benzamid.

This work was supported by grants from the NIH (P01 HL095499 and P01 HL136267 to DMP), a postdoctoral fellowship from the American Heart Association (15POST25090329), and funding from the UAB School of Medicine AMC21 program.

3.27 INCREASED SEVERITY OF RENAL ISCHEMIA REPERFUSION INJURY IN MALE VS. FEMALE MICE IS ASSOCIATED WITH GREATER EXPRESSION OF CHOLINE ACETYLTRANSFERASE IN TUBULES

Shannon Allen1, Jacob Zalewski1, Jacqui Potter1, Conor Miles2, Donald Hoover1, Aaron Polichnowski1

1Biomedical Sciences, East Tennessee State University

Activation of the cholinergic anti-inflammatory reflex has been reported to protect against renal ischemia-reperfusion (IR) injury in mice. Recent studies have documented the presence of a non-neuronal renal cholinergic system primarily within tubules, implying it could be directly targeted to reduce the incidence and severity of acute kidney injury (AKI). The goal of this study was to assess the severity of IR-induced AKI and the associated changes in the renal cholinergic system in male vs. female transgenic mice expressing green fluorescent protein (GFP) under control of the choline acetyltransferase (ChAT) promoter. AKI was induced in mice via clamping both renal pedicles for 20 minutes (bilateral IR) with body temperature precisely controlled at 37°C. First, we assessed the severity of AKI by assessing plasma creatinine (Pc), renal injury at 3 days post IR in one group of male (n=8) and female (n=8) mice and by assessing mortality rate over 7 days post IR in another group of male (n=16) and female (n=13) mice. Males exhibited greater (P<0.05) levels of Pc, 3 days post IR as compared to females (1.7±0.2 vs. 0.4±0.1 mg/dl) with parallel differences observed in the severity of tubular necrosis and protein cast formation. The mortality rate over 7 days post IR in males was 50% while no mortality was observed in females. The renal cholinergic system was assessed in separate groups of naive control mice and in mice at 3 days post IR by detecting GFP expression via Western blot and immunohistochemistry. When male and female data were combined, GFP expression in renal tissue lysates (normalized to GAPDH) was higher (P<0.05) in the 3 day post IR (0.6±0.1, n=13) group vs. naive control group (0.3±0.1, n=9). The immunohistochemistry data corroborated the Western blot data with high levels of GFP expression observed in injured tubules. In naive control mice, there was a strong tendency (P=0.07) for GFP expression in renal tissue lysates to be higher in males (0.4±0.1, n=5) vs. females (0.2±0.03, n=4). In mice examined at 3 days post IR, GFP expression in renal lysates was higher in both males (0.7±0.1, n=6, P<0.01) and females (0.4±0.04, n=7, P<0.05) when compared to their respective naive control groups. GFP expression in males at 3 days post IR was higher (P<0.05) vs. females. We conclude that male mice exhibit greater levels of renal ChAT expression at baseline vs. females and this difference is further amplified at 3 days post IR-induced AKI. The greater levels of renal ChAT expression in males 3 days post IR is likely due, in part, to greater levels of AKI-induced tubular injury. The question of whether increased tubular ChAT expression in male mice contributes to their increased severity of AKI as compared to females requires further investigation.

3.28 GENDER DIFFERENCES IN HUMAN SKIN Na+ AND MONOCYTE SALT-SENSITIVITY

Annet Kirabo1, Natalia Barbaro1, Jason Foss1, Fernando Elijovich1, Justin Van Beusecum1, Cheryl Laffer1, Mingfang Ao1, Aseel Alsouqi1, Alp Ikizler1, David Harrison1, Annet Kirabo1

1Medicine, Vanderbilt University Medical Center

Salt sensitivity increases cardiovascular mortality. Studies have focused on the role of the kidney and vasculature in salt sensitivity, however, recent data indicate that sodium accumulates in tissues and can activate immune cells. We recently found that in murine dendritic cells amiloride sensitive channels sense salt and trigger NADPH oxidase-dependent formation of isolevuglandin (IsoLG)-adducts. We tested the hypothesis that sex differences in tissue sodium accumulation affect activation of immune cells in humans. We recruited 67 subjects including pre- and post-menopausal women and men. We non-invasively quantified their skin Na⁺ using magnetic resonance imaging (MRI) and examined their peripheral blood mononuclear cells (PBMCs), obtained the same day, using flow cytometry. We found that increased skin accumulation of Na⁺ in humans is associated with a parallel increase in IsoLG-adducts (24±6 vs 38±66%, n=33, low Na⁺ vs 38±6%, n=37, high Na⁺, p<0.05) and expression of the activation marker CD83 (0.04±0.009 vs 0.12±0.04%, p=0.04) in circulating monocytes. In
addition, we found that men exhibit higher Na\(^+\) content in the skin than women (16.59 ± 1.693, n=13 men vs 12.92 ± 0.6623, n=42 women \(*p<0.05\)). Our preliminary findings also suggest that men have more IsoLG-adducts in their monocytes than women, and this seems to increase with age. These results suggest that elevated skin Na\(^+\) is associated with increased activation of human monocytes via IsoLG-adduct formation and that this is greater in men than women. Such differences in sensitivity to salt by antigen-presenting cells may explain gender disparities in hypertension.

### 3.29
**GREATER AORTIC INFLAMMATION IN MALE SHR CORRESPONDS TO VASCULAR DYSFUNCTION**

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Hypertension is a prevalent disease and an estimated 1 in 3 adults will have high blood pressure in their lifetime. Vascular dysfunction is commonly associated with hypertension and our lab has previously reported that aortas from male Spontaneously Hypertensive Rats (SHR) have enhanced vascular contraction and impaired endothelial dependent vasodilation when compared to female SHR and normotensive male and female Wistar Kyoto rat (WKY), however the mechanism mediating altered vascular function in male SHR is unknown. Based on studies implicating a role for inflammation in mediating vascular dysfunction, the goal of the current study was to test the hypothesis that indices of inflammation are greater in male SHR compared to female SHR and WKY of both sexes. In the current study, aortic T-cell profiles and HMGB1 were assessed in male and female WKY and SHR using flow cytometry (n=6 in each group) and extracellular matrix and adhesion molecules expression were measured using a PCR array (n=4). HMGB1 is a damage associated molecular pattern (DAMP) that when released from the cell triggers an inflammatory response. CD3+ and CD4+ aortic T cell counts were comparable across both sex and strain (CD3+ T cell: effect of sex p=0.55, effect of strain p=0.09, interaction p=0.55; CD4+ T cell: effect of sex p=0.45, effect of strain p=0.62, interaction p=0.56). However, SHR had fewer Tregs compared to WKY (effect of strain p<0.001), and males had fewer Tregs than females (effect of sex p=0.0002; interaction p=0.0058). Consistent with greater vascular dysfunction, male SHR had more Th17 cells (effect of strain p<0.0001; effect of sex p=0.0045; interaction p=0.05) and HMGB1 compared to all other groups (effect of strain p<0.0001; effect of sex p<0.0001; interaction p=0.0015). We also found the RNA expression of numerous extracellular matrix and adhesion molecules to be differentially upregulated by sex and strain (Table 1). Of interest, matrix metalloproteinase 12 was significantly upregulated in a sex- and strain-dependent manner. In conclusion, vascular dysfunction in male SHR is associated with increased pro-inflammatory mediators (Th17 and HMGB1). Based on the central role of HMGB1 to mediate an inflammatory response, future studies will focus on establishing a primary endothelial cell culture to directly study the effects of HMGB1 on adhesion molecule expression.

### 3.30
**PRETREATMENT WITH LOW DOSE LIPOPOLYSACCHARIDE ATTENUATES MEDULLARY CONGESTION IN MALE WKY FOLLOWING ACUTE KIDNEY INJURY.**

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Acute kidney Injury (AKI) is a sudden loss of renal function which can result in future complications or mortality, and prevalence of AKI continues to increase, specifically in hospitalized patients. Ischemia-reperfusion is a well-accepted experimental technique of AKI in rodents. Renal medullary congestion has been demonstrated to augment ischemia reperfusion (IR) in rodent models (1) and we have recently reported a sex difference in recovery from AKI, in which medullary congestion and indices of kidney function are worse in males than females at 7 days post-IR. As low grade inflammation can promote rouleaux, we hypothesized that prior exposure to LPS would worsen medullary congestion and augment AKI following IR. To test this hypothesis, we examined the effect of pretreatment with incrementing doses of lipopolysaccharide (LPS) on renal...
congestion following ischemia reperfusion. Male Wistar-Kyoto rats (WKY, 9wks) were treated with 10 (n=3), 100 (n=3), 1000 (n=3) μg/kg LPS or control (saline, n=3) for 7 days (i.p.), and then subjected to a 30 minute warm, bilateral ischemia reperfusion. Rats were allowed 24h to recover then anesthetized and humanely sacrificed. The right kidney was taken for histology and stained using Gomori’s Trichrome Stain (Thermo 87020). Blood was collected by tail vein at baseline, at days 2 and 7, and at sacrifice. C-reactive protein (CRP, Thermo ERCRP), a marker of inflammation, increased for all groups from baseline to post-IR (p<0.0001), with control treatment having the greatest increase and highest levels of CRP, and 1000μg/kg LPS demonstrating the lowest levels CRP (pinteraction=0.09), at sacrifice. In difference to our hypothesis, outer Medullary peritubular congestion (blinded scoring) showed LPS pre-treatment reduced congestion when compared to saline treated controls (% congestion: control = 80%, 10 and 100μg/kg LPS=40%, 1000μg/kg LPS= 20%). We conclude that, despite promoting inflammation, paradoxically, prior low dose LPS exposure prevents red blood cell congestion in the outer-medulla following IR. As we have recently reported that peristaltic contractions of vasa recta pericytes may prevent RBC congestion in the renal outer-medulla (2), we speculate that LPS activation of toll-like receptors of vasa recta pericytes may prime pericytes to contract preventing congestion. Further investigation of these mechanisms may lead to novel therapeutic approaches to prevent AKI.


3.31
IMMUNE CELL INFUX IN CISPLATIN-INDUCED ACUTE KIDNEY INJURY: SEX DIFFERENCES
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A sex-defined difference in response to cisplatin-induced acute kidney injury (Cp-AKI) has been described to include differences in the presence of systemic pro- and anti-inflammatory cytokines. Female mice demonstrate a significant resistance to kidney functional decline in the presence of morphological damage in a model of Cp-AKI. A stress enzyme, heme oxygenase-1 (HO-1), has been shown to be upregulated differently in males and females in response to Cp-AKI, and is known to alter the inflammatory milieu. In order to better understand the role of HO-1 in conferring a sex-based protection via modulation of inflammation, we sought to identify the lymphoid and myeloid cell populations within the kidney after Cp-AKI in male and female mice that are replete (wild-type littermates, WT) or systemically lacking HO-1 (KO) (n=1-5 mice/group). All mice were treated with 20mg/kg cisplatin or saline as vehicle and observed on day 1 or day 3 following the injections. Serum creatinine levels were obtained to confirm kidney functional decline with Cp; and weight loss was monitored over the time course. Kidney tissues were harvested and processed for histology or to obtain single cell preparations. The latter were subjected to flow cytometry to detect cells of lymphoid [B cell, NK cell, T cells (CD3+, CD4+, CD8+)] and myeloid [neutrophils, and renal resident and infiltrating immune cell populations (CD11b+Ly6Chi, CD11b+Ly6C-, CD11b+Ly6C, CD11b-F4/80-, MHCII+CD11c+, MHCII-) lineages (each expressed as a proportion of total live CD45+ cells). Proportions of neutrophils and CD8+, but not CD4+ or CD3+ T cells showed distinct differences across groups; all other cell populations were not different. By 1-way ANOVA across all groups, on day three, neutrophils were significantly higher in male KO mice relative to WT mice of both sexes. While not significant in the analysis across all groups, analysis by within group ANOVA demonstrated that male KO mice had a significant rise in proportions of neutrophils at both time points. No significant elevation was seen in neutrophils in KO females by either statistical analysis. CD8+ T cell proportions at baseline (saline-treated mice) in the different groups were higher in male KO mice relative to male and female WT mice and trended toward significance (p=0.0524) relative to female KO mice by 1-way ANOVA across all groups. CD8+ cells appeared to decrease with injury only in male WT mice by within group 1-way ANOVA; all other declines were not significant. Immune cell influx is a hallmark of Cp-AKI. Neutrophils and T cell influx are detrimental to kidney function since elimination of the cells leads to decreased measures of injury and preserved kidney function in models of AKI. These current findings highlight sex-defined differences in the immune cell complement at baseline and after injury that are altered by the presence of HO-1 and may be important modifiers of Cp-AKI leading to preferential protection in young females. We acknowledge support from the UAB-UCSD O'Brien Core Center for Acute Kidney Injury Research (NIH/NIDDK P30-DK079337) and the Interdisciplinary Training in Kidney-Related Research Grant (NIH/NIDDK T32 DK007545 (to R.B.)) for this project.
**3.32**

**SEX DIFFERENCES IN RENAL AMMONIA METABOLISM**

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Renal ammonia excretion is a critical component in maintaining acid-base homeostasis. Sex differences are well recognized as an important biological variable in many aspects of renal function. However, sex differences in renal ammonia metabolism have not been previously reported. This study's objective was to investigate sex differences in renal ammonia metabolism. We compared 4-month-old C57/BL6 male (M) and female (F) mice fed a normal diet, with measurement of plasma electrolytes, urinary ammonia excretion, morphometric analysis of renal structure, and evaluation of changes in key proteins involved in ammonia metabolism using immunoblot analysis and immunohistochemistry. Despite similar level of food intake (F, 8.9±0.9; M, 9.6±1.2 gram/day; P=NS), and thus protein intake, which is the primary determinant of endogenous acid production, female mice excreted significantly more ammonia (F, 72±23; M, 46±19 µmol/day; P<0.01) than did male mice. This difference in ammonia excretion was not due to differences in urine acidification, as urine pH did not differ significantly (F, 6.40±0.18; M, 6.36±0.14; P=NS). Titratate acid excretion (F, 53±26; M, 74±20 µmol/day; P=NS) another component of net acid excretion, did not differ significantly. Serum Na⁺, K⁺, and HCO₃⁻ did not differ significantly. There are fundamental structural differences between the female and male kidney. In the female kidney, proximal tubules account for a lower percentage of the renal cortical parenchyma than the male kidney (F, 42±3; M, 60±3%; P<0.01), whereas the collecting ducts account for a greater percentage of the renal parenchyma (F, 15.4±2.0; M, 9.6±1.6%; P<0.001). Phosphoenolpyruvate, a major proximal tubule (PT) ammonia generating protein, was significantly greater in female mice than male mice. Expression of glutamine synthetase, which recycles ammonia, was significantly greater in the PT of female mice. Expression of NBCe1, a basolateral PT transporter, recently shown to regulate PT ammonia metabolism did not differ significantly between the sexes. Expression of NHE3, which is believed to be the major mechanism of PT ammonia secretion, did not differ significantly between the sexes. Expression of NKCC2, which mediates thick ascending limb ammonia reabsorption, was significantly greater in the female kidney than the male kidney. The collecting duct secretes the majority of urinary ammonia and the Rhesus glycoproteins, Rhbg and Rhcg are the primary collecting duct ammonia transporting proteins. Rhbg was significantly greater in connecting segment cells and intercalated and principal cells in the collecting duct in the cortex and inner stripe of the outer medulla (ISOM) in female mice. Expression of Rhcg was significantly greater in female mice in connecting segment cells and in the basolateral membrane of intercalated and principal cells in the collecting of the ISOM. Thus, there are sex differences in basal ammonia metabolism that involves both renal structural differences and differences in expression of critical proteins involved in ammonia metabolism. These studies were supported by funding from the National Institute of Diabetes and Digestive and Kidney Diseases Grant R01-DK-045788 (IDW), R01-DK-107798 (IDW and JWV) and 5T32-DK-10472.

**3.33**

**SEX DIFFERENCES IN THE METABOLIC AND PHYSIOLOGICAL EFFECTS OF A HIGH FAT DIET IN THE 3XTG-AD MOUSE MODEL OF ALZHEIMER’S DISEASE**

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Obesity and factors associated with metabolic syndrome are strongly correlated to an increased risk for dementia. Specifically, over 80% of individuals with Alzheimer’s disease (AD) have either diabetes or prediabetes; therefore, it is of critical importance to understand the effects of this common comorbidity. Beta-amyloid deposits have been found in the pancreas of both humans and transgenic AD mouse models, thought to contribute to observed metabolic impairments. Additionally, high fat-diet induced obesity aggravates AD pathology, confirming the feedback loop that exists between these two conditions. In the current study, we used a high fat (HF) diet in a genetic mouse model of AD to induce prediabetes, insulin resistance and glucose intolerance in the absence of hyperglycemia, which is 3x more common than diabetes and affects 38% of Americans. In addition, since females have higher rates of dementia and faster rates of decline in cognition, sex differences in the metabolic and physiological effects of prediabetes in AD mice were explored. At three months of age, male and female wild-type (WT; B6129SF2/J) and 3xTg-AD mice were placed on either low fat (LF; 10% fat) or high fat (HF; 60% fat) diet for 3 months, then subjected to a glucose tolerance test (GTT), followed by blood and tissue collection. HF diet increased body weight gain and fat mass, and impaired glucose tolerance, to a greater degree in females compared to males, and in 3xTg-AD mice compared to WT mice. These differences in metabolic outcomes were not attributable to differences in food intake normalized to body weight. While heart mass was unaffected by diet
in WT mice, HF diet increased heart mass in 3xTg-AD mice, though this effect reached significance in females only. Additionally, HF diet reduced reproductive organ weight in 3xTg-AD females only. Liver steatosis and inflammatory cytokine expression are also being assessed. Results thus far suggest that AD females are particularly sensitive to the detrimental effects of HF diet, in line with greater cognitive impairments observed in our other studies. This study will be critical for understanding sex differences in the effects of high fat diet on metabolic and physiological outcomes that may contribute to cognitive decline in AD.

3.34
SEX DIFFERENCES IN THE CEREBRAL VASCULAR FUNCTION AND K CHANNEL ROLE IN ADULT SPRAGUE DAWLEY (SD) RATS
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The incidence of cerebrovascular disease is lower in adult women compared to adult men but the sex differences in cerebral vessel function and associated mechanisms are not clear. Using a combination of vascular, electrophysiological and biochemical approaches we explored the hypothesis that “sex differences in the cerebral vascular function in adult Sprague Dawley (SD) rats is associated with differential large conductance potassium (BK) channel subunit function”. Middle cerebral arteries (MCA) isolated from adult female rats exhibited attenuated myogenic response (decrease in vascular diameter with increase in lumen pressure). In contrast, MCA of adult male rats exhibited myogenic response (% change in diameter from 40 to 140mmHg: Females 16±8, Males -25±4, p<0.05, n=5-8). Percent myogenic tone (%MT) (calculated from active and passive diameters) is ~3.4 fold higher in females compared to their male counterparts (% MT at 40mm Hg: Females 51±6, Males 15±5, n=5-8, p<0.05). Total potassium, BK and Kv channel currents were significantly larger in female vascular smooth muscle cells (VSMCs) compared to males. Spontaneous transient outward currents (STOC) amplitude that represent BK channel function are ~1.73 fold higher in VSMCs isolated from female rats compared to males (PA: Females 90±6, Males 53±5, n=5, p<0.05). While BKα protein content is higher, BKβ1 subunit protein content as well as mRNA is lower in cerebral vessel homogenates yielding a lower BKβ1/α ratio in females than males. Endothelium-independent (Sodium nitro prusside (SNP)) relaxation is ~2.3 fold higher in female MCA compared to males (1µM SNP: Females 88±10%, Males 39±5 %, n =3-5, p<0.05). Together these results suggest that adult female MCAs exhibit attenuated myogenic response that may be associated with higher BK channel function. Also, higher BK channel function can result in greater endothelium-independent vasodilation in adult females when compared to males. In conclusion, these results may identify a mechanism with which women in adult hood are protected from cerebrovascular incidences compared to males due to their greater vasodilator capacity that is associated with higher BK channel function. Supported by AHA SDG (13SDG14000006) to Mallikarjuna R. Pabbidi.

3.35
CEREBROVASCULAR DYSFUNCTION IN THE DAHL S RAT MODEL OF SUPERIMPOSED PREECLAMPSIA
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Preeclampsia is a multi-faceted pregnancy disorder characterized by increases in blood pressure after 20 weeks of gestation coupled with either an increase in proteinuria (renal insufficiency), or other major organ involvement (liver, lung, brain). In regards to neurological complications, common symptoms include nausea, drowsiness, blurred vision, scotomata, and persistent headaches, with more severe cases leading to seizures (eclampsia), cerebral edema and intracranial hemorrhage. Our lab has recently characterized the Dahl salt sensitive rat (Dahl S) as a model of spontaneous superimposed preeclampsia, displaying characteristic symptoms of increased blood pressure, urinary protein excretion, as well as fetal demise and intrauterine growth restriction. Groups of Dahl S rats were used in different experiments during late pregnancy to test the overarching hypothesis that pregnant Dahl S rats exhibit cerebrovascular dysfunction as commonly observed in preeclamptic women. Group 1 (n=4-6): Brains were harvested and weighed on day 20, heated at 60°C for 72 hours, and re-weighed to determine brain water content (edema). Pregnant Dahl S rats have increased posterior brain dry:wet weight ratios compared to virgin littersmates (78.0±0.1 vs. 77.4±0.2%, p<0.05) with no significant difference in the anterior brain (79.2±0.2 vs. 78.8±0.3%, p=0.33). Group 2 (n=4-6): Evan’s Blue extravasation into the brain was measured to assess blood-brain barrier function. Evan’s Blue concentration was increased in both posterior (0.40±0.012 vs. 0.018±0.001 ng/g tissue/plasma concentration, p=0.07) and anterior (0.021±0.001 vs. 0.110±0.068 ng/g tissue/plasma concentration, p=0.06) brain in pregnant Dahl S rats compared to virgin littersmates, respectively.
indicating BBB dysfunction. Group 3 (n=5): Cannulated middle cerebral artery (MCA) segments were used to assess vascular permeability by measuring the fluid flux of water through the vascular wall of pressurized arteries. MCA’s from pregnant Dahl S rats showed a greater drop in intravascular pressure compared to virgin littermates (Δ=50.2±3.7 vs. 24.4±5.0mmHg, respectively, p<0.01). Together, these results suggest that the pregnant Dahl S rat, a model of superimposed preeclampsia, exhibits increases in cerebrovascular permeability which could play a role in increased cerebral edema and blood-brain barrier permeability. Therefore, pregnant Dahl S rats may be a useful model to investigate mechanisms leading to cerebrovascular abnormalities that occur during preeclampsia.

4. SESSION 2: PHYSIOLOGY AND GENDER: AGING AND SENESCENCE

4.1 SEX DIFFERENCES, AGING AND VASCULAR FUNCTION

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The incidence of coronary heart disease (CHD) in women lags behind men by 10 years for total CHD and by 20 years for more serious clinical events such as myocardial infarction and sudden death. Under age 75 years, there is a higher proportion of cardiovascular disease events due to CHD in men than in women. Indeed, between the ages of 45 and 65 years, the incidence of myocardial infarction and stroke is higher in men compared with women, in spite of no differences in the prevalence of traditional risk factors. The mechanisms responsible for age- and sex-related differences in vascular endothelial function are complex and not completely understood. Vascular endothelial cell function is critical to cardiovascular health and vitality. However, due to its unique anatomical position the endothelium is susceptible to blood-borne injury, mechanical forces and cardiovascular risk factors that can impair cell function resulting in a proatherogenic endothelial phenotype that is key in the initiation, progression and clinical complications of vascular disease. Many of the cardiovascular complications associated with age are due, at least in part, to endothelial dysfunction, particularly vasomotor dysregulation and fibrinolytic impairment. It is now recognized that the vascular endothelium is significantly influenced by circulating microparticles. The initial notion that microparticles in the circulation were merely “inert cellular debris” rapidly changed when it became apparent that circulating microparticles can trigger a proatherogenic endothelial cell phenotype by disrupting endothelial nitric oxide production, inducing endothelial inflammation, oxidative stress and senescence as well as altering apoptosis. In addition, microparticles have been shown to contribute to plaque development, thrombogenicity and instability. Microparticles are small (100 to 1000 nm diameter) anucleoid phospholipid vesicles released into the circulation by various cell types (e.g. endothelial cells, platelets, leukocytes, monocytes) in response to a myriad of triggers including: high shear stress, cellular activation stemming from proinflammatory, prothrombotic and/or proapoptotic stimuli, cellular differentiation, cell senescence and apoptosis. The potential of circulating microparticles to be vascular-protective or -destructive underscore their biological importance in cardiovascular health and disease. This seminar will review the influence of aging and sex on endothelial cell function and introduce circulating microparticles as novel mediators of age- and sex-related differences in endothelial dysfunction.

4.2 SEX DIFFERENCES IN THE EFFECTS OF PREDIABETES ON VASCULAR CONTRIBUTIONS TO DEMENTIA

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Background: Diabetes causes endothelial dysfunction and is a major risk factor for vascular contributions to cognitive impairment and dementia (VCID). Diabetic women have increased risk of VCID compared to diabetic men. Data on the effects of prediabetes, which is 3 times more common than type 2 diabetes, are lacking. Prediabetes can be modeled in mice via long-term administration of a high fat (HF) diet, which we have shown causes cognitive deficits. Young females are protected from the metabolic effects of the diet. We hypothesized the middle-aged females would lose their protection, relative to males, from the metabolic and cognitive effects of a HF diet in a mouse model of VCID.

Methods: Middle-aged (8.5 month old) male and female C57BL/6J mice were placed on a HF or control diet for 6.5 months (15 months old at the end of the study). After 3 months on the diet, they received either VCID surgery (unilateral carotid artery occlusion) or sham surgery. Body weight and glucose tolerance were monitored. After 6 months on the diet, blood flow through the left carotid was measured via ultrasound and then cognitive function was assessed in the Morris water maze. Finally, blood flow was measured via laser speckle contrast imaging. Brain were also collected and we will be performing histology to examine neuroinflammation, white matter damage, and blood brain barrier damage.
Results: HF diet caused greater increases in body weight and glucose intolerance in females than males. Blood flow through the left carotid was reduced in HF-fed mice of both sexes. Cerebral blood flow was reduced in the right hemisphere following VCID surgery, with reductions being more severe in the HF female VCID mice. Spatial memory was impaired in VCID males, regardless of diet; however, in females, either HF diet or VCID (or a combination of the two) impaired spatial memory.

Conclusions: The data show that HF-fed/prediabetic middle-aged-females have more severe metabolic and cognitive deficits than prediabetic males. This is in line with the increased risk of VCID in diabetic women compared to men. Our data suggests that prediabetes might also be a greater risk factor for VCID in women.

4.3 SEX DIFFERENCES IN THE ROLE OF THE SMOOTH MUSCLE CELL MINERALOCORTICOID RECEPTOR IN CARDIOVASCULAR AGING

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The process of vascular aging involves the development of vasomotor dysfunction and vessel stiffening, which leads to cardiovascular disease (CVD). Clinical data supports that the time course of CVD development with aging occurs differentially in males vs. females, suggesting distinct sex-specific mechanisms of vascular aging. The mineralocorticoid receptor (MR) is a steroid hormone receptor known to regulate renal sodium handling. We previously demonstrated that male mice with the MR specifically deleted from smooth muscle cells (SMC-MR-KO), have lower blood pressure, vascular tone, and vasoconstriction as they age. The purpose of this study was to determine whether there are sex differences in the role of SMC-MR in vascular aging. All evaluations of the mice took place at 3 ages: 3, 12, and 18 months, chosen as these roughly correspond to adult (3 mo.), middle age or “peri-menopause” (12 mo.) and elderly or “post-menopause” in females (18 mo.). First, we show that vascular MR increases with age differentially in male and female mice; with vascular MR increasing at 12 months of age in males but not until 18 months of age in females. This change in MR expression is accompanied by a concomitant down-regulation of vascular expression of miR-155 at 12 months in males vs. 18 months in females, which is prevented in SMC-MR-KO mice. In vitro reporter assays showed that MR transcriptionally represses miR-155 promoter activity, whereas estrogen receptor (ER) upregulates miR-155 promoter activity, and is further enhanced with estrogen treatment. These studies suggest that MR and ER regulate miR-155 in an opposite manner, and that the decline in miR-155 in females may be driven by combination of the increase in MR and a loss of estrogen. We previously identified miR-155 as a regulator of vascular target genes including the angiotensin II type 1 receptor (AT1R) and the pore-forming subunit of the L-type calcium channel (LTCC), Cav1.2, both mediators of vasoconstriction and enhanced vascular tone. Indeed, we find that AT1R- and LTCC-mediated vasoconstriction is increased at 12 months in males and 18 months in females and that these vasomotor alterations are prevented in SMC-MR-KO mice of both sexes. We next examined alterations in vascular stiffness, a hallmark of vascular aging. In vivo aortic stiffness studies, as measured by pulse wave velocity (PWV), demonstrate the same temporal difference with aging with increased vascular stiffness at 12 months in males vs. 18 months in females, that is prevented by SMC-MR-KO. We explored the contribution of SMC to stiffness via atomic force microscopy of freshly dispersed SMC from ~18 month old mice. These studies revealed that females exhibit more SMC stiffness vs. males, and that this is partially prevented by SMC-MR-KO in females only. To assess the role of fibrosis in the aging-induced alterations in stiffness, we measured carotid artery fibrosis histologically. Carotid fibrosis is increased at 12 months in males vs. 18 months in females, and partially prevented by SMC-MR-KO in males only. Together, these data suggest that the temporal difference in vascular stiffening is associated with distinct sex-specific mechanisms driving vascular stiffness with aging and that SMC-MR plays a differential role in males and females. Next, we examined the effect of aging on cardiac function in males and females by in vivo echocardiography. Cardiac function declines at 18 months in both males and females, but is attenuated by SMC-MR-KO in females only. Further characterization of overall cardiovascular function via exercise capacity testing revealed that exercise capacity declines in males at 12 months vs. 18 months in females, and is partially restored by SMC-MR-KO in males only. In summary, the time course and mechanisms of cardiovascular aging are distinct between males and females with SMC-MR playing a differential role in males versus females. Furthermore, these studies suggest that sex-specific therapies may be essential to improve CVD outcomes in the aging population.
MATERNAL AGING AND CARDIOVASCULAR DYSFUNCTION

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Pregnancy has an important influence on both short- and long-term cardiovascular outcomes for women. The age at which women experience their first pregnancy has increased, however, normal pregnancy-induced vascular adaptations may be impaired with aging. Aging is associated with cardiovascular impairments, hence the increased demands of pregnancy may exceed the aging maternal cardiovascular capacity.

Our data shows that advanced maternal age results in impaired vascular function and reduced uteroplacental perfusion, which may underlie adverse pregnancy outcomes. We used aged female rats (9.5 months; equivalent to ~35 yr old human), to investigate the impact of maternal age on pregnancy outcomes and vascular function. Delayed pregnancy reduced fertility (46%), reduced litter size (36%), caused fetal growth restriction, increased placental weight, and increased maternal systolic blood pressure (16 mmHg). Both uterine and mesenteric arteries from aged dams had greater active myogenic responses (228% and 151% respectively). Moreover, both aging and complicated pregnancies, which may include advanced maternal age, are known risk factors for later-life maternal cardiovascular disease.

Using the aged rat model, we further investigated vascular function in never pregnant (virgin), previously pregnant (postpartum) and previously mated but never delivered (nulliparous) rats at ~13.5 months of age (3 months postpartum or equivalent). Nulliparous rats had significantly reduced vasodilation responses (methylcholine [MCh] E_{max} 54±13%) vs. virgin and postpartum rats (85±4% and 85±3% respectively); suggesting a worsened vascular pathology associated with pregnancy loss. Further, in mesenteric arteries from postpartum rats, endothelium-derived hyperpolarization (EDH) mediated vasodilation was reduced and a constrictive prostaglandin effect was apparent. These data demonstrate mechanisms which may lead to worsened outcomes at an advanced maternal age; including early pregnancy termination and later life cardiovascular dysfunction.

We recently tested whether being born from a dam of advanced maternal age may constitute a prenatal stress with cardiovascular consequences for the offspring. In 4-month-old offspring (young adult), we observed impaired endothelium-dependent relaxation in males (P<0.05), but not females born from aged dams. Interestingly, there was a significant increase in nitric oxide-induced relaxation in females, but not males, born from aged dams (delta E_{max} young dam -25±12 vs. aged dam -69±8, P<0.05). Cardiac susceptibility to an ischemia-reperfusion insult was also increased in male, but not female, offspring born from aged dams (P<0.001).

These data illustrate that offspring born from aged dams have an altered cardiovascular risk profile that is sex-specific. Given the increasing trend toward delaying pregnancy, our findings have significant population and health care implications and further illustrate pregnancy as a window of opportunity to assess cardiovascular health.

This research has been funded by the generosity of the Stollery Children’s Hospital Foundation and supporters of the Lois Hole Hospital for Women through the Women and Children’s Health Research Institute, and by the Canadian Institutes of Health Research (CIHR; FS 154313 Davidge). SD is a Canada Research Chair in Maternal and Perinatal Cardiovascular Health.

increasing adiposity and play a role in the regulation of cardiovascular function, energy homeostasis and autonomic tone. Therefore, in the current study, using a post-menopausal rat model, we hypothesize that leptin, acting through central leptin receptors, augments menopausal-induced hypertension. Bilateral ovariectomized (OVX) female rats were used in these experiments. On the day the ovaries were removed, rats were instrumented with a telemetry probe for continuous monitoring of blood pressure and heart rate and an osmotic pump to deliver the leptin antagonist (LAN-6) (3µg/day, n=6) or vehicle (saline, n=6) into the lateral ventricle for four weeks. Sham animals were subjected to the same surgeries as OVX animals; however, the ovaries were not excised after opening the abdominal cavity. These animals received only vehicle infusion (SHAM-saline, n=4). Four weeks after the surgery, OVX-saline and OVX-antagonist daily food intake (58 grams) and average weight gain after 4 weeks (74 grams) were significantly greater than SHAM-saline (23 and 21 grams respectively; p<0.05). Four (4) weeks following the OVX surgery, the blood pressure of OVX-saline rats was significantly higher than SHAM-saline (106±2 mmHg vs. 96±2 mmHg respectively, P=0.0017), confirming the menopausal-induced hypertension model. The blood pressure of OVX-antagonist rats was similar to SHAM-saline (102±1 mmHg, P>0.05). To determine if the change in blood pressure in this model was due to differential activation of the sympathetic nervous system, we tested the blood pressure response to an acute injection of hexamethonium (30µg/kg, IP). The drop in blood pressure was significantly greater in OVX-saline animals, as compared to OVX-antagonist (-41±2 mmHg vs. -30±3 mmHg, P=0.021), which suggests an enhanced basal sympathetic tone in menopausal-induced hypertension due to leptin receptor activation. Taken together, these data suggest leptin, acting on its receptors in the central nervous system, modulates sympathetic nervous system activity and augments blood pressure in menopausal-induced hypertension.

The Iowa Osteopathic Educational Research Foundation supported this work

### 4.6

**BIOENERGETIC AND METABOLIC CONSEQUENCES OF THE LOSS OF OVARIAN FUNCTION IN WOMEN**

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Biological systems age at different rates and the consequences of aging in one system can influence other systems. For example, reproductive aging, which results in decreased systemic sex hormone levels, is known to increase osteoporosis risk. This is of particular concern in women, because the loss of gonadal function occurs in mid-life. The skeletal consequences of the loss of estrogen have been well studied, but potential consequences on other systems have not. There is strong preclinical evidence that loss of ovarian function triggers a disruption in energy balance that causes excess fat gain, particularly in central body regions. Some of the consequences of gonadal ablation in female rodents include insulin resistance and dyslipidemia, which can be prevented by estrogen treatment or by programmed exercise. Preclinical research has demonstrated that this disruption of energy balance involves both increased energy intake (in some species) and decreased energy expenditure. The latter reflects both the suppression of resting metabolic rate and a dramatic reduction in spontaneous physical activity; both are reversed by estradiol. To advance the translation of such findings to humans, we utilize a pharmacologic model of gonadal ablation to experimentally isolate the effects of sex hormones. Using this approach, we have demonstrated that suppressing ovarian function results in a decrease in resting metabolic rate (-50 kcal/d), which is prevented by estradiol therapy. Total energy expenditure is reduced even more dramatically (-130 kcal/d). Ovarian suppression also results in an increase in abdominal adiposity and a decrease in muscle mass, both of which are prevented by estradiol. A key question is whether regular exercise can mitigate these consequences of the loss of ovarian function. Our findings suggest that exercise can attenuate some (e.g., loss of bone), but not all (e.g., decline in resting metabolic rate), of the consequences of ovarian hormone suppression.

#### 5:  SESSION 3: SEX AND GENDER DIFFERENCES IN PHYSIOLOGY AND FUNCTION: THE HEART

##### 5.1

**SEX DIFFERENCES IN BLOOD PRESSURE HEMODYNAMICS DURING SEXUAL MATURATION AND AGING: RESULTS FROM THE SAGUENAY YOUTH STUDY**

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Blood pressure (BP) and its underlying biology show marked sex differences. These may be particularly pronounced during reproductive age when male and female sex hormones are most active. They may present as sex-specific patterns of BP hemodynamics. Here we investigated this possibility in the Saguenay Youth Study (SYS).

The SYS is a community-based study of 1000 adolescents (12-18 years, 52% females) and 650 of their middle-aged parents (36-65 years, 56% females). In each participant, we measured, beat-by-beat, BP and underlying hemodynamic factors, i.e., stroke volume (SV), total
peripheral resistance (TPR) and heart rate (HR) during a 52-minute protocol mimicking daily-life activities. We assessed the relative contributions of SV, TPR and HR to BP separately in male and female adolescents and adults. We found that SV was the main determinant of BP in all but male adults in whom it was TPR (p=0.03 to p<0.001). Consistently, in individuals with ‘high’ vs. ‘low’ BP (by median split), SV was higher in all but male adults in whom TPR was higher (p<0.001 for all). In further subset analyses, male adolescents with ‘high’ (vs. ‘low’) genetic androgenicity, and female adults in menopause (vs. pre-menopause) showed greater contribution of TPR to BP that, in both cases, this contribution was not different from that in male adults.

In conclusion, the results from the SYS suggest that marked sex differences in BP hemodynamics exist during adult reproductive age, with TPR being the main contributor of higher BP in males, whereas SV being the main contributor of higher BP in females. These sex differences emerge with male pubertal development and diminish with female menopause.

### 5.2 SEX DIFFERENCES IN HEALTHY HUMAN HEART REVEALED BY CAP ANALYSIS GENE EXPRESSION (CAGE)

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**Background:** Cardiovascular diseases remain the primary cause of death worldwide. Several epidemiologic and investigative studies have shown the evidence of sex hormones affect in cardiac electrophysiology through genomic effects (ion-channels expression) and non-genomic effects (ion-channels function modulation). However, very little is known about the molecular basis for gender-related discrepancies in cardiac electrophysiology. Due to physiologically distinct functions of atria (electrical impulse initiation) and ventricles (blood pumping), there is a difference in ion-channel expression within a heart that causes different disease susceptibilities between both sexes.

**Objective:** Compare ion channel gene expression associated with sex differences using CAGE analysis on left atrial (LA) and left ventricular (LV) human donor hearts.

**Materials and Methods:** Total RNA was extracted from left atria (LA) and left ventricle (LV) of human donor hearts n=4 males (mean age = 53.75) and n=3 females (mean age = 58.7), the cause of death was determined to be non-cardiogenic. Samples were analyzed with CAGE, which is high throughput method for transcriptome analysis that utilizes ‘cap-trapping’. The number of tags gives a frequency of usage that provides information about transcription start sites as well as transcript expression levels. Normalization of raw CAGE tag count was performed as counts per million. The two-sample t-test was used to determine statistical significance.

**Results and Discussion:** Our results confirmed higher expression of SCN5A in ventricles compared to atria. SCN5A gene encodes Nav1.5 channel α-subunit (I_{Na}) which is more abundant in working myocardium compared to nodal cells. Loss of sodium channel function is associated with Brugada phenotype that is more predominant in males. Indeed, males had a lower expression of the gene that might indicate on their higher predisposition to the disease. KCND3 that encodes for outward current potassium voltage-gated channel (I_{to}) that is the main contributing current to repolarizing phase 1 of the cardiac action potential had a significantly higher expression in female atria. KCNIP2, voltage-gated potassium channel (I_{to,i}) maintains early repolarization. During heart failure it augments Ca_{1.2} (CACNA1C) and K_{4.3} (KCNQ1). Since the hearts were healthy, high expression of KCNIP2 in atria did not affect the expression of KCND3 and CACNA1C. Estrogen is known to up-regulate CACNA1C which was indeed found in female hearts although not statistically significant for our postmenopausal female group. Mutations in potassium-channel genes, KCNH2 (I_{Ks}) and KCNQ1 (I_{Ks}), have been associated with Long QT syndrome type 2 and 1 respectively. Our results for these two ions channels did not show a statistically significant difference in expression across sexes. Interestingly, gene for inward rectifier potassium channel (I_{K1}), KCNJ3, that plays an important role in heartbeat generation is up-regulated in atria, the region with pacemaker cells. Another inwardrectifier potassium channel (I_{IACH}), KCNJ2 is up-regulated in ventricles. That demonstrates ion channel specificity to the anatomical areas of the heart and might carry a unique electrophysiological function.

**Conclusion:** In the past decade there has been a push towards sex-specific drug development since it has been established that medications affect males and females differently. Our study reveals that there are sex-dependent gene expression differences in cardiac ion channels and that CAGE approach allows high-throughput gene expression profiling which can be beneficial for gender-specific drug development and personalized medicine overall. Supported by NIH 5R01HL114395.
HEART FAILURE WITH PRESERVED EJECTION FRACTION (HFpEF) IS AUGMENTED IN OBESE MICE WITH AN XX SEX CHROMOSOME COMPLEMENT

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Objective: Heart failure with preserved ejection fraction (HFpEF) accounts for at least 50% of cardiomyopathies associated with left ventricular dysfunction. HFpEF is associated with diastolic hypertension, atrial fibrillation, obesity, age, and female sex. Alarming, given its high prevalence, there are no effective therapies for HFpEF and few experimental models that exhibit features of the human disease. Since female sex is a risk factor for HFpEF, we used a novel murine model (the four core genotypes) that enables dissection of the relative contributions of sex hormones and sex chromosomes. We also incorporated diet-induced obesity and advancing age as risk factors for HFpEF into the experimental design.

Methods and Results: Male (M, XY and XX) and female (F, XX and XY) mice (6 months of age) were fed a 60% high-fat (HF) diet for 6 months. Carotid artery catheters and radiotelemeters were implanted at week 22, mice were allowed to recover for 1 week, and then blood pressure was recorded for 7 days. Echocardiography was performed by ultrasound under anesthesia at study endpoint. There was a significant effect of sex (M>F) and genotype (XX>XY) on systolic (SBP) and diastolic blood pressures (DBP) of HF-fed mice. Within a sex, SBP and DBP were higher in XY than XX males, but a surprising opposite effect was observed in females (XX>XY SBP and DBP). Notably, left ventricular diastolic diameter (XX: M, 3.9 ± 0.1; F, 3.9 ± 0.1 mm; XY: M, 4.1 ± 0.08; F, 4.2 ± 0.06 mm; P<0.05) and volume were significantly decreased in XX compared to XY obese mice regardless of gonadal sex (P<0.05). Consistent with indices of HFpEF, ejection fraction and fractional shortening were not different between groups. However, stroke volume was decreased in XX compared to XY obese mice, regardless of gonadal sex (XX: M, 35.3 ± 2; F, 35.9 ± 3 µl; XY: M, 44.7 ± 4.4; F, 39.6 ± 2.8 µl; P<0.05). Assessment of baroreceptor activity from blood pressure records indicated that baroreceptor slope (gain) and activity were impaired in XX mice, regardless of gonadal sex. Measurements of potential HFpEF biomarkers demonstrated increased serum total TGF-β in female mice with an XX sex chromosome complement (XX, 166 ± 20; XY, 116 ± 11 ng/ml; P<0.05). Studies evaluating cardiomyocyte size and assessing cardiac collagen are underway in hearts from XX versus XY mice.

Conclusions: Using mice with differing sex chromosome complement, we have generated a murine model of HFpEF that exhibits features of the human disease, including obesity, female (XX) sex, advancing age, hypertension, diastolic stiffness, an impaired baroreceptor response, and higher serum TGF-β concentrations. Future studies will use this model to define potential therapeutic targets for HFpEF.

Funding for this project was provided by NIH HL73085 and HL107326 (LAC) and P20RR021954 (COBRE, LAC).

ESTROGEN MODULATION OF THE CARDIAC RAS IN DIASTOLIC DYSFUNCTION

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Heart failure with preserved ejection fraction predominates in postmenopausal women. Emerging epidemiologic evidence proposes that early menopause is positively associated with incident heart failure, which suggests potential protective roles for estrogens in left ventricular diastolic dysfunction (LVDD) development and disease progression. To date, there are no proven pharmacologic therapies to delay or reverse age – and female sex hormone-related LVDD; in fact, data from large trials imply that ACE-inhibitors and angiotensin II (Ang II) receptor blockers (ARBs) effectiveness may be less pronounced in women than in men receiving treatment for hypertension and heart failure. One reason for the low efficacy of ACE inhibitors and ARBs is that these agents might not directly prevent or limit Ang II intracellular formation. Additionally, chymase rather than ACE may be a primary Ang II-forming enzyme in humans. Local tissue-dependent actions of Ang II have a critical role in the progression of adverse cardiac remodeling to subsequent heart failure. Inappropriate activation or suppression of various components of the cardiac RAS have been linked to diastolic dysfunction. LV hypertrophy and increased interstitial fibrosis, induced by Ang II, impairs diastolic calcium handling and LV chamber compliance via its actions on Ang II receptors (AT1R). Loss of the counter-regulatory axis of the RAS, specifically ACE2/Ang-(1-7)/Mas receptor, further enhances the progression of diastolic dysfunction and susceptibility to heart failure by limiting its anti-hypertrophic, anti-fibrotic, and anti-oxidant effects. To this end, the objectives of the presentation will be to: 1) provide an overview of the cardiac tissue pathways to Ang II formation; 2) document the effects of ovariectomy (OVX) on the expression and activity of the cardiac RAS in female hypertensive and normotensive rodents; and 3) describe a critical RAS-related role of G protein-coupled membrane estrogen receptor (GPER) activation in the maintenance of cardiac structure and function after estrogen loss. Specifically, we found GPER cardioprotective effects include anti-proliferation of cardiac mast cells and associated attenuations of cardiac remodeling.

Specifically, we found GPER cardioprotective effects include anti-proliferation of cardiac mast cells and associated attenuations of cardiac remodeling.
chymase/Ang II along with reductions in cardiac collagen deposition and myocyte hypertrophy. Taken together, our findings support the presence of an intracellular chymase-mediated Ang II-forming system that is responsive to estrogens. The observation that cardiac tissue chymase activity correlates with worsening of diastolic function buttresses the importance of chymase rather than ACE as a critical tissue Ang II forming enzyme and its role in modulating cardiac function. These findings provide weight to an urgent need to address how sex-related impact on cellular physiology may drastically affect the effectiveness of current therapies.

Funding support R01-AG033727 and P01-HL051952

5.5 PRENATAL DIABETES AND HIGH-FAT DIET EXPOSURE IMPAIR MITOCHONDRIAL FUNCTION IN ADULT RAT CARDIOMYOCYTES

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Background: Both diabetes and obesity increase the risk of heart disease in adults, and mounting evidence shows that infants born to diabetic or obese mothers also have a higher risk of heart disease in adulthood. We have shown that newborn rats prenatally exposed to maternal diabetes and high-fat (HF) diet have diastolic and systolic dysfunction, myocardial lipid accumulation, decreased respiratory capacity, oxidative injury and mitochondrial dysfunction that mimics that of adult diabetic cardiomyopathy. However, do these adverse cardiac effects carry into adulthood? If so, is either sex more severely affected?

Objective: Determine whether prenatal exposure to maternal diabetes or HF diet impairs bioenergetics or mitochondrial function in primary isolated adult rat cardiomyocytes (ARCM).

Methods: Sprague-Dawley rats received control or HF diet 28 days before and throughout pregnancy. On gestational day 14, citrate buffer or streptozotocin was given to induce diabetes, which was then treated twice daily with sliding-scale insulin. Dams delivered controls, diabetes-exposed, HF diet-exposed and combination-exposed offspring which, on postnatal day 1, were cross-fostered and raised by healthy dams. At 12-13 months, ARCM were isolated for extracellular flux analysis (glycolytic, mitochondrial, and palmitate stress tests and permeabilized assays), live-cell confocal imaging of stress responses, mitochondrial copy number, and lipid peroxidation assays. Groups were compared using two-way ANOVA for diet, diabetes, and interaction with significance set at p≤0.05.

Results: Results to date show that male ARCM have a greater ability to oxidize fatty acids and lower glycolytic capacity than female ARCM. In both sexes, diet- and diabetes-exposed ARCM had decreased proton leak, reduced respiratory spare capacity, and impaired tolerance for uncoupler-induced stress. Confocal imaging further demonstrated these effects by showing 15-50% faster membrane potential loss in both sexes and 30-50% faster cell death in female ARCM prenatally exposed to diet and diabetes. Poor spare capacity is likely related to impaired fuel flexibility. All ARCM demonstrated poor glycolytic capacity. Diet-exposure reduced exogenous and endogenous fatty acid oxidation in males while diabetes-exposure reduced endogenous fatty acid oxidation in females. In males, diet-exposed ARCM had reduced oxidation of the complex I fuel glutamine, complex II fuel succinate, and complex IV fuel TMPD/ascorbate. In females, combination-exposed ARCM had lower oxidation of complex II succinate and complex III duroquinol. Results for mitochondrial copy number and lipid peroxidation are pending.

Conclusions: Prenatal exposure to maternal HF diet and diabetes causes sex-specific changes in mitochondrial function and fuel flexibility in ARCM. Findings highlight the role of mitochondria in fetal origins of adult cardiovascular disease.

Funding sources: NIH-NICHD K08HD078504, NIH-GMS P20GM103620, NIH-GMS P20GM103548, University of South Dakota Fathe Family Medical (UI893), Wheeler School Memorial (UI867), School of Medicine Annual (UI696), and Madsen-Jungwirth Medical (UI843) Scholarships, and Sanford Research.

7: SESSION 4: PHYSIOLOGY AND GENDER: OBESITY AND METABOLISM

7.2 PROGESTERONE UPREGULATES ENDOTHELIAL MINERALOCORTICOID RECEPTOR EXPRESSION WHICH PREDISPOSES FEMALE MICE TO OBESITY-INDUCED ENDOTHELIAL DYSFUNCTION

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Clinical data indicates that obesity ablates the protection of premenopausal women from cardiovascular disease. Compelling evidence indicates a higher efficacy of mineralocorticoid receptor (MR) blockade for the
treatment of cardiovascular disease in obese and diabetic females than in males, however, the origin of this sex-specific effect is unknown. We have shown that the adipocyte-derived hormone leptin mediates endothelial dysfunction in obese female mouse models via aldosterone-dependent activation of MR and, further, that female mice are more sensitive to aldosterone-induced endothelial dysfunction. Therefore, we hypothesized that females express higher endothelial MR (ECMR) expression than males which predisposes females to obesity-associated endothelial dysfunction. RT-PCR analysis in isolated aortic endothelial cells (magnetic cell sorting technique) of Balb/C mice revealed a higher NR3C2 (MR gene) expression in females compared to males (2.9±0.5-fold from male, P<0.05), however, no such difference was observed in non-endothelial cells (0.1±0.3-fold from male). Endothelial NR3C2 expression was further elevated in female obese mice (5.8±1.1-fold from male obese, P<0.05). Similarly, human aortic (5.4-fold from male) and adipose tissue (2.1±0.1 fold from male, P=0.07) endothelial cells derived from female patients exhibited higher NR3C2 mRNA expression than those of males. Western blotting analysis confirmed that ECMR protein expression is correspondingly elevated in aortic endothelial cells from female patients compared to those of males (0.5±0.1 male vs 1.4±0.5 female ratio/β actin). Female sex hormones suppression (ovariectomy) decreased ECMR expression in female mice (-0.8±0.2-fold from sham, P<0.05), which was restored by progesterone supplementation (-0.1±0.1-fold from sham). Increases in progesterone levels with the diestrous phase of the menstrual cycle and pregnancy were associated with a gradual increase in NR3C2 mRNA (diestrous: 1.6±0.1-fold from estrous, pregnancy day 16: 9.2±0.2-fold from estrous, P<0.05). In parallel, progesterone dose-dependently increased ECMR protein expression in human endothelial cells in vitro (P<0.05). Endothelial function was determined in male and female mice by wire myography via aorta relaxation responses to acetylcholine (10^{-9}-10^{-5} M concentration range). Increases in ECMR associated with higher progesterone levels in pregnant females were associated with an increased sensitivity to leptin-induced endothelial dysfunction in mice. In parallel, while leptin induced endothelial dysfunction in intact ECMR female mice (P<0.05), specific deletion of MR in endothelial cells protected female mice from leptin-induced endothelial dysfunction. No differences were observed in leptin-mediated endothelial function in male mice regardless of intact ECMR expression. Further, no changes in endothelium-independent relaxation responses (relaxation to sodium nitroprusside) in any groups were observed. These data indicate that progesterone drives the sex-difference in the levels of ECMR expression and predisposes female mice to leptin-induced endothelial dysfunction. In addition, these data provide a rational for the higher efficacy of MR blockade in obese and diabetic women suffering from cardiovascular disease. Supported by: NIH 1R01HL130301-01, AHA 16IRG27770047, AHA 17POST33660678 and NIH 5F32HL136191-02.

7.3 IDENTIFICATION OF SEX-SPECIFIC MIRNA AND MRNA EXPRESSION PATTERNS IN TYPE 1 DIABETES
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Type 1 Diabetes (T1D) is a chronic autoimmune disease characterized by progressive loss of insulin producing β cells. Sex biased differences exist in the incidence rate, mortality rate and severity of T1D. While T1D is more common in males, the mortality rate and severity is higher in females. The sex-specific difference may also extend into the molecular mechanisms contributing to the development of T1D and has not yet been studied for T1D.

We hypothesized that these sex-specific differences may also extend to molecular pathways activated within the β cell during the evolution of Type 1 diabetes. To address this, human islets from 5 male and 5 female donors were treated with or without cytokines (50 U/ml IL-1β and 1000 U/ml IFN-γ) for 24 hrs, to mimic the pro-inflammatory conditions of T1D. Stratified analysis based on sex was performed. mRNA and miRNA sequencing were performed using Illumina NextSeq and Ion Proton System, respectively. Partek flow, R statistical program (DESeq2 package) and Ingenuity Pathway analysis tools were utilized for analyzing the datasets. mRNAs with a fold change ≥ 2.0 and false discovery rate < 0.05 and miRNAs with a fold change ≥ 1.5 and p < 0.05 in cytokine-treated islets were considered as differentially expressed (DE). miRNA targets were predicted using TargetScan, which were then overlapped with DE mRNAs identified from islets.

Our data revealed striking sex differences in miRNA expression profiles in cytokine-treated islets. Notably, only 4 DE mRNAs were common between the two sexes, while 16 miRNAs and 25 miRNAs were unique in males and females, respectively. Classically these variations have been attributed to differences in sex chromosomes and the action of sex hormones. However, we found that > 95% of these miRNAs originated from autosomes and none of these miRNAs are known to be under the influence of sex hormones. Whereas, 1061 mRNAs were common from a total of 1667 and 1186 DE mRNAs from male and female islets, respectively. Among the significant canonical pathways, 12 pathways were common between males (total = 21; unique = 9) and females (total = 26; unique = 14) and each of these...
common pathways (Eg: Sphingosine-1-phosphate) were predicted to be regulated by unique miRNAs for males and females. Similarly, common gene ontology terms were observed to be regulated by predominantly unique miRNAs in males and females. For example, apoptosis of pancreatic cells was observed to be regulated by miR-146a-5p, miR-338-3p, miR-155-5p, miR-6891-5p, miR-296-3p, miR-181a-2-3p, miR-675-5p in males, but the same function was predicted to be regulated by miR-146a-5p, miR-194-3p, miR-22-3p, miR-320b, miR-30e-3p, miR-124-3p, miR-338-5p in females.

Taken together, our data suggests that sex-biased expression patterns among miRNAs and their upstream regulatory miRNAs may drive sex-specific activation or inhibition of molecular pathways involved in T1D pathogenesis. Understanding these differences are critical to consider in our efforts to develop biomarkers for T1D risk and heterogeneity and when designing trials of novel disease-modifying therapies.

7.4 SEX DIFFERENCES IN THE DEVELOPMENT OF RENAL INJURY ASSOCIATED WITH OBESITY

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Obesity has grown at an alarming rate within the last decade and has been associated with the development of chronic kidney disease (CKD) and end stage renal disease (ESRD). In the US, half of the ESRD population are nondiabetic and are morbidly obese. Obesity contributes to sex differences in the risk for CKD in which obese males tend to develop CKD earlier in life than obese females. To study sex differences during the development of renal disease associated with obesity our laboratory uses obese leptin receptor mutant Dahl salt-sensitive (SSlepr) mutant rats which were derived from Zinc-finger nucleases. We observed an increase in body weight in both female and male SSlepr mutant rats when compared to lean SS rats at 18 weeks of age. The SSlepr mutant strain also developed hyperinsulinemia in comparison to their lean SS counterparts. However, blood glucose in the SSlepr mutant strain remained within normal range throughout the course of the study regardless of sex. Female and male SSlepr mutant rats developed severe systolic hypertension by 18 weeks of age when compared to SS rats. Yet, the rise in arterial pressure occurred earlier in female SSlepr mutant rats than males. Protein excretion was significantly higher in the SSlepr mutant strain versus SS rats at 18 weeks of age regardless of sex. The kidneys from the SSlepr mutant strain displayed increased glomerulosclerosis and interstitial fibrosis compared to SS rats. Female and male SSlepr mutant rats had a significant increase in plasma creatinine levels compared to the SS strain suggesting the presence of severe CKD. While conducting these first set of experiments, we observed a significantly higher mortality rate in female SSlepr mutants. Therefore, in the second set of experiments, we determined the survival rate of female and male SS and SSlepr mutant rats through 18 weeks of age. The survival rate of female SSlepr mutant rats was markedly reduced compared to their male counterparts. Recently, we reported that male rats display proteinuria and podocyte injury by 6 weeks of age independent of hyperglycemia and elevations in arterial pressure. However, female SSlepr mutant rats were not examined during this same time period. Thus, we examine whether there were sex differences during the development of renal injury between 4 to 8 weeks of age. Proteinuria was significantly higher in female and male SSlepr mutant rats compared to lean SS rats at 4 weeks of age and remained elevated throughout the course of the study. When examining renal function during this time period by the clearance of FITC-sinistrin, we did not detect any strain or sex differences. The kidneys from the SSlepr mutant rats displayed significant glomerular injury and marked renal fibrosis when compared to lean SS rats during this time period. While we observed sex differences in metabolic and cardiorenal disease in older obese SSlepr mutant rats, we did not detect any sex differences in younger SSlepr mutant rats. Overall, these data indicate that the SSlepr mutant strain may be a useful model to study sex differences during the development of renal injury and CKD associated with obesity. Further studies are needed to determine the cause of the sex difference in mortality in obese SSlepr mutant rats. This research was supported by GM104357 and DK109133.

7.5 FEMALE RATS OFFERED FREE ACCESS TO LARD, SUCROSE, AND CHOW DEVELOPED FEATURES OF METABOLIC SYNDROME AND PERIUTERINE ADIPOSE TISSUE EXPANSION

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Objective: Free access to lard, sucrose solution, and chow results in the development of obesity and the metabolic syndrome within 3 weeks in male rats. The impact of obesity on adipose tissue expansion differs with each adipose depot throughout the body,
suggesting that obesity affects adipose tissue in a regional manner. We determined if free access to lard, sucrose, and chow would result in development of the metabolic syndrome and expansion (i.e. hypertrophy and hyperplasia) of peritoneal adipose tissue in female rats.

**Methods:** Two cohorts of virgin Sprague-Dawley female rats were divided into 2 weight-matched groups: 1) choice group: ad libitum access to chow, 30% sucrose solution, and lard (choice diet) and 2) chow group: ad libitum access to standard rodent chow for 3 weeks. Food intakes and body weights were recorded daily. Glucose clearance was assessed with a glucose tolerance test and insulin sensitivity was assessed with an insulin tolerance test. Fat depots and trunk blood were collected to measure visceral adiposity and triglyceride concentrations, respectively. Adipocyte morphology (cell size and count) was assessed in hematoxylin and eosin-stained peritoneal adipose tissue sections using NIS Elements software.

**Results:** Total energy intake was greater in choice rats than chow rats (1590 ± 41 vs. 1036 ± 19 kcal, p < 0.0001). Choice rats had higher percent carcass fat (10.3 ± 1.0 % vs. 4.1 ± 0.2 %, p = 0.0005) compared to chow rats, but body weight did not differ between groups (p = 0.51). Glucose tolerance and insulin sensitivity were not different in choice rats than chow rats, but choice rats had higher fasting glucose (122 ± 4 v. 110 ± 2 mg/dL, p = 0.03) compared to chow rats. Choice rats had greater visceral adiposity, serum concentrations of triglycerides (45.2 ± 3.8 vs. 31.0 ± 2.5 mg/dL, p = 0.003), and higher mean arterial pressure (95.79 ± 1.82 v. 88.55 ± 1.25 mm Hg, p = 0.01) compared to chow rats. Cross-sectional area/cell was greater in peritoneal adipose tissue from choice compared to chow rats (779.6 ± 47.6 vs. 492 ± 27.8 μm²/cell, p = 0.0001), indicating a hypertrophic response. Number of cells/unit area was fewer in peritoneal adipose tissue from choice rats (1.24 x 10³ ± 4.66 x 10² vs. 8.36 x 10³ ± 6.60 x 10⁵ cells/μm², p = 0.0002), indicating a hypoplastic response.

**Conclusion:** Free access to lard, sucrose, and chow induced some features of the metabolic syndrome. However, we did not observe glucose intolerance, as previously observed in male rats of the same strain, indicating potential sex differences in sensitivity to high-fat, high-carbohydrate diets. Choice diet-induced morphological changes in peritoneal adipose tissue may affect reproductive capacity (i.e. fertility) and pregnancy outcomes in females with preconceptional obesity.

**Funding:** UNT HSC Pilot Seed Grant

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**X MARKS THE SPOT: SEX CHROMOSOMES REGULATE HYPERCHOLESTEROLEMIA AND ATHEROSCLEROSIS**

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It is generally accepted that males have a more proatherogenic lipid profile and more coronary artery disease than females prior to menopause, when a proatherogenic lipid profile emerges in females and may exceed that of males. The majority of studies investigating sex differences in lipid profiles and atherosclerosis have focused on sex hormones. However, sex chromosome complement is the other primary biologic determinant of sex, and the X chromosome contains as much as 5% of the human genome. We used the 4 core genotype model to examine effects of a Western (WD) high fat with added cholesterol diet on hypercholesterolemia, obesity, and atherosclerosis in XX and XY male and female low density lipoprotein receptor deficient mice (Ldlr/-). Initial studies defined basal or short-term effects of the WD on whole body metabolism in male (XY and XX) and female (XX and XY) Ldlr/- mice. XX mice, regardless of gonadal sex, exhibited increased food intake compared to XY mice. Moreover, gastrointestinal fat absorption was increased in XX compared to XY mice, regardless of gonadal sex. However, liver lipoprotein secretion was not different between XX and XY mice (male or female) fed standard murine diet. When fed the WD for 3 months, XX mice (male and female) had increased body weight, and markedly higher serum cholesterol concentrations, and these effects were present even in castrated XX compared to XY mice, suggesting a prominent role for sex chromosomes. The extent of atherosclerosis was markedly increased in XX (male and female) compared to XY mice. Alterations in expression levels of key genes implicated in absorption of fat by the small intestine of XX mice, coupled with increased fat absorption and food intake, augment hypercholesterolemia and the development of atherosclerosis. These results suggest that an XX sex chromosome complement favors lipid absorption, which may contribute to proatherogenic lipid profiles and increased atherosclerosis in post-menopausal females.
8.1 LONG TERM CONSEQUENCES OF FOOD RESTRICTION ON BODY COMPOSITION AND ANGIOTENSIN SYSTEM
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Introduction: The women population is more predispose to do severe diets and people who does severe food restriction (FR) are at risk for developing cardiovascular disease during the FR period and also long term well after the FR period has ended. Abnormalities in the heart, vascular system and kidneys are common problems in individuals who have experienced FR. Unfortunately, the long-term risk for cardiovascular system due to earlier exposure to FR is an underappreciated and understudied problem and there are no known therapeutic strategies designed to prevent the increased risk after recovery from the FR period.

Methods: Female Fischer rats with 4 month old were divided in two groups, control (CT) with ad libitum diet and FR with 60% reduction in the daily food intake for 14 days. After 14 days of FR the rats received chow ad libitum for 3 months. On FR day 1 to 4 and day 14 of FR until day 4 of recovery period the rats were placed in a metabolic cage.

Aim: Determine the FR short and long-term consequences on body composition and angiotensin system.

Results: After 14 of FR the body weight (BW) was reduced by 18%, the water intake by 50%, the rats also showed a reduction in plasma volume (p<0.03), blood pressure (p=0.03), heart rate (p=0.03), kidney weight, kidney protein, high plasma Angiotensin (Ang) II (p<0.05) and angiotensinogen (p<0.001). No difference was observed in plasma aldosterone, sodium and and potassium. The rats with FR were in balance for sodium during the first 4 days of FR although for the first day the rats showed a negative balance for potassium (p<0.05) returning to the balance on the 2nd day. 3 days of FR also stopped the estrus cycle on diestrus returning to the ovarioly phase only after 7 days on ad libitum diet. The rats recovered the BW only 21 days in the reseeding protocol, although they had a higher deposition of abdominal fat after 40 days of reeding and no difference on the lean tissue. After 3 months of ad libitum diet, the rats normalized the water and food intake, however the Ang II infusion increased more the blood pressure (p<0.05), even though the blood pressure was normal.

Conclusion: The FR causes a short-term change on body composition and a high Ang system activation with a possible miscommunication between Ang-Aldo-Sodium-potassium. After 3 months of refeeding, the FR rats recovered the body weight and the blood, but had more fat deposition and pressor responsiveness to Ang II.

Acknowledgements: NIH 1R01HL119380 (KS); CAPES (AS)

8.2 PROGESTERONE UPREGULATES ENDOTHELIAL MINERALOCORTICOID RECEPTOR EXPRESSION WHICH PREDISPOSE FEMALE MICE TO OBESITY-INDUCED ENDOTHELIAL DYSFUNCTION
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Clinical data indicates that obesity ablates the protection of premenopausal women from cardiovascular disease. Compelling evidence indicates a higher efficacy of mineralocorticoid receptor (MR) blockade for the treatment of cardiovascular disease in obese and diabetic females than in males, however, the origin of this sex-specific effect is unknown. We have shown that the adipocyte-derived hormone leptin mediates endothelial dysfunction in obese female mouse models via aldosterone-dependent activation of MR and, further, that female mice are more sensitive to aldosterone-induced endothelial dysfunction. Therefore, we hypothesized that females express higher endothelial MR (ECMR) expression than males which predisposes females to obesity-associated endothelial dysfunction. RT-PCR analysis in isolated aortic endothelial cells (magnetic cell sorting technique) of Balb/C mice revealed a higher NR3C2 (MR gene) expression in females compared to males (2.9±0.5-fold from male, P<0.05), however, no such difference was observed in non-endothelial cells (0.1±0.3-fold from male). Endothelial NR3C2 expression was further elevated in female obese mice (5.8±1.1-fold from male obese, P<0.05). Similarly, human aortic (5.4-fold from male) and adipose tissue (2.1±0.1 fold from male, P=0.07) endothelial cells derived from female patients exhibited higher NR3C2 mRNA expression than those of males. Western blotting analysis confirmed that ECMR protein expression is correspondingly elevated in aortic endothelial cells from female patients compared to those of males (0.5±0.1 male vs 1.4±0.5 female ratio/β actin). Female sex hormones suppression (ovariectomy) decreased ECMR expression in female mice (~0.8±0.2-fold from sham, P<0.05), which was restored by progesterone.
supplementation (-0.1±0.1-fold from sham). Increases in progesterone levels with the diestrous phase of the menstrual cycle and pregnancy were associated with a gradual increase in NR3C2 mRNA (diestrous: 1.6±0.1-fold from estrous, pregnancy day 16: 9.2±0.2-fold from estrous, P<0.05). In parallel, progesterone dose-dependently increased ECMR protein expression in human endothelial cells in vitro (P<0.05). Endothelial function was determined in male and female mice by wire myography via aorta relaxation responses to acetylcholine (10^-9-10^-5 M concentration range). Increases in ECMR associated with higher progesterone levels in pregnant females were associated with an increased sensitivity to leptin-induced endothelial dysfunction in mice. In parallel, while leptin induced endothelial dysfunction in intact ECMR female mice (P<0.05), specific deletion of MR in endothelial cells protected female mice from leptin-induced endothelial dysfunction. No differences were observed in leptin-mediated endothelial function in male mice regardless of intact ECMR expression. Further, no changes in endothelium-independent relaxation responses (relaxation to sodium nitroprusside) in any groups were observed. These data indicate that progesterone drives the sex-difference in the levels of ECMR expression and predisposes female mice to leptin-induced endothelial dysfunction. In addition, these data provide a rational for the higher efficacy of MR blockade in obese and diabetic women suffering from cardiovascular disease.

Supported by: NIH 1R01HL130301-01, AHA 16IRG27770047, AHA 17POST33660678 and NIH 5F32HL136191-02.

8.3 CD8 T-CELLS ISOLATED FROM FEMALE MICE HAVE INCREASED IN VITRO CELL ACTIVATION IN RESPONSE TO NUTRIENT DEPRIVATION

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Nutritional status is critically important for immune cell function. T-cells from animal models of obesity have an early and critical role in inducing inflammation. Conversely, in the setting of malnutrition, T-cells have decreased effector function and proliferative capacity. We hypothesized that CD8+ T-cells isolated from females would show increased activation in response to nutrient deprivation leading to enhanced secretion of inflammatory proteins. CD8+ T-cells were isolated from the spleens of C57BL/6J wild-type mice (male=3.8±0.1 month old and female=3.6±0.1 month old; n=5/sex) and cultured in RPMI 1640 (10% FBS; 1% antibiotic/antimycotic) with T-cell activation beads (CD3ε and CD28) in 5% CO₂ at 37°C. After serum starvation in media with 0.1% FBS for 24 h, the secretome was collected and analyzed by mass spectroscopy. Proteomic analysis identified 3991 unique peptides from 3161 proteins. Of these proteins, 279 were significantly different (p<0.05 for all) and at least 2-fold different in expression between sexes (31 proteins downregulated and 248 upregulated in females compared to males). E3 ubiquitin-protein ligase, B-cell lymphoma 11b, tissue growth factor β, macrophage colony stimulating factor receptor, insulin-like growth factor binding protein, and Trp4 associated protein were the top regulators of sex differences based on fold change and p-value. Ingenuity pathway analysis indicated more than one-third (104 of the 279 proteins) mapped to networks involved in regulating cell death (↓), cell viability (↑), leukocyte trafficking (↑), angiogenesis (↑), or fibrosis (↓; arrows indicated direction of female response vs male response; p<0.05 for all). In conclusion, CD8+ T-cells isolated from females have an exacerbated response to metabolic stress induced by serum starvation. Key biological pathways were identified as regulators of sex differences in T-cell metabolic adaptation during nutrient stress.

8.4 ARGINASE 2 IS INVOLVED IN DIET-INDUCED OBESITY AND METABOLIC DYSREGULATION IN MALE AND FEMALE MICE

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Arginase is a ubiquitous enzyme, present in 2 isoforms, the cytosolic A1 or mitochondrial A2. Arginase competes with nitric oxide synthase for their common substrate L-arginine and can limit synthesis of nitric oxide (NO), which is needed to maintain normal adipocyte (AC) physiology and metabolic function. In our studies, male WT mice and mice globally lacking A2(-/-) were fed either normal chow diet (ND) or high fat, high sucrose (HFHS) diet for 16 weeks. A2 deletion blunted the HFHS-induced increases in body weight and visceral adipose tissue (VAT) weight in WT mice by 22% and 57%, respectively (p<0.05). In line with these observations, metabolic chamber studies showed that A2/- mice feed HFHS diet
also exhibited a higher respiratory exchange ratio (RER) compared to WT HFHS mice (p<0.05). A2-/- mice on HFHS diet showed a trend to an increase in energy expenditure compared to WT-HFHS (p=0.06). HFHS mice lacking A2 also exhibited a lower fasting blood glucose than WT-HFHS (p<0.05). A2 expression in ACs isolated from VAT of WT HFHS mice was elevated 4-fold compared to WT on ND. In differentiated AC (3T3-L1) exposed to high levels of palmitate (250 µM) and glucose (25 mM), A2 levels were 3-fold higher than cells in normal control media. WT-HFHS mice also exhibited reduced mRNA levels of PPAR-γ, as well as, the anti-inflammatory adipokine - adiponectin, insulin receptor substrate-1 (IRS-1) and glucose transporter (GLUT)-4 (p<0.05) vs ND controls. However, A2-/- HFHS mice showed preserved expression levels of these factors at almost control levels. A2 deletion also blunted the reduction in mitochondrial density in VAT seen in WT HFHS mice as well as the reduced AC mRNA expression of PPAR-γ coactivator-1α (PGC-1α), a regulator of mitochondrial biogenesis as well as the mRNA expression of the thermogenic markers - UCP1 and CIDE-A.

In female mice, lack of A2 produced a similar phenotype as in HFHS males, but with a more pronounced decrease in body weight (32%) and VAT weight (74%) compared to WT-HFHS (p<0.05). RER was not different between WT and A2-/- HFHS fed groups (p>0.05). However, A2 deletion significantly blunted the decrease in energy expenditure exhibited by WT group fed HFHS diet (p<0.05). In addition, fasting blood glucose was significantly lower in HFHS A2-/- mice compared to WT HFHS diet (p<0.05).

In conclusion, our study shows that A2 is critically involved in HFHS-induced obesity and metabolic abnormalities in both males and females with a more prominent role in the females.

Funding: NIH Grants EY11766 and NIH HL70215 and AHA (17PRE33660321)

We hypothesized that these sex-specific differences may also extend to molecular pathways activated within the β cell during the evolution of Type 1 diabetes. To address this, human islets from 5 male and 5 female donors were treated with or without cytokines (50 U/ml IL-1β and 1000 U/ml IFN-γ) for 24 hrs, to mimic the pro-inflammatory conditions of T1D. Stratified analysis based on sex was performed. mRNA and miRNA sequencing were performed using Illumina NextSeq and Ion Proton System, respectively. Partek flow, R statistical program (DESeq2 package) and Ingenuity Pathway analysis tools were utilized for analyzing the datasets. miRNAs with a fold change ≥ 2.0 and false discovery rate < 0.05 and miRNAs with a fold change ≥ 1.5 and p < 0.05 in cytokine-treated islets were considered as differentially expressed (DE). miRNA targets were predicted using TargetScan, which were then overlapped with DE mRNAs identified from islets.

Our data revealed striking sex differences in miRNA expression profiles in cytokine-treated islets. Notably, only 4 DE miRNAs were common between the two sexes, while 16 miRNAs and 25 miRNAs were unique in males and females, respectively. Classically these variations have been attributed to differences in sex chromosomes and the action of sex hormones. However, we found that > 95% of these miRNAs originated from autosomes and none of these miRNAs are known to be under the influence of sex hormones. Whereas, 1061 mRNAs were common from a total of 1667 and 1186 DE mRNAs from male and female islets, respectively. Among the significant canonical pathways, 12 pathways were common between males (total = 21; unique = 9) and females (total = 26; unique = 14) and each of these common pathways (Eg: Sphingosine-1-phosphate) were predicted to be regulated by unique miRNAs for males and females. Similarly, common gene ontology terms were observed to be regulated by predominantly unique miRNAs in males and females. For example, apoptosis of pancreatic cells was observed to be regulated by miR-146a-5p, miR-338-3p, miR-155-5p, miR-6891-5p, miR-296-3p, miR-181a-2-3p, miR-675-5p in males, but the same function was predicted to be regulated by miR-146a-5p, miR-194-3p, miR-22-3p, miR-320b, miR-30e-3p, miR-124-3p, miR-338-5p in females.

Taken together, our data suggests that sex-biased expression patterns among mRNAs and their upstream regulatory miRNAs may drive sex-specific activation or inhibition of molecular pathways involved in T1D pathogenesis. Understanding these differences are critical to consider in our efforts to develop biomarkers for T1D risk and heterogeneity and when designing trials of novel disease-modifying therapies.
SEX SPECIFIC UPREGULATION OF HEPATIC FGF21 EXPRESSION IN METABOLIC STATES CONTRAST BY NUTRIENT AVAILABILITY

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Many aspects of metabolic homeostasis are known to be regulated differently in males and females. Fibroblast growth factor 21 (FGF21) is a hormonal regulator of carbohydrate and lipid metabolism, which is induced by both catabolic and anabolic signals (1). Diet induced obesity (anabolic state) was demonstrated to cause sex specific upregulation of FGF21 in liver tissue (2). No previous studies have addressed the possible sexual differences in FGF21 response to fasting (catabolic state). The aim of this study was to investigate whether there is sexual dimorphism in the effects of dietary obesity and fasting on the hepatic FGF21 expression and its signaling.

Male and female C57Bl mice were subjected to 24h-fasting or to sweet-fat diet (10 weeks). Circulating FGF21 levels and hepatic expression of Fgf21 and genes involved in FGF21 signaling (Ppar-a, Pgc1, Klβ) were measured.

FGF21 plasma concentrations and hepatic Fgf21 expression were increased by the fasting and the degree of upregulation was significantly higher in the livers of female mice. PPARα gene expression, which has been reported as upstream of FGF21, also was upregulated more potently in the liver of fasted female than male mice. Reversed sexual dimorphism in upregulation of FGF21 gene expression was observed in obese mice. Obesity induced male-specific upregulation of FGF21 in liver tissue. PPARα expressions were also upregulated more potently in the liver of obese male than female mice. Consistently, higher levels of FGF21 were detected in serum obtained from obese male mice compared to that of female mice. In both fasted and obese mice, hepatic expression of Fgf21 gene did not correlate to expressions of genes involved in FGF21 signaling: PPARγ coactivator protein-1α (Pgc-1α), a key transcriptional regulator of energy homeostasis and β-klotho (Klb) – co-receptor of FGF21 receptor.

Thus, sex specific upregulation of hepatic PPARα-FGF21 endocrine signaling pathway was observed in metabolic situations contrast by nutrient availability. Upregulation of these genes was biased toward females under catabolic condition (fasting) and toward males under anabolic condition (diet induced obesity). The data suggest important role of hepatic FGF21 in the sexually dimorphic regulation of metabolic homeostasis.

8.7

FEMALE RATS OFFERED FREE ACCESS TO LARD, SUCROSE, AND CHOW DEVELOPED FEATURES OF METABOLIC SYNDROME AND PERIUTERINE ADIPOSE TISSUE EXPANSION

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Objective: Free access to lard, sucrose solution, and chow results in the development of obesity and the metabolic syndrome within 3 weeks in male rats. The impact of obesity on adipose tissue expansion differs with each adipose depot throughout the body, suggesting that obesity affects adipose tissue in a regional manner. We determined if free access to lard, sucrose, and chow would result in development of the metabolic syndrome and expansion (i.e. hypertrophy and hyperplasia) of periumarine adipose tissue in female rats.

Methods: Two cohorts of virgin Sprague-Dawley female rats were divided into 2 weight-matched groups: 1) choice group: ad libitum access to chow, 30% sucrose solution, and lard (choice diet) and 2) chow group: ad libitum access to standard rodent chow for 3 weeks. Food intakes and body weights were recorded daily. Glucose clearance was assessed with a glucose tolerance test and insulin sensitivity was assessed with an insulin tolerance test. Fat depots and trunk blood were collected to measure visceral adiposity and triglyceride concentrations, respectively. Adipocyte morphology (cell size and count) was assessed in hematoxylin and eosin-stained periumarine adipose tissue sections using NIS Elements software.

Results: Total energy intake was greater in choice rats than chow rats (1590 ± 41 vs. 1036 ± 19 kcal, p < 0.0001). Choice rats had higher percent carcass fat (10.3 ± 1% vs. 4.1 ± 0.2%, p = 0.0005) compared to chow rats, but body weight did not differ between groups (p = 0.51). Glucose tolerance and insulin sensitivity were not
different in choice rats than chow rats, but choice rats had higher fasting glucose (122 ± 4 v. 110 ± 2 mg/dL, p = 0.03) compared to chow rats. Choice rats had greater visceral adiposity, serum concentrations of triglycerides (45.2 ± 3.8 vs. 31.0 ± 2.5 mg/dL, p = 0.003), and higher mean arterial pressure (95.79 ± 1.82 v. 88.55 ± 1.25 mm Hg, p = 0.01) compared to chow rats. Cross-sectional area/cell was greater in peritoneal adipose tissue from choice compared to chow rats (779.6 ± 47.6 vs. 492.0 ± 27.8 μm²/cell, p = 0.0001), indicating a hypertrophic response. Number of cells/unit area was fewer in peritoneal adipose tissue from choice rats (1.24 x 10^5 ± 4.66 x 10^3 vs. 8.36 x 10^5 ± 6.60 x 10^5 cells/μm², p = 0.0002), indicating a hypoplastic response.

**Conclusion:** Free access to lard, sucrose, and chow induced some features of the metabolic syndrome. However, we did not observe glucose intolerance, as previously observed in male rats of the same strain, indicating potential sex differences in sensitivity to high-fat, high-carbohydrate diets. Choice diet-induced morphological changes in peritoneal adipose tissue may affect reproductive capacity (i.e. fertility) and pregnancy outcomes in females with preconceptional obesity.

Funding: UNT HSC Pilot Seed Grant

### 8.8

**PRENATAL DIABETES AND HIGH-FAT DIET EXPOSURE IMPAIR MITOCHONDRIAL FUNCTION IN ADULT RAT CARDIOMYOCYTES**

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**Background:** Both diabetes and obesity increase the risk of heart disease in adults, and mounting evidence shows that infants born to diabetic or obese mothers also have a higher risk of heart disease in adulthood. We have shown that newborn rats prenatally exposed to maternal diabetes and high-fat (HF) diet have diastolic and systolic dysfunction, myocardial lipids accumulation, decreased respiratory capacity, oxidative injury and mitochondrial dysfunction that mimics that of adult diabetic cardiomyopathy. However, do these adverse cardiac effects carry into adulthood? If so, is either sex more severely affected?

**Objective:** Determine whether prenatal exposure to maternal diabetes or HF diet impairs bioenergetics or mitochondrial function in primary isolated adult rat cardiomyocytes (ARCM).

**Methods:** Sprague-Dawley rats received control or HF diet 28 days before and throughout pregnancy. On gestational day 14, citrate buffer or streptozotocin was given to induce diabetes, which was then treated twice daily with sliding-scale insulin. Dams delivered controls, diabetes-exposed, HF diet-exposed and combination-exposed offspring which, on postnatal day 1, were cross-fostered and raised by healthy dams. At 12-13 months, ARCM were isolated for extracellular flux analysis (glycolytic, mitochondrial, and palmitate stress tests and permeabilized assays), live-cell confocal imaging of stress responses, mitochondrial copy number, and lipid peroxidation assays. Groups were compared using two-way ANOVA for diet, diabetes, and interaction with significance set at p≤0.05.

**Results:** Results to date show that male ARCM have a greater ability to oxidize fatty acids and lower glycolytic capacity than female ARCM. In both sexes, diet- and diabetes-exposed ARCM had decreased proton leak, reduced respiratory spare capacity, and impaired tolerance for uncoupler-induced stress. Confocal imaging further demonstrated these effects by showing 15-50% faster membrane potential loss in both sexes and 30-50% faster cell death in female ARCM prenatally exposed to diet and diabetes. Poor spare capacity is likely related to impaired fuel flexibility. All ARCM demonstrated poor glycolytic capacity. Diet-exposure reduced exogenous and endogenous fatty acid oxidation in males while diabetes-exposure reduced endogenous fatty acid oxidation in females. In males, diet-exposed ARCM had reduced oxidation of the complex I fuel glutamine, complex II fuel succinate, and complex IV fuel TMPD/ascorbate. In females, combination-exposed ARCM had lower oxidation of complex II succinate and complex III duroquinol. Results for mitochondrial copy number and lipid peroxidation are pending.

**Conclusions:** Prenatal exposure to maternal HF diet and diabetes causes sex-specific changes in mitochondrial function and fuel flexibility in ARCM. Findings highlight the role of mitochondria in fetal origins of adult cardiovascular disease.

Funding sources: NIH-NICHD K08HD078504, NIH-GMS P20GM103620, NIH-GMS P20GM103548, University of South Dakota Faithe Family Medical (UI893), Wheeler School Memorial (UI867), School of Medicine Annual (UI696), and Madsen-Jungwirth Medical (UI843) Scholarships, and Sanford Research.

### 8.9

WITHDRAWN

### 8.10

**HIS AND HER COMPUTATIONAL MODELS OF LONG-TERM BLOOD PRESSURE REGULATION**

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Hypertension is a global health challenge. Hypertensive men and women are typically treated with the same approach, with less effective outcome in women. To address the critical need to better understand the mechanism of blood pressure control in both men and women, we have developed sex-specific computational models of long-term blood pressure control.

The model represents sex differences in the kidney’s pressure natriuresis response, whereby increases in renal perfusion pressure lead to increases in Na+ excretion; that in turns lowers salt and water retention and reduces effective circulating volume.

Females tend to exhibit a leftward shift in the pressure-natriuresis relation relative to males. The model also includes detailed representation of the renin-angiotensin aldosterone system (RAAS), a non-sex hormonal system critical for maintaining blood pressure and effective circulating volume. Major sex differences in the RAAS have been identified, including how substrate is produced, how angiotensin interacts with receptors, and baseline aldosterone levels. Those sex differences are included in the model. Differences in renal sympathetic nervous activity (RSNA) stimulation are also included.

Using the developed model, we conclude that increased afferent arteriole resistance causes a larger increase in mean arterial pressure than other causes of hypertension. We also conclude that females are protected against hypertension caused by increased afferent arteriole resistance (a 17 mmHg increase in females vs a 22 mmHg increase in males) due to differences in RSNA sensitivity.

We also conclude that observed sex differences in anti-hypertensive drug efficacy, (i.e. that angiotensin converting enzyme inhibitors (ACEI) work better than angiotensin receptor blockers (ARB) in males while the opposite is true in females) is due to the effects of angiotensin II on renal vascular resistance when bound to the angiotensin type 2 receptor (AT2R-bound Ang II). Abolishing this effect from our model also abolishes the sex difference in drug treatment efficacy.

This research was supported by the National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, grant R01DK106102, and by the National Science Foundation, grant DMS1263995.

### 8.11 SEX DIFFERENCES IN THE ROLE OF THE SMOOTH MUSCLE CELL MINERALOCORTICOID RECEPTOR IN CARdiovascular AGING

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The process of vascular aging involves the development of vasomotor dysfunction and vessel stiffening, which leads to cardiovascular disease (CVD). Clinical data supports that the time course of CVD development with aging occurs differentially in males vs. females, suggesting distinct sex-specific mechanisms of vascular aging. The mineralocorticoid receptor (MR) is a steroid hormone receptor known to regulate renal sodium handling. We previously demonstrated that male mice with the MR specifically deleted from smooth muscle cells (SMC-MR-KO), have lower blood pressure, vascular tone, and vasoconstriction as they age. The purpose of this study was to determine whether there are sex differences in the role of SMC-MR in vascular aging. All evaluations of the mice took place at 3 ages: 3, 12, and 18 months, chosen as these roughly correspond to adult (3 mo.), middle age or “peri-menopause” (12 mo.) and elderly or “post-menopause” in females (18 mo.).

First, we show that vascular MR increases with age differentially in male and female mice; with vascular MR increasing at 12 months of age in males but not until 18 months of age in females. This change in MR expression is accompanied by a concomitant down-regulation of vascular expression of miR-155 at 12 months in males vs. 18 months in females, which is prevented in SMC-MR-KO mice. In vitro reporter assays showed that MR transcriptionally represses miR-155 promoter activity, whereas estrogen receptor (ER) upregulates miR-155 promoter activity, and is further enhanced with estrogen treatment. These studies suggest that MR and ER regulate miR-155 in an opposite manner, and that the decline in miR-155 in females may be driven by combination of the increase in MR and a loss of estrogen.

We previously identified miR-155 as a regulator of vascular target genes including the angiotensin II type 1 receptor (AT1R) and the pore-forming subunit of the L-type calcium channel (LTCC), Cav1.2, both mediators of vasoconstriction and enhanced vascular tone. Indeed, we find that AT1R- and LTCC-mediated vasoconstriction is increased at 12 months in males and 18 months in females and these vasomotor alterations are prevented in SMC-MR-KO mice of both sexes. We next examined alterations in vascular stiffness, a hallmark of vascular aging. In vivo aortic stiffness studies, as measured by...
pulse wave velocity (PWV), demonstrate the same temporal difference with aging with increased vascular stiffness at 12 months in males vs. 18 months in females, that is prevented by SMC-MR-KO. We explored the contribution of SMC to stiffness via atomic force microscopy of freshly dispersed SMC from ~18 month old mice. These studies revealed that females exhibit more SMC stiffness vs. males, and that this is partially prevented by SMC-MR-KO in females only. To assess the role of fibrosis in the aging-induced alterations in stiffness, we measured carotid artery fibrosis histologically. Carotid fibrosis is increased at 12 months in males vs. 18 months in females, and partially prevented by SMC-MR-KO in males only. Together, these data suggest that the temporal difference in vascular stiffening is associated with distinct sex-specific mechanisms driving vascular stiffness with aging and that SMC-MR plays a differential role in males and females. Next, we examined the effect of aging on cardiac function in males and females by in vivo echocardiography. Cardiac function declines at 18 months in both males and females, but is attenuated by SMC-MR-KO in females only. Further characterization of overall cardiovascular function via exercise capacity testing revealed that exercise capacity declines in males at 12 months vs. 18 months in females, and is partially restored by SMC-MR-KO in males only. In summary, the time course and mechanisms of cardiovascular aging are distinct between males and females with SMC-MR playing a differential role in males versus females. Furthermore, these studies suggest that sex-specific therapies may be essential to improve CVD outcomes in the aging population.

We studied if there is a sexual dimorphism pattern in the vascular responses in PAH, using a chronic hypoxia (CH) rat animal model, where male and female rats were exposed to 10%O2 for 2 weeks. Ageing also plays a key role in the estrogens levels, as they decrease markedly in females. Therefore this prompted us to further study the estrogens role in PAH development using both genders aged rats (>20 months old).

Young female rats showed a faster progression of PAH after 2 weeks of exposure to 10% O2, with a high increase of PAP (almost doubled; determined by right heart catheterization) and right ventricular hypertrophy (Fulton index >30%) respect to their male counterparts. Ageing didn’t affect the values of PAP or Fulton index in both genders when compared with young animals, with no differences between sexes. CH in the old animals didn’t have as much effect as it was observed in the young animals in PAP, although changes in the Fulton index were in the same direction than in the young animals, but no differences were observed between old male and female animals.

Opposite to these hemodynamic and cardiac results, the dose response carbachol-induced relaxation on preconstricted PA as indicative of vascular dysfunction showed that CH generated endothelial damage in young male rats (p<0.001), with no damage in the females (p>0.05). As for only ageing, the endothelial dysfunction appeared just in female animals (p<0.01), with no further damage after CH exposure. Ageing in males didn’t cause any significant damage before or after CH exposure (p>0.05 in both cases).

All data is suggesting a PAH sexual dimorphism in rat, with females developing PAH more rapidly than males at young ages. Conversely their PA are more prone to endothelial damage in CH. As for ageing, where estrogens levels should be decreased, effects of CH are blunted, and differences between male and female animals absent.

In conclusion we postulate that the connection between sex and pulmonary circulation rests on different vascular contractility properties.

Grants: BFU2015-70616R (MINECO-FEDER); CIBER CB06/06/0050 (ISCiii)
ABSTRACTS OF INVITED AND VOLUNTEERED PRESENTATIONS

8.13
THE ROLE OF SEX IN OXIDATIVE STRESS AND IMPLICATIONS ON VASCULAR FUNCTION AND BLOOD PRESSURES
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The abrupt loss of estrogen leads to a substantial burden on female vasculature and blood pressures (BP) and is a reason why females display a considerable increase in cardiovascular disease development and progression following menopause. It is unknown how estrogen maintains arterial compliance and BP, but may be due to the role of estrogen in increased nitric oxide bioavailability and subsequent reduction in oxidative stress (OS) levels. This link between OS and estrogen is unknown and not well studied. Reducing OS through acute dosages of antioxidants has been previously shown to improve vascular function in males with elevated OS, such as older adults and patients with coronary artery disease. It is unknown if individuals with high estrogen (young females) versus individuals with low estrogen (young males) might respond differently to decreases in OS mediated through antioxidant supplementation and increases in OS mediated through a stimulated oxidative stressor, such as hyperoxia. Therefore, the purpose of this study was to determine if estrogen plays a role in the response to elevations (oxygen supplementation) and decrements (antioxidant supplementation) in OS. Twenty males and females (10 males, 10 females; mean age = 22 years) underwent a baseline visit and then received both ascorbic acid (AOX, 2000 mg) and 100% oxygen supplementation (OXY) in a randomized, cross-over design. Peripheral and central blood pressures (brachial, aortic, and carotid) were measured using an automated sphygmomanometer and applanation tonometry (SphygmoCor) and arterial function measures were assessed using ultrasonography (Arietta 70, Aloka). Conditions were compared using a 3-way ANOVA (baseline x AOX x OXY) and followed up with independent and dependent t-tests when significant. There was an effect of sex with males displaying higher brachial pulse pressure (PP), carotid systolic BP, and carotid PP with all conditions. Males had significantly higher aortic PP at baseline and AOX (values shown as baseline; AOX; OXY [34 mmHg; 33 mmHg; 34 mmHg]) and females had significant decreases in aortic PP with AOX and significant increases in aortic PP with OXY (30 mmHg; 29 mmHg; 32 mmHg). There were no significant differences in measures of carotid beta stiffness or arterial compliance. These results demonstrate that although there were no sex differences in response to changes in OS in arterial function

measures, blood pressures were altered differently between sexes, with males consistently displaying elevated pressures. Female aortic PP was significantly affected by both AOX and OS conditions.

8.14
ESTROGEN DETERMINES THE SEX-DIFFERENCES IN ADRENERGIC VESSEL TONE REGULATION
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Introduction: Sex-specific differences in adrenergic vasoconstriction and vasorelaxation have been demonstrated in rats and humans. Although we have previously shown that differences in rats rely on endothelial β-adrenoceptors, neither translational relevance in humans nor the role of sex-hormones in endothelial β-adrenoceptor-related vessel tone regulation has been shown.

Aims: We investigated the role of endothelium in sex-specific differences of adrenergic vasoconstriction and vasorelaxation in human vessels, as well as the role of female and male sex-hormones on adrenergic vessel tone regulation in a rat model.

Methods: In human mammary arteries, obtained from the Heart Center Dresden (patient age: 50 to 70 years), vasoconstriction (norepinephrine) and vasorelaxation (isoprenaline and β₃ agonist BRL) with and without endothelium were assessed using Mulvany myography. Five weeks old female and male wistar rats were respectively ovariectomized and orchiectomized. As controls, a sham-operated, hormone substituted (2 mg/kg, twice a week) and a vehicle group of rats were examined. At age of 12 weeks, aortas were isolated for assessment of vasoconstriction and vasorelaxation. Additionally, a qRT-PCR for quantification of β-adrenoceptor mRNA levels in aorta was performed.

Results: Mammary arteries of women constricted less (P<0.05) in response of norepinephrine than arteries of men. Removal of endothelium eliminated this sex-specific difference by significantly (P<0.05) increasing vasoconstriction in arteries of women, without affecting vasoconstriction in arteries of men. Vasorelaxation caused by isoprenaline was greater (P<0.05) in mammary arteries of women compared to arteries of men. This sex-specific difference in vasorelaxation was abolished after removal of endothelium. Similar to human arteries there were sex-specific differences in vasoconstriction and relaxation in rat aorta, which was eliminated after ovariectomy in female rats. Compared to sham operated females, ovariectomy increased aortic vasoconstriction in response to norepinephrine more than 2-fold. Vasorelaxation by isoprenaline and β₃-agonist was
significantly (P<0.01) reduced after ovariectomy. Compared to vehicle, estrogen substitution largely (P<0.05) restored sex-specific differences in vasoconstriction and vasorelaxation in ovariectomized rats. Differences in vasoconstriction and vasorelaxation between sexes were diminished in presence of selective β1- and β3-adrenoceptor antagonists and L-NMMA. Consistently, mRNA levels of β1- and β3-, but not β2-adreno receptors were significantly (P<0.05) higher in aortas of sham operated females than in aortas of sham operated males. Ovariectomy abolished this difference by decreasing β1- and β3-adrenoceptor expression in female rats. Consequently, estrogen substitution in ovariectomized females largely (P<0.05) restored β1- and β3-adrenoceptor expression. Ovariectomy and testosterone treatment did not change aortic vasoconstriction and vasorelaxation nor β-adrenoceptor expression in aortas of male rats.

Conclusion: We reveal that sex-specific differences in vasoconstriction and vasorelaxation in human mammary artery are endothelium-dependent. We also demonstrate that sex-differences in a rat model are estrogen, but not testosterone-dependent. Estrogen determines these differences via regulation of vascular endothelial β1- and β3-adrenoceptor expression.

8.15
INHIBITION OF NEPRILYSIN ATTENUATES ANGII-INDUCED ABDOMINAL AORTIC ANEURYSMS (AAAS) AND ATHEROSCLEROSIS IN HYPERCHOLESTEROLEMIC MALE MICE

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Objective: Abdominal aortic aneurysm (AAA) is a symptomatic deadly vascular disease of elderly men. Plasma levels of brain natriuretic peptide (BNP), which is degraded by the metalloendpeptidase, neprilysin, have been suggested as biomarkers of incident AAA. However, it is unclear if neprilysin plays a role in AAA development. Entresto®, a neprilysin inhibitor in combination with an angiotensin receptor blocker, has demonstrated efficacy in human heart failure and is under investigation for treatment of other cardiovascular diseases. In this study, we examined the effect of the neprilysin inhibitor, sacubitril, on AngII-induced AAAs in male LDLr−/− mice.

Methods and Results: Male (8-12 weeks of age) LDLr−/− mice were fed a Western diet (Teklad TD88137) for the duration of the study. Vehicle or sacubitril (5, 1, 6 or 20 mg/kg/day) were administered by osmotic minipump for one week, and then minipumps containing vehicle or 5 (at respective doses) in combination with AngII (1,000 ng/kg/min) were implanted for 28 day delivery. Body weights were similar in all groups. Sacubitril decreased systolic blood pressure (measured by tail cuff during week 3 of AngII infusions) in a dose-dependent manner, with maximal effects 56 mg/kg/day (vehicle, 150 ± 5; S1 mg/kg/day, 142 ± 7; S6 mg/kg/day, 118 ±5; S20 mg/kg/day, 122 ± 5 mmHg). Sacubitril dose-dependently reduced suprarenal aortic lumen diameters (day 28: vehicle, 1.8 ± 0.02; S1, 1.9 ± 0.2; S6, 1.6 ± 0.2; S20, 1.2 ± 0.1 mm; P<0.05) and maximal AAA diameters at study endpoint (vehicle, 2.3 ± 0.2; S20, 1.0 ± 0.1 mm; P<0.05). AAA incidence (89% in vehicle-infused mice) was significantly reduced by S20 mg/kg/day (20%). Similarly, sacubitril reduced atherosclerosis in a dose-dependent manner (vehicle, 8 ± 1.3; S1, 9.2 ±1.1; S6, 5.6 ±1.4; S20, 2.7 ± 0.4 % lesion surface area; P<0.05). Interestingly, AngII-induced reductions in plasma renin concentrations were reversed by sacubitril (vehicle, 3.5 ±0.1; S20, 22.3 ± 3.2 ng/ml; P<0.05).

Conclusions: These results demonstrate that inhibition of neprilysin protects against AngII-induced atherosclerosis and AAAs in male LDLr−/− mice. Future studies will determine mechanism of action in neprilysin inhibition, and whether combination with an angiotensin receptor blocker is an effective therapeutic in the prevention and treatment of atherosclerosis and AAA progression.

Funding: These studies were supported by the National Institutes of Health Heart Lung and Blood Institute (R01 HL107326; LC) and from the American Heart Association (YA; predoctoral fellowship 14PRE20030018).

8.16
WITHDRAWN

8.17
WITHDRAWN

8.18
SEX DIFFERENCES IN VASCULAR REACTIVITY AND BIOMARKERS OF INFLAMMATION IN OFFSPRING OF DAMS EXPOSED TO PERINATAL HIGH SALT DIET.

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Hypertension is an important risk factor for cardiovascular diseases and excess of dietary salt is the most common environmental factor that contributes to the development of hypertension (Meneton et al., 2005). However, in utero factors have been implicated in the pathogenesis of hypertension (Alexander, 2006). To assess the effect of perinatal high salt diet (HSD) on the offspring, pregnant Sprague-Dawley rats were exposed to perinatal normal (0.3%) or high (8%) salt diet from day
1 of pregnancy till term. The following experiments were conducted in the male and female offspring at 12 weeks of age: Blood pressure (BP) was measured via arterial cannulation under urethane and α-chloralose anesthesia (5ml/kg body weight i.p) using ADInstruments powerlab. Isolated abdominal aorta reactivity to noradrenaline (NA) and acetylcholine (ACh) in the presence or absence of endothelial nitric oxide synthase (eNOS) inhibitor; L-nitro arginine (L-NA) was determined. Serum concentrations of C-reactive proteins (CRP), TNF-α and IL-6 was also measured using commercially available kits. Perinatal HSD elevated BP (p< 0.05) in both sexes but to a greater magnitude in male when compared with female offspring. Basal vascular tone was higher in the male offspring from dams fed a perinatal HSD when compared with male from dams fed a normal salt diet and female from dams fed a HSD. Female offspring from dams fed a HSD has a higher contractile response to NA before and after eNOS inhibition by L-NA when compared to male. However, male offspring from dams fed a perinatal HSD exhibit impaired vasorelaxation response to ACh both in the presence or absence of L-NA when compared with females. High salt diet increased CRP, TNF-α and IL-6 in both the male and female offspring, but the magnitude is higher in males when compared with females. Findings from this study suggest that perinatal exposure of dams to a high salt diet causes hypertension via mechanisms involving vascular function impairment as well as systemic and vascular inflammation. However, this effect of perinatal HSD is more pronounced in male offspring suggesting sexual disparity in the effect on offspring, of perinatal exposure of dams to HSD.

References:

8.19
NITRIC OXIDE HELPS MAINTAIN THE BUFFERING CAPACITY OF PERIVASCULAR ADIPOSE TISSUE IN FEMALE DAHL SS IN RESPONSE TO A HIGH FAT DIET DESPITE INCREASES IN BLOOD PRESSURE AND VASCULAR INFLAMMATION
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Local inflammation in perivascular adipose tissue (PVAT) is linked to high fat diet (HFD)-induced increases in BP and vascular dysfunction in males. There is limited data on the impact of HFD on BP or vascular function in females. Thus the goal of this study was to test the hypotheses that HFD will 1) increase BP and vascular inflammation and 2) PVAT will exacerbate HF diet-induced vascular dysfunction in female DSS. 6-wko female DSS were fed a normal-fat diet (NFD; 7.2% fat) or HFD (35% fat) diet for 10 weeks, and BP was measured by telemetry. At 16 wko, aortic rings (+/- PVAT) were mounted for isometric myography and cumulative concentration response curves to phenylephrine (PE) or acetylcholine (Ach) were generated in the absence or presence of the nonselective nitric oxide synthase (NOS) inhibitor L-N6-Nitroarginine methyl ester (LNAME) or polyethylene glycol (PEG)-catalase. In separate rats, aortic T cells were measured by flow cytometry. HFD increased BP (mmHg: 176 ± 8 HFD vs 130 ± 4 NFD, P<0.001) and led to greater numbers of total aortic T cells (P=0.05), T cell activation (P=0.002), and pro-inflammatory Th17 cells (P=0.002) compared to NFD. There was no change in anti-inflammatory T regulatory cells (P=0.67). HFD alone had no effect on vascular function. Although the presence of PVAT did not increase relaxation to Ach, it did attenuate PE-induced constriction [Area Under Curve (AUC): effect of PVAT P<0.01] regardless of diet. HFD is known to promote oxidative stress via increased production of reactive oxygen species, including H2O2. Interestingly, Peg-catalase uncovered a PVAT-mediated vasoconstritor with HFD (AUC: effect of diet P=0.015; effect of PegCat: P=0.17; interaction: P=0.0091) while LNAME increased force generation to PE in the presence of PVAT regardless of diet (effect of LNAME P=0.047; effect of diet: P=0.17). Thus, in contrast to what has been shown in male DSS following a HFD in other studies, vascular function is maintained in female DSS; and PVAT enhanced the vasodilatory capacity of the aorta regardless of diet. Further, our data suggests that overproduction of NO rather than H2O2 plays a role in maintaining the anti-contractile effect of PVAT in response to a HFD. Future studies will determine 1) which NOS isoform contributes to the enhanced buffering capacity of PVAT in response to a HFD and 2) whether or not this capacity is mediated by the endothelium using endothelium intact and denuded vessels.

8.20
AFFERENT ARTERIOLAR RESPONSIVENESS TO ENDOTHELIN RECEPTOR ACTIVATION: DOES SEX MATTER?
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The pathogenesis of hypertension is distinct between men and women. Endothelin-1 (ET-1) is a potential contributor to sex-differences in the pathophysiology of hypertension. ET-1 is known to participate in blood
pressure regulation through activation of endothelin A (ET\textsubscript{A}) and endothelin B (ET\textsubscript{B}) receptors in both the renal tubule and vasculature. However, little is known about sex-differences in ET-1 dependent renal microvascular reactivity. Our lab previously reported that renal medullary ET-1 reduces medullary blood flow in male, but not female rats. Orchietomy (ORX) eliminated ET-1 dependent decreases in medullary blood flow, but ovariectomy (OVX) had no apparent effect on this sex difference. Thus, we hypothesized that sex and sex steroids regulate the afferent arteriolar responses to ET receptor activation. To test that, we used 15-17 week old male and female Sprague Dawley rats subjected to gonadectomy or sham surgery. Three weeks later, kidneys from those rats were prepared for assessment of renal microvascular responses to ET-1 (ET\textsubscript{A} and ET\textsubscript{B} agonist, 10\textsuperscript{-12} to 10\textsuperscript{-8}M) and sarafotoxin 6c (S6c, ET\textsubscript{A} agonist, 10\textsuperscript{-12} to 10\textsuperscript{-8}M) using the blood-perfused juxtamedullary nephron preparation. Baseline afferent arteriolar diameter at 100 mmHg averaged 15.3±0.3 and 14.6±0.3 μm for sham male and female rats, respectively (n=12, each). Gonadectomy had no significant effect on baseline arteriolar diameter. In sham males, ET-1 produced significant concentration-dependent decreases in afferent arteriolar diameter, with 10\textsuperscript{-8}M ET-1 decreasing diameter by 84±1 % (n=6). Similarly, ET-1 induced concentration-dependent vasoconstrictor responses in sham female rats, with 10\textsuperscript{-8}M ET-1 decreasing the diameter by 82±1 % (n=6). The vasoconstrictor responses to ET-1 within the afferent arteriole were unchanged by ORX or OVX. In addition, ET\textsubscript{B} receptor activation by S6c induced a concentration dependent decline in the afferent arteriolar diameter, with 10\textsuperscript{-8}M S6c decreasing diameter by 77±3 and 76±3 % in sham male and female rats, respectively (n=6, each). These data do not support our original hypothesis and suggest that sex or sex hormones do not significantly influence afferent arteriolar reactivity to ET receptor activation. They further suggest that reported sex differences of the renal ET-1 system on blood pressure are most likely mediated through renal tubular activity of the ET\textsubscript{A} and ET\textsubscript{B} receptors as we have previously reported.

**Background:** Despite the well-established sex-specific differences in the incidence of bronchopulmonary dysplasia (BPD), the molecular mechanism(s) behind these are not completely understood. Human pulmonary microvascular endothelial cells (HPMECs) provide a robust in vitro model for the study of endothelial cell physiology and function. Alterations in fibroblast phenotype may underlie some of the changes observed in babies with BPD, which is characterized by impaired alveolarization and vascular growth. Notch pathway activation leads to fibroblast activation and proliferation in response to changes in oxygen and is also to modulate angiogenesis.

**Objective:** To elucidate the sex-specific differences in male and female human neonatal pulmonary microvascular endothelial cells at normoxic and hyperoxic conditions and delineate the differences in Notch pathway activation and test the hypothesis that male fibroblasts would display a greater pro-fibrotic phenotype and greater Notch activation upon exposure to hyperoxia.

**Methods:** The HPMEC cells (18-22 weeks gestation; 3 male and 3 female) were obtained from ScienCell (Carlsbad, California). They were cultured in endothelial cell media per protocol. Cells from passage from 3 to 6 were used for experiments. Murine lung fibroblasts were isolated from 7-day old male and female mice. Cells (passage 2 and 3) from three different isolations were used. For hyperoxic exposure, confluent murine fibroblasts were incubated in 95% O	extsubscript{2} and 5% CO	extsubscript{2} at 37 °C for upto 72 hours. Normoxia was air and 5% CO	extsubscript{2}. Cell proliferation (thymidine incorporation), viability (trypan blue exclusion), cell migration, angiogenesis, proliferation (PCR array) and Notch pathway mediators (RT-PCR and immunocytochemistry and western blot) were analyzed. Data were analyzed using 2-way ANOVA.

**Results:** Hyperoxia exposure decreased cell viability and proliferation markedly in male HPMECs at 48 and 72 h (P<0.01). HPMECs had significantly higher cell migration when assessed by the scratch assay. Even at baseline normoxic conditions, female HPMECs formed detailed 3D plexus structures and showed greater sprouting compared to similarly maintained male endothelial cells. Upon exposure to hyperoxia, there was decreased expression of Dll4 (delta like ligand 4) in female endothelial cells both at the mRNA and protein level. In normoxic conditions, proliferation was significantly higher in female lung fibroblasts (P<0.01). Upon exposure to hyperoxia, cell proliferation was arrested both in male and female fibroblasts (P<0.01), but cell viability was preserved. PCA analysis demonstrates that male and female are very different in their fibrosis phenotype at baseline in room air. In response to hyperoxia, male and female fibroblasts demonstrate distinct changes in

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**3.21**

**SEX-SPECIFIC DIFFERENCES IN PRIMARY NEONATAL LUNG FIBROBLASTS AND MICROVASCULAR ENDOTHELIAL CELLS EXPOSED TO HYPEROXIA IN VITRO: IMPLICATIONS FOR BRONCHOPULMONARY DYSPLASIA (BPD)**

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fibrosis-related genes. Female fibroblasts from normoxia and hyperoxia are clustered closer in the PCA analysis as compared to the males. Many pro-fibrotic genes related to the TGF-beta pathway were downregulated in females, while pro-fibrotic genes such as IL-13 and EGF were exclusively upregulated in males. Notch pathway activation was noted in male fibroblasts with greater expression and nuclear localization of NICD and increased expression of activated Notch in lung fibroblasts by western blot.

Conclusion: The results indicate that sex differences exist between male and female neonatal pulmonary fibroblasts and endothelial cells in vitro at baseline and after hyperoxia exposure. Differential Notch pathway activation upon hyperoxia exposure may modulate these sex-specific differences. These differences could explain in part the mechanisms behind sex-specific differences in BPD.

8.22
SERELAXIN INFUSION DOES NOT ATTENUATE THE DEVELOPMENT OF HYPERTENSION IN A MOUSE MODEL OF SYSTEMIC LUPUS ERYTHEMATOSUS
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Systemic lupus erythematosus (SLE) is an autoimmune disease that most commonly affects women of reproductive age. Sex steroid hormones have long been thought to contribute to disease susceptibility; however, little is known about other sex-specific factors, including the peptide hormone relaxin, in the progression of SLE. While the role of relaxin in SLE is unclear, there is indirect evidence suggesting that reduced relaxin levels may contribute to some complications of the disease. Because relaxin has known vasodilatory and cardiovascular protective effects, we tested the hypothesis that administration of relaxin would attenuate the development of hypertension and renal injury in a murine model of SLE. Serelaxin (human recombinant relaxin-2, 0.5 mg/kg/day, Novartis) or vehicle (citrate buffer) was administered via osmotic mini-pumps (Alzet 1002) in female NZBWF1 (SLE) mice (aged 30 weeks) and NZW (control) mice (aged 31 weeks) for a total of 24 days (pumps were replaced after 14 days). NZBWF1 mice are an established mouse model of SLE that shares many of the characteristics of SLE as seen in humans, including a female sex bias, increased circulating autoantibodies, hypertension and renal injury. Uterine weights were collected at the termination of the study as in vivo confirmation of bioactivity of the serelaxin in NZBWF1 and NZW mice. Serelaxin-treated NZW mice had significantly increased uterine weights (p < 0.05) compared to vehicle-treated NZW mice, but there was no difference in uterine weights between vehicle- and serelaxin-treated NZBWF1 mice, suggesting the control NZW mice were more responsive to serelaxin than the SLE mice. Mean arterial pressure was measured over two consecutive days at the conclusion of the study in conscious mice via carotid artery catheters. Preliminary data suggest that a four-week administration period of serelaxin was not sufficient to attenuate the development of hypertension in this mouse model of SLE. Mean arterial pressure was significantly increased (p < 0.05) in both vehicle- (135 ± 6 mmHg, n=7) and serelaxin-treated NZBWF1 mice (138 ± 5 mmHg, n=5) compared to vehicle or serelaxin treated NZW mice (109 ± 3, n=7, and 113 ± 4 mmHg, n=7). Urinary albumin, a marker of renal injury, was determined by dipstick assay at the termination of the study, and the prevalence of albuminuria was similar between vehicle- and serelaxin-treated NZBWF1 mice with 55.5% (n=9) of serelaxin-treated NZBWF1 mice and 45.5% (n=11) of vehicle-treated NZBWF1 mice with albumin > 100 mg/dL. Although serelaxin infusion does not attenuate the development of hypertension or urinary albumin excretion under these experimental conditions, the effects of serelaxin on other cardiovascular risk factors including endothelial dysfunction during SLE have not been tested. Therefore, future experiments will determine if serelaxin will improve the endothelial dysfunction observed in NZBWF1 mice and will ultimately provide further insight into potential cardiovascular protective effects of serelaxin.

8.23
PURINOCEPTOR-DEPENDENT REGULATION OF SODIUM EXCRETION IS SEXUALLY DIMORPHIC
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Premenopausal women have a lower risk of hypertension and renal disease compared to age-matched men. Recently, prominent roles have been assigned to P2Y2 and P2Y4 purinoceptor subtypes in promoting sodium excretion, implicating dysfunction of these receptors as potential contributors to hypertension. We recently reported that activation of P2Y2 and P2Y4 receptors in the renal medulla by UTP promotes sodium excretion in male rats. In intact females, UTP did not stimulate sodium excretion while ovariectomy unmasked UTP-induced natriuresis. These observations led us to hypothesize that intact females have higher basal renal medullary activity of P2Y2 and P2Y4 receptors in regulating sodium excretion.
compared to male and ovariectomized (OVX) rats. To test that, we determined (i) P2Y$_2$ and P2Y$_4$ mRNA and protein expression in the inner medulla from male, intact female and OVX Sprague Dawley rats and (ii) the effect of inhibiting medullary purinoreceptors (P2 receptors) on sodium excretion in those rats. We found that P2Y$_2$ and P2Y$_4$ mRNA expression was higher in the inner medulla from females compared to males (1.00±0.09 vs. 0.70±0.05 and 1.00±0.22 vs. 0.29±0.05, respectively, P<0.5, n=5-10). These sex differences in P2Y$_2$ and P2Y$_4$ mRNA expression were eliminated by ovariectomy (0.60±0.06 and 0.29±0.04, respectively, p<0.5, n=5,8). Consistently, Western blots on inner medullary lysates showed that intact females have higher expression of P2Y$_2$ receptor, compared to males. In anesthetized rats, medullary P2 receptor inhibition by suramin (P2 receptor antagonist, 750 µg/kg/min) significantly attenuated sodium excretion in intact females (0.4±0.1 vs. 0.9±0.2 µmol/min, P<0.5, n=7), but not in male or OVX rats. To test whether estradiol (E$_2$) increases the expression of P2Y$_2$ and P2Y$_4$ receptors, we subjected cultured mouse inner medullary collecting duct cells (mIMCD3) to different concentrations of E$_2$(0, 10, 100 and 1000 nM). We found that E$_2$ dose-dependently increased the expression of P2Y$_2$ and P2Y$_4$ mRNA in mIMCD3. These data suggest that females have enhanced P2Y$_2$ and P2Y$_4$-dependent regulation of sodium excretion in the renal medulla, compared to male and OVX rats, at least partially via an E$_2$-dependent mechanism. This pathway may contribute to facilitated renal sodium handling in premenopausal females.

8.24
DIFFERENTIAL PROTEIN EXPRESSION OF RENAL DOPAMINE RECEPTORS BUT SIMILAR AT$_2$R ACTIVITY IN SALT-SENSITIVE MALE AND FEMALE C57BL/6J MICE
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Male C57Bl/6J mice are salt-sensitive that is related, in part, to the functional state of the renal dopamine D$_1$ receptor (D$_1$R). However, sex-related difference on salt sensitivity in mice is not well documented. Therefore, we studied the effect of sodium intake on blood pressure (BP), renal dopamine receptors, angiotensin type 1 receptor (AT$_1$R), and sodium transporters in male and female C57Bl/6J mice. Similar to previous reports in male mice, the BP (telemetry) of female mice (n=4) was also increased by 4% NaCl diet (1 wk) relative to 0.4% NaCl diet which was associated with a decrease in serum renin but an increase in renal AT$_1$R protein. The salt-induced increase in BP was ameliorated by 18-20 mm Hg with the AT$_1$R antagonist, candesartan (1 mg/kg/day, subcutaneously administered with osmotic mini-pumps, 1 wk), in both sexes on high salt intake (6% NaCl) (4 groups, n=5/group). Except for the lower body weight of female mice, relative to male mice, there were no sex differences in food/water intake, urinary excretions of water, Na$^+$, K$^+$, and Cl$^-$, and serum concentrations of creatinine, Na$^+$, K$^+$, and Cl$^-$. Relative to vehicle, candesartan caused a similar decrease in renal protein expressions of sodium-hydrogen exchanger isoform 3, sodium-potassium-2 chloride cotransporter, sodium-chloride cotransporter, and a$b$ and g epithelial sodium channel but not type 2 sodium phosphate cotransporter and a1Na$^+$K$^+$ATPase in male and female mice on high salt diet. However, candesartan increased renal D$_3$R (144±9, % of vehicle group) and D$_2$R (151±6%), but not D$_1$R, D$_2$R or D$_3$R protein expression in female mice but increased renal D$_3$R protein expression (240±44%) only in male mice. We conclude that the salt sensitivity of BP and AT$_1$R activity in C57Bl/6J mice is not sex-related. However, the amelioration of high salt induced-hypertension by AT$_1$R blockade may be mediated by a differential increase in renal dopamine receptor subtype expression but a similar reduction in renal apical sodium transporters in male and female C57Bl/6 mice.

8.25
RENAL ISCHEMIA REPERFUSION INJURY IN A PIG MODEL REVEALS GENDER SPECIFIC EXPRESSED GENES AS POTENTIAL NEW BIOMARKERS OF RENAL INJURY/REGENERATION PROCESSES DRIVING TO CHRONIC KIDNEY DISEASE.
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Kidney diseases are a global public health problem, that is reaching epidemic proportions. Renal ischemia/reperfusion injury (IRI) is a major cause of acute kidney injury (AKI) leading to injury of proximal tubule epithelial cells (PTEC). After injury, the kidney can either regenerate or be engaged in remodeling processes driving to fibrosis and chronic kidney disease (CKD). Men are more prone to AKI and CKD than women and it is accepted that androgens participate on that. The molecular mechanisms involved in regeneration as well as in gender related outcomes upon injury remain to be elucidated. We postulate that the identification of differentially expressed genes in male and female kidney pigs, both in basal and in IRI conditions might unravels...
genes and pathways useful to understand the different outcomes observed in men and women. We aim to provide new candidate repair regulators able to promote restoration of kidney function. Renal IRI was performed in female and male pigs and mice to identify genes of translational relevance for humans that could be also studied in mouse models. Pre-ischemic, ischemic and post-ischemic kidney tissues from male and female pigs were collected for microarray assays. Moreover, systems biology-based mathematical models for the analysis of microarray data were conducted. The most promising targets that exhibit sexual dimorphism along the injury/regeneration process have been selected and characterized. The mRNA levels, the protein expression and localization in the kidney have been assessed. The early results strongly suggest that the selected targets are potentially androgen regulated. In order to further study the molecular mechanisms of these targets, in vitro IRI models of pig and human PTEC cultured cells are currently under development. This work was supported in part by grants from Ministerio de Economía y Competitividad (SAF2014-59945-R and SAF2017-89989-R to A. Meseguer) and Red de Investigación Renal REDinREN (12/0021/0013 to A. Meseguer). Meseguer’s research group holds the Quality Mention from the Generalitat de Catalunya (2014 SGR).

**8.26**

**FEMALE RATS WITH PREEXISTING CKD EXHIBIT IMPAIRED RECOVERY FROM AKI AND THE SUBSEQUENT DEVELOPMENT OF PROTEINURIA**

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Interactions between acute kidney injury (AKI) and chronic kidney disease (CKD) predispose to the development of end-stage renal disease (ESRD). A previous study from our group showed that such deleterious AKI-CKD interactions include impaired recovery from AKI and the development of de novo mechanisms of CKD progression in male rats with preexisting CKD. The goal of this study was to determine if impaired recovery from AKI is also observed in female rats with preexisting CKD. We induced two levels of CKD in 10-12 week old male and female Sprague-Dawley rats by performing either 50% renal mass reduction via a right uninephrectomy (UNX, n=14, 6 females) or 75% renal mass reduction via a right UNX + surgical excision of 1/2 of the left kidney (3/4 NX, n=12, 6 females). Rats recovered for two weeks to allow for completion of compensatory adaptations in renal size and function. Rats were then subjected to 35 minute ischemia-reperfusion (IR)-induced AKI under isoflurane anesthesia with core body temperature maintained at 37°C. Blood samples were obtained prior to IR and at 48 hours, 7 days, 14 days and 28 days post IR to assess plasma creatinine (Pcr). A 24-hour urine collection was performed in a subset of rats prior to IR and at 28 days post IR to assess proteinuria. At the end of the study, kidneys were fixed in paraformaldehyde and paraffin embedded sections were stained with H&E to assess renal pathology and tubular vimentin expression was assessed using immunohistochemistry. Tubular vimentin expression 28 days post IR identifies sublethally injured tubules that have failed to redifferentiate, which is a robust index of impaired recovery. Vimentin expression was semiquantitatively on a scale from 0-4 with 0 representing no tubular vimentin staining and 4 representing tubular vimentin staining in >75% of tubules. Vimentin scoring was conducted in a blinded fashion. The severity of AKI, based on Pcr levels 48 hours post AKI, was similar between UNIX vs. 3/4 NX groups within both males and females. The severity of AKI was lower (P<0.05) in females vs. males with 3/4 NX (1.9±0.3 vs. 3.0±0.4 mg/dl) but not significantly different between females vs. males with UNIX (1.6±0.6 vs. 2.7±0.4 mg/dl). While minimal injury and vimentin expression (0.7±0.2) was observed in female rats with UNIX, females with 3/4 NX exhibited greater (P<0.05) tubular vimentin staining (1.3±0.3), tubular injury and fibrosis 28 days post IR. Moreover, recovery of Pcr, over 28 days post IR was delayed in females with 3/4 NX vs. UNIX, which is also indicative of impaired recovery from AKI. Finally, female rats with 3/4 NX developed substantial (P<0.05) increases in proteinuria 28 days post IR as compared to pre-IR levels (195±57 vs. 62±20 mg/day) while proteinuria was similar at 28 days post IR vs. pre-IR in female rats with UNIX (21±6 vs. 23±5 mg/day). Similar to our previous study, males with 3/4 NX exhibited impaired recovery from AKI and the development of substantial proteinuria 28 days post AKI as compared to males with UNIX. In conclusion, these data support previous studies documenting resistance to IR-induced AKI in female vs. male rats. However, our data indicate that preexisting CKD of greater than 50% renal mass reduction predisposes female rats to impaired recovery from AKI and the subsequent development of mechanisms of CKD progression, similar to male rats.

**8.27**

**ORAL L-ARGININE TREATMENT SIGNIFICANTLY INCREASES RENAL TREGS IN FEMALE DOCA SALT HYPERTENSIVE RATS**

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The immune system plays a critical role in the development of hypertension. Our lab has previously reported that there is a sex difference in the renal T cell profile in DOCA salt hypertension, with males having
more pro-inflammatory Th17 cells, whereas females have more anti-inflammatory regulatory T cells (Tregs). DOCA treated males also have a greater blood pressure (BP) response to DOCA than females, and we propose this is due to the relative abundance of Tregs in the female. We have also previously reported that an intact nitric oxide (NO) system is required for Treg maintenance in hypertension, and the DOCA model has been characterized by impaired NO. In the current study, we hypothesized that treatment with L-arginine, a substrate for NO production, would increase renal Tregs and attenuate the sex difference in hypertension in male and female DOCA treated rats. Briefly, male and female Sprague Dawley rats (n=5-15, 10 wks of age) were uninephrectomized and subcutaneously implanted with a DOCA pellet (200 mg/rat, 60-d time release) and given 0.9% NaCl to drink ad libitum. A subset of rats were treated with L-arginine (L-arg, 350 mg/kg/d, via drinking water). BP was measured by tail cuff plethysmography at baseline and after 3 wks of DOCA treatment. After 3 wks of treatment, kidneys were processed for flow cytometric analysis of T cells (CD3+ T cells, CD3+CD4+ T cells, CD3+CD4+FOXP3+ Tregs, and IL-10+CD3+ T cells). BP was comparable between treatment groups within each sex at baseline, although males had a higher BP than females (Table, effect of sex, p<0.001, 2-Way ANOVA). DOCA treatment increased BP in all groups compared to baseline values (Table, effect of DOCA, p=0.002). L-arginine treatment attenuated DOCA-induced increases in BP in both sexes, although this effect was greater in females (Table, effect of L-arginine, p=0.02, interaction of sex and L-arginine, p=0.03). Females had more Tregs than males, and L-arginine treatment increased Tregs in females alone (Table, effect of sex, p<0.01, effect of L-arg, p<0.01, interaction, p<0.01, 2-Way ANOVA). Interestingly, despite the Treg numbers remaining unchanged with L-arg, IL-10 increased in both sexes to a similar extent (Table, effect of sex, p=0.29, effect of L-arg, p=0.03, interaction, p=0.93, 2-Way ANOVA). Future studies will examine additional mechanisms by which L-arginine supplementation increases Tregs in females.

<table>
<thead>
<tr>
<th></th>
<th>BP Baseline (mmHg)</th>
<th>BP Wk 3 (mmHg)</th>
<th>CD3</th>
<th>CD4</th>
<th>Tregs</th>
<th>IL-10</th>
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</thead>
<tbody>
<tr>
<td>M DOC A</td>
<td>153±2</td>
<td>225±2</td>
<td>0.9±0</td>
<td>.1</td>
<td>65±1</td>
<td>3.4±0</td>
</tr>
<tr>
<td>M DOC A + L-Arg</td>
<td>153±1</td>
<td>208±1</td>
<td>1.2±0</td>
<td>.2</td>
<td>72±2</td>
<td>3.7±0</td>
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<tr>
<td>F DOC A</td>
<td>142±2</td>
<td>216±2</td>
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<td>.1</td>
<td>61±1</td>
<td>3.9±0</td>
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<tr>
<td>F DOC A + L-Arg</td>
<td>140±3</td>
<td>180±3</td>
<td>0.6±0</td>
<td>.1</td>
<td>64±1</td>
<td>6.6±0</td>
</tr>
</tbody>
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8.28

NECROX-5 ABOLISHED MATURATION INDUCED SEX DIFFERENCES IN BLOOD PRESSURE (BP) IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR).

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It is well established that hypertension is accompanied by cell death, however, less is known regarding the role of cell death in blood pressure (BP) control. Necrosis is an uncontrolled pathologic form of cell death that is associated with inflammation. Therefore, the goal of the current study was to test the hypothesis that necrosis contributes to the development of hypertension in SHR. Initial studies measured renal necrosis in 13 wk old male and female SHR (n=9) using flow cytometry. Male SHR had greater renal necrosis compared to female SHR (3.7±0.4% vs. 0.4±0.2% of total renal cells, respectively; p<0.0001). Additional male and female SHR were then randomized to receive vehicle or Necrox-5 from 6-12 weeks of age (1 mg/kg via IP injection twice weekly; n=4); Necrox-5 is a cell permeable inhibitor of necrosis that blocks oxidative stress induced necrotic cell death. BP was measured weekly via tail-cuff and via telemetry from 10-13 wks of age. Following 6 wks of treatment, kidneys
were isolated and necrosis was measured by flow cytometry. Treatment with Necrox-5 beginning at 6 wks of age attenuated maturation-induced increases in BP in male SHR (Necrox-5: 152±11 vs Control: 177±4 mmHg; p=0.07). BP in female SHR was not altered by chronic Necrox-5 treatment (Necrox-5: 152±14 vs. Control: 146±11 mmHg; p=0.72). In addition, the sex difference in BP apparent in control rats was abolished by Necrox-5 treatment. Consistent with these results, Necrox-5 treatment for 6 wks decreased renal necrosis (Necrox-5: 1.5±0.5% vs Control: 3±0% of total renal cells), only in males. Taken together, our data suggest that greater cell death in male SHR compared to female SHR contributes to sex differences in BP by exacerbating age-related increases in BP in males.

8.29

ANDROGEN INFLUENCE ON RENAL FIBROSIS ASSOCIATED WITH PYELONEPHRITIS

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Renal scarring after pyelonephritis is linked to long-term health risks for hypertension and chronic kidney disease. Testosterone signaling through the androgen receptor increases susceptibility to, and severity of, uropathogenic Escherichia coli (UPEC) pyelonephritis in both male and female mice (1), while anti-androgen therapy is protective against severe UTI (2). Mice with severe pyelonephritis develop renal fibrosis and scarring (3). This work elucidates the molecular mechanisms of renal fibrosis in androgenized female C3H/HeN and C57BL/6 mouse backgrounds, and determines how these pathways are altered by the presence of testosterone. C3H/HeN mice feature vesicoureteral reflux (VUR), which allows for severe pyelonephritis and widespread renal fibrosis. C57BL/6 mice do not have VUR, but still exhibit alterations in renal fibrosis markers and display scarring following upper-tract UTI. We demonstrate that renal fibrosis after pyelonephritis involves both the TGF-β/Activin A and Hedgehog pathways, with altered local expression of proteins in the Smad family, TGFβ1, Activin A, and Gli1. Elevated circulating testosterone levels drive Ly6C+ monocyte recruitment to the kidney in the uninfected state and upon urinary tract inoculation with UPEC. Our results are consistent with a model in which testosterone increases recruitment of Ly6C+ monocytes, and that these cells are activated in the presence of UPEC during renal infection, driving local expression of pro-inflammatory and pro-fibrotic markers and thereby promoting fibrosis and renal scar formation.


8.30
COLLECTING DUCT NOS1, SPECIFICALLY NOS1β, IS CRITICAL FOR MAINTAINING FLUID-ELECTROLYTE BALANCE IN BOTH MALES AND FEMALES

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¹University of Alabama at Birmingham

Over the past few years, we have reported that collecting duct (CD) nitric oxide synthase-1 (NOS1) is necessary for maintaining fluid-electrolyte balance during high sodium intake. Genetic deletion of NOS1 (specifically the splice variant NOS1β) in the CD, results in inappropriate sodium handling, and a salt-sensitive increase in blood pressure in male mice. The purpose of this study was to determine if CD NOS1β is also a critical mediator in maintaining fluid-electrolyte balance in female mice. Similar to male mice, NOS1β is specifically expressed in CD. Female CD NOS1 knockout (CDNOS1KO) mice were randomly assigned to receive either low sodium (LS, <0.01%), normal sodium (NS, 0.16%), or high sodium (HS, 1.6%) diet for 1 week. Food and water intake was similar between littermate control and CDNOS1KO mice. When switched from a LS to HS diet, female CDNOS1KO mice produced significantly less urine (3.4 ± 0.4 vs 4.9 ± 0.1 ml/day, p = 0.04) and excreted less sodium on day 1 of HS compared to controls (1.7 ± 0.2 vs 2.5 ±0.1 mmol/day, p = 0.02). However, by day 2 of HS, urine production and sodium excretion were similar between the genotypes. In male mice, we found a similar sodium handling defect, but with a delay of 4 days to come into sodium balance after the HS challenge. Mean arterial blood pressure was also significantly higher in CDNOS1KO females than controls while on HS (113.5±1.3 vs 107.6±1.6 mmHg, p = 0.046), and very similar to what we observed in male CDNOS1KO. Glomerular filtration rate (GFR) was determined by FITC-sinistrin clearance in conscious mice. In male and female, CDNOS1KO and controls, GFR was similar on LS (male control 260.9 ± 9.8 vs CDNOS1KO 242.7 ± 6.3 μl/min; female control 238.9 ± 7.0 vs CDNOS1KO 280.7 ± 12.4 μl/min) and NS diets (male control 224.2 ± 6.4 vs CDNOS1KO 218.0 ± 10.4 μl/min; female control 258.0 ± 16.6 vs CDNOS1KO 325.1 ± 42.1 μl/min) . However, on day 1 of HS diet, GFR
significantly increased in controls compared to CDNOS1KO mice (males: 329.8 ± 12.4 vs 255.1 ± 22.8 μl/min, p = 0.03; females: 296.7 ± 14.1 vs 244.0 ± 10.8 μl/min, p = 0.001). Male control and CDNOS1KO had similar GFR on day 7 of HS compared to GFR on LS and NS (251.3 ± 16.0 vs 290.6±26.7μl/min). However, female control and CDNOS1KO had significantly higher GFR (323.2 ± 29.7 and 358.0 ± 32.4 μl/min) compared to LS and NS. In conclusion, CD NOS1β is critical for maintaining fluid-electrolyte balance and blood pressure control during high salt feeding. However, female mice return to sodium balance much quicker than males, although blood pressure remains high. Interestingly in both sexes, acute (within 24 hr) changes in GFR are dependent on CD NOS1β activation, but chronic (7 day) changes in GFR are independent of CD NOS1β. Moreover, salt-dependent increases in GFR are maintained in female mice but not male mice. Future experiments will determine the mechanisms behind the sex and salt dependent changes in GFR. Funding KO1DK105038 to KAH and HL136267, HL69999, AHA24450002 to JSP.

8.31 TISSUE-SPECIFIC ESTROGEN RECEPTOR PROFILING USING DROPLET DIGITAL PCR

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Estrogen signals via th ree identified receptors ERα, ERβ, and the G Protein-Coupled Estrogen Receptor (GPER). The relative contribution of each receptor to estrogenic signaling may elucidate the disparate effects of this sex hormone across tissues. Moreover, a novel PCR technology termed droplet digital PCR (ddPCR) now allows direct comparison of multiple target sequences due to absolute transcript quantification. Therefore, we hypothesized that utilization of this new technology would reveal tissue- and sex-specific differences in mRNA for the three estrogen receptors and aromatase. The following tissues were collected from Sprague-Dawley rats (6 female, 6 male) at 13 weeks of age: reproductive (gonads, uterus, mammary gland), cardiovascular (heart, aorta, kidney, adrenal gland), and brain (somatosensory cortex, hippocampus, and prefrontal cortex). GPER expression was relatively stable across all tissues in both sexes, ranging from 14-113 copies/ng RNA, an approximate 8-fold difference. ERα and ERβ were more variable although relatively stable within each organ system. ERα displayed a range of 4.5-614 copies/ng RNA, a fold change of ~136 while ERβ ranged from 0.2-83 copies/ng RNA or 415-fold. Both ERβ and aromatase were highly expressed only in the ovary, with slightly higher levels detected in the brain. Significant sex differences were broadly absent except for renal ERα (female 206 vs. male 614 copies/ng RNA, p<0.001), somatosensory ERα (8.5 vs. 4.5 copies/ng RNA, p<0.001), gonadal ERβ (83 vs. 0.30 copies/ng RNA, p<0.01), and gonadal GPER (5.5 vs 48 copies/ng RNA, p<0.001). Cardiovascular tissues showed a predominance of ERα followed by GPER, while ERβ was nearly undetectable. In contrast, GPER was the predominant transcript in the three selected brain regions, with similarly low levels of ERα and ERβ. While the data revealed surprisingly few sex differences, significant differences were found in the range of receptor mRNA across tissues as well as the estrogen receptor profile between organ systems. These data provide an overview of estrogen expression at the organ level, but future studies are needed on cell-specific receptor profiles. In conclusion, estrogen receptor profiling will enhance understanding on the mechanisms by which estrogen elicits tissue-specific effects. Funding: NIH HL133619 (SHL), COBRE P30GM103337.

8.32 A STUDY ON THE INCIDENCE AND TYPES OF TWINNING IN THE SOUTH INDIAN POPULATION DR. R. SHAKTHI KUMARAN, CRRI FOR ABSTRACT-BASED TRAINEE TRAVEL AWARDS

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The study was undertaken to know the true incidence and types of twinning or other multiple births in Vijayawada under the following protocol- incidence of twinning, multiple births, frequency of twinning and maternal aging with gravidity of mother, sex incidence of twin and the zygosity of twins. In the present study, there is 0.6% incidence of twinning.

8.33 T CELL SPECIFIC KNOCKDOWN OF ESTROGEN RECEPTOR A DOES NOT ELIMINATE PREMENOPAUSAL PROTECTION FROM ANGIOTENSIN II-INDUCED HYPERTENSION, BUT DOES IMPACT RENAL T CELL EXPRESSION OF CD28 AND CTLA-4

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1Department of Physiology, University of Arizona, 2Department of Immunobiology, University of Arizona, 3Arizona Center on Aging, University of Arizona

There is extensive evidence that the immune system is required for the development of angiotensin II (Ang II) induced hypertension in males. In contrast, we have shown that premenopausal females are protected from T cell-mediated Ang II hypertension. However, following
menopause, female protection from T cell-mediated Ang II hypertension is lost. Adoptive transfer of CD3\(^{+}\) T cells into postmenopausal Rag-1\(^{-}\) mice significantly increased Ang II-induced SBP (SBP \(\Delta 28 \pm 3\) mmHg; \(p<0.05\)), whereas in the absence of T cells, the Ang II-induced systolic blood pressure (SBP) response in postmenopausal Rag-1\(^{-}\) mice was similar to premenopausal mice (SBP \(\Delta 12 \pm 2\) mmHg). Thus, we hypothesize that the loss of estrogen in postmenopausal mice eliminates female protection against T cell-mediated hypertension. Further, we examined if T cell-specific estrogen receptor alpha (ER\(\alpha\)) signaling is required for premenopausal protection from T cell-mediated hypertension. CD3\(^{+}\) T cells were purified from ER\(\alpha\) null mice (CD3\(^{+}\)ER\(\alpha\)KO) or wild-type mice (CD3\(^{+}\)WT) and adoptively transferred into premenopausal female Rag-1\(^{-}\) mice (no T/B cells). Ang II was infused via osmotic mini-pump for 14 days (490 ng/kg/min). Similar to our previous studies, premenopausal female Rag-1\(^{-}\) mice were resistant to T cell-mediated Ang II hypertension following T cell transfer from wild type mice (SBP: CD3\(^{+}\)WT/Ang \(\Delta 6 \pm 5\) mmHg). Additionally, there was no significant difference in SBP when ER\(\alpha\)KO T cells were adoptively transferred (SBP: CD3\(^{+}\)ER\(\alpha\)KO/Ang \(\Delta 5 \pm 4\) mmHg). Flow cytometric analysis of renal CD4\(^{+}\), CD8\(^{+}\), and regulatory Foxp3\(^{+}\) T cells identified that the absence of ER\(\alpha\) increases expression of the costimulatory receptor CD28 in all three T cell subtypes (CD3\(^{+}\)ER\(\alpha\)KO/Ang vs. CD3\(^{+}\)WT/Ang: CD4\(^{+}\) 138\%, CD8\(^{+}\) 144\%, Foxp3\(^{+}\) 141\%; \(p<0.05\)). Further, the absence of ER\(\alpha\) significantly reduced Ang II-induced expression of the anti-inflammatory CTLA-4 receptor on renal regulatory Foxp3\(^{+}\) T cells (CD3\(^{+}\)ER\(\alpha\)KO/Ang vs. CD3\(^{+}\)WT/Ang: Foxp3\(^{+}\) 67\%; \(p<0.05\)). Our studies show that menopause (loss of estrogen signaling) increases female susceptibility to T cell-mediated hypertension. However, T cell-specific ER\(\alpha\) signaling does not seem to play a key role in premenopausal protection from T cell-mediated Ang II hypertension, but may impact anti-inflammatory pathways that regulate T cells.

Funding: T32HL007249, R01HL131834

8.34

**SEX SHAPES CANCER CACHEXIA AND THE RESPONSE TO THERAPEUTIC BLOCKING OF ACVR2B LIGANDS IN THE GENETICALLY ENGINEERED KPC MOUSE MODEL OF PANCREATIC DUCTAL ADENOCARCINOMA**

**Xiaoling Zhong\(^{1}\), Jianguo Liu\(^{1}\), Ashok Narasimhan\(^{1}\), Leonidas Koniaris\(^{1}\), Teresa Zimmers\(^{2}\)**

\(^{1}\)Surgery, Indiana University

**Background:** Women and men are genetically different and this difference influences other levels of biological organization (cell, organ, organ system and organism). Sex has been recognized to impact clinical manifestation and therapeutic effects in diseases including heart disease; yet little information is available for sex differences in cachexia, a metabolic syndrome characterized by progressive fat/muscle loss and associated fatigue and dysmobility. PDAC is among the most lethal of malignancies in part because patients with PDAC have the highest rates and greatest severity of cachexia. Pre-clinical studies indicate that blocking cachexia improves quality of life and survival in PDAC. Activin is elevated in the circulation of patients with PDAC-associated cachexia; thus inhibition of Activin receptor signaling has been trialed for cachexia in patients with pancreatic cancer, with unclear benefit.

**Hypothesis:** Biological differences between males and females affects the manifestation of PDAC cachexia and the response to therapies.

**Objective:** Characterize sex-specific phenotypes in PDAC cachexia and in response to anti-cachexia therapy.

**Strategy:** We characterized cachexia in the genetically engineered mouse model of PDAC, KPC (LSL-KrasG12D;LSL-Trp53R172H;Pdx1-Cre). We monitored spontaneous tumor initiation and changes in body weight and body composition over the course of tumor growth up to 33 weeks of age. We used the ACVR2B/Fc, a soluble receptor-chimera trap for Activins and related proteins, to block the ACVR2B-mediated signaling and assessed the response of male vs. female mice to the soluble receptor. Collected tumors and organs were analyzed for sex differences in RNA and protein expression.

**Results:** Male and female KPC mice had a same median PDAC tumor latency of 17 weeks of age, similar tumor growth dynamics, and same median survival of 25 weeks. ACVR2B/Fc did not significantly inhibit tumor growth in either sex. However, sex-specific differences in body weight loss, organ wasting, and response to ACVR2B/Fc were observed. Male KPC ceased gaining weight and fat mass by 17 weeks of age. Organs, including skeletal and cardiac muscle, fat, and kidney started to waste from early stages of tumor growth and wasting became more severe at late stage. Organ wasting was mostly prevented by ACVR2B/Fc treatment. In contrast, female KPC ceased fat mass gain by 20 weeks but did not lose overall body weight despite identical tumor growth/latency. Organ wasting was less severe in females at early stages, but progressed to severe cachexia quickly at late stage, similar to males. However, prominent differences in the heart and kidney wasting persisted, indicating that some factors in females may specifically protect these two organs from wasting throughout tumor progression. Unlike males, female KPC mice essentially did not respond to ACVR2B/Fc, and organ mass was not preserved. Consistent with these differences at the level of organ mass, differential activation of known catabolic signals and their downstream markers was observed in male vs. female.
As well, RNA-sequencing of the skeletal muscle from male and female KPC euthanized at early or late cachexia, with or without ACVR1B/Fc-treatment, revealed many differentially activated pathways. Further analysis is underway.

Conclusions: We demonstrate that sex impacts the manifestations of PDAC-associated cachexia, including onset and severity, and the response to anti-cachexia therapy. The KPC model recapitulates many of the phenotypic and genotypic features of human cancer cachexia and could be useful for testing sex differences in cachexia therapies.

Effect of Oleanolic Acid on Lipid Metabolism in Neonatal Rats with Metabolic Syndrome

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For the past two decades, emergence of the metabolic syndrome, which is a modern epidemic with frightful consequences to the health of humans worldwide [1]. Diet is an important integral part of human health and livelihood. For instance, fructose intake has increased at an alarming rate and its intake is linked to the epidemic of obesity and diabetes [2, 3]. Lipid synthesis and oxidation are two processes major processes of metabolism. [4]. Lipid accumulation may add to the development of metabolic disorders such as obesity, type 2 diabetes, insulin resistance and cardiovascular related diseases [5]. The use of medicinal plants to curb human ailments has been in use for some time in African and Asian countries. Oleanolic acid is a pentacyclic triterpenoid complex which possesses many promising pharmacological activities, such as hepatoprotective, anti-inflammatory, antioxidant, and anticancer activities. However, there is limited information about direct influence of oleanolic acid on mechanism of antidiabetic activity of oleanolic acid, hence it the reason to undertake this research to determine the effect of oleanolic acid on lipid metabolism in fructose induced Sprague dawley rats.

Materials and Methods: This study was conducted using 40 male pups of Sprague Dawley rats. Rats were randomly divided into five groups namely the Control (CON), Oleanolic acid (OA), High fructose (HF), Oleanolic acid and High fructose (OA+HFD) and Metformin and High fructose (MET+HFD). The experiment was conducted in accordance with protocols approved by the Animal Ethics Screening Committee (AESC) of the University of Witwatersrand, Johannesburg, South Africa (AESC approval number 2014/47/D).

Gas Chromatography Mass Spectrometry (GS-MS) was used to determine expression of polyunsaturated fatty acid carried out according to modified method of Association of Official Analytical Chemists AOAC (2005). Fatty acids are identified by comparing their retention times to the retention times of the suitable standard using Aligent 7890A Gas Chromatograph. Real Time Polymerase Chain Reaction (qRT-PCR) was performed using the PowerUp SYBR Green master mix (Applied Biosystems, Life Technologies) according to the manufacturer’s protocol.

Results: These results indicate that expression of Oleanolic acid was suppressed in HFD group however this was reversed when HFD was accompanied by oleanolic acid administration, indicating promising influence of OA in ameliorating metabolic syndrome. It was interesting to see EPA increasing, the increases observed suggests, activation of the desaturation/elongation pathway. In high fructose diet increased levels of arachidonic acid higher than in treated samples, metformin showed a threefold decrease compared to HFD group.

Conclusions: This study demonstrates that dietary modification may delay or prevent the transition from developing metabolic syndrome or its related complications. The use of oleanolic acid has showed great significant increase in upregulating the expression of eicosapentaenoic acid (EPA) while being downregulated in arachidonic acid. Oleanolic acid has increased the expression lipid oxidation genes (CPT-1) and decreased the expression of lipid synthesis (FAS). Therefore, oleanolic acid can be used to ameliorate development of metabolic disorders and its related complications.

3.36
EFFECTS OF TENDER COCONUT WATER ON THE CORONARY ARTERY OF MALE DIABETIC WISTAR RATS
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Diabetes is a major risk factor for cardiovascular disease (CVD). Tender coconut water (TCW) is the liquid endosperm of coconut fruit and it is rich in minerals (Potassium, calcium and magnesium), Vitamins (B and C) which are known to reduce the risk of developing Coronary Heart Disease (Anurag and Rajamohan 2003). It is also a major source of the amino acid L-arginine (Boges and Bode-Boger, 2001). TCW have been reported to have antioxidant and cardio-protective effect (Nnodim et. al., 2013). However, there are very few studies on its cardio-protective effect in diabetes. Therefore the aim of this study was to investigate the effects of Tender coconut water on the coronary artery of male diabetic Wistar rats. Twenty (20) male Wistar rats were selected for this study. The rats in each group were allowed access to feed and water ad libitum. All experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. Diabetes mellitus was induced in rats by a single intraperitoneal injection of streptozotocin (STZ) (60mg/kg body weight) dissolved in 0.1M sodium citrate buffer (pH 4.5). Rats in group A “control” were injected with corresponding volumes of the citrate buffer equivalent to the volume of STZ administered intraperitoneally. Rats found to have blood glucose levels between ≥ 250 mg/dl were considered diabetic and were randomly assigned into groups B, C and D. Diabetes was allowed to develop and stabilize in these STZ-treated rats over a period of 72 hours. Before the induction of diabetes, all the rats were fasted for 16-h (overnight), but still allowed free access to water throughout. At the end of the 16-h fasting period – taken as 0 time (i.e., 0h) – blood glucose levels (initial glycemia, G₀) were determined and recorded. Same procedure was repeated weekly after induction of diabetes and Fasting blood glucose levels (G₁, G₂, G₃ and G₄) of the fasted normal (control), and other experimental rats were recorded. Rats in group A served as control; group B served as untreated diabetic group; diabetic rats in group C received 1IU/kg/day of Humulin subcutaneously and group D diabetic rats received 1ml/100g/day of TCW from freshly harvested Tender coconuts. The experimental period lasted for four weeks after initial glycemia. The results obtained showed that there was a significant reduction (P<0.05) in the Fasting Blood glucose concentration of diabetic rats treated with Humulin and TCW compared with untreated diabetic rats. There was a significant (P<0.05) increase in serum TG, TC, LDL-C and VLDL-C of untreated diabetic rats compared with diabetic rats treated with Humulin and TCW and control while HDL-C and AAI of untreated diabetic rats were significantly (P<0.05) decreased compared with diabetic rats treated with Humulin and TCW and control. Histological studies of the heart of untreated diabetic rats showed coronary artery with focal intimal ulceration, lumen obstruction and hypertrophy of the muscular wall. The wall of the coronary artery of Diabetic rats treated with TCW and Humulin appear normal compared with the control. Histochemistry of elastin fibers showed that there was a strong positive stain for elastin in the wall of the coronary artery of rats in control and those of diabetic rats treated with Humulin and TCW respectively. The wall of the coronary artery of untreated diabetic rats appear negative to elastin stain. The coronary artery of Wistar rats induced with diabetic and treated with humulin and TCW showed normal vascular wall as compared with control. The implication of the result is that Tender coconut water has both glucose lowering and cardio-protective properties in diabetic rats.

References

3.37
THERAPEUTIC ROLE OF INTRAPARTUM PDE-5 INHIBITION ON BLOOD PRESSURE AND RENAL INJURY IN OFFSPRING OF PREECLAMPTIC RATS
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Up to 10% of pregnancies are complicated by preeclampsia. As a result, up to 15 million US citizens today are offspring of preeclamptic pregnancies. Offspring of preeclamptic pregnancies have increased blood pressure (BP) during childhood and nearly double the risk of stroke later in life. The Barker hypothesis proposes that the adverse intrauterine environment created by preeclampsia programs fetal tissues and organs to develop high BP from early childhood. Animal models of hypertension have demonstrated the ability of
various therapies such as nitric oxide (NO) donors to reprogram hypertension. Sildenafil citrate, a phosphodiesterase-5 (PDE-5) inhibitor, prolongs the NO signaling cascade and improves the maternal syndrome of preeclampsia; however, determination of optimal timing, effectiveness, and safety during perinatal use have yet to be reported. This project tests the hypothesis that use of a PDE-5 inhibitor during preeclamptic pregnancy improves the long-term BP and renal injury in the offspring. Female Dahl S rats on a 0.3% salt diet, a previously characterized spontaneous model of superimposed preeclampsia, were mated and treated orally with sildenafil (50 mg/kg/day), labetalol (currently used to manage hypertension in preeclamptic patients, 10 mg/kg/day), or vehicle from gestational day 10 to delivery. Dams were placed on normal rat chow at delivery throughout weaning at four weeks of age. At seven weeks of age, male and female offspring were acclimated to restraints for four days before tail cuff BP measurement on day five. The process was repeated at 11 weeks of age for analysis of the time-dependent change in systolic BP. Systolic BP increased in Dahl S rats of untreated mothers as expected; however, the rise in BP was abolished in offspring from sildenafil treated dams (VEH: +28 mmHg ±7; SLD: -7 mmHg ±5, p=0.007). This protective effect was not elicited by treatment with labetalol (+13 mmHg ±5). Tubulointerstitial scarring was measured in 4 µm kidney sections stained with Masson’s trichrome (Nikon 55I microscope with DS-Fi1 S-Meg Color C digital camera and Nis-Elements Image-analysis software version 3.03) from Dahl S offspring of sildenafil and vehicle-treated dams at age three months. Tubulointerstitial scarring is increased in male Dahl S offspring of untreated mothers as compared with offspring of sildenafil treated dams (Area: VEH: 9 ±0.6%; SLD: 5 ±0.6%, p=0.006), but no changes were observed in kidney sections from female rats. Urine was collected via 24-hour metabolic cage for measurement of urinary protein (Bradford assay). No significant differences in urinary protein excretion were observed in either male or female offspring. These data support the hypothesis that use of a PDE-5 inhibitor during preeclamptic pregnancy improves the long-term BP and renal injury in the offspring.

8.38

EFFECTS OF PRENATAL SILDENAFIL TREATMENT ON LONG-TERM CARDIOVASCULAR FUNCTION IN OFFSPRING FROM DAHL SALT-SENSITIVE RATS

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Introduction: Fetal growth restriction (FGR) is associated with increased risk for cardiovascular disorders in later life. Cardiovascular disease is the most common cause of death worldwide. Previous studies report that prenatal sildenafil improves pregnancy outcomes, such as birthweight, in FGR animal models; however, whether sildenafil treatment is protective against long-term cardiovascular disease in these offspring is unknown.

Objective: We hypothesize that prenatal sildenafil reduces blood pressure and endothelial dysfunction in FGR offspring from Dahl salt-sensitive (SS) rats on normal salt intake.

Methods: Sildenafil citrate (60 mg/kg/day) or control gel diet was administered from gestational day 10 until birth. Birthweight and litter size were measured (treated n=10; untreated n=8 dams). Telemetry devices (DSI) were implanted via the femoral artery to measure mean arterial pressure (MAP) from weeks 5-8 in the offspring (treated n=12; untreated n=4). Aortic rings were isolated from 10 week old offspring to assess vascular sensitivity (logEC50) to endothelial-dependent (acetylcholine) and independent (sodium nitroprusside, SNP) vasorelaxation (treated n=10; untreated n=10). Data shown as mean ± S.E.M.

Results: No sex differences were observed in any variables; therefore, data were pooled between males and females. Sildenafil improved birthweight (treated 6.8±0.2; untreated 6.2±0.1 g; p=0.02) without significantly changing viable litter size (treated 9.6±0.9; untreated 8.2±0.3; p=0.023). While MAP at 5 weeks was similar between groups (treated 107±1; untreated 108±2 mmHg; p=0.55), there was a trend towards lower MAP in prenatally treated offspring at 8 weeks (treated 116±1; untreated 120±2 mmHg; p=0.09). Aortas from offspring of treated dams displayed enhanced sensitivity to acetylcholine (logEC50: treated -7.4±0.3; untreated -6.6±0.3 mol/L; p=0.03), but not to SNP (treated -8.2±0.3; untreated -7.9±0.2 mol/L; p=0.43).

Discussion: Prenatal sildenafil treatment improves birthweight in a model of FGR. In young adult offspring, there was a trend towards a sex-independent lowering of blood pressure and increased endothelium-dependent relaxation.
8.39

TESTOSTERONE SUPPLEMENTATION IN POSTMENOPAUSAL HYPERTENSIVE RATS

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The indication of testosterone supplements (T) is increasing in the aging population of the US. It is useful in relieving both the physical and psychological symptoms of androgen insufficiency in clinically affected women and men. However, while in aging men T may have increased risk for hypertension and cardiovascular events, in aging women, its effect is not clear.

**Aim:** In the present study, we determined whether T would affect blood pressure and induce endocrine-metabolic disorders in a model of postmenopausal (PM) hypertension.

**Methods:** Aging female spontaneously hypertensive rats (PMR), aged 18-22 mos (n=4/grp), were randomized into two groups and treated with testosterone propionate (18 mg/silastic pellet, SC: PMR+T) or placebo (PMR+P) for six weeks, respectively. After four weeks of treatment, we implanted telemetry probes, and after two weeks of recovery, we measure the mean arterial pressure (MAP) and heart rate (HR). Also, to evaluate whether T affects body composition, we analyzed body weight, lean and fat mass by ECHO MRI once a week for three weeks.

**Results:** After six weeks of treatment, MAP was higher in PMR+T than controls (PMR+T: 190±2 mmHg, n=4 vs. PMR+P: 174±2 mmHg, n=4, p<0.05, respectively). While T has no effect on body weight or lean mass, fat mass was reduced in PMR+T (PMR+P: 24±2 g vs. PMR+T: 17±1 g, n=4, p<0.05). These data suggest that testosterone supplements in postmenopausal women may improve some metabolic syndrome parameters, but measurement and maintenance of blood pressure control is imperative. Supported by NIH-R01HL66072, PO1HL51971 (JFR), 14POST18640015 (ROM), P20GM121334 (to JFR and ROM).

8.40

HYPOGONADAL HYPERTENSION IN MALE SPRAGUE-DAWLEY RATS IS REVERSED BY TESTOSTERONE REPLACEMENT THERAPY, WHICH DOWN-REGULATES RENIN-ANGIOTENSIN SYSTEM MESSAGE EXPRESSION

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Acutely, testosterone (TES) and other androgens are efficacious vasodilators, both in vitro and in vivo; however, their long-term effects on arterial blood pressure (BP) are unclear. We previously reported that castration (CsX) produced hypertension in male rats, and that TES replacement therapy (TRT) was antihypertensive and normalized BP. Thus, long-term effects of endogenous TES and exogenous TRT on BP and renin-angiotensin system (RAS) function were studied in intact (InT) and castrated (CsX) male Sprague-Dawley (SD) and Testicular-feminized male (Tfm, androgen receptor defective) rats (12-13 wk old). Weekly measurements of systolic BP (tail cuff plethysmography) revealed a progressive rise in BP over 10 wks in CsX (108 ± 0.9 vs. 139 ± 1.2 mmHg), while BP remained stable in InT (109 ± 3.1 vs. 115 ± 0.5). During the next 5 weeks, half of CsX received TRT (CsX+TES-enanthate-replaced; 1.75 mg/kg, 2X/wk). BP gradually declined to normal in CsX+TES replaced rats (115 ± 1.2), while BP remained elevated in CsX (141 ± 1.2) and normal in InT (113 ± 0.3). In separate CsX+SD rats, treatment with Losartan (LST; 250 mg/L drinking water) prevented development of hypertension at 10 wks (95 ± 0.8 CsX+LST vs. 139 ± 1.2 in CsX). During the next 5 weeks with TRT, BP declined in CsX+TES (113 ± 1.3) and remained lower in CsX+LST (99 ± 0.4). In Tfm, CsX resulted in a similar rise in BP (108 ± 0.6 vs. 139 ± 0.4 mmHg), and TRT reduced BP to a similar extent. Real-time PCR (rt-PCR) of kidney from InT, CsX, and CsX+TES rats revealed that CsX increased expression of renin (45.5%), AT1 receptor (AT1R; 38%), and angiotensin converting enzyme (ACE; 239%) mRNA, while TRT normalized expression of renin, AT1R, and ACE mRNA to levels of InT rats. In contrast, CsX reduced expression of angiotensinogen (Angt) mRNA (59%) while TRT restored Angt mRNA to 78% of InT rats. 24 hr urine output (UO) in InT-SD was 48.3 ml/kg at baseline, 34.3 at 10 wks, 38.4 at 12 wks, and 34.8 at 15 wks. UO in CsX-SD was 47.6, 30.3, 25.2, and 27.7; UO in CsX+TRT was 46.1, 29.2 36.2, and 29.5. These data suggest that: 1) endogenous androgens (TES) exert antihypertensive effects in male SD and Tfm rats; 2) the antihypertensive effect of TES appear to involve a diuretic effect on the kidney, which is non-genomically mediated; and 3) these antihypertensive effects may involve TES-induced reductions in RAS expression in the kidney. (State of Texas)
compared to age-matched men. Since we know that high salt diets contribute to these major health problems, it is important to understand sex differences in renal control of salt and water balance. Our lab has a long-standing interest in the renal endothelin (ET-1) system that includes a major role in renal excretion of high salt diets. Focusing primarily on rat models, we observed some basic sex differences in the renal medulla, the location with very high ET-1 expression and activity. Urinary ET-1 excretion, a reflection of intrarenal production, is enhanced in response to a high salt diet and is consistently higher in females compared to males. Genetic or pharmacological blockade of the ET\_B receptor results in salt-dependent hypertension. The ratio of ET\_A to ET\_B receptors is much lower in female compared to male rats. Exogenous administration of ET-1 produces a natriuretic response in female, but not male rats. At the same time, ET-1 reduces medullary blood flow in males, but not females. This could be explained by lower ET\_A-dependent vasoconstriction in females that would allow for a more robust natriuresis. One of the more unexpected findings from our lab in recent years has been the observation that the adaptation of a high salt diet is more rapid in female compared to male rats. As classically explained in textbooks, it takes several days for male rats to come into balance when transferred from a low to a high salt diet. In contrast, female rats come into balance within the very first day of the high salt diet despite eating an identical amount of salt as males. This fits with more recent findings that the response to an acute high salt load in a model of salt-dependent hypertension – ET\_B receptor deficiency – female rats excrete the salt load faster than males, which is dependent upon the time of day that the salt load is given. We have continued to explore how other natriuretic pathways, that is, the purinergic system, contribute to salt handling via activation of the ET-1 system. We observed that activation of P2Y\_2 and P2Y\_4 receptors in the renal medulla by UTP promotes Na\(^+\) excretion in male rats. In intact females, UTP did not stimulate Na\(^+\) excretion while ovariectomy unmasked UTP-induced natriuresis. An ET\_B antagonist inhibited purinergic dependent natriuresis. Since the purinergic and ET\_B systems work via inhibition of the epithelial Na channel (ENaC), it is interesting to note that expression of the alpha subunit of ENaC is higher in female compared to male animals. However, we have observed that the natriuretic response to ENaC inhibition is greater in male compared to female rats. Collectively, these observations demonstrate clear differences in sodium handling by renal medullary structures in the kidney. Our lab continues to explore potential mechanisms that can explain sex differences in handling of sodium. We posit that a better understanding of these sex-specific pathways can provide one of the most fundamental aspects of personalized medicine, sex-based therapy in hypertension and renal disease.

9.2 SEX-SPECIFIC REGULATION OF SIRTUIN-3 MEDIATES DIFFERENCES IN ISCHEMIA-REPERFUSION KIDNEY INJURY

**Background:** While the pathogenesis of ischemic acute kidney injury (AKI) is better defined, the therapeutic options remain limited. Sex influences susceptibility to kidney ischemia-reperfusion injury (IRI), and sex hormones play a crucial role. We have previously shown that a pathway from stanniocalcin-1 (STC1) mediated activation of AMPK to induction of mitochondrial sirtuin-3 (SIRT3) suppresses ROS generation and confers resistance to kidney IRI. Our observations reveal increased baseline kidney expression of STC1, activated AMPK, and SIRT3 in female mice vs. males, and we hypothesize that SIRT3 protects from IRI and mediates the observed sexual dimorphism in response to injury.

**Methods:** We subjected wild-type (WT) and SIRT3 transgenic (Tg) male or female mice to bilateral kidney IRI (clamping of renal pedicles for 30 minutes). A group of male or female WT mice were treated with testosterone by subcutaneous implantation of a 200 mg (21-day release) testosterone pellet for 2 weeks. Cultured HEK 293T cells were treated with 17β-estradiol, testosterone or vehicle.

**Results:** We observed higher kidney expression of STC1, mitochondrial SIRT3 and activity of AMPK in WT female mice compared with males. While there was age-dependent decline in kidney SIRT3 and AMPK activity, differential expression in males and females persisted. Aged 6 months-old SIRT3 Tg male mice display less tubular vacuolization vs. similarly aged WT male mice. Compared with WT male mice, SIRT3 Tg male mice demonstrated resistance to 30-minutes of kidney IRI characterized by: improved survival; preserved creatinine clearance (CrCl); decreased morphological damage and ROS production. SIRT3 Tg male mice tolerated IRI with survival and kidney function impairment similar to WT females. WT or SIRT3 Tg female mice display no measurable change in kidney function with 30-minutes of kidney IRI. In WT female mice, kidney mitochondrial SIRT3 expression correlates with both plasma estradiol and testosterone levels; in WT male mice, kidney mitochondrial SIRT3 expression correlates with only plasma testosterone level. Testosterone administration to aged 6 months-old WT male mice increased plasma testosterone ~4-fold, caused kidney injury (decreased CrCl and increased tubular vacuolization), and decreased kidney mitochondrial SIRT3 expression (with no effect on whole cell SIRT3). Testosterone treatment to WT female mice caused no measurable kidney injury, but increased
whole cell and mitochondrial SIRT3 expression; possibly due to an associated increase in plasma estradiol level. In cultured HEK cells, estradiol increased whole cell and mitochondrial SIRT3 protein expression, and SIRT3 mRNA in a dose-dependent manner. Testosterone decreased mitochondrial SIRT3 protein expression in a dose-dependent manner, but had no effect on whole cell SIRT3 protein expression and SIRT3 mRNA. Estradiol also increased estrogen receptor-β and estrogen related receptor-α mRNA expression.

**Conclusion:** The data suggest that: 1) SIRT3 ameliorates kidney IRI, and decreased SIRT3 expression in males mediates the increased susceptibility to ischemic injury; 2) sex steroids regulate mitochondrial SIRT3 expression; estrogen via transcriptional regulation and testosterone via inhibition of mitochondrial targeting.

**Funding:** This work was supported by Career Development Award #IK2 BX002912 and Merit Award #BX002006 from the United States (U.S.) Department of Veterans Affairs Biomedical Laboratory Research and Development Program, and a generous gift from Dr. and Mrs. Harold Selzman.

### 9.3

**SEX DIFFERENCES IN RENAL SODIUM TRANSPORTERS**

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Pre-menopause, females have lower blood pressure, blunted hypertensive response to angiotensin II infusion (AngII-HTN), and a leftward shift in pressure natriuresis compared to males. Veiras *et al* (1) examined whether the female advantage was associated with sex dependent pattern of transporters' abundance and/or activation along the nephron, using carefully validated quantitative immunobLOTS and physiological assays of renal function in rats. Female proximal tubule (PT) exhibited differences in transporters, claudin-2 and water channels (AQP1) indicative of less PT reabsorption in females versus males. Supporting these differences physiologically, females excreted a saline load more rapidly and exhibited both lower bicarbonate reabsorption (determined by micropercuture) and higher volume flow from the PT (determined by lithium clearance) than males. Along the distal nephron and collecting duct, sodium transporters, channels and claudin-7 were more abundant and activated in females associated with lower baseline plasma [K+] and increased Na-Cl cotransporter (NCC) phosphorylation in females indicating acute regulation of the lower K+ set point. The findings suggest lower PT reabsorption in female rats expedites excretion of a saline load and provokes higher NCC and ENaC abundance and activation which may increase K+ secretion and re-set plasma K+ at a lower level. Similar sex dependent profiles were evident in female versus male C57BL/6 mice except for lower PT AQP1 in females and no sex dependence of the response to saline challenge.

The Layton lab (2) generated a sex-specific computational model of solute and transport in the rat PT that accounts for the sex differences in abundance levels of the apical and basolateral transporters, in single-nephron glomerular filtration rate, and in tubular dimensions. Model simulations predict more reabsorption of the filtered volume and Na+ by male PT (71%) than female PT (39%). The lower fractional volume reabsorption in female can be attributed to their smaller transport area and lower AQP1 expression level. The latter also results in a larger contribution of the paracellular pathway to water transport. The lower fractional Na+ reabsorption in female is due primarily to their smaller transport area and lower Na+/H+-exchanger (NHE3) and claudin-2 expression levels. Notably, Na+/glucose cotransporter 2 expression levels are 2.5-fold higher in female which the simulations suggest may compensate for lower PT tubular transport area to achieve a similar hyperglycemic tolerance as male.

Studies on the effects of Angiotensin II-hypertension (AngII-HTN) and high salt diet (HSD) in females vs. males are nearing completion. In males, AngII-HTN depresses PT NHE3 and raises DT NCC-P while in females AngII-HTN increases PT NHE3 and does not raise NCC-P. HSD in males raises NHE3-P and depresses NCC, NCC-P. In females, HSD elicits a more robust natriuresis associated with lower PT NHE3 at baseline coupled to reduced distal transporter NCC, NKCC activation. The studies in AngII and HSD indicate sex specific differences in regulatory patterns that indicate careful attention should be paid to therapeutic effects of diuretics and RAS inhibitors in females vs males.

**References:**


9.4 ORAL L-ARGININE TREATMENT SIGNIFICANTLY INCREASES RENAL TREGS IN FEMALE DOCA SALT HYPERTENSIVE RATS
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The immune system plays a critical role in the development of hypertension. Our lab has previously reported that there is a sex difference in the renal T cell profile in DOCA salt hypertension, with males having more pro-inflammatory Th17 cells, whereas females have more anti-inflammatory regulatory T cells (Tregs). DOCA treated males also have a greater blood pressure (BP) response to DOCA than females, and we propose this is due to the relative abundance of Tregs in the female. We have also previously reported that an intact nitric oxide (NO) system is required for Treg maintenance in hypertension, and the DOCA model has been characterized by impaired NOS. In the current study, we hypothesized that treatment with L-arginine, a substrate for NO production, would increase renal Tregs and attenuate the sex difference in hypertension in male and female DOCA treated rats. Briefly, male and female Sprague Dawley rats (n=5-15, 10 wks of age) were uninephrectomized and subcutaneously implanted with a DOCA pellet (200 mg/rat, 60-d time release) and given 0.9% NaCl to drink ad libitum. A subset of rats were treated with L-arginine (L-arg, 350 mg/kg/d, via drinking water). BP was measured by tail cuff plethysmography at baseline and after 3 wks of DOCA treatment. After 3 wks of treatment, kidneys were processed for flow cytometric analysis of T cells (CD3⁺T cells, CD3⁺CD4⁺ T cells, CD3⁺CD4⁺FOXP3⁺ Tregs, and IL-10⁺CD3⁺ T cells). BP was comparable between treatment groups within each sex at baseline, although males had a higher BP than females (Table, effect of sex, p<0.001, 2-Way ANOVA). DOCA treatment increased BP in all groups compared to baseline values (Table, effect of DOCA, p=0.002). L-arginine treatment attenuated DOCA-induced increases in BP in both sexes, although this effect was greater in females (Table, effect of L-arginine, p=0.02, interaction of sex and L-arginine, p=0.03). Females had a more Tregs than males, and L-arginine treatment increased Tregs in females alone (Table, effect of sex, p<0.01, effect of L-arg, p<0.01, interaction, p<0.01, 2-Way ANOVA). Interestingly, despite the Treg numbers remaining unchanged with L-arg, IL-10 increased in both sexes to a similar extent (Table, effect of sex, p=0.29, effect of L-arg, p=0.03, interaction, p=0.93, 2-Way ANOVA). Future studies will examine additional mechanisms by which L-arginine supplementation increases Tregs in females.

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<th>BP Baseline (mmHg)</th>
<th>BP Wk 3 (mmHg)</th>
<th>CD3</th>
<th>CD4</th>
<th>Tregs</th>
<th>IL-10</th>
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9.5 SEX DIFFERENCES IN RENAL ISCHEMIA-REPERFUSION INJURY
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Postischemic organ dysfunction is influenced by gender and sexual steroids in several organs. Renal ischemia-reperfusion injury leading to acute renal failure continues to be an important clinical problem, especially in situations where the kidney is subjected to periods of warm ischemia, such as suprarenal aortic surgery and renal transplantation. The initial ischemic injury of the kidney is one of the most important risk factors for acute and chronic transplant dysfunction.

What is the impact of gender and sexual steroids on the development of renal damage following ischemic injury? We have first reported that sex and sex hormones influence the susceptibility to renal ischemia/reperfusion injury, since female rats have better survival rates and improved renal recovery following ischemic insult. Since the first report of gender related differences in renal ischemia/reperfusion injury several studies was performed to investigate the background and pathophysiology of this difference. While these studies explored many
different pathways and described several different answers to ischemia/reperfusion injury the exact pathophysiology is still not known. The importance to answer this question is not only to know the pathophysiology but to develop therapeutic possibilities in human settings. In this review we summarize the recent knowledge about renal ischemia/reperfusion injury and treatment possibilities.

9.6
SEX DIFFERENCES IN RENAL AMMONIA METABOLISM
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Renal ammonia excretion is a critical component in maintaining acid-base homeostasis. Sex differences are well recognized as an important biological variable in many aspects of renal function. However, sex differences in renal ammonia metabolism have not been previously reported. This study's objective was to investigate sex differences in renal ammonia metabolism. We compared 4-month-old C57/B6 male (M) and female (F) mice fed a normal diet, with measurement of plasma electrolytes, urinary ammonia excretion, morphometric analysis of renal structure, and evaluation of changes in key proteins involved in ammonia metabolism using immunoblot analysis and immunohistochemistry. Despite similar level of food intake (F, 8.9±0.9; M, 9.6±1.2 gram/day; P=NS), and thus protein intake, which is the primary determinant of endogenous acid production, female mice excreted significantly more ammonia (F, 72±23; M, 46±19 μmol/day; P<0.01) than did male mice. This difference in ammonia excretion was not due to differences in urine acidification, as urine pH did not differ significantly (F, 6.40±0.18; M, 6.36±0.14; P=NS). Titratable acid excretion (F, 53±26; M, 74±20 μmol/day; P=NS) another component of net acid excretion, did not differ significantly. Serum Na+, K+, and HCO3 did not differ significantly. There are fundamental structural differences between the female and male kidney. In the female kidney, proximal tubules account for a lower percentage of the renal cortical parenchyma than the male kidney (F, 42±3; M, 60±3%; P<0.01), whereas the collecting ducts account for a greater percentage of the renal parenchyma (F, 15.4±2.0; M, 9.6±1.6%; P<0.001). Phosphoenoypyruvate, a major proximal tubule (PT) ammonia generating protein, was significantly greater in female mice than male mice. Expression of glutamine synthetase, which recycles ammonia, was significantly greater in the PT of female mice. Expression of NBCe1, a basolateral PT transporter, recently shown to regulate PT ammonia metabolism did not differ significantly between the sexes. Expression of NHE3, which is believed to be the major mechanism of PT ammonia secretion, did not differ significantly between the sexes. Expression of NCC2, which mediates thick ascending limb ammonia reabsorption, was significantly greater in the female kidney than the male kidney. The collecting duct secretes the majority of urinary ammonia and the Rhesus glycoproteins, Rhbg and Rhcg are the primary collecting duct ammonia transporting proteins. Rhbg was significantly greater in connecting segment cells and intercalated and principal cells in the collecting duct in the cortex and inner stripe of the outer medulla (ISOM) in female mice. Expression of Rhcg was significantly greater in female mice in connecting segment cells and in the basolateral membrane of intercalated and principal cells in the collecting of the ISOM. Thus, there are sex differences in basal ammonia metabolism that involves both renal structural differences and differences in expression of critical proteins involved in ammonia metabolism. These studies were supported by funding from the National Institute of Diabetes and Digestive and Kidney Diseases Grant R01-DK-045788 (IDW), R01-DK-107798 (IDW and JWV) and ST32-DK-10472.

9.7
SEX DIFFERENCES IN THE REGULATION OF BLOOD PRESSURE BY THE CIRCADIAN CLOCK PROTEINS PER1 AND BMAL1 IN C57BL/6J MICE
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Blood pressure (BP) exhibits a circadian rhythm in healthy individuals in which there is a peak during the active period and a decrease during the rest phase. The core transcriptional mechanism of the circadian clock consists of four proteins: BMAL1, CLOCK, CRYPTOCHROME (CRY), and PERIOD (PER). BMAL1 and CLOCK interact with E-box response elements in the promoters of clock-controlled genes, including those for PER and CRY, to regulate expression. PER and CRY act in a negative feedback loop to inhibit the actions of BMAL1 and CLOCK. To date, every clock gene mutant mouse that has been tested has exhibited some type of BP phenotype, yet these studies have exclusively been performed in male mice. We have previously demonstrated that male global PER1 knockout mice exhibit non-dipping hypertension in response to a high salt (HS) diet plus mineralocorticoid (DOCP) treatment (Solocinski et al. Acta Physiol. 2017). Curtis et al. demonstrated that male global BMAL1 knockout mice exhibited a BP that was ~100 mm Hg less than control mice and did not have a detectable rhythm. The goal of the present study was to test whether female global PER1 KO mice and female...
kidney-specific BMAL1 KO mice exhibit a BP phenotype relative to littermate controls. We also tested the sensitivity of the female mice to the HS/DOCP treatment. The results demonstrate that both PER1 and BMAL1 contribute to the regulation of BP in a sex-specific manner that may be independent of dietary salt and mineralocorticoid.

11.2 BETA-CAROTENE METABOLISM IN THE MATERNAL HEART DURING PREGNANCY
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Pregnancy-related deaths associated with cardiovascular diseases have recently increased in the U.S. with a higher occurrence among African American and Hispanic women. Low intake of fruits and vegetables, the main source of vitamin A, is a hallmark of poor nutrition that is manifested in these populations that are mainly affected by gestational cardiac complications. Many signaling pathways have been associated with the physiological hypertrophy (remodeling) of the heart that occurs during pregnancy ². However, how these pathways are activated and influenced during pregnancy has yet to be fully understood. Retinoic acid, the active form of vitamin A that functions as a transcriptional regulator, has been implicated in cardiac remodeling in the adult ², but whether or not retinoids (vitamin A and its derivatives) are essential during the cardiac hypertrophy of pregnancy is still unknown. Preliminary data from our laboratory revealed a small but significant decrease in Dhrs3 (Dehydrogenase reductase 3) and Lrat (Lecithin:retinol acyltransferase) in the heart of pregnant wild-type mice at 14.5 dpc, suggesting that during gestation, heart retinoid metabolism may be shunted towards retinoic acid formation rather than storage. Thus, if cardiac retinoid acid synthesis is favored in wild-type mice during pregnancy, the active vitamin A metabolite may play a potential role in sustaining maternal cardiac hypertrophy (remodeling).

Beta-carotene, the most abundant dietary precursor of vitamin A, can be cleaved asymmetrically by the mitochondrial beta-carotene 9',10'-oxidase (BCO2) to generate beta-apo-10’-carotenal, which can serve as a precursor of retinoids, but may also antagonize retinoid acid action per se ³. Preliminary observations in our lab indicate that cardiac mRNA levels of Bco2, which is the only carotenoid cleavage enzyme expressed in the adult mammalian heart ⁴, were elevated in wild-type pregnant mice at mid-gestation. Moreover, in the absence of BCO2 (Bco2-/− mice) the maternal heart failed to enlarge during pregnancy. Notably during pregnancy, the heart of Bco2-/− mice showed significantly reduced retinyl ester levels compared to wild-type pregnant mice. Based on these premises, we hypothesized that BCO2 may contribute to the physiological hypertrophy of the maternal heart during pregnancy. Current studies aim at further understanding the effects of the lack of murine BCO2 on retinoid metabolism and cardiac function in the maternal heart. Understanding the role of carotenoid and retinoid metabolism in this process will allows us to ultimately design dietary preventative measures to potentially decrease adverse cardiac function during pregnancy.


11.3 VASCULAR CHANGES IN THE POSTMENOPAUSAL FEMALE
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The number of years women live past menopause has drastically increased over the last century. A decline in estrogen induces hypertension and arterial stiffness, accelerating end organ damage and heart failure. In contrast, adverse cardiovascular outcomes have been reported in response to currently available menopausal hormone therapy, indicating that new treatment strategies are needed. Our goal is to elucidate estrogen receptor pharmacology to allow the development of more selective pharmaceuticals that provide cardiovascular protection. We focus our efforts on the vascular effects of the G protein-coupled estrogen receptor (GPER). GPER is membrane-bound and activates acute intracellular signaling pathways, distinguishing it from the nuclear estrogen receptors ERα and ERβ.
Moreover, this receptor is unique in that it does not impact traditional estrogenic actions in reproductive tissues but induces protective cardiovascular effects, indicating a potential drug target. Activation of GPER attenuates aortic remodeling, and GPER deletion increases pulse wave velocity, an in vivo indicator of arterial stiffness. GPER also decreases vascular oxidative stress via regulation of NOX4. Moreover, we find that vascular GPER expression decreases during aging, which may further promote vascular disease and worsen the response to menopausal hormones. Current studies in the lab are assessing the impact of sex and aging on not only GPER but expression profiles of all estrogen receptors in order to give a clearer picture of the contribution of each signaling pathway in different tissues. Our overall goal is to promote the improvement of menopausal hormonal therapy and therefore quality of life in aging women.

**11.4 FEMALE RATS WITH PREEXISTING CKD EXHIBIT IMPAIRED RECOVERY FROM AKI AND THE SUBSEQUENT DEVELOPMENT OF PROTEINURIA**

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Interactions between acute kidney injury (AKI) and chronic kidney disease (CKD) predispose to the development of end-stage renal disease (ESRD). A previous study from our group showed that such deleterious AKI-CKD interactions include impaired recovery from AKI and the development of de novo mechanisms of CKD progression in male rats with preexisting CKD. The goal of this study was to determine if impaired recovery from AKI is also observed in female rats with preexisting CKD. We induced two levels of CKD in 10-12 week old male and female Sprague-Dawley rats by performing either 50% renal mass reduction via a right uninephrectomy (UNX, n=14, 6 females) or 75% renal mass reduction via a right UNX + surgical excision of 1/2 of the left kidney (3/4 NX, n=12, 6 females). Rats recovered for two weeks to allow for completion of compensatory adaptations in renal size and function. Rats were then subjected to 35 minute ischemia-reperfusion (IR)-induced AKI under isoflurane anesthesia with core body temperature maintained at 37°C. Blood samples were obtained prior to IR and at 48 hours, 7 days, 14 days and 28 days post IR to assess plasma creatinine (P Cr). A 24-hour urine collection was performed in a subset of rats prior to IR and at 28 days post IR to assess proteinuria. At the end of the study, kidneys were fixed in parafomaldehyde and paraffin embedded sections were stained with H&E to assess renal pathology and tubular vimentin expression was assessed using immunohistochemistry. Tubular vimentin expression 28 days post IR identifies sublethally injured tubules that have failed to redifferentiate, which is a robust index of impaired recovery. Vimentin expression was semiquantitated on a scale from 0-4 with 0 representing no tubular vimentin staining and 4 representing tubular vimentin staining in >75% of tubules. Vimentin scoring was conducted in a blinded fashion. The severity of AKI, based on P Cr levels 48 hours post AKI, was similar between UNX vs. 3/4 NX groups within both males and females. The severity of AKI was lower (P<0.05) in females vs. males with 3/4 NX (1.9±0.3 vs. 3.3±0.3 mg/dl) but not significantly different between females vs. males with UNX (1.7±0.6 vs. 2.7±0.4 mg/dl). While minimal injury and vimentin expression (0.7±0.2) was observed in female rats with UNX, females with 3/4 NX exhibited greater (P<0.05) tubular vimentin staining (1.3±0.3), tubular injury and fibrosis 28 days post IR. Moreover, recovery of P Cr over 28 days post IR was delayed in females with 3/4 NX vs. UNX, which is also indicative of impaired recovery from AKI. Finally, female rats with 3/4 NX developed substantial (P<0.05) increases in proteinuria 28 days post IR as compared to pre-IR levels (195±57 vs. 62±20 mg/day) while proteinuria was similar at 28 days post IR vs. pre-IR in female rats with UNX (21±4 vs. 23±5 mg/day). Similar to our previous study, males with 3/4 NX exhibited impaired recovery from AKI and the development of substantial proteinuria 28 days post AKI as compared to males with UNX. In conclusion, these data support previous studies documenting resistance to IR-induced AKI in female vs. male rats. However, our data indicate that preexisting CKD of greater than 50% renal mass reduction predisposes female rats to impaired recovery from AKI and the subsequent development of mechanisms of CKD progression, similar to male rats.

**11.5 LONG-TERM SEQUELAE OF PREECLAMPSIA: A CLINICAL PERSPECTIVE**

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Preeclampsia complicates 5-7 % of pregnancies and remains a leading cause of fetal growth restriction, premature birth, as well as infant and maternal morbidity and mortality. It is primarily a disease of the vascular endothelium. Accordingly, women with a history of preeclampsia have now been documented to have an increased risk for the development of hypertension, stroke, coronary artery disease as well as end stage renal disease later in life. This increased vascular risk is likely mediated by abnormalities in vascular physiology, including significant impairments in flow-mediated vasodilatation as well as increased arterial stiffness that
remain present into the postpartum period, and are most marked in women with a history of early onset, severe preeclampsia accompanied by fetal growth restriction, suggesting that vascular dysfunction may, in fact, be the predisposing factor for both abnormal placentation as well as the development of vascular disease in later life. It is critical that clinicians recognize the relationship between placental disease and future vascular disease to ensure those women most at risk are appropriately counselled and targeted with risk reduction strategies.

11.6
ANGIOTENSIN II INDUCES A PRO-INFLAMMATORY SHIFT IN THE SPLENIC CD4⁺ T CELL PROTEOME IN MENOPAUSAL MICE
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Premenopausal female mice are protected against Angiotensin II-induced hypertension, however this protection against Ang II-induced hypertension is lost following the onset of menopause. T cells are required for the development of Ang II hypertension in male mice and we have shown that premenopausal females are protected from T cell-mediated hypertension. This protection is also lost in menopausal mice. The purpose of this study was to utilize a bottom-up shotgun proteomics-based approach to examine how CD4⁺ T cell activation and pro-inflammatory signaling is modified in Ang II-infused menopausal mice compared to premenopausal counterparts. 10-week-old C57BL/6J female mice received intraperitoneal injections of 4-vinylcyclohexene diepoxide (VCD; sesame oil vehicle) for 20 consecutive days to induce menopause. Cyclicality was monitored daily to determine the day of onset of menopause. Ang II (800ng/kg/min) was infused for 14 days via osmotic minipump into VCD-treated menopausal (Meno/Ang II) or vehicle-treated premenopausal (Ang II) mice. After 14 days of Ang II infusion, splenic CD4⁺ T cells were isolated and purified via negative immunomagnetic selection. CD4⁺ purity was measured via flow cytometry and protein was obtained from these cells. Splenic CD4⁺ T cell protein samples were fractioned via SDS PAGE prior to trypsin digestion and Zip Cleaning on C18 columns. These peptide samples were analyzed via label-free MS/MS tandem mass spectrometry and were subsequently identified and quantified using Mascot and Progenesis software. 7,123 proteins were identified from the peptide samples. From this protein list, 5,857 proteins were identified by more than one unique peptide sequence and were used for subsequent analysis. 964 proteins were differentially expressed between control, Ang II and Meno/Ang II groups (p < 0.05). Of the 964 differentially expressed proteins, 350 were significantly different between Ang II and Control, while 639 proteins were differentially expressed between Meno/Ang II and Control, and 248 between Meno/Ang II and Ang II. Gene Ontology (GO) enrichment of the 964 differentially expressed proteins was assessed using Perseus software and the DAVID database. Ang II infusion resulted in the overexpression of 220 GO biological pathways (p < 0.05), including positive regulation of cell adhesion (5.1-fold enrichment), negative regulation of interleukin-6 production pathway (4.5-fold enrichment), and negative regulation of cell cycle arrest (4.1-fold enrichment). Overall, expression of proteins positively regulating cell adhesion and negatively regulating interleukin 6 were decreased in the Ang II group versus control and were further decreased in the Meno/Ang II group (see attached table). Proteins associated with negative regulation of cell cycle arrest were equally increased by Ang II in premenopausal and menopausal female mice, suggesting an increase in CD4⁺T cell proliferation. These results demonstrate that Ang II induces a significantly greater shift in the splenic CD4⁺ T cell proteome in female mice after menopause. This shift results in a proteomic profile favoring the proliferation and migration of pro-inflammatory T cells in postmenopausal females.

11.7
LEPTIN ADMINISTRATION LOWERS REGULATORY T CELLS AND ACCELERATES THE DEVELOPMENT OF HYPERTENSION IN AN EXPERIMENTAL MODEL OF AUTOIMMUNE DISEASE
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Systemic lupus erythematosus (SLE) is a prototypic multisystem autoimmune disorder that predominately
affects women during childbearing years. SLE is characterized by a loss of immunological tolerance and the expansion of autoreactive T and B lymphocytes, leading to the production of autoantibodies. The autoantibody production leads to downstream chronic inflammation resulting in high rates of hypertension, renal injury, and cardiovascular disease in SLE patients. The immunomodulatory adipocytokine leptin plays a key role in the maintenance and development of inflammation, in part by promoting the expansion of proinflammatory T helper 1 (Th1) cells and inhibiting the differentiation of regulatory T cells (Treg). Although women have higher circulating leptin levels than men, and the levels are even higher in women with SLE, it is unclear whether leptin plays a direct role in the pathogenesis of SLE. In the present study, we hypothesized that administration of leptin would lower circulating Treg and accelerate the development of hypertension in a female mouse model of SLE. To test this hypothesis, 30 week old female SLE (NZBWF1, n=21) and control (NZW, n=25) mice were implanted with microosmotic pumps to continuously deliver recombinant mouse leptin at a rate of 0.5 mg/kg/day or vehicle (0.9% NaCl) for four weeks. Body composition was assessed using Echo MRI and fat mass, as a percentage of body mass, was not changed in control mice (20.9±1.1% Control-vehicle vs. 19.6±1.2% Control-leptin p=0.99), but was significantly lower in SLE-leptin treated mice (26.5±1.6% SLE-vehicle vs. 16.2±3.1% SLE-leptin, p<0.05). Circulating levels of anti-dsDNA IgG autoantibodies, a marker of SLE disease activity, were higher in SLE mice compared to controls (0.45±0.1 Control-vehicle vs. 1.3±0.3 SLE-vehicle OD450, p<0.01), but the administration of leptin did not significantly increase anti-dsDNA IgG production (0.98±0.2 SLE-vehicle vs. 1.3±0.1 SLE-leptin OD450, p=0.58). Plasma levels of IgG isotypes were also analyzed, and circulating levels of IgG2a were higher in SLE mice as compared to control (0.25±0.006 mg/mL Control-vehicle vs. 0.79±0.2 mg/mL SLE-vehicle, p<0.01), and were further increased in SLE mice administered leptin (0.79±0.2 SLE-vehicle vs. 1.2±0.05 SLE-leptin, p<0.05), suggesting increased Th1 activity. Circulating CD4+FoxP3+ Treg as assessed by flow cytometry, were lower in SLE mice than in control mice (3.6±0.5% Control-vehicle vs. 1.1±0.2% SLE-vehicle, p<0.05), as previously reported by our laboratory. Leptin administration further decreased the levels of circulating Treg in SLE mice (1.1±0.2% SLE-vehicle vs. 0.52±0.1% SLE-leptin, p<0.05). Mean arterial pressure (MAP; mmHg), measured in conscious mice by carotid catheter, was higher in SLE mice than in control mice (113±3 Control-vehicle vs. 128±3, p=0.06), and leptin further increased blood pressure in SLE mice (SLE-leptin: 134±3; p<0.01 vs. Control-vehicle and Control-leptin). Taken together, these data suggest that SLE mice may have enhanced sensitivity to leptin and that increased leptin enhances Treg1 responses and decreases Treg. In addition, therapeutics aimed at modulating leptin activity could have potential benefit for patients with SLE.

12: SESSION 7: SEX AND GENDER DIFFERENCES IN PHYSIOLOGY AND FUNCTION: THE VASCULATURE

12.1 IMPACT OF SEX ON VASCULAR FUNCTION IN CARDIOVASCULAR AND METABOLIC DISORDERS

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Obesity is a major risk factor for cardiovascular disease in males and females. Whether obesity triggers cardiovascular disease via similar mechanisms in both the sexes is, however, unknown. In males, the adipokine leptin highly contributes to obesity-related cardiovascular disease by increasing sympathetic activity. Females secrete 3× to 4× more leptin than males, but do not exhibit high sympathetic tone with obesity. Nevertheless, females show inappropriately high aldosterone levels that positively correlate with adiposity and blood pressure (BP). We hypothesized that leptin induces hypertension and endothelial dysfunction via aldosterone-dependent mechanisms in females. Leptin control of the cardiovascular function was analyzed in female mice sensitized to leptin via the deletion of protein tyrosine phosphatase 1b (knockout) and in agouti yellow obese hyperleptinemic mice (Ay). Hypersensitivity to leptin (wild-type, 115 ± 2; protein tyrosine phosphatase 1b knockout, 124 ± 2 mm Hg; P<0.05) and obesity elevated BP (a/a, 113 ± 1; Ay, 128 ± 7 mm Hg; P<0.05) and impaired endothelial function. Chronic leptin receptor antagonist restored BP and endothelial function in protein tyrosine phosphatase 1b knockout and Ay mice. Hypersensitivity to leptin and obesity reduced BP response to ganglionic blockade in both strains and plasma catecholamine levels in protein tyrosine phosphatase 1b knockout mice. Hypersensitivity to leptin and obesity significantly increased plasma aldosterone levels and adrenal CYP11B2 expression. Chronic leptin receptor antagonism reduced aldosterone levels. Furthermore, chronic leptin and mineralocorticoid receptor blockade reduced BP and improved endothelial function in both leptin-sensitized and obese hyperleptinemic female mice. Together, these data demonstrate that leptin induces hypertension and endothelial dysfunction via aldosterone-dependent mechanisms in female mice and suggest that obesity leads to cardiovascular disease via sex-specific mechanisms.
12.2

ESTROGEN DETERMINES THE SEX-DIFFERENCES IN ADRENERGIC VESSEL TONE REGULATION

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Introduction: Sex-specific differences in adrenergic vasoconstriction and vasorelaxation have been demonstrated in rats and humans. Although we have previously shown that differences in rats rely on endothelial β-adrenoceptors, neither translational relevance in humans nor the role of sex-hormones in endothelial β-adrenoceptor-related vessel tone regulation has been shown.

Aims: We investigated the role of endothelium in sex-specific differences of adrenergic vasoconstriction and vasorelaxation in human vessels, as well as the role of female and male sex-hormones on adrenergic vessel tone regulation in a rat model.

Methods: In human mammary arteries, obtained from the Heart Center Dresden (patient age: 50 to 70 years), vasoconstriction (norepinephrine) and vasorelaxation (isoprenaline and β3 agonist BRL) with and without endothelium were assessed using Mulvany myography. Five weeks old female and male wistar rats were respectively ovariectomized and orchiectomized. As controls, a sham-operated, hormone substituted (2 mg/kg, twice a week) and a vehicle group of rats were examined. At age of 12 weeks, aortas were isolated for assessment of vasoconstriction and vasorelaxation. Additionally, a qRT-PCR for quantification of β-adrenoceptor mRNA levels in aorta was performed.

Results: Mammary arteries of women constricted less (P<0.05) in response of norepinephrine than arteries of men. Removal of endothelium eliminated this sex-specific difference by significantly (P<0.05) increasing vasoconstriction in arteries of women, without affecting vasoconstriction in arteries of men. Vasorelaxation caused by isoprenaline was greater (P<0.05) in mammary arteries of women compared to arteries of men. This sex-specific difference in vasorelaxation was abolished after removal of endothelium. Similar to human arteries there were sex-specific differences in vasoconstriction and relaxation in rat aorta, which was eliminated after orchiectomy in female rats. Compared to sham operated females, ovariectomy increased aortic vasoconstriction in response to norepinephrine more than 2-fold. Vasorelaxation by isoprenaline and β3-agonist was significantly (P<0.01) reduced after ovariectomy. Compared to vehicle, estrogen substitution largely (P<0.05) restored sex-specific differences in vasoconstriction and vasorelaxation in ovariectomized rats. Differences in vasoconstriction and vasorelaxation between sexes were diminished in presence of selective β1- and β3-adrenoceptor antagonists and L-NMMA. Consistently, mRNA levels of β1- and β3-, but not β2-adrenoreceptors were significantly (P<0.05) higher in aortas of sham operated females than in aortas of sham operated males. Ovariectomy abolished this difference by decreasing β1- and β3-adrenoreceptor expression in female rats. Consequently, estrogen substitution in ovariectomized females largely (P<0.05) restored β1- and β3-adrenoceptor expression. Orchietomy and testosterone treatment did not change aortic vasoconstriction and vasorelaxation nor β-adrenoceptor expression in aortas of male rats.

Conclusion: We reveal that sex-specific differences in vasoconstriction and vasorelaxation in human mammary artery are endothelium-dependent. We also demonstrate that sex-differences in a rat model are estrogen, but not testosterone-dependent. Estrogen determines these differences via regulation of vascular endothelial β1- and β3-adrenoceptor expression.

12.3

GENOTYPE CONTRIBUTES TO SEX DIFFERENCES IN MICRO- AND MACROVASCULAR ENDOTHELIAL PHENOTYPE

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Among humans, at the whole-body level, while both sexes may be in homeostasis, how they achieve that state appears to differ by sex. It is well known that in females the state of pregnancy results in profound changes in fluid distribution with resulting changes in hemodynamics (Duvekot et al., 1993; Mudrovic et al., 2017). One supposition is that because females must possess the mechanisms to reversibly alter fluid distributions to accommodate pregnancy, the multiple factors regulating fluid and solute flux between the vasculature and metabolizing tissue will likewise differ by sex. In animal studies we and other have demonstrated profound differences in the basal levels of protein and/or fluid flux in some portions of the microvasculature and not others (Huxley et al., 2004, 2007, 2010, Sasaki et al., 2007, Wang et al., 2010). These same studies also demonstrated varied sex-dimorphism in permeability responses to a variety of vasoactive agents under conditions where the reproductive hormone levels were known or not varying suggesting a role for genomic control of barrier function. To assess the role of genomic sex a comprehensive study of endothelial cell (EC) phenotype was undertaken of passage 4.4 EC derived from age-matched sexually mature rat aorta (macrovascular) and skeletal muscle (microvessel) maintained under identical conditions of culture and low,
unvarying levels of reproductive hormones. The data demonstrated that both genomic sex and position in the vasculature played distinct roles in EC morphology, growth, wound healing, lactate production, messenger RNA, and expression of key proteins (including sex hormone receptors for estrogen (ERα and ERβ) and androgen; barrier proteins PECAM-1 and VE-CAD; αvβ3 and N-Cadherin influencing matrix interactions; ICAM-1 and VCAM-1 mediating EC/white cell adhesion). A hierarchy of variable importance was unveiled when the EC growth data were analyzed as precision improved assuming EC homogeneity < Sex < Vessel Origin < Sex and Vessel Origin. Many identified sex differences are subtle and easily ignored. In the aggregate, though, they can profoundly alter phenotype, especially under conditions of pregnancy, exercise, and disease states ranging from diabetes to heart failure. Overall, ignoring either sex (and/or age) is inappropriate and will prevent the design and implementation of appropriate interventions to present, ameliorate, or correct vascular dysfunction. Supported by NIH NIDDK R01 DK095501

References:


12.4

**NITRIC OXIDE HELPS MAINTAIN THE BUFFERING CAPACITY OF PERIVASCULAR ADIPOSE TISSUE IN FEMALE DAHL SS IN RESPONSE TO A HIGH FAT DIET DESPITE INCREASES IN BLOOD PRESSURE AND VASCULAR INFLAMMATION**

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Local inflammation in perivascular adipose tissue (PVAT) is linked to high fat diet (HFD)-induced increases in BP and vascular dysfunction in males. There is limited data on the impact of HFD on BP or vascular function in females. Thus the goal of this study was to test the hypotheses that HFD will 1) increase BP and vascular inflammation and 2) PVAT will exacerbate HF diet-induced vascular dysfunction in female DSS. 6-wko female DSS were fed a normal-fat diet (NFD; 7.2% fat) or HFD (35% fat) diet for 10 weeks, and BP was measured by telemetry. At 16 wko, aortic rings (+/- PVAT) were mounted for isometric myography and cumulative concentration response curves to phenylephrine (PE) or acetylcholine (Ach) were generated in the absence or presence of the nonselective nitric oxide synthase (NOS) inhibitor L-Nω-Nitroarginine methyl ester (LNAME) or polyethylene glycol (PEG)-catalase. In separate rats, aortic T cells were measured by flow cytometry. HFD increased BP (mmHg: 176 ± 8 HFD vs 130 ± 4 NFD, P<0.001) and led to greater numbers of total aortic T cells (P=0.05), T cell activation (P=0.002), and pro-inflammatory Th17 cells (P=0.002) compared to NFD. There was no change in anti-inflammatory T regulatory cells (P=0.67). HFD alone had no effect on vascular function. Although the presence of PVAT did not increase relaxation to Ach, it did attenuate PE-induced constriction [Area Under Curve (AUC): effect of PVAT P<0.01] regardless of diet. HFD is known to promote oxidative stress via increased production of reactive oxygen species, including H2O2. Interestingly, Peg-catalase uncovered a PVAT-mediated vasoconstrictor with HFD (AUC: effect of diet: P=0.015; effect of PegCat: P=0.17; interaction: P=0.0091) while LNAME increased force generation to PE in the presence of PVAT regardless of diet (effect of LNAME: P=0.047; effect of diet: P=0.17). Thus, in contrast to what has been shown in male DSS following a HFD in other studies, vascular function is maintained in female DSS; and PVAT enhanced the vasodilatory capacity of the aorta regardless of diet. Further, our data suggests that overproduction of NO rather than H2O2 plays a role in maintaining the anti-contractile effect of PVAT in response to a HFD. Future studies will determine 1) which NOS isoform contributes to the enhanced buffering capacity of PVAT in response to a HFD and 2) whether or not this capacity is mediated by the...
endothelium using endothelium intact and denuded vessels.

12.5
FUNCTIONAL IMPLICATIONS OF SEXUAL DIMORPHISM OF TRANSPORTER PATTERNS ALONG THE RAT NEPHRON
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The goal of this study is to investigate the functional implications of the sexual dimorphism in transporter patterns along the nephron. To do so, we began by developing sex-specific computational models of solute and transport in the proximal convoluted tubule of the rat kidney. The models account for the sex differences in expression levels of the apical and basolateral transporters, in single-nephron glomerular filtration rate, and in tubular dimensions. Model simulations predict that 70.6 and 38.7% of the filtered volume is reabsorbed by the proximal tubule of the male and female rat kidneys, respectively. The lower fractional volume reabsorption in female can be attributed to their smaller transport area and lower Na+/H+ exchanger (NHE3) and claudin-2 expression levels. Notably, unlike most Na+ transporters, whose expression levels are lower in female, Na+/glucose cotransporter 2 (SGLT2) expression levels are 2.5-fold higher in female. Model simulations suggest that the higher SGLT2 expression in female may compensate for its lower tubular transport area to achieve a similar hyperglycemic tolerance as male.

This research was supported by the National Institutes of Health: National Institute of Diabetes and Digestive and Kidney Diseases, grant R01DK106102 to AT Layton and R01DK083785 to AA McDonough.

12.6
AFFERENT ARTERIOLE RESPONSIVENESS TO ENDOTHELIN RECEPTOR ACTIVATION: DOES SEX MATTER?
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The pathogenesis of hypertension is distinct between men and women. Endothelin-1 (ET-1) is a potential contributor to sex-differences in the pathophysiology of hypertension. ET-1 is known to participate in blood pressure regulation through activation of endothelin A (ETa) and endothelin B (ETb) receptors in both the renal tubule and vasculature. However, little is known about sex-differences in ET-1 dependent renal microvascular reactivity. Our lab previously reported that renal medullary ET-1 reduces medullary blood flow in male, but not female rats. Orchiectomy (ORX) eliminated ET-1 dependent decreases in medullary blood flow, but ovariectomy (OVX) had no apparent effect on this sex-difference. Thus, we hypothesized that sex and sex steroids regulate the afferent arteriolar responses to ET receptor activation. To test that, we used 15-17 week old male and female Sprague Dawley rats subjected to gonadectomy or sham surgery. Three weeks later, kidneys from those rats were prepared for assessment of renal microvascular responses to ET-1 (ETa and ETb agonist, 10^-12 to 10^-8 M) and sarafotoxin 6c (S6c, ETb agonist, 10^-12 to 10^-8 M) using the blood-perfused juxtamedullary nephron preparation. Baseline afferent arteriolar diameter at 100 mmHg averaged 15.3±0.3 and 14.6±0.3 μm for sham male and female rats, respectively (n=12, each). Gonadectomy had no significant effect on baseline arteriolar diameter. In sham males, ET-1 produced significant concentration-dependent decreases in afferent arteriolar diameter, with 10^-8 M ET-1 decreasing diameter by 84±1 % (n=6). Similarly, ET-1 induced concentration-dependent vasoconstrictor responses in sham female rats, with 10^-8 M ET-1 decreasing the diameter by 82±1 % (n=6). The vasoconstrictor responses to ET-1 within the afferent arteriole were unchanged by ORX or OVX. In addition, ETb receptor activation by S6c induced a concentration dependent decline in the afferent arteriolar diameter, with 10^-8 M S6c decreasing diameter by 77±3 and 76±3 % in sham male and female rats, respectively (n=6, each). These data do not support our original hypothesis and suggest that sex or sex hormones do not significantly influence afferent arteriolar reactivity to ET receptor activation. They further suggest that reported sex differences of the renal ET-1 system on blood pressure are most likely mediated through renal tubular activity of the ETa and ETb receptors as we have previously reported.
THE ROLE OF THE CEREBROVASCULATURE IN ISCHEMIC STROKE INJURY AND RECOVERY: SEX AND DIABETES INTERACTIONS

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Over 30 million Americans who have diabetes are not only at a 2 to 6-fold higher risk of having an acute ischemic stroke but also suffer from poor recovery and greater physical and cognitive disability. Furthermore, diabetic patients are more likely to bleed into the brain (hemorrhagic transformation, HT), especially with the use of tissue plasminogen activator (tPA), which remains to be the only pharmacological treatment for ischemic stroke. The lack of understanding on how increased bleeding occurs and influences the restorative and regenerative processes within the neurovascular networks hindered the development of new therapeutic strategies for stroke. While clinically it is known that women have poorer functional outcomes, the inadequate inclusion of female animals coupled with the limited use of diabetic models in preclinical stroke research has further deepened this gap.

For neurorestorative strategies to be effective and facilitate functional recovery, a supportive microenvironment is needed. Undoubtedly, cerebral microvasculature is a key component of this reparative & restorative niche. Our early studies in male diabetic animals showed that a) there is robust pathological neovascularization of the brain in a lean and moderate model of type 2 diabetes, b) a reperfusion injury superimposed on this pathology amplifies HT and worsens neurological deficits without increasing infarct size, and c) in the presence of HT, toll like receptor TLR4, a major mediator of innate inflammatory response, is upregulated in the ischemic region and especially in cerebral microvasculature. While testing the hypotheses that any form of bleeding into the brain is detrimental for stroke recovery and excess iron attenuates neurovascular restoration via the activation of TLR-4, an administrative supplement enabled us to incorporate female animals to our ongoing studies in male rats. Our exciting results suggest that 1) young diabetic female rats lose the neuroprotection typically seen in control female animals and develop greater HT than in controls and even diabetic male rats; 2) matrix metalloprotease (MMP)-3, an enzyme known to cause HT and to be regulated by TLR4, is increased to a greater degree in cerebral microvessels of female diabetic rats; 3) while male diabetic animals show significant loss of cerebrovasculature by activation of multiple cell death pathways in the recovery period, female diabetic animals do not, but rather undergo phenotypic changes in endothelial cells resembling endothelial-mesenchymal transition, EndMT, a process associated with scarring and impaired healing, and 4) in the long-term, diabetes worsens sensorimotor and cognitive recovery in both sexes.

Thus, our current knowledge of the impact of diabetes on cerebrovascularization in both sexes will be reviewed with a focus on stroke injury and recovery.

ANDROGENS IN CARDIOVASCULAR HEALTH AND DISEASE

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Androgens have differential effects whether in men or women. For example, most chronic diseases, such as heart disease, obesity, chronic kidney disease, cancer, cause reductions in androgen levels in men. What is not clear is whether the reduction in androgens in men leads to chronic diseases or whether men with chronic diseases experience reduced androgen levels since there have been no serial longitudinal studies in men to address this question. In obese male animals, for example, androgen supplements reduce body weight, increase activity, improve insulin resistance with reductions in plasma insulin and leptin, decrease hyperlipidemia, reduce inflammation with reductions in cytokine levels. But androgen supplements increase blood pressure in the males. In female animals, androgen supplements increase food intake and body weight, increase leptin, cause insulin resistance and hyperlipidemia, and inflammation. Androgen supplements in females also cause elevated blood pressure. Elevated levels of androgens in women are symptomatic of polycystic ovary syndrome. In both women and female with increased androgen levels, reproductive issues are prevalent. If female animals become pregnant, their male offspring have reductions in plasma testosterone, but female offspring do not develop symptoms of polycystic ovary syndrome or hyperandrogenemia. The mechanisms by which androgens cause increases in blood pressure in males and females are likely different and are the focus for future research. These studies are supported by NIH R01HL16072, R01HL69194, P01HL55971, P20GM121334, and R01HL135089.
13.2

GROUP IV CYTOSOLIC PHOSPHOLIPASE A\(_2\,\alpha\) IS REQUIRED FOR 6\(\beta\)-HYDROXYTESTOSTERONE MEDIATED ANGIOTENSIN II INDUCED HYPERTENSION IN MALE MICE

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Previously we showed that cytochrome P450 (CYP) 1B1-testosterone derived metabolite 6\(\beta\)-hydroxytestosterone (6\(\beta\)-OHT), by acting as a permissive factor, contributes to the development of angiotensin II (Ang II)-induced hypertension in male mice. Also, we reported that Ang II-induced hypertension is mediated by group IV cytosolic phospholipase A\(_2\,\alpha\) (cPLA\(_2\,\alpha\)) activation, resulting in arachidonic acid (AA) release, and generation predominantly of eicosanoids with pro-hypertensive effects. This study was performed to investigate the interaction of CYP1B1 and cPLA2\(_\alpha\)/AA system by testing the hypothesis that 6\(\beta\)-OHT contributes to Ang II-induced hypertension by promoting cPLA\(_2\,\alpha\) activation and generation of eicosanoids with pro-hypertensive effects. Male intact or castrated (Cas) Cyp1b1\(^{+/+}\)/cPLA2\(_\alpha\)\(^{+/+}\), Cyp1b1\(^{-/-}\), and cPLA2\(_\alpha\)\(^{-/-}\) mice (8 weeks old, n=4-5) were infused with Ang II (700 ng/kg/min) and injected with 6\(\beta\)-OHT (15 \(\mu\)g/g, i.p. every 3\(^{rd}\) day), for 2 weeks, and systolic blood pressure (SBP) was measured by tail cuff. In Cyp1b1\(^{+/+}\)/cPLA2\(_\alpha\)\(^{+/+}\) mice, castration or CYP1B1 gene disruption minimized the Ang II-induced increase in SBP (127±3 and 148±3 vs. 188±3 mmHg, respectively, P<0.05). Ang II infusion in 6\(\beta\)-OHT, but not its vehicle (DMSO, 50ml) treated Cyp1b1\(^{+/+}\) mice increased SBP (189±15 vs.148±3 mmHg, P<0.05); this increase was minimized by the AA metabolism inhibitor, 5,8,11,14-eicosatetraynoic acid (25 mg/kg, i.p. every 3\(^{rd}\) day) (140±4 mmHg, P<0.05). Ang II infusion with 6\(\beta\)-OHT treatment increased SBP in Cas cPLA2\(_\alpha\)\(^{+/+}\) mice, but not in Cas cPLA2\(_\alpha\)\(^{-/-}\) mice (176±7 vs. 122±2 mmHg, P<0.05).

Treatment with antagonists of prostaglandin (PG) E2 receptors EP1 (SC19220) and EP3 (L-798106) (28 \(\mu\)g/g, s.c. every 2\(^{nd}\) day) attenuated the Ang II-induced increase in SBP in 6\(\beta\)-OHT treated Cas cPLA2\(_\alpha\)\(^{+/+}\) mice (123±4, 123±6 vs. 189±5 mmHg, respectively, P<0.05). These data suggest that 6\(\beta\)-OHT contributes to Ang II-induced increase in SBP via cPLA2\(_\alpha\) activation, the release of AA and generation of eicosanoids, most likely PGE2 that exerts pro-hypertensive effects by stimulating EP1 and EP3 receptors. Therefore, the development of agents that selectively inhibit the cPLA2\(_\alpha\) activity or block EP1 and EP3 receptors could be useful in treating hypertension and its pathogenesis.

13.3

PRETREATMENT WITH LOW DOSE LIPOPOLYSACCHARIDE ATTENUATES MEDULLARY CONGESTION IN MALE WKY FOLLOWING ACUTE KIDNEY INJURY.

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Acute kidney Injury (AKI) is a sudden loss of renal function which can result in future complications or mortality, and prevalence of AKI continues to increase, specifically in hospitalized patients. Ischemia-reperfusion is a well-accepted experimental technique of AKI in rodents. Renal medullary congestion has been demonstrated to augment ischemia reperfusion (IR) in rodent models (1) and we have recently reported a sex difference in recovery from AKI, in which medullary congestion and indices of kidney function are worse in males than females at 7 days post-IR. As low grade inflammation can promote rouleaux, we hypothesized that prior exposure to LPS would worsen medullary congestion and augment AKI following IR. To test this hypothesis, we examined the effect of pretreatment with incrementing doses of lipopolysaccharide (LPS) on renal congestion following ischemia reperfusion. Male Wistar-kyoto rats (WKY, 9wks) were treated with 10 (n=3), 100 (n=3), 1000 (n=3) \(\mu\)g/kg LPS or control (saline, n=3) for 7 days (i.p), and then subjected to a 30 minute warm, bilateral ischemia reperfusion. Rats were allowed 24h to recover then anesthetized and humanely sacrificed. The right kidney was taken for histology and stained using Gomori’s Trichrome Stain (Thermo 87020). Blood was collected by tail vein at baseline, at days 2 and 7, and at sacrifice. C-reactive protein (CRP, Thermo ERCRP), a marker of inflammation, increased for all groups from baseline to post-IR (\(P_{\text{treatment}}<0.0001\)), with control treatment having the greatest increase and highest levels of CRP, and 1000\(\mu\)g/kg LPS demonstrating the lowest levels CRP (\(P_{\text{interaction}}=0.09\)), at sacrifice. In difference to our hypothesis, outer Medullary peritubular congestion (blinded scoring) showed LPS pre- treatment reduced congestion when compared to saline treated controls (% congestion: control=80%, 10 and 100\(\mu\)g/kg LPS=40%, 1000\(\mu\)g/kg LPS= 20%). We conclude that, despite promoting inflammation, paradoxically, prior low dose LPS exposure prevents red blood cell congestion in the outer-medulla following IR. As we have recently reported that peristaltic contractions of vasa recta pericytes may prevent RBC congestion in the renal outer-medulla (2), we speculate that LPS activation of toll-like receptors of vasa recta pericytes may prime pericytes to contract preventing congestion. Further investigation of these mechanisms may lead to novel therapeutic approaches to prevent AKI.


13.4 ANDROGEN INFLUENCE ON RENAL FIBROSIS ASSOCIATED WITH PYELONEPHRITIS

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Renal scarring after pyelonephritis is linked to long-term health risks for hypertension and chronic kidney disease. Testosterone signaling through the androgen receptor increases susceptibility to, and severity of, uropathogenic Escherichia coli (UPEC) pyelonephritis in both male and female mice (1), while anti-androgen therapy is protective against severe UTI (2). Mice with severe pyelonephritis develop renal fibrosis and scarring (3). This work elucidates the molecular mechanisms of renal fibrosis in androgenized female C3H/HeN and C57BL/6 mouse backgrounds, and determines how these pathways are altered by the presence of testosterone. C3H/HeN mice feature vesicoureteral reflux (VUR), which allows for severe pyelonephritis and widespread renal fibrosis. C57BL/6 mice do not have VUR, but still exhibit alterations in renal fibrosis markers and display scarring following upper-tract UTI. We demonstrate that renal fibrosis after pyelonephritis involves both the TGF-β/Activin A and Hedgehog pathways, with altered local expression of proteins in the Smad family, TGFβ1, Activin A, and Gli1. Elevated circulating testosterone levels drive Ly6C+ monocyte recruitment to the kidney in the uninfected state and upon urinary tract inoculation with UPEC. Our results are consistent with a model in which testosterone increases recruitment of Ly6C+ monocytes, and that these cells are activated in the presence of UPEC during renal infection, driving local expression of pro-inflammatory and profibrotic markers and thereby promoting fibrosis and renal scar formation.


13.5 HYPOGONADISM IN MALES: ONE SIZE DOES NOT FIT ALL

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In recent years, we have evaluated the effect of androgens on cardiometabolic risks factors in diverse male rodent models. We reported that long-term testosterone supplementation in obese male Zucker rats causes an increase in blood pressure; despite improvement of other cardiovascular risk factors, such as dyslipidemia, insulin resistance and inflammation. Also, we reported that testosterone upregulates intrarenal angiotensin system in an animal experimental model of salt sensitive hypertension. More recently, we observed a differential effect on blood pressure of testosterone supplementation in young versus old male spontaneously hypertensive rats. The negative effect of endogenous androgens in the cardiovascular system had been reported in other animal experimental models of hypertension. However, in clinical studies performed with hypogonadal males, the role of endogenous and exogenous testosterone upon cardiovascular system remains still unclear. The recent 2018 Clinical Guideline for “Testosterone Therapy in Men With Hypogonadism” from the Endocrine Society states that testosterone replacement is contraindicated in hypogonadal men with uncontrolled heart failure, myocardial infarction or stroke within the last 6 months. In specific populations such as in subjects with Klinefelter syndrome or Diabetes, testosterone have clear beneficial effects on cardiometabolic risk factors. More research is needed to elucidate the complex effects of testosterone upon cardiometabolic risk factors across different populations.
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2019 The Interface of Mathematical Models and Experimental Physiology: Organ Function from the Microvascular Perspective
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