

**2007 APS Conference**  
**Sex Steroids and Gender in Cardiovascular-Renal**  
**Physiology and Pathophysiology**

**APS Council**

*President*

**Hannah V. Carey**

Barbara E. Goodman  
Joey P. Granger  
Gary C. Sieck

*Past President*

**Dale J. Benos**

Susan M. Barman  
James W. Hicks  
Dee U. Silverthorn

*President-Elect*

**Irving H. Zucker**

Irving G. Joshua  
David M. Pollock  
J. Michael Wyss

*Ex officio Members*

Kim E. Barrett  
Martin Frank  
Michael A. Portman

Curt D. Sigmund  
Peter D. Wagner

Thomas A. Pressley  
Kenneth M. Baldwin  
William T. Talman

**Conference Organizers**

**Jane F. Reckelhoff (Chair)**

Univ. of Mississippi Med. Ctr.

**Joey P. Granger (Vice-chair)**

Univ. of Mississippi Med. Ctr.

**Kathryn Sandberg**

Georgetown Univ.

**Anna F. Dominiczak**

Univ. of Glasgow, UK.

**Carmen Hinojosa-Laborde**

Univ. of Texas Hlth. Sci. Ctr.,  
San Antonio

**J. Michael Wyss**

Univ. of Alabama at  
Birmingham

**John Stallone**

Texas A&M Univ.  
Col. of Vet. Med

**Acknowledgements**

The Conference Organizers and The American Physiological Society gratefully recognize the generous financial support provided through unrestricted educational grants from:

**NIH, National Heart, Lung, and Blood Institute**  
**Bristol-Myers Squibb Company**  
**Transoma**

**2007 APS Conference**  
**Sex Steroids and Gender in Cardiovascular-Renal Physiology and Pathophysiology**  
**August 9-12, 2007, Hyatt Regency Austin on Town Lake**

<b>Thursday, August 9</b>	<b>Friday, August 10</b>	<b>Saturday, August 11</b>	<b>Sunday, August 12</b>
<p>3:00 PM <b>Registration</b></p> <p>6:00 - 8:00 PM <b>Opening Reception</b></p> <p>8:00 - 9:00 PM Symposium I: <b>Sex Steroids in Clinical and Epidemiological Studies</b> Participants : <b>Jane Reckelhoff</b>, (Chair) Univ. of Mississippi Med. Ctr. <b>Pamela Ouyang</b>, Johns Hopkins Bayview Med. Ctr. <b>Sharon Silbiger</b>, Montefiore Hosp.</p>	<p>7:00 AM <b>Registration</b></p> <p>8:00 - 9:40 AM Symposium II: <b>Update on Sex Steroid Receptors and Cardiovascular Diseases</b> Participants: <b>Pascale Lane</b>, (Chair) Univ. of Nebraska Med. Ctr. <b>Matthias Barton</b>, Univ. Hosp. of Zurich, Switzerland <b>Chawnshang Chang</b>, Univ. of Rochester <b>Theo Pelzer</b>, Univ. of Wuerzburg, Germany <b>John Cidlowski</b>, NIEHS, NIH</p> <p>9:40 - 10:00 AM Break</p> <p>10:00 – 11:00 AM Symposium III: <b>Sex Steroids and Vascular Function</b> Participants: <b>John Stallone</b>, (Chair) Texas A&amp;M Univ. Col. of Vet. Med., College Station <b>Sue Duckles</b>, Univ. of California, Irvine <b>Raouf Khalil</b>, Harvard Univ.</p> <p>11:00 AM – 1:00 PM <b>Selected Oral Presentations</b> <b>J. Michael Wyss</b>, (Chair) Univ. of Alabama at Birmingham</p> <p>1:00 - 4:00 PM FREE TIME</p> <p>2:00 – 4:00 PM <b>Career Workshop</b></p> <p>4:00 - 6:00 PM <b>Poster Session</b></p> <p>6:00 - 8:00 PM FREE TIME</p> <p>8:00 - 9:00 PM Symposium IV: <b>Sex Steroids and Metabolic Syndrome</b> Participants: <b>Carmen Hinojosa-Laborde</b>, (Chair) Univ. of Texas Hlth. Sci. Ctr., San Antonio <b>Domenic Sica</b>, VA Commonwealth Univ. <b>Carolyn Bondy</b>, NICHD, NIH</p>	<p>7:00 AM <b>Registration</b></p> <p>8:00 - 9:45 AM Symposium V: <b>Sex Steroids, the Renin-Angiotensin System and Hypertension</b> Participants: <b>Kathryn Sandberg</b>, (Chair) Georgetown Univ. <b>Mark Chappell</b>, Wake Forest Univ. <b>Judith Miller</b>, Univ. of Toronto <b>Edwin Jackson</b>, Univ. of Pittsburgh <b>J. Michael Wyss</b>, Univ. of Alabama at Birmingham</p> <p>9:45 - 10:00 AM Break</p> <p>10:00 AM - 12:00 PM Symposium VI: <b>Sex Steroids and Target Organ Injury</b> Participants: <b>David Pollock</b>, (Chair) Med. Col. of Georgia <b>Douglas Bowles</b>, Univ. of Missouri, Columbia <b>Christine Maric</b>, Georgetown Univ. <b>Chris Baylis</b>, Univ. of Florida, Gainesville <b>Virginia Miller</b>, Mayo Clinic &amp; Foundation <b>Raymond Quigley</b>, Univ. of Texas Southwestern</p> <p>12:05 PM – 1:00 PM <b>Selected Oral Presentations</b> <b>Virginia Miller</b>, (Chair) Mayo Clinic &amp; Foundation</p> <p>1:00 - 4:00 PM FREE TIME</p> <p>2:00 – 4:00 PM <b>Career Workshop</b></p> <p>4:00 - 6:00 PM <b>Poster Session</b></p> <p>7:00 PM <b>Conference Banquet and Awards Presentations</b></p>	<p>8:00 AM <b>Registration</b></p> <p>9:00 - 10:30 AM Symposium VII: <b>Sex Steroids, Pregnancy, Pre-eclampsia, and Fetal Programming</b> Participants: <b>Barbara Alexander</b>, (Chair) Univ. of Mississippi Med. Ctr. <b>Bridget Brosnihan</b>, Wake Forest Univ. <b>Joey Granger</b>, Univ. of Mississippi Med. Ctr. <b>S. Ananth Karumanchi</b>, Harvard Univ. Med. Sch.</p>

**Location:**

The 2007 APS Conference, Sex Steroids and Gender in Cardiovascular-Renal Physiology and Pathophysiology will be held August 9-12, 2007 at the Hyatt Regency Austin, 208 Barton Springs Road, Austin, TX 78704, telephone (512) 477-1234, FAX: (512) 480-2069.

**Onsite Registration Hours:**

Thursday, August 9 .....3:00—9:00 PM  
 Friday, August 10 ..... 7:00 AM—1:00 PM  
 Friday, August 10 ..... 4:00 PM— 8:30 PM  
 Saturday, August 11 ..... 7:00 AM—1:00 PM  
 Saturday, August 11 ..... 4:00 PM— 6:00 PM  
 Sunday, August 12 .....8:00—10:00 AM

**On-Site Registration Fees:**

APS Member..... \$350  
 Retired Member ..... \$235  
 Nonmember..... \$400  
 Postdoctoral..... \$290  
 Student ..... \$235

*The registration fee includes entry into all scientific sessions, opening reception and banquet.*

**Payment Information:**

Registrants may pay by institutional or personal check, traveler's check, MasterCard, VISA or American Express. Checks must be payable to "The American Physiological Society" and drawn on a United States bank payable in US dollars.

**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. Nonmember 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 four years of this meeting, 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.**

**Press:**

Press badges will be issued at the APS registration desk, only 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.

**Continuing Medical Education (CME):**

The Federation of American Societies for Experimental Biology is accredited by the Accreditation Council for Continuing Medical Education to sponsor continuing medical education for physicians.

Category I Continuing Medical Education (CME) credits will be offered at this meeting. All CME applications must be completed online after the meeting has concluded. Please visit the APS website in August for more details on submitting your CME application at: [www.the-aps.org/austin](http://www.the-aps.org/austin). For the purposes of Continuing Medical Education credits toward the American Medical Association Physician's Recognition Award, the 2007 APS Conference: Sex Steroids and Gender in Cardiovascular-Renal Physiology and Pathophysiology is jointly sponsored by the Federation of American Societies for Experimental Biology. There is a \$45 application fee, payable upon submission of the form. Please include your payment with the completed CME form. For more information, contact the FASEB Office of Scientific Meetings and Conferences at 301-634-7010.

**Program Objective:**

Upon completing the program, participants should gain more knowledge in the field of sex steroids and how they affect genders in the physiology of the cardiovascular-renal systems. The goal of the conference is to accumulate together a critical mass of scientists who have interests in sex steroids in physiology of the cardiovascular and renal systems to promote the exchange of ideas and potential collaborations in the future.

**Target Audience:**

The intended audience for this meeting includes all levels of researchers working in the field of sex steroids, particularly as it pertains to the cardiovascular and renal systems.

*This meeting has been made possible through the generous support from:*

*NIH, National Heart, Lung, and Blood Institute*

*Bristol-MyersSquibb Company*

*Transoma*

## DAILY SCHEDULE

### THURSDAY, AUGUST 9, 2007

#### Symposium I

#### 1.0 SEX STEROIDS IN CLINICAL AND EPIDEMIOLOGICAL STUDIES

Thur., 8:00 - 9:00 PM, Texas Ballroom II/III.

Chair: **Jane Reckelhoff**, *Univ. of Mississippi Med. Ctr.*

8:00 PM **1.1** Introduction. **Jane Reckelhoff**. *Univ. of Mississippi Med. Ctr.*

8:05 PM **1.2** The Latest on the Cardiovascular Effects of Hormone Therapy in Postmenopausal Women. **Pamela Ouyang**. *Johns Hopkins Bayview Med. Ctr.*

8:30 PM **1.3** Sex Steroids and Renal Disease in Humans. **Sharon Silbiger**. *Montefiore Hosp., Bronx.*

### FRIDAY, AUGUST 10, 2007

#### Symposium II

#### 2.0 UPDATE ON SEX STEROID RECEPTORS AND CARDIOVASCULAR DISEASES

Fri., 8:00 - 9:40 AM, Texas Ballroom II/III.

Chair: **Pascale Lane**, *Univ. of Nebraska Med. Ctr.*

8:00 AM **2.1** Introduction. **Pascale Lane**. *Univ. of Nebraska Med. Ctr.*

8:05 AM **2.2** Sex Steroid Receptors and Atherosclerosis. **Matthias Barton**. *Univ. Hosp. of Zurich, Switzerland.*

8:25 AM **2.3** Increased Hepatic Steatosis and Insulin Resistance in Mice Lacking Hepatic Androgen Receptor via Modulation of PTP1 $\beta$ /PPAR $\alpha$  Involved in Lipid and Glucose Homeostasis. **Chawnschang Chang**. *Univ. of Rochester.*

8:45 AM **2.4** Estrogen Receptor Studies Using Transgenic Animals. **Pascale Lane**. *Univ. of Nebraska Med. Ctr.*

9:05 AM **2.5** Redundant, Divergent or Opposing Functions of Estrogen Receptor Subtypes ER $\alpha$  and ER $\beta$  in Hypertension, Cardiac Hypertrophy and Heart Failure? **Theo Pelzer**. *Univ. of Wuerzburg, Germany.*

9:25 AM **2.6** The Glucocorticoid Receptor: One Gene, Many Proteins - New Mechanisms for Tissue Specific Anti-Inflammatory Actions of Glucocorticoids in Health and Disease. **John Cidlowski**. *NIEHS, NIH.*

*Plan to Attend the Welcome and Opening Reception*

*Thursday, August 9*

*6:00 - 8:00 PM*

*Foothills II Ballroom*

#### Symposium III

#### 3.0 SEX STEROIDS AND VASCULAR FUNCTION

Fri., 10:00 - 11:00 AM, Texas Ballroom II/III.

Chair: **John Stallone**, *Texas A&M Univ. Col. of Vet. Med., College Station.*

10:00 AM **3.1** Introduction. **John Stallone**. *Texas A&M Univ. Col. of Vet. Med., College Station.*

10:05 AM **3.2** Estrogen and Mitochondria: A New Paradigm for Vascular Protection? **Sue Duckles**. *Univ. of California, Irvine.*

10:25 AM **3.3** Sex Steroids and Vascular Responses in Aging. **Raouf Khalil**. *Harvard Univ.*

10:45 AM **3.4** Rapid Nongenomic Effects of Androgens on the Vascular Wall. **John Stallone**. *Texas A&M Univ. Col. of Vet. Med., College Station.*

#### Oral Presentations I

#### 4.0 SELECTED ORAL PRESENTATIONS

Fri., 11:00 AM - 1:00 PM, Texas Ballroom II/III.

Chair: **J. Michael Wyss**, *Univ. of Alabama at Birmingham.*

11:05 AM **4.1** Dihydrotestosterone Modulates Cerebral Vascular Tone in Part by Enhancing COX-2. **Rayna Gonzales**. *Univ. of California, Irvine. (5.13).*

11:12 AM **4.2** Early Diabetic Kidney Damage in the Mouse VCD Model of Menopause. **Maggie Keck**. *Univ. of Arizona. (10.11).*

## DAILY SCHEDULE

- 11:19 AM **4.3** Sex Differences in the Response to Vasoactive Substances in Early Uncontrolled Diabetes. **Adam Mitchell.** *Georgetown Univ.* (10.12).
- 11:26 AM **4.4** Does Gender Influence Cardiovascular and Renal Responses to Water Immersion? **Donald Watenpaugh.** *Univ. of North Texas Hlth. Sci. Ctr.* (5.24).
- 11:34 AM **4.5** Sex Differences in Diabetic Renal Remodeling: Effects of Growth Hormone. **Jennifer Rogers.** *Georgetown Univ.* (10.13).
- 11:41 AM **4.6** Female Gender Protects Obese Rats from Nephropathy of the Metabolic Syndrome. **Jesus Dominguez.** *Vet. Affairs Med. Ctr. and Indiana Univ. Med. Ctr.* (10.15).
- 11:48 AM **4.7** Developmental Changes of Autonomic Control of Heart Rate in the Conscious Behaving Rat: State and Sex Influences. **Carie Reynolds.** *Univ. of Florida.* (5.7).
- 11:55 AM **4.8** Differential Expression of Neprilysin and Angiotensin Converting Enzyme 2 may Contribute to Decreased Organ Damage in the Female Hypertensive mRen2.Lewis Rat. **Karl Pendergrass.** *Wake Forest Univ.* (10.3).
- 12:02 PM **4.9** Testosterone Modulates Heart Rate Variability in Swine. **April Durtschi.** *Univ. of Missouri, Columbia.* (5.9).
- 12:09 PM **4.10** Effect of Estrogens and Selective Estrogen Receptor Modulators on Vascular Reactivity in the Perfused Mesenteric Vascular Bed. **Connie Mark.** *Univ. of South Dakota.* (5.20).
- 12:16 PM **4.11** Testosterone Supplements Promote Renal Injury and Exacerbate Hypertension in Aging SHR. **Radu Iliescu.** *Univ. of Mississippi Med. Ctr.* (10.4).
- 12:23 PM **4.12** Sex Differences in the Relationship Between Rat Mesenteric Venular Protein Leakage and Tissue Protein Clearance. **Rie Sasaki.** *Univ. of Missouri, Columbia.* (5.21).
- 12:30 PM **4.13** Testosterone Mediates Hypertension and Renal Injury in Dahl Rats

Despite High Sodium Diet-mediated Decrease in Testosterone Levels. **Licy Yanes.** *Univ. of Mississippi Med. Ctr.* (10.6).

- 12:38 PM **4.14** Population Extremes-based Approach Defines Gender Differences in Adrenergic and Renal Genes Contributing to Blood Pressure. **Brinda Rana.** *Univ. of California, San Diego.* (10.7).

- 12:45 PM **4.15** Effect of Age and Estrogen Loss on Estrogen Receptor Alpha and Beta in Kidney of Dahl Salt Sensitive Rats. **Ma Eugenia Davila.** *Univ. of Texas Hlth. Sci. Ctr. at San Anotnio.* (10.8).

*Don't forget....  
Pick up your Banquet Ticket by  
10:00 AM on FRIDAY  
This banquet is free but you MUST  
have a ticket for entry*

### Career Workshop

#### **MAKING A GREAT IMPRESSION AT A SCIENTIFIC MEETING: PRESENTING YOUR POSTER, PRESENTING YOURSELF**

Fri., 2:00 - 4:00 PM, Texas Ballroom II/III.

A special workshop presented by the APS Career Opportunities in Physiology Committee.

### Poster Session

#### **5.0 SEX STEROIDS IN HEART AND VASCULAR FUNCTION**

Fri., 4:00 - 6:00 PM, Texas Ballroom I.

### Board #

- 1 **5.1** Sex-based Differences in the Architecture of the Hypertrophied Heart in Salt Stressed Borderline Hypertensive Rats. **J. Krontiris-Litowitz, and R. Fulton.** *Youngstown State Univ., and Belmont Tech. Col., Clairsville.*
- 2 **5.2** Estrogen Receptor Alpha Interacts with  $17\beta$ -hydroxysteroid Dehydrogenase Type 10 and Regulates its Enzymatic Activity in the Heart. **T. Pelzer, and V. Jazbutyte.** *Univ. of Wuerzburg, Germany.*

## DAILY SCHEDULE

Board #		Board #	
3	<b>5.3</b> Characterization and Functional Analysis of the 5'-flanking Region of the ER $\alpha$ Gene in the Human Heart. <b>S. Fritschka, S. Mahmoodzadeh, and V. Regitz-Zagrosek.</b> <i>Charité Univ., Berlin, Germany.</i>	12	<b>5.12</b> Cutaneous Venoarteriolar Response is not Impaired in Postural Orthostatic Tachycardia Syndrome. <b>T. Van Gundy, S. Shibata, R. Shook, M. Sandgarten, J. Hastings, B. Levine, and Q. Fu.</b> <i>The Inst. for Exercise and Environ. Med., Dallas.</i>
4	<b>5.4</b> Voluntary Exercise Induces Sex-Specific Physiological Cardiac Remodeling. <b>S. Brokat, K. Cantow, N. Ehrenberg, A. Kühne, J. Thomas, and V. Regitz-Zagrosek.</b> <i>Charité Univ., Berlin, Germany.</i>	13	<b>5.13</b> Dihydrotestosterone Modulates Cerebral Vascular Tone in Part by Enhancing COX-2. <b>R. Gonzales, D. Krause, and S. Duckles.</b> <i>Univ. of California, Irvine.</i>
5	<b>5.5</b> Comparison of the Second Derivative Photoplethysmography a-a Interval Method and Electrocardiogram R-R Interval Method. <b>T. Kikuchi, Y. Sano, S. Urushidani, and J. Abo.</b> <i>Tokyo Univ. of Marine Sci., and Tech., Japan.</i>	14	<b>5.14</b> Gender and Circulating Endothelial Progenitor Cell Number and Apoptosis. <b>B. Stauffer, O. MacInearney, G. Hoetzer, E. Kushner, J. Cech, C. Westby, and C. DeSouza.</b> <i>Univ. of Colorado, Denver, and Univ. of Colorado, Boulder.</i>
6	<b>5.6</b> Cardiac Size and Plasma Volume: Potential Mechanisms for POTS? <b>Q. Fu, J. Hastings, S. Shibata, T. Van Gundy, R. Shook, and B. Levine.</b> <i>Presbyterian Hosp. of Dallas and Univ. of Texas Southwestern Med. Ctr.</i>	15	<b>5.15</b> Gender Differences in Endothelial Functions, Vascular Estrogen Receptors, eNOS and Triglycerides. <b>S. Chou, Y. Wang, S. Tsau, and Y. Lau.</b> <i>Chang Gung Univ., Taiwan.</i>
7	<b>5.7</b> Developmental Changes of Autonomic Control of Heart Rate in the Conscious Behaving Rat: State and Sex Influences. <b>C. Reynolds, C. Ward, N. Doperalski, D. Fuller, and L. Hayward.</b> <i>Univ. of Florida.</i>	16	<b>5.16</b> Estradiol Inhibits Apoptotic Signaling and Microvascular Endothelial Cell Hyperpermeability. <b>E. Childs, B. Tharakan, and F. Hunter.</b> <i>Texas A&amp;M Univ. Hlth. Sci. Ctr. Col. of Med. /Scott and White Hosp.</i>
8	<b>5.8</b> Testosterone Modulates Heart Rate and QT Interval in Swine. <b>A. Durtschi, and L. Rubin.</b> <i>Univ. of Missouri, Columbia.</i>	17	<b>5.17</b> Gender Modulation of Venous Function in Spontaneously Hypertensive Rats. <b>D. Martin, R. Redetzke, E. Vogel, C. Mark, and K. Eyster.</b> <i>Univ. of South Dakota.</i>
9	<b>5.9</b> Testosterone Modulates Heart Rate Variability in Swine. <b>A. Durtschi, L. Rubin, and J. Dodam.</b> <i>Univ. of Missouri, Columbia.</i>	18	<b>5.18</b> Sex Differences in the Response to Vasoconstrictor and Vasodilator Substances in Chronically Stressed Male and Female Rats. <b>A. Mitchell, J. Rogers, A. Myers, Z. Zukowska, and S. Mulroney.</b> <i>Georgetown Univ.</i>
10	<b>5.10</b> Role of Oxidative Stress in Parity-Induced Coronary Dysfunction. <b>S. Kaufman, and H. Tawfik.</b> <i>Univ. of Alberta, Canada.</i>	19	<b>5.19</b> Estrogen Decreases Mitochondrial Oxidative Stress in Human Brain Microvascular Endothelial Cells. <b>A. Razmara, S.P. Duckles, L. Sunday, C. Stirone, X. Wang, D.N. Krause, and V. Procaccio.</b> <i>Univ. of California, Irvine.</i>
11	<b>5.11</b> Analysis of the Effect of E2 on the Human MMP2 Expression in HT1080 Cells: A Promoter Based Analysis. <b>E. Dworatzek, S. Mahmoodzadeh, and V. Regitz-Zagrosek.</b> <i>Charité Univ., Berlin, Germany.</i>		

- Board #
- 20 **5.20** Effect of Estrogens and Selective Estrogen Receptor Modulators on Vascular Reactivity in the Perfused Mesenteric Vascular Bed. **C. Mark, R. Tatchum-Talom, D. Martin, and K. Eyster.** *Univ. of South Dakota.*
- 21 **5.21** Sex Differences in the Relationship Between Rat Mesenteric Venular Protein Leakage and Tissue Protein Clearance. **R. Sasaki, S. Bingham, and V. Huxley.** *Univ. of Missouri, Columbia.*
- 22 **5.22** Sex Difference in the Regulation of Muscle Blood Flow During Static Handgrip Exercise. **M. Kawamoto, K. Morimoto, and A. Takamata.** *Nara Women's Univ., Japan.*
- 23 **5.23** Skeletal Muscle Arterioles Demonstrate Sexual Dimorphism with Respect to Macromolecule Transvascular Exchange Pathways. **V. Huxley.** *Univ. of Missouri, Columbia.*
- 24 **5.24** Does Gender Influence Cardiovascular and Renal Responses to Water Immersion? **D. Watenpaugh, B. Pump, P. Bie, and P. Norsk.** *Univ. of North Texas Hlth. Sci. Ctr., Danish Aerospace Med. Ctr. of Res., Copenhagen, Denmark.*
- 25 **5.25** Effect of Estrogen Replacement on Osmoregulatory and Central Angiotensin II-induced Fluid Regulation in Ovariectomized Rats. **K. Torii, K. Morimoto, and A. Takamata.** *Nara Women's Univ., Nara, Japan.*
- 26 **5.26** Aldosterone Excretion During Chronic Intermittent Hypoxia in Male and Female Rats. **C. Hinojosa-Laborde, T. Craig, C. Mehring, T. Cunningham, and S. Mifflin.** *Univ. of Texas Hlth. Sci. Ctr. at San Antonio.*
- 27 **5.27** Genome-Wide Scan for Genetic Determinants of Alcohol and Tobacco Use in French Canadian Families. **M. Nikpay, O. Seda, J. Tremblay, E. Merlo, D. Gaudet, T. Kotchen, A. Cowley, and P. Hamet.** *Univ. of Montreal, Canada, and Med. Col. of Wisconsin.*

Symposium IV

- 6.0 SEX STEROIDS AND METABOLIC SYNDROME**  
Fri., 8:00 - 9:00 PM, Texas Ballroom II/III.
- Chair: **Carmen Hinojosa-Laborde,** *Univ. of Texas Hlth. Sci. Ctr., San Antonio.*
- 8:00 PM **6.1** Sex Differences in Sleep Apnea. **Carmen Hinojosa-Laborde.** *Univ. of Texas Hlth. Sci. Ctr. at San Antonio.*
- 8:20 PM **6.2** Sex Differences in Metabolic Syndrome and Cardiovascular Disease. **Domenic Sica.** *Virginia Commonwealth Univ.*
- 8:40 PM **6.3** The Parental Origin of the X Chromosome and Metabolic Risk Factors. **Carolyn Bondy.** *NICHD, NIH.*

**SATURDAY, AUGUST 11, 2007**

Symposium V

- 7.0 SEX STEROIDS, THE RENIN-ANGIOTENSIN SYSTEM AND HYPERTENSION**  
Sat., 8:00 - 9:45 AM, Texas Ballroom II/III.
- Chair: **Kathryn Sandberg,** *Georgetown Univ.*
- 8:00 AM **7.1** Introduction. **Kathryn Sandberg.** *Georgetown Univ.*
- 8:05 AM **7.2** Sex Differences in Angiotensin II, Angiotensin Converting Enzyme 2 and Angiotensin-(1-7). **Mark Chappell.** *Wake Forest Univ.*
- 8:25 AM **7.3** Gonadal Hormone-independent Sex Chromosome Effects in Angiotensin II-induced Hypertension. **Kathryn Sandberg.** *Georgetown Univ.*
- 8:45 AM **7.4** Role of the RAS in Sex Differences in Hypertension. **Judith Miller.** *Univ. of Toronto, Canada.*
- 9:05 AM **7.5** 2-Methoxyestradiol: A Safe and Effective Cardiorenal Protective Hormone Therapy for Women and Men. **Edwin Jackson.** *Univ. of Pittsburgh.*
- 9:25 AM **7.6** The Role of Estrogens and Polyphenols in Hypertension and Diabetes. **J. Michael Wyss.** *Univ. of Alabama at Birmingham.*

## DAILY SCHEDULE

### Symposium VI

- 8.0 SEX STEROIDS AND TARGET ORGAN INJURY**  
Sat., 10:00 AM - 12:00 Noon, Texas Ballroom II/III.
- Chair: **David Pollock**, *Med. Col. of Georgia*.
- 10:00 AM **8.1** Introduction. **David Pollock**. *Med. Col. of Georgia*.
- 10:05 AM **8.2** Sex Steroids, Coronary Smooth Muscle, Atherosclerosis and Restenosis. **Douglas Bowles**. *Univ. of Missouri, Columbia*.
- 10:25 AM **8.3** Critical Role for ET<sub>B</sub> Receptors in Attenuating the Response to Environmental Stress in Female, but not Male Rats. **David Pollock**. *Med. Col. of Georgia*.
- 10:45 AM **8.4** Sex, Diabetes and Renal Injury. **Christine Maric**. *Georgetown Univ.*
- 11:05 AM **8.5** Sex, NO and Aging. **Chris Baylis**. *Univ. of Florida*.
- 11:25 AM **8.6** Sex Steroids, Platelet Aggregation and Inflammation. **Virginia Miller**. *Mayo Clinic & Fdn.*
- 11:45 AM **8.7** Androgens Stimulate Proximal Tubule Transport. **Raymond Quigley**. *Univ. of Texas Southwestern Med. Ctr.*

### Oral Presentations II

- 9.0 SELECTED ORAL PRESENTATIONS**  
Sat., 12:05 - 1:00 PM, Texas Ballroom II/III.
- Chair: **Virginia Miller**, *Mayo Clinic & Fdn.*
- 12:05 PM **9.1** Reduced Uterine Perfusion Pressure Increases Soluble Flt-1 Expression in Pregnant Rats. **Jeffrey Gilbert**. *Univ. of Mississippi Med. Ctr. (10.23)*.
- 12:12 PM **9.2** Sleep Deprivation and Nocturnal Urine Output-Gender Difference in the Effect. **B. Mahler**. *Aarhus Univ. Hosp., Denmark. (10.18)*.
- 12:19 PM **9.3** Analysis of the Effect of E2 on the Human MMP2 Expression in HT1080 Cells: A Promoter Based Analysis. **Elke Dworatzek**. *Charité Univ., Berlin, Germany. (5.11)*.
- 12:26 PM **9.4** Elevated Agonistic Auto-antibodies to the Angiotensin Type 1 Receptor in Response to Placental

Ischemia and TNF Alpha in Pregnant Rats. **Babbette LaMarca**. *Univ. of Mississippi Med. Ctr. (10.24)*.

- 12:34 PM **9.5** Characterization and Functional Analysis of the 5'-flanking Region of the ERα Gene in the Human Heart. **Stephen Fritschka**. *Charité Univ., Berlin, Germany. (5.3)*.
- 12:41 PM **9.6** Vasomotor Sympathetic Neural Control is Enhanced in Early Pregnant Women. **Qi Fu**. *Presbyterian Hosp. of Dallas. (10.26)*.

### Career Workshop

**WRITING YOUR FIRST PAPERS: THE "INS" AND "OUTS" OF AUTHORSHIP**  
Sat., 2:00 - 4:00 PM, Texas Ballroom II/III.

A special workshop presented by the APS Career Opportunities in Physiology Committee.

### Poster Session

- 10.0 SEX STEROIDS IN HYPERTENSION, DIABETES, AND PREGNANCY**  
Sat., 4:00 - 6:00 PM, Texas Ballroom I.
- Board #
- 28 **10.1** Aldosterone/NaCl-induced Hypertension: The Role of Gender, Sex Hormones and Central Reactive Oxygen Species. **B. Xue, A. K. Johnson, and M. Hay**. *Univ. of Iowa*.
- 29 **10.2** MPA but not Drospirenone Aggravates Renal Injury in Aldosterone-salt Treated Rats. **T. Pelzer, and P. A. A. Loza**. *Univ. of Wuerzburg, Germany*.
- 30 **10.3** Differential Expression of Nprilysin and Angiotensin Converting Enzyme 2 May Contribute to Decreased Organ Damage in the Female Hypertensive mRen2.Lewis Rat. **K.Pendergrass, B. Westwood, and M. Chappell**. *Wake Forest Univ.*
- 31 **10.4** Testosterone Supplements Promote Renal Injury and Exacerbate Hypertension in Aging SHR. **R. Ilescu, L. L. Yanes, J. C. Sartori-Valinotti, and J. F. Reckelhoff**. *Univ. of Mississippi Med. Ctr.*

## DAILY SCHEDULE

Board #		Board #	
32	<b>10.5</b> Sex Differences in Renal 11 $\beta$ -Hydroxysteroid Dehydrogenase Type 2 Immunoreactivity in Rat Kidneys. <b>D. Roesch, M. Shi, C. Ecelbarger, and K. Sandberg.</b> <i>Georgetown Univ.</i>	41	<b>10.14</b> Role of Estrogens in Postmenopausal Obesity and Hypertension. <b>L. Fortepiani, and H. Zhang.</b> <i>Univ. of Texas Hlth. Sci. Ctr. at San Antonio, and Univ. of Mississippi Med. Ctr.</i>
33	<b>10.6</b> Testosterone Mediates Hypertension and Renal Injury in Dahl Rats Despite High Sodium Diet-mediated Decrease in Testosterone Levels. <b>L. L. Yanes, R. Ilescu, J. C. Sartori-Valinotti, H. Zhang, D. Romero, and J. F. Reckelhoff.</b> <i>Univ. of Mississippi Med. Ctr.</i>	42	<b>10.15</b> Female Gender Protects Obese Rats from Nephropathy of the Metabolic Syndrome. <b>J. Dominguez, and K. Kelly.</b> <i>Vet. Affairs Med. Ctr. and Indiana Univ. Med. Ctr.</i>
34	<b>10.7</b> Population Extremes-based Approach Defines Gender Differences in Adrenergic and Renal Genes Contributing to Blood Pressure. <b>B. Rana, P. Insel, N. Schork, and D. O'Connor.</b> <i>Univ. of California, San Diego.</i>	43	<b>10.16</b> Gender-Dependent Metabolic and Renal Effects of 2-Hydroxyestradiol in Obese Diabetic ZSF <sub>1</sub> Rats. <b>S. Tofovic, S. Bastacky, and E. Jackson.</b> <i>Univ. of Pittsburgh Sch. of Med.</i>
35	<b>10.8</b> Effect of Age and Estrogen Loss on Estrogen Receptor Alpha and Beta in Kidney of Dahl Salt Sensitive Rats. <b>M. E. Davila, T. Craig, and C. Hinojosa-Laborde.</b> <i>Univ. of Texas Hlth. Sci. Ctr. at San Antonio.</i>	44	<b>10.17</b> Dysregulated Estradiol Metabolism in Pre-eclampsia. <b>S. Tofovic, E. Jackson, and G. Tofovic.</b> <i>Univ. of Pittsburgh Sch. of Med., Univ. St. Cyril and Methodius Sch. of Med., Skopje, Macedonia.</i>
36	<b>10.9</b> Sexual Dimorphic Regulation of AQP2 in DOCA-Salt Hypertension. <b>S. Masilamani, C. Berry, T. Musselman, and Z. Zhang.</b> <i>Virginia Commonwealth Univ.</i>	45	<b>10.18</b> Sleep Deprivation and Nocturnal Urine Output-Gender Difference in the Effect. <b>B. Mahler, K. Kamperis, S. Hagstroem, E. Radvanska, S. Rittig, and J.C. Djurhuus.</b> <i>Aarhus Univ. Hosp., Denmark.</i>
37	<b>10.10</b> Role of Hydrogen Peroxide in Mediating Hypertension and Proteinuria in Female SHR. <b>J. C. Sartori-Valinotti, W. Dorsett-Martin, R. Ilescu, L. L. Yanes, and J. F. Reckelhoff.</b> <i>Univ. of Mississippi Med. Ctr.</i>	46	<b>10.19</b> Gender Bias Toward a Functional Subclass of Myelinated Visceral Afferent. <b>B. Li, and J. Schild.</b> <i>Indiana Univ. Perdue Univ. Indianapolis.</i>
38	<b>10.11</b> Early Diabetic Kidney Damage in the Mouse VCD Model of Menopause. <b>M. Keck, M. J. Romero-Aleshire, Q. Cai, P. B. Hoyer, and H. L. Brooks.</b> <i>Univ. of Arizona.</i>	47	<b>10.20</b> Estrogen Alters Myosin Heavy Chain Isoform Expression of Rat Vaginal Smooth Muscle. <b>M. Basha, T. Wang, J. Lassman, R. Moreland, A. J. Wein, and S. Chacko.</b> <i>Drexel Univ. Col. of Med. and Univ. of Pennsylvania.</i>
39	<b>10.12</b> Sex Differences in the Response to Vasoactive Substances in Early Uncontrolled Diabetes. <b>A. Mitchell, A. Myers, and S. Mulroney.</b> <i>Georgetown Univ.</i>	48	<b>10.21</b> Sex Hormones Contribute to Gender Differences in Programmed Hypertension Induced by Placental Insufficiency in the Rat. <b>N. Ojeda, D. Grigore, E. Robertson, and B. Alexander.</b> <i>Univ. of Mississippi Med. Ctr.</i>
40	<b>10.13</b> Sex Differences in Diabetic Renal Remodeling: Effects of Growth Hormone. <b>J. Rogers, C. Maric, K. Sandberg, and S. Mulroney.</b> <i>Georgetown Univ.</i>	49	<b>10.22</b> Sex Differences in Renal Function of Betamethasone-Treated Sheep: A Model of Fetal Programming. <b>T. Y. Gwathmey, L. Tang, J. Figueroa, M. Chappell, and J. Rose.</b> <i>Wake Forest Univ.</i>

## DAILY SCHEDULE

- Board #  
50 **10.23** Reduced Uterine Perfusion Pressure Increases Soluble Flt-1 Expression in Pregnant Rats. **J. Gilbert, B. LaMarca, S. Babcock, K. Cockrell, and J. Granger.** *Univ. of Mississippi Med. Ctr.*
- 51 **10.24** Elevated Agonistic Auto-antibodies to the Angiotensin Type 1 Receptor in Response to Placental Ischemia and TNF Alpha in Pregnant Rats. **B. LaMarca, R. Dechend, G. Wallukat, and M. Llinas.** *Univ. of Mississippi Med. Ctr., and Oberarzt HELIOS Clinic, Berlin, Germany.*
- 52 **10.25** Altered Cerebral Vascular Function in Response to Reductions in Uterine Perfusion in Pregnant Rats. **M. Ryan, G. McLemore Jr., J. Granger, and B. LaMarca.** *Univ. of Mississippi Med. Ctr.*
- 53 **10.26** Vasomotor Sympathetic Neural Control is Enhanced in Early Pregnant Women. **Q. Fu, S. Shibata, T. Van Gundy, J. Hastings, and B. Levine.** *Presbyterian Hosp. of Dallas, and Univ. of Texas Southwestern Med. Ctr.*
- 54 **10.27** Ability to Buffer Changes in pH During Ischemia – Are There Sex Differences in the Newborn Heart? **D. Quaglietta, M. P. Belanger, and C. Wittnich.** *Univ. of Toronto, Canada.*
- 55 **10.28** Orthotopic Liver Transplantation in Newborns—Lower Success Rates from Female Donors and Why Ischemic Metabolism May Play a Role. **D. Quaglietta, M. P. Belanger, and C. Wittnich.** *Univ. of Toronto, Canada.*
- 56 **10.29** The Role of Sexual Process in the Regulation of Stress-induced Cardiovascular Responses in Rat. **Z. Ghodarzi, N. Hydarieh, A. Khorami, S. Charkh-kar, M. Behnava, and A. Vahabzadeh.** *Iran Univ. of Med. Sci., Tehran, Iran.*
- 57 **10.30** Aortic Coarctation-induced Hypertension During Pregnancy: A Model of Pre-eclampsia in Rats. **A. Adeagbo, N. L. Alsip, J. J. D. Lucca.** *Univ. Louisville Med. Sch.*

- Board #  
58 **10.31** IUGR Alters COX-2 Expression through Steroid Signaling and Affects 11- $\beta$ -Hydroxysteroid Dehydrogenase Type 2 Chromatin Structure in the Rat Kidney. **M. Baserga, M. Hale, X. Yu, Q. Fu, C. Callaway, R. McKnight, and R. Lane.** *Univ. of Utah.*

## SUNDAY, AUGUST 12, 2007

### Symposium VII

- 11.0 SEX STEROIDS, PREGNANCY, PRE-ECLAMPSIA, AND FETAL PROGRAMMING**  
Sun., 9:00 - 10:30 AM, Texas Ballroom II/III.
- Chair: **Barbara Alexander,** *Univ. of Mississippi Med. Ctr.*
- 9:00 AM **11.1** Introduction. **Barbara Alexander.** *Univ. of Mississippi Med. Ctr.*
- 9:05 AM **11.2** Ang-(1-7) and ACE2 in Human Pregnancy. **K. Bridget Brosnihan.** *Wake Forest Univ.*
- 9:25 AM **11.3** Pathophysiology of Hypertension During Pre-eclampsia. **Joey Granger.** *Univ. of Mississippi Med. Ctr.*
- 9:45 AM **11.4** Pre-eclampsia and Angiogenic Factors. **S. Ananth Karumanchi.** *Harvard Univ. Med.Sch.*
- 10:05 AM **11.5** Sex Differences in Fetal Programming of Cardiovascular Disease. **Barbara Alexander.** *Univ. of Mississippi Med. Ctr.*

*This meeting has been made possible through the generous support from:*

*NIH, National Heart, Lung, and Blood Institute  
Bristol-MyersSquibb Company  
Transoma*

**2007 APS Conference  
Sex Steroids and Gender in Cardiovascular-Renal  
Physiology and Pathophysiology**

**Abstracts of Invited and Contributed Presentations**

1.0	Sex Steroids in Clinical and Epidemiological Studies .....	12
2.0	Update on Sex Steroid Receptors and Cardiovascular Diseases.....	12
3.0	Sex Steroids and Vascular Function .....	13
5.0	Sex Steroids in Heart and Vascular Function .....	13
6.0	Sex Steroids and Metabolic Syndrome .....	17
7.0	Sex Steroids, the Renin-Angiotensin System and Hypertension .....	17
8.0	Sex Steroids and Target Organ Injury .....	18
10.0	Sex Steroids in Hypertension, Diabetes, and Pregnancy .....	19
11.0	Sex Steroids, Pregnancy, Pre-elampsia, and Fetal Programming .....	23
<b>Author Index .....</b>		<b>24</b>

# 2007 APS Conference: Sex Steroids and Gender in Cardiovascular-Renal Physiology and Pathophysiology

## ABSTRACTS OF INVITED AND VOLUNTEERED PRESENTATIONS

### 1.0: SEX STEROIDS IN CLINICAL AND EPIDEMIOLOGICAL STUDIES

#### 1.2

#### THE LATEST ON THE CARDIOVASCULAR EFFECTS OF HORMONE THERAPY IN POSTMENOPAUSAL WOMEN

Pamela Ouyang<sup>1</sup>

<sup>1</sup>Cardiology/School of Medicine, Johns Hopkins University, 4940 Eastern Ave, Baltimore, MD, 21224.

Women have lower coronary heart disease (CHD) risk than men though this is attenuated after menopause. Whether estrogen loss contributes to this is debated. The increased androgenic state in postmenopausal women is associated with an adverse metabolic risk profile. The effects of estrogen on endothelial function, vasodilation, adhesion molecules and lipids could result in cardiovascular protection. This, along with observational studies data support potential CHD benefit from estrogen replacement therapy. However, the randomized placebo-controlled trials of conjugated equine estrogen (CEE) and medroxyprogesterone, and of unopposed CEE, show no reduction in CHD events and an increased stroke risk. It was postulated that selective estrogen receptor modulators might provide both CHD and breast cancer protection. However, the Raloxifene Use for the Heart Trial showed no cardiovascular protection from raloxifene. The explanation for the different findings of randomized and observational trials include healthy individual bias explaining the CVD benefit seen in observational studies, the physiologic effects of different types and doses of estrogen therapy, potential adverse cardiovascular effects of progesterone, and different vascular effects dependent on the timing of hormone therapy related to menopause, and resulting from the degree of underlying vascular disease and changes in hormone receptors due to the age of the woman. Studies are ongoing to test some of these hypotheses.

#### 1.3

#### SEX STEROIDS AND RENAL DISEASE IN HUMANS

Sharon Silbiger<sup>1</sup>

<sup>1</sup>Dept. of Medicine/Division of Nephrology, Albert Einstein College of Medicine/Montefiore Medical Center, 111 E. 210th St, Centennial 3, Bronx, New York, 10467.

In a manner similar to animals, men with a variety of renal diseases progress to end-stage renal failure at a rate faster than women. This rate is independent of blood pressure control or serum lipids levels. The exact physiologic mechanisms underlying this gender disparity are unclear, but are speculated to include gender differences in renal and systemic hemodynamics, glomerular size and/or number and the direct local effects of sex hormones. In rodents models of renal disease, hormonal manipulation such as ovariectomy in females, castration in males or the supplementation of estrogens or testosterone, modulates the course of renal disease. In these models, in general, estrogens have been found to be "protective", while testosterone has been found to be "detrimental". Naturally, such specific parallel data in humans is unavailable. Based on our meta-analysis evaluating the effect of gender on the renal disease progression rate in Membranous nephropathy, Polycystic kidney disease, IgA nephropathy and Chronic renal disease of mixed etiology, we can conclude that males with these underlying diseases have a more fulminant course. The role of gender on the course of human diabetic nephropathy is less clear, but data suggest that diabetic nephropathy in males may progress at a faster rate than this disease in women. REFERENCES: Neugarten J, Acharya, Silbiger SR. Effect of gender on the progression of nondiabetic renal disease: a meta-analysis. *J Am Soc Nephrol* 11:319-329, 2000. Silbiger S, Neugarten J. The role of gender in the progression of renal disease. *Adv Renal Repl Ther* 10:3-14, 2003.

### 2.0: UPDATE ON SEX STEROID RECEPTORS AND CARDIOVASCULAR DISEASES

#### 2.2

#### SEX STEROID RECEPTORS AND ATHEROSCLEROSIS

Matthias Barton<sup>1</sup>

<sup>1</sup>Internal Medicine, University Hospital Zurich, Ramistrasse 100, Zurich, 8091, Switzerland.

Clinical manifestations related to atherosclerotic vascular disease show marked gender differences with regard to time of onset and clinical presentation. Although endogenous sex hormones, particularly 17 $\beta$ -estradiol, non-selective estrogen receptor agonist, have been implicated in cardiovascular protection, the underlying mechanisms are unknown. In contrast to endogenous hormones, hormone therapy with oral contraceptives and with equine estrogens in postmenopausal women is associated with increased venous thrombotic events. Of note, testosterone exerts its effects not only via binding to the androgen receptor, but also after conversion to 17 $\beta$ -estradiol by aromatase. The exact roles of sex steroids and individual sex steroid receptors in atherogenesis and vascular homeostasis is still largely unclear. This information will be important for the understanding of vascular activities and clinical side effects of sex hormones. Most recent data suggest that natural estrogens and receptor-elective ligands for individual estrogen receptors have specific functions in arterial and venous vascular beds in humans and animals possibly offering new therapeutic opportunities. (Support: SNF 32-058426.99, 3232-058421.99, 32-108258/1). Traupe T., Stettler C, Li H.G., Haas E., Bhattacharya I., Minotti R., Barton M. (2007) Distinct roles of estrogen receptors alpha and beta mediating acute vasodilation of epicardial coronary arteries. *Hypertension* (in press); Haas E., Meyer M.R., Schurr U., Bhattacharya I., Minotti R., Nguyen H.H., Heigl A., Lachat M., Genoni M., Barton M. (2007) Differential effects of 17 $\beta$ -estradiol on function, ER $\alpha$ , ER $\beta$  and GPR30 expression in arteries and veins of patients with atherosclerosis. *Hypertension* (in press). [www.athero.ch](http://www.athero.ch).

#### 2.3

#### INCREASED HEPATIC STEATOSIS AND INSULIN RESISTANCE IN MICE LACKING HEPATIC ANDROGEN RECEPTOR VIA MODULATION OF PTP1 $\beta$ /PPAR $\alpha$ INVOLVED IN LIPID AND GLUCOSE HOMEOSTASIS

Chawnsang Chang<sup>1</sup>, Hung-Yun Lin<sup>2</sup>, Ruey-Shen Wang<sup>2</sup>, I-Chen Yu<sup>2</sup>, Ning-Chun Liu<sup>2</sup>, Wen-Lung Ma<sup>3</sup>, Janet D. Sparks<sup>2</sup>, Shuyuan Yeh<sup>2</sup>

<sup>1</sup>Pathology, and Urology, the Cancer Center, and George Whipple Lab for Cancer Research, University of Rochester Medical Center, 601 Elmwood Ave, Box 626, Rochester, NY, 14642,

<sup>2</sup>Pathology, University of Rochester Medical Center, 601 Elmwood Ave, Box 626, Rochester, NY, 14642.

Early studies showed that whole body androgen receptor (AR) knockout (T-AR<sup>-/-</sup>) mice with hypogonadism exhibit insulin resistance. However, the detailed mechanism how androgen/AR

signaling regulates insulin sensitivity in individual organs remains unclear. Here we generated hepatic AR knockout (H-AR<sup>-/-</sup>) mice and found obese male H-AR<sup>-/-</sup> mice, but not female H-AR<sup>-/-</sup> mice, fed with high fat diet had hepatic steatosis with insulin resistance, whereas lean male H-AR<sup>-/-</sup> mice fed with normal chow exhibited normal insulin sensitivity. Mechanism dissection found increased hepatic steatosis in obese male H-AR<sup>-/-</sup> mice could be due to decreased fatty acid  $\beta$ -oxidation and increased de novo lipid synthesis that may come from decreased PPAR $\alpha$  and increased SREBP1c expressions with its downstream target genes expressions. Furthermore, reduced insulin sensitivity with PI3k activity and increased PEPCK expression in obese male H-AR<sup>-/-</sup> could be due to increased PTP1b expression. Together, hepatic AR alone might play vital roles to prevent the development of insulin resistance and hepatic steatosis. AR agonists that specifically target hepatic AR might be developed to provide better strategies for treatment of metabolic syndromes in men.

#### 2.4

#### ESTROGEN RECEPTOR STUDIES USING TRANSGENIC ANIMALS

Pascale Lane<sup>1</sup>

<sup>1</sup>Pediatrics, University of Nebraska Medical Center, 982169 Nebraska Medical Center, Omaha, NE, 68198-2169.

The development of targeted disruptions in the murine genes for estrogen receptors (ER)  $\alpha$  and  $\beta$  in the 1990s has provided many lessons regarding the role of estrogens in various organ functions. While reproductive tract abnormalities in females were anticipated, reduced fertility in males also occurred. Lack of ER $\alpha$  eliminates neuroendocrine feedback, resulting in high circulating levels of both estrogens and androgens in female mice with ovaries. In mice, the kidney is the most ER $\alpha$ -regulated organ outside of the neuroendocrine system. ER $\alpha$  knockout mice (ERKO) show no differences in adult kidney size in males, while female ERKO mice show greater kidney weights than wild-type littermates, similar to values seen in males. No differences in glomerular size have been noted in this model; however, glomerular growth induced by diabetes is suppressed in female ERKO mice, suggesting a sex-specific effect of this genotype on the diabetic state. Another model of kidney enlargement, compensatory kidney growth 48 hours after uninephrectomy, also shows suppression in ERKO females. These effects may be mediated by the lack of ER $\alpha$  or via increased stimulation of ER $\beta$ . No renal phenotype has been described for the ERKO mouse, although this animal has not yet been studied in the same manner. ERKO mice do show hypertension and vascular dysfunction with aging, so renal effects of this genotype seem likely. Study strategies to determine effects of each receptor include studying females after ovariectomy to remove elevated hormone levels, and the use of selective estrogen receptor stimulation or blockade. Much has been learned from ERKO mice, and much more can be determined using this tool. (Supported in part by NIH DK59869).

#### 2.5

#### REDUNDANT, DIVERGENT OR OPPOSING FUNCTIONS OF ESTROGEN RECEPTOR SUBTYPES, ER $\alpha$ AND ER $\beta$ IN HYPERTENSION, CARDIAC HYPERTROPHY AND HEART FAILURE?

Theo Pelzer<sup>1</sup>

<sup>1</sup>Medicine I, University of Wuerzburg, Josef Schneider Str. 2, Wuerzburg, D-97080, Germany.

Gender and ageing are determinants for the incidence and the prognosis of cardiovascular diseases including hypertension, cardiac hypertrophy and chronic heart failure. Clinical observations on a low incidence of cardiovascular disease in women during their reproductive age that is lost with the decline of sex hormone levels after menopause served as the starting point for clinical and basic research into estrogens as class of potentially cardioprotective hormones. In support of this concept, both estrogen receptor subtypes, ER $\alpha$  and ER $\beta$ , are expressed throughout the cardiovascular system and mediate important vascular functions such as NO-dependent vasorelaxation. Importantly, the myocardium represents another established target for female sex hormones. But against this evidence and the initial dogma, clinical endpoint trials on the primary or secondary prevention of coronary artery disease including the HERS and WHI trials have failed. Thus, a better understanding on the role of estrogens and progestogens in the cardiovascular system is warranted to (1) understand the mechanism(s) of gender effects in heart disease, (2) to improve the safety of hormone replacement therapy in women with a co-existing cardiovascular morbidity and (3) to develop innovative ER and PR ligands that are safe, efficacious and suitable to treat heart diseases in women. This presentation will provide an update on cardiovascular function in ER $\alpha$  and ER $\beta$  knock out mice and on the properties of novel subtype selective agonists for ER $\alpha$  (16 $\alpha$ -LE2) and ER $\beta$  (8 $\beta$ -VE2) and novel progestins with anti-mineralocorticoid functionality that were tested in different animal models of hypertension, cardiac hypertrophy and chronic heart failure.

#### 2.6

#### THE GLUCOCORTICOID RECEPTOR: ONE GENE, MANY PROTEINS-NEW MECHANISMS FOR TISSUE SPECIFIC ANTI-INFLAMMATORY ACTIONS OF GLUCOCORTICOIDS IN HEALTH AND DISEASE

John Cidlowski<sup>1</sup>, Nick Lu<sup>1</sup>, Christine Jewell<sup>1</sup>, Onard Schoneveld<sup>1</sup>, Danielle Duma<sup>1</sup>, Kathy Gross<sup>1</sup>, Javier Revollo<sup>1</sup>, Robert Oakley<sup>1</sup>

<sup>1</sup>Laboratory of Signal Transduction, NIEHS, 111 TW Alexander Dr., Research Triangle Park, NC, 27709.

Glucocorticoids are necessary for life after birth and regulate numerous homeostatic functions in man, including glucose homeostasis, protein catabolism, skeletal growth, respiratory function, inflammation, development, behavior and apoptosis. They are also one of the most prescribed classes of anti-inflammatory drugs in the world. Our understanding of how one hormone or drug regulates all of these diverse processes is limited, although most of these actions are thought to be mediated via the glucocorticoid receptor, which is a product of a single gene. However, recent studies in our laboratory have shown that multiple glucocorticoid receptor isoforms are produced from one gene via combinations of alternative mRNA splicing and alternative translation initiation. In addition these glucocorticoid receptor isoforms are subject to several post-translational modifications including ubiquitination, phosphorylation and sumoylation which also modulate receptor function. In this lecture, we will show that these GR receptor isoforms regulate specific subsets of genes and selectively regulate distinct cellular functions such as apoptosis. Finally, we will also describe new studies on the human glucocorticoid receptor  $\alpha$  protein whose expression is associated with various states of glucocorticoid resistance in human inflammatory disease. Reference: Lu, N. Z. and Cidlowski, J.A. (2006). Glucocorticoid receptor isoforms generate transcription specificity. *TRENDS in Cell Biology*, Vol. 16, No.6.

# 2007 APS Conference: Sex Steroids and Gender in Cardiovascular-Renal Physiology and Pathophysiology

## ABSTRACTS OF INVITED AND VOLUNTEERED PRESENTATIONS

### 3.0: SEX STEROIDS AND VASCULAR FUNCTION

#### 3.2 ESTROGEN AND MITOCHONDRIA: A NEW PARADIGM FOR VASCULAR PROTECTION?

Sue Duckles<sup>1</sup>, A Razmara<sup>1</sup>, DN Krause<sup>1</sup>, V Procaccio<sup>2</sup>

<sup>1</sup>Pharmacology, Univ Calif, Irvine, Sch Med, Irvine, CA, 92697, <sup>2</sup>Pediatrics, Univ Calif, Irvine, Sch Med, Irvine, CA, 92697.

There is much evidence that E decreases the incidence of cardiovascular disease and prolongs lifespan. Mitochondrial reactive oxygen species (ROS) cause lasting mutations of mitochondrial DNA. Thus the mitochondrial theory of aging links aging, exercise and diet. E receptor  $\alpha$  was detected in mitochondria. Therefore we investigated whether E alters mitochondrial function in brain blood vessels isolated from ovariectomized female rats, treated 3 weeks with or without 17- $\beta$  E by subcutaneous implant. Nuclear respiratory factor-1 protein, a primary regulator of nuclear gene-encoded mitochondrial genes, was significantly increased by E. Treatment with E increased levels of mitochondrial proteins: E receptor  $\alpha$ , cytochrome c, subunits I and IV of complex IV, and MnSOD. E treatment increased activity of mitochondrial citrate synthase and complex IV, key rate limiting steps in energy production. In cultured human brain microvascular endothelial cells, 10 nM E decreased mitochondrial superoxide production and increased mitochondrial aconitase activity, an enzyme inactivated by mitochondrial superoxide. MnSOD protein, mRNA and activity were unchanged. We find that E modulates mitochondrial function in two ways: increasing energy-producing capacity and decreasing ROS production. The effectiveness of E against age-related cardiovascular disorders, including stroke, may arise in part from hormonal effects on mitochondrial function. However, E may be unable to reverse existing disease. E-mediated increases in mitochondrial efficiency may contribute to the longer lifespan of women. NIH RO1 HL50775.

#### 3.3 SEX STEROIDS AND VASCULAR RESPONSES IN AGING

Raouf Khalil<sup>1</sup>

<sup>1</sup>Brigham & Women's Hospital, Boston, MA, 02115.

Cardiovascular disease (CVD) is less in pre- than post-menopausal women, suggesting vascular benefits of estrogen (E2). In adult female animals, E2 promotes endothelium-dependent vascular relaxation via the NO, prostacyclin and hyperpolarization pathways. Also, E2 receptors (ER) inhibit [Ca<sup>2+</sup>]<sub>i</sub>, protein kinase C and perhaps Rho kinase-dependent vascular smooth muscle (VSM) contraction. However, HERS, HERS-II and WHI clinical trials did not support the experimental findings, and demonstrated adverse CV events of hormone therapy (HT) in aging women. The lack of vascular benefits of HT may be related to the hormone used, the ER, or subject's CV condition or age. Experiments on vascular strips from aging (16 month) female-SHR have shown reduction in ER-mediated endothelial NO production, and decreased inhibitory effects of E2 on Ca<sup>2+</sup> entry in VSM. The age-related decrease in ER-mediated vascular relaxation may explain the decreased effects of HT on CVD in aging women. New HT strategies should examine natural E2 and phytoestrogens for potential benefits over synthetic E2. Transdermal E2 may be more effective than oral E2, and specific ER modulators (SERMs) could maximize the vascular benefits, with little side effects on breast cancer. Variants of vascular ER should be screened for genetic polymorphism and postmenopausal decrease in amount or downstream signaling mechanisms. HT during menopausal transition could be more effective than in late menopause. Progesterone, testosterone or their specific modulators, combined with E2, may provide alternative HT strategies. Thus, HT type, dose, route of administration and timing should be customized depending on the subject's CV condition and age, and thereby enhance the vascular benefits of HT in aging women. (HL-65998, HL-70659).

#### 3.4 RAPID NONGENOMIC EFFECTS OF ANDROGENS ON THE VASCULAR WALL

John N. Stallone<sup>1</sup>

<sup>1</sup>Michael E. DeBakey Institute and Department of Veterinary Physiology & Pharmacology, Texas A&M University, College of Veterinary Medicine, College Station, TX, 77843-4466.

Our laboratory has demonstrated that TES produces rapid, nongenomic vasorelaxation that is endothelium-, sex-, and androgen receptor-independent; however, little is known about TES mechanisms in vascular smooth muscle (VSM). Therefore, TES mechanisms in rat aortic and mesenteric arteriolar (MA) VSM were examined. In aorta, TES induced full relaxation (100%, EC50 36±4  $\mu$ M), while reactivity to dihydrotestosterone, the nonpolar ester TES-enanthate, TES-hemisuccinate conjugated to BSA, and the excretory metabolite androsterone exhibited a fundamentally different rank order of efficacy/potency than in reproductive tissues. In MA, TES produced vasorelaxation at physiological concentrations (50±7%, EC50 2.3±0.5 nM). Precontraction of vessels with 80 mM KCl markedly reduced maximal response to TES (by 91±4%). 4-aminopyridine (4-AP, 2 mM) inhibited TES-induced relaxation by 65±5%. Pretreatment of MA with ICI 182,780 (1  $\mu$ M) did not alter TES effects. In whole-cell patch clamped MA myocytes, TES increased outward K<sup>+</sup> current >tenfold; this was nearly eliminated by 4-AP. In single MA myocytes, TES activated the fluorescent nitric oxide (NO) indicator DAF-2DA; ICI 182,780 (1  $\mu$ M) did not alter this effect, while L-NMMA (10  $\mu$ M) eliminated it. These data suggest that: 1) TES exerts a direct vasorelaxing effect on VSM at physiological concentrations which involves, at least in part, activation of the Kv channel, perhaps through the NO signal cascade; and 2) TES-induced vasorelaxation is a structurally-specific effect of the androgen molecule, which is enhanced in more polar analogs with lower permeability to the VSM cell membrane, and does not require aromatization to estrogen. Supported by NIH: HL-080402.

### 5.0: SEX STEROIDS IN HEART AND VASCULAR FUNCTION

#### 5.1 SEX-BASED DIFFERENCES IN THE ARCHITECTURE OF THE HYPERTROPHIED HEART IN SALT STRESSED BORDERLINE HYPERTENSIVE RATS

Johanna Krontiris-Litowitz<sup>1</sup>, Rita Fulton<sup>2</sup>

<sup>1</sup>Biological Sciences, Youngstown State University, One University Plaza, Youngstown, Ohio, 44555, <sup>2</sup>Biology, Belmont Technical College, 120 Shannon Place, St. Clairsville, Ohio, 43950.

The Borderline Hypertensive Rat (BHR), the first generation offspring of a mating between a female spontaneously hypertensive rat and male normotensive Wistar-Kyoto rat, exhibits borderline hypertension and left ventricular hypertrophy at maturity. If exposed to an environmental stressor or dietary stressor such as sodium, BHR will develop hypertension. In these experiments we investigate the effect of elevated dietary sodium on the perivascular collagen deposition (PVC) in the hearts of 20 male and 20 female BHR. Half of the male and female BHR were fed a high salt diet (8% NaCl) for 12 weeks and half were fed a normal salt diet (0.6% NaCl). At the end of the experiment the animals were euthanized and the hearts harvested and analyzed. Animals on a high salt diet exhibited an increase in systolic pressure (p<.05) and ventricular hypertrophy (p<.05 n=20, 20). The PVC fraction in the ventricles of high salt females was significantly less than in high salt males (p<.05, n=6.6). Additionally, the distribution of PVC was regulated differently in males than females. PVC deposits in males were thicker than females in both control and high sodium animals (p<.05, p<.05, n= 6.6). Males also appeared to be sensitive to dietary sodium, exhibiting a significantly decreased PVC thickness when on a high sodium diet (p<.001, n= 6.6). In contrast, PVC deposits in females on a high sodium diet were not significantly thicker than their control counterparts. These studies suggest that there is a gender-based difference in perivascular collagen deposition in the hypertrophied heart and that dietary sodium may influence the reorganization of perivascular collagen differently in males and females.

#### 5.2 ESTROGEN RECEPTOR ALPHA INTERACTS WITH 17 $\beta$ -HYDROXYSTEROID DEHYDROGENASE TYPE 10 AND REGULATES ITS ENZYMATIC ACTIVITY IN THE HEART

Theo Pelzer<sup>1</sup>, Virginija Jazbutyte<sup>2</sup>

<sup>1</sup>Medicine I, University of Wuerzburg, Josef Schneider Str. 2, Wuerzburg, D-97080, Germany, <sup>2</sup>Department of Medicine I, University of Wuerzburg, Josef Schneider Str. 2, Wuerzburg, 97080, Germany.

Background: Studies in MCF7 cells have indicated that both estrogen receptor subtypes, ER $\alpha$  and ER $\beta$  localize not only to the nucleus and the cytosol but also to the mitochondrial compartment. Because estrogens might influence cardiac energy homeostasis that depends critically on mitochondrial function, we determined whether heart mitochondria contain ER $\alpha$  protein and whether ER $\alpha$  may act on the heart also via protein-protein interactions. Results: Confocal studies in cardiac myocytes localized ER $\alpha$  to the nucleus and to the cytosol in a pattern that overlapped partially with that of cardiac mitochondria. Candidate protein-protein interaction partners of ER $\alpha$  including 17 $\beta$ -hydroxysteroid dehydrogenase type 10 (17 $\beta$ -HSD10) were identified by two-hybrid screens using the ER $\alpha$  LBD as a bait. 17 $\beta$ -HSD10 co-localized with ER $\alpha$  in heart mitochondria and was co-precipitated with ER $\alpha$  (and vice versa) in pull-down experiments. The enzymatic activity of endogenous 17 $\beta$ -HSD10 was analyzed spectrophotometrically in cardiac mitochondrial fractions. 17 $\beta$ -HSD10 was expressed to comparable amounts in the mitochondria of ER $\alpha$  KO and WT hearts. But 17 $\beta$ -HSD10 activity was higher in control compared to KO animals. Conclusion: Heart mitochondria contain ER $\alpha$  protein. ER $\alpha$  may modulate estrogen metabolism via protein-protein interactions with 17 $\beta$ -HSD10 which catalyses the conversion of 17 $\beta$ -estradiol to estrone.

#### 5.3 CHARACTERIZATION AND FUNCTIONAL ANALYSIS OF THE 5'-FLANKING REGION OF THE ER $\alpha$ GENE IN THE HUMAN HEART

Stephan Fritschka<sup>1</sup>, Shokoufeh Mahmoodzadeh<sup>1</sup>, Vera Regitz-Zagrosek<sup>1</sup>

<sup>1</sup>Center for Cardiovascular Research, Gender in Medicine, Charite, Hessische Str. 3-4, Berlin, 10115, Germany.

Estrogen receptor (ER)-mediated effects have been associated with the modulation of myocardial hypertrophy, but the regulation of ER expression in the human heart has not yet been analyzed. It has been shown that the human ER $\alpha$  (ER $\alpha$ ) mRNA is transcribed from at least seven different promoters with unique 5'-untranslated regions (UTRs), which are utilized in a cell- and tissue-specific manner. Therefore, we determined the 5'-UTR promoter variants of the ER $\alpha$  gene in left ventricular biopsies from patients with dilated cardiomyopathy and control hearts using 5'-RACE and PCR. The promoter activity of the most frequent promoter variant was investigated by transfection experiments in a human cardiomyocyte cell line followed by Luciferase reporter assay. The PCR-based results showed that four 5'-UTR variants, namely A, B, C and F are expressed in the human heart, and furthermore pointed to the predominance of the F-promoter variant. Luciferase reporter assays with the F-promoter variant revealed regulatory regions, containing enhancer and suppressor elements. Database analysis showed the presence of several putative consensus sequences for transcription factors including CDP, NF-Kappa and NF-KappaB within the suppressor region. Site directed mutagenesis within the CDP- and NF-Kappa-binding sequences greatly increased the promoter activity. The results indicate that the expression of ER $\alpha$ -gene is predominantly controlled by the F-promoter and the expression of ER $\alpha$ -gene might be regulated by the CDP and NF-kappa-family of transcription factors in the human heart.

#### 5.4 VOLUNTARY EXERCISE INDUCES SEX-SPECIFIC PHYSIOLOGICAL CARDIAC REMODELING

Sebastian Brokat<sup>1</sup>, Kathleen Cantow<sup>1</sup>, Nadine Ehrenberg<sup>1</sup>, Arne Kühne<sup>1</sup>, Jenny Thomas<sup>1</sup>, Vera Regitz-Zagrosek<sup>1</sup>

<sup>1</sup>Center for Cardiovascular Research, Charité Berlin, Hessische Str. 3-4, Berlin, 10115, Germany.

**Background:** Physical activity leads to sex-specific protection of the heart against cardiovascular diseases. Therefore it is of great interest to identify how the sex of an individual determines physiological hypertrophy. **Methods:** Male and female mice, exercised for 5½ weeks on a wheel, were characterized by echocardiography to evaluate morphological and physiological cardiac adaptation in response to voluntary physical activity. RNA was isolated from the left ventricle and analyzed by quantitative PCR. **Results:** We found that female mice revealed a higher exercise performance (9.2km/d vs. 6.4km/d in males, p<.0001). Females showed a greater increase in LV mass (15% vs. 5% in males, p=0.007) in comparison to their sex-matched sedentary controls.  $\beta$ / $\alpha$ -MHC ratio was decreased by 41% in running females (p=0.035) but unaltered in males. Exercise led to a decrease by 20% of CTGF mRNA in females (p=0.036) but not in males. Furthermore CTGF mRNA was inversely correlated with LV/TL ratio only in females (p=0.045). IGF1 mRNA in exercised females was significantly higher than in males (p=0.011) and strongly correlated with daily running distance and time (p<.0001). **Conclusions:** Moderate long-term exercise provokes a sex-dependent cardiac adaptation. Female

# 2007 APS Conference: Sex Steroids and Gender in Cardiovascular-Renal Physiology and Pathophysiology

## ABSTRACTS OF INVITED AND VOLUNTEERED PRESENTATIONS

mice develop a stronger hypertrophy. In opposition to pathological hypertrophy, this adaptation is characterized by anti-fibrotic gene expression pattern and IGF1 dependent pathways which are known to mediate physiological hypertrophy via PI3K. Therefore our findings can be of importance to explain the sex bias of physical activity as a protective cardiovascular factor.

### 5.5

#### COMPARISON OF THE SECOND DERIVATIVE PHOTOPLETHYSMOGRAPHY a-a INTERVAL METHOD AND ELECTRO-CARDIOGRAM R-R INTERVAL METHOD

Toshiki Kikuchi<sup>1</sup>, Yuji Sano<sup>2</sup>, Shinsuke Urushidani<sup>1</sup>, Junichi Abo<sup>1</sup>

<sup>1</sup>Graduate School, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Japan, <sup>2</sup>Marine Science, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Japan.

Objective: To examine the relationship between two methods for the assessment of autonomic nervous activity: the Second Derivative Photoplethysmography a-a Interval Method (SDPTG method), and Electrocardiogram R-R Interval Method (ECG method). Methods: 10 healthy males aged 22.1 (SD 2.0) years volunteered as subjects. ECG (standard techniques) and SDPTG (obtained from three sites: (A) forehead, (B) left middle fingertip, and (C) 1st digit MP joint of left foot), were simultaneously measured for 5 minutes. Power spectrum of heart rate and pulse beat variability was obtained by the maximum entropy method. Results: The average value of the a-a interval measured at site A was significantly correlated with R-R interval, at 978.8 (53.6) vs. 978.8 (52.9) ms,  $r=0.99$ ,  $p<0.001$ . There was no significant difference between the two intervals. As for the value of low-frequency peak power (LF: 0.04-0.15 Hz), high-frequency peak power (HF: 0.15-0.40 Hz) and LF:HF ratio, there were significant correlations between the value measured at site A using the SDPTG method and the value obtained from ECG method ( $r>0.99$ ,  $p<0.001$ ). The same results were obtained between the value measured at site B and the value obtained from ECG and between the value measured at site C and the value obtained from ECG. Conclusions: At three measurement sites, the SDPTG method for the assessment of autonomic nervous activity may serve as a replacement for the ECG method.

### 5.6

#### CARDIAC SIZE AND PLASMA VOLUME: POTENTIAL MECHANISMS FOR POTS?

Qi Fu<sup>1</sup>, Jeffrey Hastings<sup>1</sup>, Shigeki Shibata<sup>1</sup>, Tiffany VanGundy<sup>1</sup>, Robin Shook<sup>1</sup>, Benjamin Levine<sup>1</sup>

<sup>1</sup>Exercise Physiology, IEEM, Presbyterian Hospital of Dallas and UT Southwestern Medical Center in Dallas, 7232 Greenville Ave., Suite 435, Dallas, TX, 75231.

We tested the hypothesis that Postural Orthostatic Tachycardia Syndrome (POTS) is associated with a small cardiac size and a low plasma volume. Eight young female POTS patients, 15 healthy women and 11 men, matched for age and body mass index, were studied. All subjects consumed a constant diet three days prior to study. Heart rate (HR) and blood pressure (BP) responses were assessed during 10 minutes of standing. Plasma volume (PV) was measured by the Carbon-Monoxide rebreathing technique. Left ventricular (LV) mass was determined using MRI. POTS patients had the greatest elevation in HR after 10 minutes of standing compared with healthy women and men (AHR,  $32\pm 10$  beats/min vs  $18\pm 12$  and  $12\pm 6$ , between groups  $P<0.001$ ). Systolic BP was greater in men than in women and POTS patients ( $P<0.001$ ), but diastolic BP was not different between groups in the supine and upright positions ( $P=0.134$ ). POTS patients had the smallest normalized PV ( $41\pm 6$  mL/kg vs  $48\pm 7$  and  $50\pm 7$ , between groups  $P=0.008$ ) and normalized LV mass ( $45\pm 6$  g/m<sup>2</sup> vs  $58\pm 8$  and  $80\pm 6$ , between groups  $P<0.001$ ) compared with healthy women and men. However, normalized PV did not differ between healthy women and men ( $P=0.516$ ), while normalized LV mass was significantly smaller in women than in men ( $P<0.001$ ). Multiple linear regression analysis showed that HR responses could be predicted from a linear combination of both normalized LV mass and PV in all subjects ( $R=0.618$ ,  $P<0.001$ ). These results suggest that there is a sex-specific difference in cardiac size, even in the healthy population. This difference is exaggerated in female patients with POTS. It seems likely that a small cardiac size coupled with a low plasma volume can be important contributor(s) to the pathophysiology of POTS.

### 5.7

#### DEVELOPMENTAL CHANGES OF AUTONOMIC CONTROL OF HEART RATE IN THE CONSCIOUS BEHAVING RAT: STATE AND SEX INFLUENCES.

Carie Reynolds<sup>1</sup>, Christopher Ward<sup>1</sup>, Nicholas Doperalski<sup>2</sup>, David Fuller<sup>2</sup>, Linda Hayward<sup>1</sup>

<sup>1</sup>Physiological Sciences, University of Florida, 1600 SW Archer Rd. B2-5, Gainesville, Florida, 32610, <sup>2</sup>Physical Therapy, University of Florida, Box 100154, UFHSC, Gainesville, Florida, 32610.

Investigations into the postnatal development of autonomic control of the cardiovascular system have suggested that neonatal heart rate (HR) is primarily controlled by the sympathetic nervous system until postnatal day 16. At this critical developmental period, the parasympathetic system begins to influence HR control, paralleling changes in respiration. Though studies have examined this development in depth, the interaction of sex and sleep states on the development of HR control has not been investigated. The current study examined these factors on normal postnatal developmental changes in the cardiorespiratory interaction. At days 10, 17 & 28, Sprague Dawley pups were instrumented with ECG leads and placed in a plethysmograph where respiration rate (RR) and HR was measured for one hour. Heart variability (HRV) was determined by spectral analysis of heart beat to beat interval during different sleep/wake states. Preliminary data suggests that RR is significantly lower in all pups during sleep irrespective of sex at 28 days compared to 10 days of age. At day 17, HRV appeared to be greater in females irrespective of sleep stage versus to males. Indices of increased parasympathetic tone were greater in females compared to males at this time point. These preliminary findings suggest that male rat pups may develop tonic vagal tone more slowly than females. Supported by FL-Dept of Health.

### 5.8

#### TESTOSTERONE MODULATES HEART RATE AND QT INTERVAL IN SWINE

April Durtschi<sup>1</sup>, Leona Rubin<sup>1</sup>

<sup>1</sup>Biomedical Sciences, University of Missouri, E102 Vet Med Bldg, 1600 E Rollins Dr, Columbia, MO, 65211.

QT interval, the duration between the Q wave and T wave of an ECG heart beat, provides an in-vivo measure of cardiac ventricular repolarization. Women have a longer QT interval, even when corrected (cQT) for heart rate (HR) than men and thus, are at a higher risk for arrhythmias.

Clinical data suggests testosterone shortens QT interval. Using a swine model with regulated sex hormone levels, the purpose of this study was to determine whether the male sex steroid hormone, testosterone influences regulation of HR and QT. Methods: Adult Yucatan swine were divided into 3 groups: control intact (IM), castrated (CM), and castrated with testosterone replacement (MHR) with topical AndroGel (10mg/d). In addition, we examined the effects of 3 weeks of treatment with an androgen inhibitor, flutamide (FLUT, 500mg q12h). HR and QT intervals were calculated from 24-hour ambulatory ECG recordings prior to and after treatment. cQT was calculated using the Bazett's formula and measured from 50 heart beats during both an active time period (10:00 AM) and a resting time period (1:00 AM) to represent data from high and low HR. Results: CM and FLUT had lower average 24-hour HR ( $79\pm 3.4$ ,  $80\pm 1.2$  bpm, respectively) compared to pre-treatment IM ( $92\pm 3$ ,  $90\pm 3.5$  bpm respectively) and MHR ( $94\pm 3.2$  bpm). cQT interval of CM  $94\pm 7.5$  msec was significantly increased compared to IM ( $363\pm 7$  msec) or MHR ( $364\pm 8$  msec). FLUT also exhibited increased cQT interval compared to paired, pre-treatment control males ( $410\pm 12$  msec). These data suggest that 1) castration alters HR and cQT, and 2) testosterone appears to mediate the effects of castration as a) testosterone therapy maintains HR and cQT levels comparable to IM, and b) androgen receptor blockade with FLUT altered HR and cQT comparable to CM.

### 5.9

#### TESTOSTERONE MODULATES HEART RATE VARIABILITY IN SWINE

April Durtschi<sup>1</sup>, Leona Rubin<sup>1</sup>, John Dodam<sup>1</sup>

<sup>1</sup>Biomedical Sciences, University of Missouri, 1600 E Rollins Dr, Columbia, MO, 65211.

Heart rate variability (HRV) analysis, variation in beat to beat intervals of heart rate, reflects autonomic nervous system activity and can be used as an assessment of cardiovascular disease. Control of HRV differs between males and females with females exhibiting greater parasympathetic influence. To date, the influence of androgens on HRV has not been examined. This study tests the hypothesis that androgens influence HRV. Methods: 24-hour ECG recordings were obtained from adult Yucatan pigs in the following groups: normal males (NM, n=15), castrated males (CM, n=8), and castrated males treated daily with topical testosterone (AndroGel, 10 mg) (MHR, n=7). HRV was interpreted with time domain analysis. A second group of swine was treated for 3 weeks with placebo, following by 3 weeks with androgen receptor blocker, flutamide (500mg twice daily (FLUT, n=3)). Results: Overall 24-hour HRV (SDNN) and long term HRV (SDANN) were greater in CM ( $207.2\pm 14$ ,  $150.5\pm 11.7$ ) than in NM ( $162.89\pm 18.6$ ,  $83\pm 3.4$ ). Testosterone replacement restored SDNN comparable to NM, but SDANN remained higher than NM ( $99\pm 12.2$ ). There were no differences in short-term HRV (RMSSD) between any group. Diurnal variation in HRV also existed with an increased nighttime SDNN ( $172.7\pm 8.2$ ) and SDANN ( $83.3\pm 11$ ) in MC vs NM ( $121\pm 7.3$ ,  $41.5\pm 3.4$ ). Similar data were obtained using natural paired sampling and also revealed a difference in nighttime RMSSD between NM prior to ( $80.8\pm 9.1$ ) and after castration ( $107.7\pm 8.8$ ). Similar to castration, treatment with flutamide increased SDNN ( $207.5\pm 13.1$ ) and SDANN ( $173.3\pm 12.4$ ) compared to pre-treatment ( $186.6\pm 18$ ,  $141.3\pm 15.4$ ). These data indicate that testosterone does modulate HRV with greatest effects on long term variability. Study supported by NASA and T32RR007004.

### 5.10

#### ROLE OF OXIDATIVE STRESS IN PARITY-INDUCED CORONARY DYSFUNCTION

Susan Kaufman<sup>1</sup>, Huda Tawfik<sup>1</sup>

<sup>1</sup>Physiology, University of Alberta, 473 HMRC, Edmonton, AB, T6G 2S2, Canada.

Multiparity is associated with increased risk of cardiovascular disease in postmenopausal women. During pregnancy, there is increased oxidative burden. However, it is not clear whether this persists long-term. We examined the effect of parity on coronary artery function, and on vascular reactive oxygen species (ROS) and nitric oxide levels (NO), in multiparous and age-matched virgin rats. Vascular reactivity to acetylcholine (ACh) was measured by wire myography using small coronary arteries precontracted with U46619, in the presence of superoxide dismutase (SOD) or FeTPPs (peroxynitrite scavenger). Oxidative stress was measured using the luminol derivative L-012, and NO was measured by the Griess reaction. There was reduced ACh-mediated maximal relaxation in coronary arteries from multiparous rats (Parous:  $49.2\pm 2.9$  %; Virgins:  $94.6\pm 3.1$  %;  $P<0.05$ ). ACh-mediated relaxation was abolished in both groups by L-NAME. Superoxide anion formation was increased in parous rats by  $171.77\pm 42.29$  % URL (vs. virgins). Incubation of parous vessels with 100U/ml SOD or 10  $\mu$ M FeTPPs improved maximal vascular relaxation to ACh ( $76.6\pm 3.0$  % and  $65.8\pm 4.2$  % respectively). Parity also reduced eNOS protein levels in aorta, and reduced total nitrite/nitrate levels in heart tissue from  $1.72\pm 0.27$  to  $0.98\pm 0.12$   $\mu$ M/mg protein. In addition, coronary arteries from parous rats exhibited reduced maximal relaxation to the NO donor SNAP (Parous:  $73.5\pm 2.8$  % vs. Virgin:  $96.8\pm 1.8$  %). These data suggest that pregnancy causes long-term coronary vascular dysfunction through decreased NO availability and increased ROS formation. Funding: CIHR.

### 5.11

#### ANALYSIS OF THE EFFECT OF E2 ON THE HUMAN MMP2 EXPRESSION IN HT1080 CELLS: A PROMOTER BASED ANALYSIS

Elke Dworzatzek<sup>1</sup>, Shokoufeh Mahmoodzadeh<sup>1</sup>, Vera Regitz-Zagrosek<sup>1</sup>

<sup>1</sup>Center for Cardiovascular Research, Gender in Medicine, Charite, Hessische Str. 3-4, Berlin, 10115, Germany.

Background: It is known that MMP-2 plays an important role in cardiac remodeling and is regulated by E2 in different cell lines. Therefore we study the regulation of the human MMP-2 gene and the molecular mechanism involved in the E2-dependent control of the MMP-2 promoter. Methods: To understand the regulation of the human MMP2 gene, a series of MMP-2 promoter constructs of varying length were transiently transfected in HT1080. MMP-2 promoter-activity was analyzed by luciferase reporter assays. To investigate the effect of E2 on the MMP-2 promoter activity, MMP-2 promoter constructs were co-transfected with the human ERA in HT1080 cells. After treatment with E2 or vehicle, luciferase reporter assays were carried out. To evaluate direct regulation of E2 on the MMP-2 promoter EMSA was performed. Results: We identified within the human MMP-2 promoter sequence two sections, which show most luciferase activity, suggesting that these regions may contain enhancer elements. The co-transfection experiments in HT1080 cells showed an increase in MMP-2 promoter activity in the presence of ERA and a significantly decrease when the transfected cells were treated with E2. The luciferase reporter assays and EMSA suggest that E2 exerts its inhibitory effect upon binding sites located in the proximal region of the human MMP-2 promoter. Conclusion: Our study shows that ERA is capable to diminish the MMP-2 promoter activity in response to E2 in HT1080 cells. A deficiency or excess of E2 may cause a dysregulation of the ECM turnover in the human heart.

# 2007 APS Conference: Sex Steroids and Gender in Cardiovascular-Renal Physiology and Pathophysiology

## ABSTRACTS OF INVITED AND VOLUNTEERED PRESENTATIONS

### 5.12

#### CUTANEOUS VENOARTERIOLEAR RESPONSE IS NOT IMPAIRED IN POSTURAL ORTHOSTATIC TACHYCARDIA SYNDROME

Tiffany Van Gundy<sup>1</sup>, Shigeki Shibata<sup>1</sup>, Robin Shook<sup>1</sup>, Marla Sandgarten<sup>1</sup>, Jeffrey Hastings<sup>1</sup>, Benjamin Levine<sup>1</sup>, Qi Fu<sup>1</sup>

<sup>1</sup>Exercise Physiology, The Institute for Exercise and Environmental Medicine, 7232 Greenville Ave, suite 435, Dallas, TX, 75231.

Postural Orthostatic Tachycardia Syndrome (POTS) is characterized by excessive tachycardia during orthostasis, which appears to be a compensatory mechanism for a small stroke volume. One of the major factors affecting stroke volume is the amount of venous return which may be determined in part by the venoarteriolar response (VAR). We tested the hypothesis that POTS is associated with an impaired cutaneous VAR. Thirteen female POTS patients, 13 healthy females, and 10 healthy males, age (16-51 yr.) and body mass index matched, were studied. Skin blood flow (SkBF, laser-Doppler flowmetry), was measured in the calf at heart level and during leg dependency of 17-36 cm below the heart for two minutes each while supine. Blood pressure (BP, Portapres) and heart rate (HR, ECG) were monitored continuously. HR was higher in POTS patients when compared to both healthy females and males (79±12 bpm vs. 67±7 vs. 60±7, P<0.001). Between groups, BP was not different at baseline and during VAR, however diastolic BP increased slightly during VAR in males (61±4 mmHg baseline vs. 64±4 VAR, P=0.015). VAR, determined by the relative decrease in SkBF, was if anything, slightly greater between POTS patients and healthy females (39±15% vs. 28±12, P=0.056) and not different than males (45±15%, P=0.275). However VAR was lower in healthy females compared with males (P=0.015). These results suggest that POTS is not associated with an impaired VAR. Other mechanisms rather than this cutaneous local axon reflex may contribute to this syndrome. Supported by NIH K23 (HL075283) and the GCRC grant (RR00633).

### 5.13

#### DIHYDROTESTOSTERONE MODULATES CEREBRAL VASCULAR TONE IN PART BY ENHANCING COX-2

Rayna Gonzales<sup>1</sup>, Diana Krause<sup>1</sup>, Sue Duckles<sup>1</sup>

<sup>1</sup>Pharmacology, University of California Irvine School of Medicine, Med Surg II, Room 350/351, Irvine, California, 92697.

Sex steroids modulate cerebral vascular responses: testosterone augments and estrogen attenuates vascular tone. Therefore, we investigated the effect of the more potent testosterone metabolite, dihydrotestosterone (DHT), on cerebral vascular function. Cerebral arteries were isolated from orchietomized male rats treated chronically (3 wk) with either DHT 25 mg pellets (DHT25), DHT 45 mg pellets (DHT45), or placebo (ORX). In isolated, pressurized middle cerebral artery segments (MCA) passive diameters were not different among groups; however, vascular tone was significantly greater in DHT25 vessels compared to ORX. In contrast, vascular tone in DHT45 MCA was not different from ORX. Following endothelial removal, myogenic tone in DHT25 and DHT45 MCA was suppressed compared to ORX. The NOS inhibitor, L-NAME (100 µM), constricted all arteries with no differences among groups. Indomethacin (non-selective cyclooxygenase inhibitor, 10 µM) in the presence of L-NAME dilated DHT25 and DHT45 MCA, but constricted ORX MCA. Chronic DHT had no effect on eNOS or COX-1 protein levels measured by Western blot; however, COX-2 protein and NFκB activation were greater in DHT25 and DHT45 cerebral vessels. In conclusion, DHT has a dose dependent effect on vascular tone in cerebral vessels. At both doses effects of DHT involved COX-2-dependent constriction. AHA National Scientist Development Award (RJG).

### 5.14

#### GENDER AND CIRCULATING ENDOTHELIAL PROGENITOR CELL NUMBER AND APOPTOSIS

Brian Stauffer<sup>1</sup>, Owen MacLearney<sup>2</sup>, Greta Hoetzer<sup>2</sup>, Erich Kushner<sup>2</sup>, Jennifer Cech<sup>2</sup>, Christian Westby<sup>2</sup>, Christopher DeSouza<sup>2</sup>

<sup>1</sup>Medicine/Cardiology, University of Colorado, 4200 E. 9th Ave, B139, Denver, CO, 80262, <sup>2</sup>Integrative Physiology, University of Colorado at Boulder, UCB 354, Boulder, CO, 80309.

Clinical interest in bone marrow-derived circulating endothelial progenitor cells (EPCs) has increased due to their importance in vascular repair processes as well as their emerging role as a biomarker of cardiovascular risk. We have previously shown that EPC clonogenic and migratory capacity are higher in middle-aged women than men, suggesting gender-related functional phenotypic differences with EPCs. In the present study we tested the hypothesis that: 1) circulating EPC number is higher and 2) EPC susceptibility to apoptosis is lower in middle-aged women vs men. EPCs were isolated from peripheral blood samples collected from 42 healthy, sedentary adult humans: 21 men (M: age 58±1 yr) and 21 women (W: 67±1 yr). EPC number was determined by flow cytometry and apoptotic tendency by active caspase-3 concentrations. There was no difference in % of CD45 low/CD34/VEGF-R2/CD133 positive cells between M (0.0019±0.0005%) and W (0.0016±0.0005%). Although, M demonstrated higher (55%; P<0.05) basal EPC caspase-3 activity vs W, active caspase-3 levels in response to the apoptotic stimulant staurosporine was not different (2.5±0.2 vs 2.2±0.2 ng/mL; P<0.30). In contrast to our hypothesis, these results demonstrate no gender-related differences in circulating EPC number and apoptosis susceptibility in middle-aged and older adult humans.

### 5.15

#### GENDER DIFFERENCES IN ENDOTHELIAL FUNCTIONS, VASCULAR ESTROGEN RECEPTORS, ENOS AND TRIGLYCERIDES

Shih-Hsuan Chou<sup>1</sup>, Yu-Ren Wang<sup>1</sup>, Shiu-Ling Tsau<sup>1</sup>, Ying-Tung Lau<sup>1</sup>

<sup>1</sup>Department of Physiology, Chang Gung University, 259 Wen-Hwa 1st Road, Tao Yuan, 333, Taiwan.

The lower incidence of cardiovascular disease in women than men has been, in part, correlated with estrogen's protective effects on endothelial functions. We test the hypothesis that whether the higher estrogen level in female rats exhibited higher vascular nitric oxide (NO) content and acetylcholine-induced endothelial dependent relaxation (EDR) through up-regulation of estrogen receptors (ER) and endothelial NO synthase (eNOS). Our data showed that acetylcholine-induced EDR and vascular NO content were significantly higher in female compared to male rats. Quantitative real-time RT-PCR analysis demonstrated that mRNA expression of ER-α was significantly higher in female compared to male rats, but ER-β and eNOS did not differ between genders. Several lines of evidence indicated that the lower bioactive NO in vascular tissue was partially correlated with higher serum triglycerides. We further examined several biochemical parameters and found that serum triglyceride was significantly lower in female than male rats. Thus, the higher vascular NO output and endothelial function (EDR) in female rats correlated with higher expression of ER-α and lower level of serum triglyceride but not eNOS mRNA. (Supported by CMRPD 140152 N5C95-2320-B-182-030 to Y.T. Lau).

### 5.16

#### ESTRADIOL INHIBITS APOPTOTIC SIGNALING AND MICROVASCULAR ENDOTHELIAL CELL HYPERPERMEABILITY

Ed Childs<sup>1</sup>, Binu Tharakan<sup>1</sup>, Felicia Hunter<sup>1</sup>

<sup>1</sup>Surgery, Texas A&M Health Science Center College of Medicine and Scott and White Hospital, 2401 South 31st Street, Temple, TX, 76508.

Disruption of endothelial cell-cell junction following ischemia-reperfusion (IR) has been shown to increase vascular hyperpermeability. Recent studies have also demonstrated that activation of the apoptotic signaling cascade is involved in endothelial dysfunction, which may result in hyperpermeability. We hypothesized that mitochondrial (intrinsic) apoptotic signaling pathway is involved in vascular hyperpermeability in endothelial cells and factors that inhibit this pathway may prevent hyperpermeability. The purpose of this study was to identify if estrogens such as 17β-estradiol, that are known to inhibit intrinsic apoptotic signaling, are effective in preventing hyperpermeability. Rat lung microvascular endothelial cells (RLMEC) grown on Transwell membranes as monolayers were treated with 17β-estradiol (1 or 10 nM) followed by pro-apoptotic BAK (BH3) peptide (5µg/ml) transfection. The permeability changes were determined based on FITC-albumin flux across the monolayer. Cytosolic cytochrome c levels were measured by ELISA. BAK (BH3) transfection induced monolayer hyperpermeability (p < 0.05). 17β-Estradiol (10 nM) attenuated BAK (BH3)-induced hyperpermeability (p < 0.05). Further, BAK (BH3) transfection induced cytochrome c release to the cytosol. 17β-Estradiol (10 nM) inhibited BAK (BH3)-induced cytochrome c release. This suggests 17β-estradiol as an important regulator of vascular permeability in RLMEC through cytochrome c-induced apoptotic signaling cascade.

### 5.17

#### GENDER MODULATION OF VENOUS FUNCTION IN SPONTANEOUSLY HYPERTENSIVE RATS.

Doug Martin<sup>1</sup>, Rebecca Redetzke<sup>1</sup>, Erin Vogel<sup>1</sup>, Connie Mark<sup>1</sup>, Kathleen Eyster<sup>1</sup>

<sup>1</sup>Sanford School of Medicine, University of South Dakota, 414 East Clark St., Vermillion, SD, 57069.

Our previous work suggested that increased venous tone contributes to the onset of hypertension. We also showed that male sex steroids amplified venous tone (18%) during hypertension development. This study tested the hypothesis that endogenous estrogens reduce venous tone in the female spontaneously hypertensive rat (SHR). Female SHR rats (5 weeks old) underwent sham operation (Sham) or ovariectomy (OVX). At 10 weeks of age, mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP) were recorded in chronically instrumented conscious rats. MCFP, an index of venous tone, was calculated during brief right atrial balloon-induced circulatory arrest. Postsynaptic adrenergic responsiveness was assessed by constructing cumulative dose response curves to intravenous norepinephrine (NE) infusion. MAP was not significantly affected by ovariectomy (Sham 127±6 vs OVX 130±3 mm Hg). Conversely, MCFP was moderately, but significantly, increased in OVX SHR by 14% (Sham 5.2±0.2 vs OVX 5.9±0.2 mm Hg). Ganglionic blockade markedly decreased MAP and MCFP, however these responses were not different between sham (MAP: -60±8 mm Hg; MCFP: -2.1±0.3 mm Hg) and OVX (MAP: -54±4 mm Hg; MCFP: -1.9±0.2 mm Hg) SHR. NE dose dependently increased MAP and MCFP. There were no marked differences in these responses between Sham and OVX rats. Accordingly we conclude that endogenous ovarian hormones effect a modest reduction in venous tone that does not involve adrenergic mechanisms. Supported by NIH HLBI 63053 and 69886.

### 5.18

#### SEX DIFFERENCES IN THE RESPONSE TO VASOCONSTRICTOR AND VASODILATOR SUBSTANCES IN CHRONICALLY STRESSED MALE AND FEMALE RATS

Adam Mitchell<sup>1</sup>, Jennifer Rogers<sup>1</sup>, Adam Myers<sup>1</sup>, Zofia Zukowska<sup>1</sup>, Susan Mulrone<sup>1</sup>

<sup>1</sup>Physiology and Biophysics, Georgetown University, 3900 Reservoir Road NW, Washington, DC, 20007.

Chronic stress, which increases plasma NPY, has been reported to increase atherosclerotic-like lesions following angioplasty. While this is intriguing, whether there are sex differences in response to stress in normal vasculature is unknown. Stress has been shown to reduce circulating estrogen and testosterone levels, with the loss of estrogen potentially having a negative vascular effect. Adult male and female rats were separated into control and cold-water stress groups. After two-weeks, mesenteric arteriolar vascular reactivity to the vasoconstrictor PE and vasodilator ACh was performed on a wire myograph. Stress increased plasma NPY in both male and female rats (P<0.05 vs sham). An inverse response to ACh was observed between the sexes; vessels from stressed males had a 115% increase in ACh response compared to controls (P<0.05), versus a 56% decrease in females (P<0.05). While stress had no effect on PE reactivity in either sex, L-NAME incubation before PE challenge resulted in an increase in PE reactivity in the stressed female but not male. This indicates that vessels from the stressed female, but not stressed male, increase constitutive NO production versus control non-stressed vessels. Finally, NPY incubation before PE resulted in a decrease in sensitivity (-25% from controls) in stressed male vessels, while vessels from stressed females increased the responsiveness to NPY (+35% from controls). These findings support the concept that chronic stress induces early sex-related changes in vascular remodeling, which may put the stressed female at greater risk for developing vascular disease. (supported by NIH R01 DK064916 and the National Kidney Foundation of the National Capital Area).

### 5.19

#### ESTROGEN DECREASES MITOCHONDRIAL OXIDATIVE STRESS IN HUMAN BRAIN MICROVASCULAR ENDOTHELIAL CELLS

A. Razmara<sup>1</sup>, S.P. Duckles<sup>1</sup>, L. Sunday<sup>1</sup>, C. Strone<sup>1</sup>, X. Wang<sup>1</sup>, D.N. Krause<sup>1</sup>, V. Proccacio<sup>2</sup>

<sup>1</sup>Pharmacology, Univ Calif, Irvine, Sch Med, Irvine, CA, 92697, <sup>2</sup>Pediatrics, Univ Calif, Irvine, Sch Med, Irvine, CA, 92697

Mitochondrial reactive oxygen species (ROS) and endothelial dysfunction are key paradigms in cerebrovascular pathophysiology. 17β-estradiol (E) enhances efficiency of mitochondrial energy production and suppresses mitochondrial ROS in cerebral blood vessels. Cultured human brain microvascular endothelial cells (HBMEC) were exposed to 10 nM E for 24 hr, and mitochondrial superoxide measured with MitoSOX Red dye. E decreased mitochondrial superoxide in an E-receptor dependent manner. Raloxifene suppressed mitochondrial superoxide, but tamoxifen did not. Aconitase activity is a functional indicator of mitochondrial ROS production as the iron-sulfur core of aconitase is inactivated by superoxide. E increased aconitase activity, confirming less mitochondrial ROS production. Enzyme reactivation with reducing agents showed that E did not affect enzyme levels per se. E had no effect on Mn superoxide dismutase mRNA, protein, or enzyme activity. E increased levels of the peroxisome proliferator-activated receptor-gamma

# 2007 APS Conference: Sex Steroids and Gender in Cardiovascular-Renal Physiology and Pathophysiology

## ABSTRACTS OF INVITED AND VOLUNTEERED PRESENTATIONS

coactivator-1 family of transcriptional co-activators in an ER-mediated manner, suggesting that mitochondrial ROS may be decreased by E via these co-activators. The ability of E to decrease cerebral endothelial mitochondrial ROS and protect against damage to mitochondrial DNA may contribute to differences in lifespan as well as stroke morbidity and mortality between men and women. NIH ROI HL50775.

### 5.20

#### EFFECT OF ESTROGENS AND SELECTIVE ESTROGEN RECEPTOR MODULATORS ON VASCULAR REACTIVITY IN THE PERFUSED MESENTERIC VASCULAR BED.

Connie Mark<sup>1</sup>, Rabelais Tatchum-Talom<sup>1</sup>, Doug Martin<sup>1</sup>, Kathleen Eyster<sup>1</sup>

<sup>1</sup>Basic Biomedical Sciences, University of South Dakota, 414 E Clark St, Vermillion, SD, 57069.

Estrogen and SERMs such as raloxifene (RAL) and tamoxifen (TAM) acutely relax arteries, but the long term effects of estrogens and SERMs on vascular reactivity in the mesenteric vasculature are unknown. In this study, we used an isolated perfused mesenteric vascular bed technique to investigate the effect of chronic treatment of estrogens and SERMs on vascular reactivity of the mesenteric bed. Female Sprague Dawley rats were ovariectomized (ovx) at 4 weeks of age. After 2 week recovery the rats were treated by gavage with vehicle (CTL, 2-hydroxypropyl- $\beta$ -cyclodextrin), ethinyl estradiol (EE), estradiol benzoate (EB), equilin (EQ), TAM, or RAL for 3 weeks. Mean arterial blood pressure (BP, mmHg) was significantly increased in EQ (134 $\pm$ 4) and decreased in TAM (104 $\pm$ 4) compared to CTL (117 $\pm$ 4). EQ, EE, and TAM treatment increased the perfusion pressure response to KCl induced depolarization compared to CTL. The ED<sub>70mmHg</sub> for KCl was significantly lower for EQ and TAM vs. CTL. EQ and TAM treatment increased the perfusion pressure responses to norepinephrine (NE) compared to CTL. The ED<sub>125mmHg</sub> for NE was significantly lower for EE vs CTL and higher for EB vs. CTL. EQ and EE treatment increased the perfusion pressure at the maximal dose of serotonin (5HT) compared to CTL. The ED<sub>30mmHg</sub> for 5HT was significantly lower for EQ vs. CTL. These data demonstrate that chronic treatment with estrogens and SERMs affect vascular reactivity in the mesenteric vascular bed. Supported by NIH HL 69886 & 63053.

### 5.21

#### SEX DIFFERENCES IN THE RELATIONSHIP BETWEEN RAT MESENTERIC VENULAR PROTEIN LEAKAGE AND TISSUE PROTEIN CLEARANCE

Rie Sasaki<sup>1</sup>, Susan Bingaman<sup>1</sup>, Virginia Huxley<sup>1</sup>

<sup>1</sup>Medical Pharmacology & Physiology, University of Missouri - Columbia, National Center for Gender Physiology, MA415 Med Sci Bldg, Dept of Med Pharm & Physiol, Columbia, MO, 65212.

We tested whether protein flux from individual venules was indicative of net protein clearance from the rat mesenteric tissue, whether high dose insulin alters this relationship, and whether it does so differently with sex and age. Following intravenous injection of Alexa 594<sup>T</sup> labeled bovine serum albumin (Alexa-BSA) the exposed mesenteric vasculature was suffused for 30mins with bicarbonate-buffer solution (BBS), then 75mins BBS with or without 10-7M porcine insulin. Microvascular BSA flux for a postcapillary venule was assessed by fluorescence microscopy (indicated as leak index; LI). The suffusate flowing off the mesentery was collected and Alexa-BSA concentration was measured. No correlation existed between protein leakage (LI) and clearance (indicated by %BSA of total protein in suffusate) in the adult males either with or without insulin treatment. Protein leakage and clearance were negatively correlated in juvenile males treated with ( $r=-0.78$ ;  $p=0.01$ ) or without ( $r=-0.71$ ;  $p=0.03$ ) insulin. A positive correlation existed between protein leakage and clearance ( $r=0.80$ ;  $p=0.01$ ) for adult females treated with insulin. In conclusion, the handling of proteins by individual venules and overall mesenteric tissue can differ significantly depending not only on the sex of the animal, but also its maturity and metabolic status. NIH-HL078816 & HL075186, RR017353, NASA-NNJ05HF37G, AHA0615515Z(RS).

### 5.22

#### SEX DIFFERENCE IN THE REGULATION OF MUSCLE BLOOD FLOW DURING STATIC HANDGRIP EXERCISE.

Mariko Kawamoto<sup>1</sup>, Keiko Morimoto<sup>1</sup>, Akira Takamata<sup>1</sup>

<sup>1</sup>Department of Environmental Health, Nara Women's University, Kitauoya Nishimachi, Nara, 630-8506, Japan.

There exists sex difference in the vasodilatory response to reactive hyperemia and flow-mediated vasodilation (FMD) is larger in females than males. We hypothesize that muscle blood flow response to active muscle is different between females and males because of sex difference in endothelial function. To elucidate the sex difference in the regulation of blood flow to the active muscle during exercise, we examined the regulation of muscle blood flow during static handgrip exercise (20 % MVC) in female and male subjects. In the female subjects, we conducted experiments three times; menstrual (M), ovulatory (O), and luteal (L) phases. FMD in the brachial artery was larger in females than males, and in females FMD tended to be larger during O phase than the other phases. The percent increase in blood flow during exercise in females during O phase was higher than in males. Mean arterial pressure increased by about 15 mmHg in males, but did not significantly increase in females regardless of phases. The increase in vascular conductance during exercise in females during O phase was higher than in males. Thus, the muscle blood flow during exercise is regulated differently between males and females. The contribution of increased perfusion pressure is significant in males, but peripheral vasodilation contributes almost all to the increase in blood flow to the muscle in females. In addition, the magnitude of vasodilation was influenced by menstrual cycle. These results suggest sex and menstrual cycle affect the regulation of muscle blood flow to the exercising muscle.

### 5.23

#### SKELETAL MUSCLE ARTERIOLES DEMONSTRATE SEXUAL DIMORPHISM WITH RESPECT TO MACROMOLECULE TRANSVASCULAR EXCHANGE PATHWAYS.

Virginia Huxley<sup>1</sup>

<sup>1</sup>Med Pharm Physiol, University of Missouri-Columbia, MA415 HSC, Columbia, MO, 65212.

We have demonstrated that sex can be a primary determinant of basal coronary microvessel permeability (Ps, 10<sup>7</sup> cm/s) and, independently a determinant of the magnitude and direction of response to acute vasoactive stimulants or chronic adaptation to exercise. In this study we hypothesized that arterioles (art) isolated from skeletal muscle (*triceps brachii*) of adult male (M) and female (F) pigs would not differ with respect to basal Ps, but like coronary arterioles, would differ with respect to the change in Ps (APs) in response to adenosine (ADO, 10<sup>-5</sup> M). Ps

to porcine serum albumin (PSA, 29 F & 19 M) and alpha lactalbumin (a-lact, 29 F & 13 M) were measured in the absence and presence of ADO. Not only was sex without influence on Ps but there was no difference in Ps to PSA and a-lact despite the difference in size (66.5 vs 14 kD). While ADO was without influence on Ps to PSA in either M or F, Ps to a-lact was increased in arterioles from F and decreased in arterioles from M ( $p<0.05$ ). In aggregate the basal Ps data to the two proteins and the responses to ADO are consistent with the interpretation that under basal conditions these molecules use different routes to traverse the vessel wall. Further, the sex influences the mechanisms whereby ADO alters the paracellular pathway used by a-lact while being without influence on the pathway used by PSA. These data differ from the results observed in the heart. In both organs the data support sexual dimorphism in the regulation of exchange and mechanisms regulating volume homeostasis. Supported by NIH PO1 HL 52490; RO1 HL078816 and NASA NNJ05HF37G.

### 5.24

#### DOES GENDER INFLUENCE CARDIOVASCULAR AND RENAL RESPONSES TO WATER IMMERSION?

Donald Watenpaugh<sup>1</sup>, Bettina Pump<sup>2</sup>, Peter Bie<sup>2</sup>, Peter Norsk<sup>2</sup>

<sup>1</sup>Integrative Physiology, University of North Texas Health Science Center, 1521 Cooper St, Fort Worth, Texas, 76104, <sup>2</sup>Physiology, Danish Aerospace Medical Centre of Research, Tagensvej, Copenhagen, Denmark.

Human female responses to water immersion remain poorly studied when compared to the huge body of literature obtained from male subjects. We hypothesized that women and men exhibit similar cardiovascular and renal responses to thermoneutral water immersion (WI) to neck level after adjustment of renal responses per kg body weight. Ten women and nine men underwent two sessions in random order: 1) seated non-immersed for 5.5 h (control); and 2) WI to neck level for 3 h, with subjects sitting non-immersed for 1.5 h pre- and 1 h post-WI. We measured left atrial diameter, heart rate, arterial pressure, urine volume and osmolality, and urinary endothelin, urodilatin, sodium, and potassium excretion. No significant gender differences emerged in cardiovascular responses. Women and men also exhibited largely similar renal responses to WI after adjustment for body mass. However, female urodilatin excretion per kg during WI was 128% greater than men ( $P < 0.05$ ). Also, women exhibited a delayed kaliuretic response to WI relative to men, and women excreted 24% less potassium per kg during the third h of WI. Men may excrete more potassium than women during WI because men possess greater lean body mass (potassium per kg body weight). Alternatively, anti-kaliuretic effects of urodilatin may be responsible. Selected responses of men to WI may be cautiously extrapolated to women, yet urodilatin and potassium excretion responses exhibit gender differences.

### 5.25

#### EFFECT OF ESTROGEN REPLACEMENT ON OSMOREGULATORY AND CENTRAL ANGIOTENSIN II-INDUCED FLUID REGULATION IN OVARECTOMIZED RATS

Kayo Torii<sup>1</sup>, Keiko Morimoto<sup>1</sup>, Akira Takamata<sup>1</sup>

<sup>1</sup>Department of Environmental Health, Nara Women's University, Kitauoya Nishimachi, Nara, 630-8506, Japan.

We examined the effect of estradiol (E2) replacement in ovariectomized rats on the fluid intake and c-Fos expression of the arginine vasopressin (AVP) neurons at the paraventricular (PVN) and supraoptic (SON) nuclei in response to iv hypertonic saline infusion and icv angiotensin II (ANGII) injection. We also examined the c-Fos expression induced by these challenges at the organum vasculosum of the lamina terminalis (OVLT), subfornical organ (SFO) and median preoptic nucleus (MnPO). E2 replacement significantly attenuated osmotically-induced fluid intake and c-Fos expression of AVP neurons at the PVN and SON. E2 replacement did not affect the c-Fos expression at the osmosensitive regions, such as OVLT, SFO or MnPO following systemic osmotic challenge, suggesting that E2 does not modify osmoreceptor sensitivity. E2-effect on osmoregulation might be located on the pathway between osmoreceptors and the centers generating thirst and AVP secretion, or on these centers directly. E2 replacement also attenuated fluid intake and AVP neuron's activity induced by icv ANGII injection, and also attenuated the c-Fos expression at the SFO and MnPO, indicating that the central sensitivity to ANGII is attenuated by E2 replacement. Thus, the reduced central sensitivity to ANGII might be at least in part involved in the attenuated osmoregulatory responses by E2, because central ANGII plays an important role in osmoregulatory thirst and AVP release.

### 5.26

#### ALDOSTERONE EXCRETION DURING CHRONIC INTERMITTENT HYPOXIA IN MALE AND FEMALE RATS

Carmen Hinojosa-Laborde<sup>1</sup>, Teresa Craig<sup>1</sup>, Cindy Mehring<sup>1</sup>, Tom Cunningham<sup>2</sup>, Steve Mifflin<sup>2</sup>

<sup>1</sup>Anesthesiology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX, 78229, <sup>2</sup>Pharmacology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX, 78229.

We have shown that chronic intermittent hypoxia (CIH) increases blood pressure in male (M), but not female (F) rats. This protection in F is dependent on F sex hormones because ovariectomized (OVX) females responded to CIH with an increase in blood pressure similar to M. CIH in M and OVX suppressed pressure-natriuresis/diuresis, while CIH in F enhanced pressure-natriuresis/diuresis. We hypothesize that F sex hormones facilitate renal sodium and water excretion during CIH by suppressing aldosterone production. We investigated the effect of CIH on aldosterone excretion in M (n=8), F (n=6) and OVX (n=6) rats. Rats were exposed to 7 days of CIH, defined as continuous cycles of 3 minutes of room air (21% O<sub>2</sub>) and 3 minutes of 10% O<sub>2</sub> for 8 hours/day. Urine volume and aldosterone levels were measured during the CIH periods of the day. Following a similar increase in aldosterone excretion on the first day of CIH in all groups, aldosterone excretion subsequently decreased in F (-29%) and M (-8%). In contrast, CIH resulted in a sustained increase in aldosterone excretion in OVX (+13%). The reduction in aldosterone excretion in F was significantly different from M and OVX ( $p<0.05$ ). We conclude that CIH reduces aldosterone excretion in females, and this effect is dependent on the presence of female sex hormones. We speculate that female sex hormones protect against elevations in blood pressure associated with CIH by facilitating renal sodium and water excretion mediated by a suppression of aldosterone production.

### 5.27

#### GENOME-WIDE SCAN FOR GENOMIC DETERMINANTS OF ALCOHOL AND TOBACCO USE IN FRENCH CANADIAN FAMILIES

M. Nikpay<sup>1</sup>, O. Seda<sup>1</sup>, J. Tremblay<sup>1</sup>, E. Merlo<sup>2</sup>, D. Gaudet<sup>3</sup>, T. Kotchen<sup>4</sup>, A. Cowley<sup>4</sup>, P. Hamel<sup>1</sup>

# 2007 APS Conference: Sex Steroids and Gender in Cardiovascular-Renal Physiology and Pathophysiology

## ABSTRACTS OF INVITED AND VOLUNTEERED PRESENTATIONS

<sup>1</sup>CRCHUM, CHUM, Montreal, QC, H1W 4A4, Canada, <sup>2</sup> Polymtl, , Montreal, QC, H3C 3A7, Canada, <sup>3</sup>, CHS, , Montreal, Quebec, G7H 5H6, Canada, <sup>4</sup>Physiology, Med. Col. of Wisconsin, Milwaukee, WI, 53226.

We investigated the genomic factors in alcohol and tobacco use in a cohort of 120 families with at least 1 sib pair affected by hypertension and dyslipidemia from the Saguenay-Lac-Saint-Jean region. Phenotyping was performed by questionnaire. Joint sex-specific and non-specific linkage and association analyses were carried out with dense map (3 haplotypes per cM;  $r^2 > 0.4$ ) generated by merging 58000 SNPs and 437 microsatellites. Alcohol and smoking were strongly correlated with sex. Several loci with strong evidence of linkage and association were identified (LOD score  $> 3$ , P-value  $< 0.001$ ). A common locus on chromosome (chr) 1 was found for alcohol and tobacco use. Fine mapping for alcohol revealed a LD block located downstream of LPHN2 gene; female-specific SNPs were also uncovered inside the block for smoking. Moreover, on chr 1 at 233 cM, where we previously reported a QTL for diastolic blood pressures (DBP), a locus for smoking emerged. We noted SNPs inside GRID2 gene on chr 4; male-specific analyses revealed associated SNPs inside this gene for alcohol. At 168-180 cM on chr 3, where we previously observed loci for night and pre-math stress DBP, we uncovered a locus for alcohol. Along with the results of segregation mating tables indicated a pattern of X-linked inheritance for alcohol; SNPs emerged within POU3F4 gene on X chromosome. Besides, female-specific SNPs were found for smoking inside HTR2C gene. Common loci were identified for alcohol and tobacco use and for BP, suggesting common genomic determinants pointing to novel mechanisms of BP modification by these substances and the importance of sex. This work was supported by the NIH, CIHR, HSFC and the GENESIS Team.

### 6.0: SEX STEROIDS AND METABOLIC SYNDROME

#### 6.1 SEX DIFFERENCES IN SLEEP APNEA

Carmen Hinojosa-Laborde<sup>1</sup>  
<sup>1</sup>Anesthesiology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX, 78229-3900.

Sleep apnea (SA) is characterized by repetitive episodes of apnea-induced arterial hypoxemia during sleep. Repeated exposure to SA results in an increase in arterial pressure during waking hours, and disturbances in sodium and volume homeostasis. SA also associates with other cardiovascular risk factors that are components of metabolic syndrome such as obesity, dyslipidemia, and insulin resistance. Pre-menopausal women have a lower occurrence of SA than males, while in post-menopausal women the incidence is the same as in males. However, the cardiovascular complications associated with SA represent a significant health problem in both men and women. The mechanisms for the cardiovascular and renal effects of SA are not well understood, but evidence suggests that activation of the sympathetic nervous system, and the renin-angiotensin-aldosterone system are contributing factors. Chronic intermittent hypoxia (CIH) is a model for the repetitive bouts of apnea-induced arterial hypoxemia that occur during SA. Using this model, we have studied blood pressure, sodium and water homeostasis, renal pressure natriuresis, and aldosterone production in male and female rats during a 7 day exposure to CIH. We have demonstrated significant sex differences in the response to CIH. These findings and the clinical observations of sex differences in the effects of SA underscore the importance of further research of the mechanism of these sex-dependent responses. Reference: Hinojosa-Laborde C and Mifflin SW. (2005) Sex Differences in Blood Pressure Response to Intermittent Hypoxia in Rats. *Hypertension*, 46:1016-1021.

#### 6.3 THE PARENTAL ORIGIN OF THE X CHROMOSOME AND METABOLIC RISK FACTORS

Carolyn Bondy<sup>1</sup>  
<sup>1</sup>Develop. Endocrinology Br., NICHD, NIH, Bldg. 10 CRC, Rm 1-3330, Bethesda, MD, 20892-1103.

Some important gender disparities in metabolic profile and longevity aren't explained by sex steroid effects. Despite random inactivation of the 2<sup>nd</sup> X chromosome in females, the finding of a distinct phenotype in 45,X females with Turner syndrome (TS) indicates that the second X chromosome is important for normal female development. Our study of women with TS suggests that parental imprinting of X-linked genes involved in body composition and lipid metabolism contributes to gender differences in risk for atherosclerosis. Normal men are monosomic for  $X_{mat}$  while women are mosaic for  $X_{mat}$  and  $X_{pat}$ . If X-chromosome gene or genes that prevent visceral fat accumulation were imprinted (silenced) on  $X_{mat}$  and selectively expressed from  $X_{pat}$ , that could explain some of the observed differences between male and female risk profiles. To test this hypothesis, we compared regional fat distribution and lipid profile in women monosomic for  $X_{pat}$  vs. women monosomic for  $X_{mat}$ . Although BMI and total body fat were similar in our age-matched groups, women with a single maternally inherited X-chromosome ( $X_{mat}$ ) had more than twice as much visceral fat vs. women with a paternal X ( $P=0.001$ , JAMA, 2006). Lipid profile was also significantly more atherogenic in the  $X_{mat}$  group of women. This male-type fat distribution and lipid profile in 45,  $X_{mat}$  women supports the view that differential X-chromosome gene dosage, determined by genomic imprinting, contributes to the excess mortality from ischemic heart disease in 46,  $X_{mat}Y$  men as well as 45,  $X_{mat}$  women. The identification of these genes clearly is of clinical importance.

### 7.0: SEX STEROIDS, THE RENIN-ANGIOTENSIN SYSTEM AND HYPERTENSION

#### 7.2 SEX DIFFERENCES IN ANGIOTENSIN II, ANGIOTENSIN CONVERTING ENZYME 2 AND ANGIOTENSIN-(1-7)

Mark Chappell<sup>1</sup>  
<sup>1</sup>Hypertension Center, Wake Forest University, Medical Center Blvd, Winston Salem, NC, 27157-1032.

The mRen2.Lewis (mRen) is a congenic hypertensive model that exhibits sex-dependent differences in blood pressure and tissue injury. We tested the hypothesis as to whether the tissue renin-angiotensin system (RAS) is enhanced in the male versus female mRen. Age-matched hemizygous male [ $200 \pm 4$  mmHg] and female mRen [ $146 \pm 7$  mmHg] were utilized in these

studies. Renal cortical Ang II content was 2-fold higher in the male mRen [ $9.2 \pm 0.4$  vs.  $4.2 \pm 0.3$  fmol/mg]. Conversely, cortical levels of Ang-(1-7) were lower in the male strain [ $1.1 \pm 0.5$  vs.  $3.4 \pm 0.8$  fmol/mg]. To determine the potential enzymatic contribution, we assessed cortical ACE2 and neprilysin activities as both enzymes are capable of degrading Ang II and producing Ang-(1-7). Neprilysin was 3 fold higher in the female cortex [ $919 \pm 73$  vs.  $290 \pm 51$  fmol/min/mg] consistent with the higher expression of Ang-(1-7). Surprisingly, ACE2 activity was significantly higher in the male mRen [ $69 \pm 3$  vs.  $31 \pm 3$  fmol/min/mg]. We conclude that the gender differences in the mRen strain involves differential regulation of the RAS that may favor Ang II over Ang-(1-7) in the kidney. However, increased ACE2 may reflect a compensatory response to the greater pressure and tissue injury in the male. NIH HL-56973, HL-51952 & AHA-Grant-in-Aid. References: Chappell MC, Yamaleyeva LI, Westwood BM. Estrogen and Salt-Sensitivity. *Am J Physiol*: 291: H2166-H2172,2006.

#### 7.3 GONADAL HORMONE-INDEPENDENT SEX CHROMOSOME EFFECTS IN ANGIOTENSIN II-INDUCED HYPERTENSION

K. Sandberg<sup>1</sup>, H. Ji<sup>1</sup>, W. Zheng<sup>1</sup>, A. Arnold<sup>2</sup>, X. Wu<sup>1</sup>  
<sup>1</sup>Medicine, Georgetown Univ., Ste 232 Bldg D, 4000 Reservoir Rd, NW, Washington, DC, 20057, <sup>2</sup>Physiological Science, UCLA, Rm 4117, 621 Charles E. Young Dr, Los Angeles, CA, 90095.

Men have higher blood pressures (BP) and a higher incidence of hypertension than women up to the 6<sup>th</sup> decade of life after which point, women catch up. To determine if sex chromosomes contribute to this sex difference in BP independently of the gonadal hormone milieu, we studied the magnitude of angiotensin II (Ang II)-induced hypertension in the Sry transgenic mouse on an MF-1 background. The Sry gene is the testis-determining factor and thus its presence (phenotypic male) or absence (phenotypic female) determines an individual's sex. The Sry gene was moved from the Y chromosome to an autosome resulting in 4 genotypes: 2 females [XX and XY (lack Sry)] and 2 males [XY<sup>Sry</sup> and XX<sup>Sry</sup>] differing only in their sex chromosomal complement, respectively. Six weeks after birth, both the males and females were castrated. At 6-8 mo, radiotransmitters were inserted and baseline recordings were made for 3 days after the mice had recovered from surgery (1 week). Once a stable baseline was established, Ang II was infused by osmotic minipump (800ng/Kg/min) and BP was recorded by radiotelemetry at 10s intervals for 10 min/hour from 8 pm to 6 am for 8 d. A mixed effect statistical model was used to compare the differences in BP (DBP) between basal and Ang II treatment. The DBP was significantly higher in the XX compared to the XY<sup>Sry</sup> female ( $p < 0.013$ ) [DBP $\pm$ SEM (mm Hg): XX,  $36.8 \pm 7.6$ ; XY<sup>Sry</sup>,  $26.5 \pm 7.8$ ; XY<sup>Sry</sup>,  $26.3 \pm 8.0$ ; XX<sup>Sry</sup>,  $32.8 \pm 6.1$ ;  $n=9-11$ ]. These data suggest that under conditions of gonadal steroid deficiency such as menopause, the XX sex chromosomal complement makes the female more susceptible to hypertension than the XY male. Supported by NIH grant AG19291.

#### 7.4 ROLE OF THE RAS IN SEX DIFFERENCES IN HYPERTENSION

Judith Miller<sup>1</sup>  
<sup>1</sup>Nephrology, Toronto General Hospital, 8N-828, 585 University Avenue, Toronto, Ontario, M5G 2N2, Canada.

Sexual dimorphism in arterial pressure (AP) exists in human and animal models. Hypertension is more prevalent in post-menopausal compared to pre-menopausal women. The RAS likely plays a prominent role. Estrogen induces increased circulating RAS components in women and in users of oral contraceptives (OCs) and hormone replacement therapy (HRT); estrogen induces downregulation of ACE activity and AT1 receptors, and upregulation of AT2 receptors. Human studies document RAS functional differences between the sexes and between high and low estrogen states: The luteal phase is characterized by elevated circulating RAS components, a blunted response to Ang II, and an inability to maintain AP in response to simulated orthostasis. Women exhibit augmented hemodynamic responses to RAS blockade. Exogenous estrogens also impact RAS function: OC users exhibit elevated AP which is abolished by RAS blockade and increased AT1 receptor expression compared to non-users; HRT induces discordant Ang II and AP responses to orthostasis in post-menopausal women. The Nitric Oxide (NO) pathway as a counterregulatory system may offer insight into sexual dimorphism in AP, in that women who are users and non-users of OCs exhibit differences in response to NO system stimulation. Funding Source: Canadian Institutes of Health Research. (Miller JA, Cherney DZ, Duncan JA et al: Gender differences in the response to renin angiotensin system blockade. *J Am Soc Nephrol* 17:2554-2560, 2006; Harvey PJ, Morris BL, Miller JA et al: Estradiol induces discordant angiotensin and blood pressure responses to orthostasis in healthy post-menopausal women. *Hypertension* 45:399-405, 2005).

#### 7.5 2-METHOXYESTRADIOL: A SAFE AND EFFECTIVE CARDIORENAL PROTECTIVE HORMONE THERAPY FOR WOMEN AND MEN

Edwin Jackson<sup>1</sup>, Stevan Tofovic<sup>1</sup>, Claude Piche<sup>2</sup>, Raghvendra Dubey<sup>3</sup>  
<sup>1</sup>Center for Clinical Pharmacology, University of Pittsburgh, 100 Technology Drive, Suite 450, Pittsburgh, PA, 15219, <sup>2</sup>Clinical Development and Regulatory Affairs, PR Pharmaceuticals, Inc., 1716 Heath Parkway, Fort Collins, CO, 80524, <sup>3</sup>Department of Obstetrics and Gynecology, Clinic for Endocrinology, University Hospital Zurich, D217, NORD-1, Frauenklinik, Zurich, CH-8091, Switzerland.

2-Methoxyestradiol (2ME) is a non-estrogenic metabolite of estradiol. In smooth muscle cells, cardiac fibroblasts and mesangial cells 2ME potently inhibits cell growth. Moreover, 2ME reduces endothelin-1 production, yet increases prostacyclin biosynthesis. In rats with the metabolic syndrome, treatment with 2-hydroxyestradiol, the precursor of 2ME, decreases body weight, improves vascular endothelial function, decreases nephropathy, exerts antidiabetic actions and lowers blood pressure and blood cholesterol. Also, 2ME protects against nephrotoxins and pulmotoxins. The beneficial effects of 2ME are such that it makes sense to evaluate the drug's potential as a safe and effective hormone therapy for both women and men. 2ME is not feminizing in men nor would it be expected to increase the risk of uterine or breast cancer in women. Indeed, 2ME is anti-carcinogenic. Although the pharmacokinetic properties of 2ME are challenging, progress has been made in developing a long-acting formulation of 2ME that provides pharmacologic levels of the drug in humans for several weeks after a single dose. An interesting finding in the phase I studies with this preparation is that approximately 25% of men have significant basal levels of 2ME as determined by state-of-the-art mass spectrometric methods. In conclusion, 2ME may play an important physiological role and is an important molecule to consider in the quest for a safe and effective hormone therapy.

# 2007 APS Conference: Sex Steroids and Gender in Cardiovascular-Renal Physiology and Pathophysiology

## ABSTRACTS OF INVITED AND VOLUNTEERED PRESENTATIONS

7.6

### THE ROLE OF ESTROGENS AND POLYPHENOLS IN HYPERTENSION AND DIABETES.

J. Wyss<sup>1</sup>

<sup>1</sup>Cell Biology, Univ. Alabama at Birmingham, 1900 University Blvd. THH 950, Birmingham, AL, 35294.

Cognitive loss, hypertension, cardiovascular disease, diabetes and stroke increase dramatically after menopause. In SHR, estrogen deprivation increases hypertension, insulin resistance and stroke. We have demonstrated that in female ovariectomized SHR three polyphenols (i.e., genistein [soy], puerarin [kudzu] and proanthocyanidins [grape]) blunt hypertension, cognitive decline and insulin resistance. Of these, puerarin is most easily tracked, largely because the C-glucoside remains unmetabolized as it crosses the gut/blood barrier, circulates and is eliminated. Puerarin blunts <50% of the blood pressure rise that occurs in the non-treated SHR on a high salt diet. Suppression of both sympathetic nervous system activity and superoxide production play a role in these effects. Puerarin also reduces blood glucose, insulin and leptin in the SHR. Glucose tolerance and glucose sensitivity are improved by <20% in chronic studies and by about 50% when the puerarin and glucose are administered simultaneously. In the later effects puerarin appears to act via Na-dependent glucose transporters. Both of these effects occur in young and aged SHR, but there are several changes in the effects with age. Clearly, treatment of the animals is always beneficial, but the greatest effects occur when the animals are treated prior to 12-15 months of age. (NIH/ODS P50AT000477; NINDS P30NS047466 and P30NS057098; Peng, et al., Antihypertensive and cognitive effects of grape polyphenols. *Amer J Physiol*, 289:771, 2005. Meezan E, et al., Contrasting effects of puerarin and daidzin on glucose homeostasis in mice. *J Agric Food Chem*, 53:8760, 2005.)

## 8.0: SEX STEROIDS AND TARGET ORGAN INJURY

8.2

### SEX STEROIDS, CORONARY SMOOTH MUSCLE, ATHEROSCLEROSIS AND RESTENOSIS

Doug Bowles<sup>1</sup>

<sup>1</sup>Biomedical Sciences, Dalton Cardiovascular Research Center and National Center for Gender Physiology, University of Missouri, E102 Veterinary Medicine, 1600 E Rollins, Columbia, Mo, 65211.

Sex differences in the prevalence of coronary artery disease (CAD) have led to the belief that testosterone increases the risk of CAD in men. However, recent clinical studies have failed to support a detrimental effect of testosterone on CAD or carotid atherosclerosis in men. On the contrary, epidemiological and clinical trials indicate that low testosterone levels in men are associated with a higher risk of cardiovascular disease. Accumulation of smooth muscle cells (SMC) in the intima is a hallmark of coronary atherosclerosis and restenosis, reflecting the balance between proliferation and apoptosis. We have recently shown that 1) PKC $\delta$  levels are higher in coronary smooth muscle cells (CSMC) of males, 2) endogenous testosterone increases PKC $\delta$  protein levels in CSMC and 3) both testosterone and dihydrotestosterone (DHT) increase PKC $\delta$  expression and activity in CSMC *in vitro*. PKC $\delta$  has been shown to be anti-proliferative and pro-apoptotic in other cells types. Accordingly we have shown that testosterone induced a PKC $\delta$ -dependent G1/S phase cell cycle arrest and stimulated apoptosis in CSMC, providing a potential mechanistic basis for observed effects of testosterone on coronary vasculoproliferative diseases. Using a swine model of coronary restenosis we found both intimal to medial ratio (I/M) and I/M normalized to rupture index (RI) were increased in castrated males, but not those with testosterone replacement, compared to intact males. Thus, both *in vitro* and *in vivo* data support beneficial effects of endogenous T on post-angioplasty restenosis in males. The effect of testosterone on complex atherosclerotic lesions is likely context dependent, e.g. vulnerable plaque vs. stable plaque, warranting caution when extrapolating to human clinical outcomes. Support: NIH HL 071574 and NASA.

8.3

### CRITICAL ROLE FOR ET<sub>B</sub> RECEPTORS IN ATTENUATING THE RESPONSE TO ENVIRONMENTAL STRESS IN FEMALE, BUT NOT MALE RATS

David M. Pollock, Jennifer C. Sullivan, Jeffrey A. Bobo, Gerard D'Angelo, and Jennifer S. Pollock

Vascular Biology Center, Medical College of Georgia, Augusta, GA 30912-2500.

The endothelin (ET) system contributes to blood pressure regulation through both vascular and renal tubular mechanisms with ET<sub>A</sub> and ET<sub>B</sub> receptors having contrasting effects. Genetic ET<sub>B</sub> receptor deficiency or pharmacological blockade of the ET<sub>B</sub> receptor results in salt-dependent hypertension. When ET<sub>B</sub> receptor deficient rats were placed on a high salt diet, female rats were observed to have a greater increase in blood pressure compared to male rats when assessed by the tail cuff method. When we sought to confirm these observations using telemetry, the gender difference was no longer observed. This led us to hypothesize that female ET<sub>B</sub> receptor deficient rats have an exaggerated pressor response to acute stress such as would occur during the tail cuff procedure. Male and female ET<sub>B</sub> receptor deficient rats along with receptor intact control rats were implanted with telemetry transmitters to monitor blood pressure and heart rate changes in response to an acute stressor. Rats were placed in a restrainer and subjected to a 3 min period of air jets directed at their head. We observed that the total pressor response (area under curve) to air jet stress was greater in ET<sub>B</sub> deficient female (67±6 mmHg/3min, n=6) compared to male rats (50±5 mmHg/3min, n=5, p<0.05) while there was no gender difference in controls. After a high salt diet (8% NaCl) for 3 weeks, both male and female ET<sub>B</sub> receptor deficient rats had similar increases in baseline blood pressure (165±10 and 160±5 mmHg during the 4<sup>th</sup> week of high salt in male vs. female, respectively). Air jet stress increased pressure more in the female rats (61±9 mmHg/3min), an effect that was again more pronounced compared to male rats (37±9 mmHg/3min, n=5, p<0.05). These studies highlight an important gender difference between ET<sub>B</sub> receptor function in the response to stress and suggest that the ET<sub>B</sub> receptor functions to attenuate the stress response in female, but not male rats.

8.4

### SEX, DIABETES AND RENAL INJURY

Christine Maric<sup>1</sup>

<sup>1</sup>Medicine, Georgetown University Medical Center, 394 Bldg D, 4000 Reservoir Road NW, Washington, DC, 20057.

The incidence and progression of renal disease is lower in women than in men, however, in the setting of diabetes, this relationship no longer exists. While the data are inconclusive, mainly due to inadequate analyses, the current view is that the incidence and progression of diabetic renal complications in women is either equal or exceeds that in men. Our studies have suggested that diabetes is associated with reduced estradiol levels (1), which may explain why the female gender is lost as a protective factor in diabetes. We have recently shown that supplementation with 17 $\beta$ -estradiol is renoprotective in attenuating and reversing albuminuria and renal structural damage in the streptozotocin-induced rat model of diabetic renal disease, suggesting that restoring estradiol levels in diabetes protects from the development of the disease.

While estradiol is generally believed to be renoprotective in the non-diabetic kidney, testosterone is thought to contribute to non-diabetic renal disease. Interestingly, in the setting of diabetes, this no longer holds. Similar to diabetic women, who exhibit low circulating levels of estradiol, diabetic men exhibit decreased levels of testosterone. Our studies in an experimental model have shown that absence of testosterone contributes to more rapid progression and more severe renal damage in diabetes. Collectively, these observations suggest that sex hormones play a significant role in the pathophysiology of diabetic renal disease and that it may not be the absolute levels of hormones, but rather the relative ratio of androgens to estrogens that determines the overall contribution and effect of the hormone in the diabetic kidney. 1. Wells CC, Riazzi S, Mankhey RW, Bhatti F, Ecelbarger C, Maric C. *Gender Medicine* 2: 237-247, 2005. 2. Mankhey RW, Bhatti F, Maric C. *AJP-Renal Physiol* 288: 399-400, 2005.

8.5

### SEX, NO AND AGING

Chris Baylis<sup>1</sup>

<sup>1</sup>Physiology, Univ Florida, 1600 SW Archer, Gainesville, Florida, 32610.

With advancing age the kidney shows falls in GFR due to renal vasoconstriction and structural damage. This usually occurs slowly and does not cause severe renal impairment unless additional insults are superimposed. There is a pronounced sexual dimorphism in kidney aging with females protected. Pre-menopausal females produce more NO than men and total NO production falls with age, although there is no clinical data on sex differences. NO is a major factor in regulation of vascular tone, growth and structural integrity and becomes deficient with advancing age, as endothelial dysfunction develops. Although the substrate, L-arginine is maintained, the concentration of the circulating endogenous nitric oxide synthase (nNOS) inhibitor ADMA increases with age; this is delayed in women. There may be falls in vascular eNOS abundance, decreased membrane association and decreased stimulatory protein-protein interactions with age as well the cumulative oxidative stress of aging promoting superoxide rather than NO formation by the eNOS. Within the kidney, declines in abundance and activity of the neuronal (nNOS) correlate with development of disease in the male rat, whereas in the protected female, renal nNOS abundance is maintained. The impact of sex steroids is complex and includes NO stimulatory and direct antifibrotic actions of estrogens and possible damaging effects of androgens, although in man this is not clear cut. Taken together, it is likely that age-dependent declines in NO generation contribute to age-dependent kidney damage. REFERENCE: Baylis C. Changes in renal hemodynamics and structure in aging kidney; sexual dimorphism and the NO system *Exp Gerontol* 40: 271-278, 2005.

8.6

### SEX STEROIDS, PLATELET AGGREGATION AND INFLAMMATION

Virginia Miller<sup>1</sup>, Muthuvel Jayachandran<sup>1</sup>, Kazumori Kashimoto<sup>1</sup>, John A. Heit<sup>1</sup>, Whyte G. Owen<sup>1</sup>

<sup>1</sup>Dept. of Surgery, Physiol. & Bioengineering, Mayo Clinic Col. of Med., 200 First St., SW, Rochester, MN, 55905.

In women, risk of cardiovascular disease and thrombosis increases exponentially at menopause suggesting that estrogen may influence both processes. Infection also increases risk for venous thrombosis. Platelet activation is required for formation of thrombin, development of arterial vascular lesions, and activation of leukocytes. Hormones and infection-associated factors, i.e. lipopolysaccharide (LPS), will influence the phenotype of circulating platelets through gene transcription and translation in platelet precursors, megakaryocytes. Genetic polymorphisms in receptors which bind hormones or LPS will cause variation in platelet characteristics. For example, platelets were more reactive in mice lacking estrogen receptor beta whereas platelets derived from mice lacking toll-like receptor 4 were less responsive to thrombin compared to their wild type counterparts, respectively. Therefore, a platelet procoagulant phenotype may be characteristic of an individual which could predispose that individual to risk for adverse thrombotic events under certain environmental influences, for example, with hormone therapy or infection. The concept of using platelet phenotype to define thrombotic risk for individuals is a new and is being tested in women enrolled in an ancillary study to the Kronos Early Estrogen Prevention Study (KEEPES), a study of hormone therapy to prevent progression of cardiovascular disease in early menopausal women. Reference: Miller, V.M., et al., *Estrogen therapy and thrombotic risk*. *Pharmacology & Therapeutics*, 2006. 111(3): p. 792-807.

8.7

### ANDROGENS STIMULATE PROXIMAL TUBULE TRANSPORT

Raymond Quigley<sup>1</sup>

<sup>1</sup>Pediatrics, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, Texas, 75390-9063.

Disrupting the enzyme CYP4A14 in mice leads to hypertension, which is more severe in the male mice and appears to be due to androgen excess. Androgens are known to increase expression of angiotensinogen, but the effect of androgens on proximal tubule transport is unknown. PCTs from knockout (KO) and wild type (SV/129) mice were perfused *in vitro*. Volume absorption was elevated in tubules from the KO mice as compared to the wild type mice (1.11±0.06 vs 0.77±0.12 nl/min/mm, p<0.05). Expression of the sodium-proton antiporter (NHE3) was found to be higher in brush border membrane vesicles (BBMV) from KO mice than that in wild type mice. To determine if this effect could be reproduced in a model of androgen excess, male Sprague-Dawley rats were given dihydrotestosterone (DHT) injections IP for ten days. The PCT volume reabsorptive rate was significantly higher in treated rats than in control rats given vehicle injections (4.57 ± 0.31 vs. 3.31 ± 0.23 nl/mm • min, p < 0.01). Luminally perfusing with either enalaprilat or losartan decreased the PCT reabsorptive rate in DHT treated rats to a significantly greater degree than in control vehicle injected rats. The renal expression of angiotensinogen and BBMV protein abundance of NHE3 was higher in the DHT treated animals using Northern analysis. DHT treated rats had higher blood pressures and lower serum angiotensin II levels than the control rats. These results suggest that androgens may directly upregulate the proximal tubule renin-angiotensin system, increase the volume reabsorptive rate, and thereby increase extracellular volume and blood pressure and secondarily decrease serum angiotensin II levels.

# 2007 APS Conference: Sex Steroids and Gender in Cardiovascular-Renal Physiology and Pathophysiology

## ABSTRACTS OF INVITED AND VOLUNTEERED PRESENTATIONS

### 10.0: SEX STEROIDS IN HYPERTENSION, DIABETES, AND PREGNANCY

#### 10.1

##### ALDOSTERONE/NaCl-INDUCED HYPERTENSION: THE ROLE OF GENDER, SEX HORMONES AND CENTRAL REACTIVE OXYGEN SPECIES

Baojian Xue<sup>1</sup>, Alan Kim Johnson<sup>2</sup>, Meredith Hay<sup>3</sup>  
<sup>1</sup>Psychology, University of Iowa, 11 Seashore Hall E, Iowa City, Iowa, 52242, <sup>2</sup>Psychology and Cardiovascular Center, University of Iowa, 11 Seashore Hall E, Iowa City, Iowa, 52242, <sup>3</sup>Psychology and Molecular Physiology & Biophysics, University of Iowa, 11 Seashore Hall E, Iowa City, Iowa, 52242.

Elevated aldosterone (Aldo) contributes to hypertension and vascular and renal injury. Little is known about the role of sex differences in Aldo/salt-induced hypertension and the role of central reactive oxygen species (ROS) in this form of high blood pressure. The purpose of the present study was to test the hypothesis that male and female Sprague-Dawley rats respond differently to subcutaneous infusion of Aldo (0.75 µg/h, 28 days) combined with 1% NaCl as the sole drinking fluid and the effects in males of superoxide mimetic treatment. Blood pressure was measured by DSI telemetry. Aldo+1% NaCl-treated male rats progressively developed hypertension (101.6±2.8 to 124.6±3.6, Δ23.2±3.7 mmHg, n=6), but blood pressure in females was not different from controls (98.4±1.7 to 101.8±3.5, Δ3.4±1.4 mmHg, n=4). Gonadectomy augmented Aldo+1% NaCl-induced hypertension in females (Δ17.1±2.3 mmHg, n=3) but had no additional effect in males (Δ20.9±2.2 mmHg, n=4). Systemic infusion of 17β-estradiol (250 µg/day) totally blocked Aldo+1% NaCl-induced hypertension in intact males (Δ-1.5±2.4 mmHg, n=3). In males, central, but not peripheral infusions of tempol (200 nmol/kg/min), prevented the development of hypertension (Δ6.0±1.9 mmHg n=4 vs Δ17.6±2.0 mmHg, n=3). These results indicate that estrogen may play a protective role in the development of Aldo+1% NaCl-induced hypertension and that central ROS are involved in the development of Aldo+1% NaCl-induced hypertension. (NIH HL-59676; HL-62261; HL-14388; DK-66086).

#### 10.2

##### MPA BUT NOT DROSPIRENONE AGGRAVATES RENAL INJURY IN ALDOSTERONE-SALT TREATED RATS

Theo Pelzer<sup>1</sup>, Paula Anahi Arias Loza<sup>1</sup>

<sup>1</sup>Medicine I, University of Wuerzburg, Josef Schneider Str. 2, Wuerzburg, D-97080, Germany. Background: Clinical trials on the prevention of cardiovascular disease by combined hormone replacement therapy (HRT) revealed negative or neutral results. The HERS and WHI trials employed medroxyprogesterone acetate (MPA), which binds not only to the progesterone receptor (PR) but transactivates also the androgen- (AR), the glucocorticoid- (GR) and the mineralocorticoid receptor (MR). In contrast, the recently developed progestin drospirenone (DRO) is not only devoid of partial AR and GR agonist activities but also possesses a strong anti-mineralocorticoid functionality. The aim of this study was to compare the effect of different hormone replacement regimes including 17β-estradiol (E2) plus either MPA or DRO in uninephrectomized rats receiving chronic aldosterone infusion plus a high salt diet for 8 weeks (AST rats). Elevated water consumption, sodium uptake and renal potassium excretion in AST rats increased further by co-treatment with MPA whereas DRO attenuated fluid and sodium turnover in AST rats. Only MPA but not DRO increased kidney mass and caused extensive inflammatory kidney injury resulting in glomerular and peritubular fibrosis. MPA but not DRO enhanced the expression of regulatory NADPH oxidase subunits including p67phox as well as eNAC alpha expression. DRO completely prevented kidney hypertrophy as well as glomerular and tubular damage. In conclusion, MPA aggravates and DRO attenuates renal injury in aldosterone salt treated rats that relates to enhanced ROS generation. We conclude that drospirenone confers nephroprotective effects under conditions of excess MR activation that are superior to MPA.

#### 10.3

##### DIFFERENTIAL EXPRESSION OF NEPRILYSIN AND ANGIOTENSIN CONVERTING ENZYME 2 (ACE2) MAY CONTRIBUTE TO DECREASED ORGAN DAMAGE IN THE FEMALE HYPERTENSIVE mRen2.LEWIS RAT

Karl Pendergrass<sup>1</sup>, Brian Westwood<sup>1</sup>, Mark Chappell<sup>1</sup>

<sup>1</sup>Hypertension and Vascular Research Center, Wake Forest University, Medical Center Blvd, Winston-Salem, North Carolina, 27157-0001.

The mRen2.Lewis (mRen) strain exhibits a gender difference in blood pressure and tissue injury. At 14 weeks of age, males exhibit higher blood pressure to that of the females [200 ± 4 vs. 146 ± 7 mm Hg; p<0.01; n=5-6], as well as greater cardiac hypertrophy [4.0 ± 0.1 vs. 3.2 ± 0.1 mg/g] and increased proteinuria [156 ± 32 vs. 29 ± 3 mg protein/kg/day; p<0.01; n=5-6]. Serum C-reactive protein, a marker for inflammation was 1.3 fold higher in the male mRen compared to females [595 ± 20 vs. 468 ± 31 µg/mL; p<0.01; n=6]. The aim of the present study evaluated the enzymatic pathways in cardiac and renal tissue that contribute to the gender differences in organ damage. We utilized a sensitive method to detect the metabolism of angiotensin peptides (Ang), Ang II to Ang-(1-7) for ACE2 and Ang I to Ang-(1-7) for neprilysin. Cardiac ACE2 activity was 1.4 fold higher in male mRen than females [19.3 ± 0.9 vs. 14.3 ± 0.9 fmol/min/mg; p<0.01; n=4]. In renal cortical membranes, ACE2 activity was 1.7 fold higher [69.4 ± 2.9 vs. 40.8 ± 3.1 fmol/min/mg, p<0.01; n=4]. In contrast, neprilysin activity in the renal cortex was 3 fold higher in the female mRen [919 ± 73 vs. 290 ± 51 fmol/min/mg, p<0.01; n=4]. We conclude that the higher expression of neprilysin may confer a protective mechanism in the female kidney through: 1) the enhanced metabolism of Ang II; and 2) the increased formation of Ang-(1-7). A preferred pathway for Ang I to Ang-(1-7) in the female mRen kidney may negate the reliance on ACE2 given the reduced levels of Ang II as the ACE2 substrate. In contrast, the higher ACE2 activity in the male mRen may reflect a compensatory mechanism for the lower expression of neprilysin and the greater degree of hypertension and tissue injury. Supported by NIH grants HL56973, HLS56973, & HL51952.

#### 10.4

##### TESTOSTERONE SUPPLEMENTS PROMOTE RENAL INJURY AND EXACERBATE HYPERTENSION IN AGING SHR

Radu Iliescu<sup>1</sup>, Licy L. Yanes<sup>1</sup>, Julio C. Sartori-Valinotti<sup>1</sup>, Jane F. Reckelhoff<sup>1</sup>

<sup>1</sup>Physiology and Biophysics, University of Mississippi Medical Center, 2500 North State St, Jackson, MS, 39216.

Testosterone supplements are commonly prescribed to aging men, who are frequently hypertensive and have increased risk of developing hypertensive renal injury. Endogenous androgens may promote hypertension and renal disease. Spontaneously hypertensive rats (SHR)

develop androgen-dependent hypertension at a young age but are relatively protected from any hypertensive renal injury until after 9 months of age. We hypothesized that testosterone supplementation of aging male SHR exacerbates hypertension and renal injury. Intact, castrated (at 8 months) and testosterone-supplemented male SHR (n=4/group) were followed from 9 to 13 months of age, when blood pressure (BP, indwelling arterial catheters) and urinary protein excretion were determined. Testosterone supplementation with Silastic® pellets led to approximately 2-fold increase of plasma testosterone concentration. At 13 months of age, BP was significantly higher in testosterone supplemented male SHR as compared with intact rats (199±2 vs 188±3 mmHg, p<0.05) whereas castration did not alter BP levels (187±6 mmHg). Testosterone supplemented SHR excreted more urinary protein than intact rats (150±8 vs 96±8 mg/day, p<0.05) and castration reduced proteinuria (34±6 mg/day). These results suggest that testosterone supplementation of aging male SHR promotes renal injury and thereby exacerbates hypertension. On the other hand, removal of endogenous androgens later in life (8 months) prevents the development of hypertensive renal injury without altering established hypertension.

#### 10.5

##### SEX DIFFERENCES IN RENAL 11β-HYDROXYSTEROID DEHYDROGENASE TYPE 2 IMMUNOREACTIVITY IN RAT KIDNEYS

Darren Roesch<sup>1</sup>, Min Shi<sup>1</sup>, Carolyn Ecelbarger<sup>1</sup>, Kathryn Sandberg<sup>1</sup>

<sup>1</sup>Center for the Study of Sex Differences in Health, Aging, and Disease, Georgetown University, 4000 Reservoir Rd, NW, Rm 393 Bldg D, Washington, DC, 20007.

Female control rats, as compared to male control rats, exhibit increased renal abundances of aldosterone-sensitive electrolyte transporters, suggesting that constitutive mineralocorticoid activity is increased in female as compared to male rats. The purpose of this study was to test the hypothesis that decreased renal abundance of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) contributes to increased constitutive mineralocorticoid activity in female rats. Vehicle or aldosterone (200 µg/day) was infused in male and female rats fed a 1% NaCl diet for 4 weeks. Four groups were studied: male control rats (n=5), male rats infused with aldosterone (n=6), female control rats (n=6), and female rats infused with aldosterone (n=6). There were significant effects of sex (p<0.001) and treatment (p<0.001) on renal 11β-HSD2 abundance as determined by Western blot. 11β-HSD2 abundance was 1.00±0.02 absorbance units (AU) in male control rats and was significantly (p<0.001) less in female control rats (0.63±0.04 AU). Aldosterone infusion did not significantly change 11β-HSD2 abundance in male rats, but in female rats, aldosterone infusion significantly reduced 11β-HSD2 abundance to 0.39±0.05 AU. The results of this study demonstrate that renal 11β-HSD2 abundance is decreased in female as compared to male rats. We propose that reduced 11β-HSD2 abundance contributes to increased constitutive activation of mineralocorticoid receptors by endogenous glucocorticoids in female as compared to male rats.

#### 10.6

##### TESTOSTERONE MEDIATES HYPERTENSION AND RENAL INJURY IN DAHL RATS DESPITE HIGH SODIUM DIET-MEDIATED DECREASE IN TESTOSTERONE LEVELS

Licy Yanes<sup>1</sup>, Radu Iliescu<sup>1</sup>, Julio Sartori-Valinotti<sup>1</sup>, Huimin Zhang<sup>1</sup>, Damian Romero<sup>2</sup>, Jane Reckelhoff<sup>1</sup>

<sup>1</sup>Physiology and Biophysics, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS, 39216, <sup>2</sup>Medicine, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS, 39216.

Recent epidemiological studies suggest that low levels of plasma testosterone (PT) are associated with cardiovascular diseases in men. However, a cause-effect relationship between the two parameters has not been conclusively established. High sodium (HS) diet in Dahl salt sensitive rats (DS) causes hypertension and renal damage. To test the hypothesis that HS diet reduces PT in DS males and yet castration protects against hypertension and renal damage, DS males (5/group) were challenged with HS diet for 3 weeks, starting at 4, 6, 9 and 15 weeks of age. Age matched animals on low sodium (LS) diet were used as control. At the end of the experiment PT was measured by RIA. On LS diet, PT increased at 11 weeks and remained constant until 15 weeks of age. On HS diet, PT did not rise at 11 weeks and remained significantly lower compared to high sodium group up to 22 weeks of age. Other group of intact and castrated DS male rats (11/group) maintained on LS diet were challenged with a HS diet for 4 weeks. Mean arterial pressure was measured by radiotelemetry. Renal injury was determined by urinary albumin excretion by ELISA and glomerular sclerosis by histology. Castration significantly attenuated HS diet induced hypertension and renal injury. Our study suggests that although HS diet decreases PT in males DS, androgens still play a major role in the pathogenesis of hypertension and renal injury associated with HS diet.

#### 10.7

##### POPULATION EXTREMES-BASED APPROACH DEFINES GENDER DIFFERENCES IN ADRENERGIC AND RENAL GENES CONTRIBUTING TO BLOOD PRESSURE

Brinda Rana<sup>1</sup>, Paul Insel<sup>2</sup>, Nicholas Schork<sup>1</sup>, Daniel O'Connor<sup>3</sup>

<sup>1</sup>Psychiatry, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA, 92093, <sup>2</sup>Pharmacology, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA, 92093, <sup>3</sup>Medicine, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA, 92093.

Recent studies indicate that gender impacts genetic expression of complex traits & diseases. To investigate the differential contribution of genetic variants to BP in men vs. women we exploited the power of sampling subjects from the top and bottom 5th percentiles of BP from a community-based sample of >53,000 people in a health maintenance program. With a sample of 611 men and 656 women White-Americans, we had >90% power to detect genes contributing as little as 3% to trait (BP) variation. We assayed for >60,000 genotypes in the subjects including 48 SNPs at 33 autosomal and 2 X-linked genes in adrenergic and renal pathways that regulate BP and observed different effects on BP in males and females: In females, polymorphisms at β1-adrenergic receptor (AR) and α2A-AR contributed to BP, while in males polymorphisms at β2-AR and angiotensinogen (AGT) were associated. Combination of SNPs within a single gene (haplotypes) also influenced BP differentially in males and females: An α2A-AR haplotype influenced BP in females, while two AGT haplotypes were associated with increased BP in males. The results thus reveal gender-specific effects of SNPs and haplotypes that determine BP in Caucasian-Americans and demonstrate the power of an approach based on population extremes for identifying genetic contributors to physiological (e.g. cardiovascular) traits, even ones that can be influenced by multiple genes with small contributions to variance. Moreover, the findings imply that gender should be taken into account in the development of genotype-based diagnostic and therapeutic treatment for hypertension.

# 2007 APS Conference: Sex Steroids and Gender in Cardiovascular-Renal Physiology and Pathophysiology

## ABSTRACTS OF INVITED AND VOLUNTEERED PRESENTATIONS

### 10.8

#### EFFECT OF AGE AND ESTROGEN LOSS ON ESTROGEN RECEPTOR ALPHA AND BETA IN KIDNEY OF DAHL SALT SENSITIVE RATS

Ma. Eugenia Davila<sup>1</sup>, Teresa Craig<sup>1</sup>, Carmen Hinojosa-Laborde<sup>1</sup>

<sup>1</sup>Anesthesiology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX, 78229-3900.

We have shown that renal estrogen receptors alpha and beta (ERa and ERb) from female Dahl salt sensitive (DSS) rats are affected by either age or ovariectomy (OVX). At middle age, there is an increase in ERb compared to young rats, while old age was associated with an increase in both ERa and ERb. OVX in young DSS rats was associated with a decrease in ERa and an increase in ERb. Estrogen replacement in OVX rats reversed the over expression of ERb, but had no effect on ERa. In this study we determined the combined effect of aging and estrogen loss on renal ERa and ERb. We compared young (4 months), middle age (12-13 months) and old (15-18 months) DSS rats that were OVX, and OVX with estrogen replacement (OVX+E), which were initiated at 2 months of age. Western blot analysis was used to determine ERa and ERb levels reported as optical density. In OVX rats, renal ERa at middle age (0.20±0.01) and old (0.22±0.03) were significantly greater ( $p < 0.01$ ) than young (0.05±0.01), while ERb was similar in young (0.29±0.01), middle age (0.26±0.02) and old (0.24±0.02). In OVX+E, renal ERa at middle age (0.19±0.04) was significantly greater than young (0.04±0.01) and old (0.07±0.01) rats, while ERb was similar in young (0.13±0.02), middle age (0.14±0.09) and old (0.10±0.01). These results indicate that renal ERa levels increased with age in OVX and OVX+E. In contrast, renal ERb was not altered by aging, in OVX and OVX+E. We conclude that renal ERa levels are affected by aging, while renal ERb are affected by circulating estrogen.

### 10.9

#### SEXUAL DIMORPHIC REGULATION OF AQP2 IN DOCA-SALT HYPERTENSION

Shyama Masilamani<sup>1</sup>, Collin Berry<sup>1</sup>, Teddy Musselman<sup>1</sup>, Zheng Zhang<sup>1</sup>

<sup>1</sup>Internal Medicine/Nephrology, VCU Medical Center, 1101 East Marshall St., Sanger Hall 8-056, Box 980160, Richmond, Virginia, 23298-0160.

Hypertension is more prevalent and more severe in men than pre-menopausal women. We used the DOCA-salt hypertensive rat model to examine if a sexual dimorphic relationship is seen in the development of hypertension in this model. Further, we determined if an altered role of aquaporin 2 (AQP2) regulation occurs in this sex-associated difference in the regulation of blood pressure in this hypertensive model. Radio-telemetry units were implanted for measurement of mean arterial blood pressure in conscious, unrestrained DOCA-salt hypertensive male and female rats. Chronic measurement of blood pressure in conscious DOCA-salt rats demonstrated that intact male rats had higher blood pressures compared to intact females (intact male=164±3; intact female=136±4,  $p < 0.05$ ) thereby replicating the differential between men and women. Twelve days following induction of hypertension, AQP2 expression was determined by semi quantitative immunoblotting of kidney homogenates from cortex. In cortical homogenates, a down regulation of AQP2 was seen in DOCA-salt intact female compared to intact male rats (intact male = 100±6; intact female = 53±6, % of control,  $p < 0.05$ ). This down regulation of AQP2 expression may in part be involved with the diminished severity of hypertension in female DOCA-salt rats compared to their male counterparts.

### 10.10

#### ROLE OF HYDROGEN PEROXIDE IN MEDIATING HYPERTENSION AND PROTEINURIA IN FEMALE SHR

Julio Sartori-Valinotti<sup>1</sup>, Wanda Dorsett-Martin<sup>2</sup>, Radu Iliescu<sup>1</sup>, Licy Yanes<sup>1</sup>, Jane Reckelhoff<sup>1</sup>

<sup>1</sup>Physiology and Biophysics, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS, 39216, <sup>2</sup>Surgery, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS, 39216.

Reactive oxygen species (ROS) such as superoxide anion (O<sub>2</sub><sup>-</sup>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) are implicated in the development of hypertension. Males exhibit greater oxidative stress (OS) and blood pressure (BP) than females. O<sub>2</sub><sup>-</sup> is important in the maintenance of hypertension in male SHR, since the superoxide dismutase mimetic tempol and the NADPH oxidase inhibitor apocynin reduces the BP in male but not female SHR. In light of these observations, we hypothesized that H<sub>2</sub>O<sub>2</sub>, rather than O<sub>2</sub><sup>-</sup>, is responsible for mediating the hypertension in female SHR and that treatment with the glutathione peroxidase mimetic ebselen will reduce both BP and proteinuria. Methods: Female SHR (245-300 g) were assigned to receive either vehicle (5% Carboxymethylcellulose) or ebselen (7.5mg/kg) twice a day by gavage for ten days. BP was recorded by telemetry. Proteinuria was assessed prior to administration of the drug and at the end of the experimental protocol. Results: Ebselen has no effect on BP (last 24 hrs MAP [mmHg]: 132±1 vs. 127±1 control) or protein excretion (2.8±0 vs. 3.8±1 mg/24hrs, vs. control). These data suggest that H<sub>2</sub>O<sub>2</sub> is not important in the hypertension of female SHR. Taken together with our previous observations, it is likely that OS mediates hypertension in male but not female SHR.

### 10.11

#### EARLY DIABETIC KIDNEY DAMAGE IN THE MOUSE VCD MODEL OF MENOPAUSE

Maggie Keck<sup>1</sup>, Melissa J. Romero-Aleshire<sup>1</sup>, Qi Cai<sup>1</sup>, Patricia B. Hoyer<sup>1</sup>, Heddyven L. Brooks<sup>1</sup>

<sup>1</sup>Physiology, University of Arizona, 1501 N Campbell Ave, PO Box 245051, Tucson, AZ, 85724.

Perimenopause, the 5-10 years preceding menopause in women, is increasingly recognized as a critical period in the development and treatment of many diseases. The extent to which ovarian hormones impact the development of diabetic nephropathy is not well studied. We hypothesized that changes in ovarian hormones across the menopausal transition promote the development of diabetic kidney damage. We used the 4-vinylcyclohexene diepoxide (VCD) model of menopause, which mimics the perimenopause period (PE) and postmenopausal (PO) ovarian androgen production, in combination with the streptozotocin (STZ) model of diabetes. B6C3F1 female mice were injected with STZ during PE or 2 weeks PO; 6 weeks later kidneys were processed for microarray and real-time PCR or immunohistochemistry. Blood glucose was elevated in PO diabetic mice compared to PE and cycling diabetic mice (336 ± 31 vs 238 ± 32 and 218 ± 37 mg/dL). Proliferating cell nuclear antigen protein expression, a marker of early kidney damage, was greater in PO diabetic than in cycling diabetic mice ( $P < 0.05$ ). Using microarray, we identified 66 genes that are differentially expressed in diabetic kidneys dependant on hormonal status. Midkine (Mdk) was identified by the microarray as increased 1.68 fold in PO diabetic kidneys compared cycling diabetic kidneys ( $P < 0.05$ ). Mdk is regulated by estrogen, and Mdk knockout mice develop less severe diabetic nephropathy. These data suggest changes in ovarian hormone production across the menopausal transition promote the development of diabetic kidney disease. Funding: NIH AG-021948 to PBH.

### 10.12

#### SEX DIFFERENCES IN THE RESPONSE TO VASOACTIVE SUBSTANCES IN EARLY UNCONTROLLED DIABETES

Adam Mitchell<sup>1</sup>, Adam Myers<sup>1</sup>, Susan Mulrony<sup>1</sup>

<sup>1</sup>Physiology and Biophysics, Georgetown University, 3900 Reservoir Road NW, Washington, DC, 20007.

Diabetes is associated with development of vascular disease, and we hypothesize that there are early changes in vascular reactivity in the diabetic animal that are sex-related. Adult male and female S-D rats were divided into sham, diabetic (STZ), or STZ+GH to mimic the human diabetic milieu. After eight weeks, mesenteric arteriolar vascular reactivity to PE and ACh was performed on a wire myograph. STZ caused a profound decrease in the vasodilatory response to ACh in both male and female vessels, independent of GH. While there was no change in the constrictor response to PE in vessels from male diabetic rats, PE response increased 103% ( $P < 0.05$  vs sham) in vessels from female STZ rats, which was prevented in STZ+GH rats (ns from sham). In the presence of L-NAME (to block NO), female sham had a larger change in PE reactivity than STZ (7- vs 5-fold), indicating a reduction of NO in the STZ rat. This contrasted with the male, with STZ producing more NO than sham. In both sexes, STZ+GH increased NO. The effects of GH on vascular reactivity affirm its importance in the rat model of human diabetes. Together, these findings support the hypothesis of early vascular changes in diabetes, which may include a loss of endothelial function in female vasculature before major changes are observed in the male, suggesting females may be more susceptible to early vascular disease development. (Supported by NIH R01 DK064916 and National Kidney Foundation of the National Capital Area).

### 10.13

#### SEX DIFFERENCES IN DIABETIC RENAL REMODELING: EFFECTS OF GROWTH HORMONE

Jennifer Rogers<sup>1</sup>, Christine Maric<sup>2</sup>, Kathryn Sandberg<sup>2</sup>, Susan Mulrony<sup>2</sup>

<sup>1</sup>Physiology and Biophysics, Georgetown University, BSB Rm 256, 3900 Reservoir Rd, Washington, DC, 20007, <sup>2</sup>Physiology, Georgetown University, 3900 Reservoir Rd, Washington, DC, 20007.

While GH levels are elevated in human diabetes, pulsatile GH release is abolished in the streptozotocin (STZ) diabetic rat. We hypothesize that replacement GH will result in a more human-like disease process including early renal remodeling, which will be exacerbated in the male kidneys. Male and female S-D rats were divided into three groups; Sham, STZ (55 mg/kg ip), and STZ+GH (2.5µg GH, 2X daily) and sacrificed after 8 weeks. The Glomerular Sclerotic Index (GSI) was increased in female STZ kidneys (0.79±0.06 vs 0.20±0.03 in Sham,  $P < 0.05$ ), without additional effects of GH (0.87±0.07); kidneys from male STZ rats, however, had an increased GSI (0.90±0.02 vs 0.26±0.05,  $P < 0.05$ ), and there was a greater effect in STZ+GH (1.08±0.11,  $P < 0.05$  vs STZ). Males also exhibited a greater GH response in the Tubular Interstitial Fibrotic Index (TIFI): STZ increased the TIFI in both male and female rats from Sham [F (1.21±0.06 vs 0.44±0.07); M (1.37±0.03 vs 0.54±0.02),  $P < 0.01$ ] and while GH significantly increased the female TIFI (1.67±0.05,  $P < 0.05$  vs STZ) the male response was significantly higher than the female (1.99±0.09,  $P < 0.05$  vs F STZ+GH). STZ increased albuminuria, [F (0.42±0.08 vs 11.3±2.71 mg/dL); M (10.38±0.82 vs 1.96±0.07 mg/dL)  $P < 0.01$ ], but again, while GH had no additional effects in females (8.62±1.78 mg/dL), in males there was a marked increase in the presence of GH (16.15±1.67,  $P < 0.05$  vs STZ). This data supports our hypothesis that GH is important in diabetic renal disease, and has a preferential effect on male kidneys to exacerbate renal remodeling, that is not observed in females. Funded by R01DK064916.

### 10.14

#### ROLE OF ESTROGENS IN POSTMENOPAUSAL OBESITY AND HYPERTENSION

L. Fortepiani<sup>1</sup>, H. Zhang<sup>2</sup>

<sup>1</sup>Physiology, UTHSCSA, 7300 Floyd Curl Dr., San Antonio, TX, 78229, <sup>2</sup>Physiology, UMMC, 2500 N. State St., Jackson, MS, 39216.

Protection against cardiovascular and renal disease in women is lost when they reach menopause. The incidence of obesity also increases in this low estrogen environment, which can raise blood pressure (BP) by activating the renin angiotensin system (RAS) or via leptin induced increases in sympathetic nervous system (SNS) activity. We hypothesize that estrogen deficiency after menopause leads to obesity related metabolic changes that may activate vasopressor systems, contributing to obesity induced postmenopausal hypertension and renal disease. We used 8 month old intact female and ovariectomized (ovx) rats, with or without estrogen replacement. OvX rats exhibit higher BP than aged matched intact and estrogen repleted female (126.2±3 vs 110.6±2 and 102.6±3 mmHg respectively). However renal function and hemodynamics indexes remained unchanged. Body weight (BW) gain with age was 2 fold higher in ovx compared to intact females and it was accompanied by increases in metabolic indexes, leptin (70%) and blood glucose (35%). We also found that RAS, determined as plasma renin activity, and SNS, determined as renal norepinephrine, were also increased after ovx by 16% and 39% respectively. All these hormonal and metabolic changes were reverted by estrogen administration. In summary, ovx in old female rat increased BW, leptin and elevated BP together with the activation of RAS and renal SNS. These effects were completely abolished with estrogen replacement. These results suggest that estrogen deficiency in aged female rats may trigger the development of obesity and postmenopausal hypertension in the absence of renal disease. This work was supported by NIH AG029250-01 award.

### 10.15

#### FEMALE GENDER PROTECTS OBESE RATS FROM NEPHROPATHY OF THE METABOLIC SYNDROME

Jesus Dominguez<sup>1</sup>, Katherine Kelly<sup>2</sup>

<sup>1</sup>Medicine, VAMC and IUMC, VAMC, N111, 1481 W. 10th St., Indianapolis, Indiana, 46202, <sup>2</sup>Medicine, IUMC, 950 Walnut St., R2-202, Indianapolis, Indiana, 46202.

Renal vasculopathy and interstitial fibrosis are more prevalent in men, while premenopausal women seem protected. We hypothesized that female sex (either from gender-specific or estrogen-specific effects) protects kidneys of rats with the metabolic syndrome. These experiments were conducted according to APS guiding principles for the care and use of animals. We fed ad-lib obese male (OM) and female (OF) ZSF<sub>1</sub> hybrid rats two separate diets: 27% protein (diet A: OMA, n = 10 and OFA, n = 13) or 10% protein (diet B: OMB, n = 3 and OFB, n = 14) for 36 weeks: Hyperglycemia (mM; M±SE) was present in all: OMA, 19±4; OFA, 10±2; OMB, 32±1; and OFB, 19±1. Body weight (BW, gm) in OMA, 659±23; was higher than OFA, 580±16 ( $p < 0.01$ ); highest in OMB, 847±28 ( $p < 0.01$ ); while OFB was 575±10. Kidney

# 2007 APS Conference: Sex Steroids and Gender in Cardiovascular-Renal Physiology and Pathophysiology

## ABSTRACTS OF INVITED AND VOLUNTEERED PRESENTATIONS

weight (gm/100 gm BW) was highest in OMA:  $0.51 \pm 0.02$  ( $p < 0.01$ ); and equal in all others:  $0.31 \pm 0.01$ ;  $0.42 \pm 0.03$ , and  $0.35 \pm 0.01$ . Interstitial fibrosis (fibrosis index: 1-4) was worse in OMA,  $3.8 \pm 0.03$  than OFA  $1.6 \pm 0.5$  ( $p < 0.01$ ), OMB,  $1.9 \pm 0.10$ , and OFB,  $1.1 \pm 0.2$ . Kidney TGF $\beta$ 1 (pg/mg prot.) was highest in OMA  $710 \pm 65$  ( $p < 0.01$ ) than OFA,  $295 \pm 60$ ; OMB,  $370 \pm 40$ ; and OFB,  $270 \pm 50$ . Serum creatinine ( $\mu$ M) was highest in OMA:  $100 \pm 16$ , and equal in all others:  $46 \pm 1$ ;  $50 \pm 3$ ; and  $42 \pm 1$ . Renal fibrosis and failure was more severe in OMA than in OFA eating the higher protein diet. In contrast, kidneys in OMB and OFB were relatively unaffected when eating the lower protein diet. Hyperglycemia did not influence the renal outcome. We conclude that: Female gender offers protection from renal injury and fibrosis in obese rats on high protein diets. The diet composition was a critical determinant for this type of nephropathy, while hyperglycemia by itself was less significant.

### 10.16

#### GENDER-DEPENDENT METABOLIC AND RENAL EFFECTS OF 2-HYDROXYESTRADIOL IN OBESE DIABETIC ZSF<sub>1</sub> RATS.

Stevan Tofovic<sup>1</sup>, Sheldon Bastacky<sup>2</sup>, Edwin Jackson<sup>3</sup>

<sup>1</sup>Department of Medicine, University of Pittsburgh School of Medicine, 100 Technology Drive, Pittsburgh, PA, 15219, <sup>2</sup>Department of Pathology, University of Pittsburgh School of Medicine, 200 Lothrop Street, Pittsburgh, PA, 15213, <sup>3</sup>Department of Pharmacology, University of Pittsburgh School of Medicine, 100, Technology Drive, Pittsburgh, PA, 15219.

Previously we have demonstrated that 2-hydroxyestradiol (2HE), an estradiol metabolite with some estrogenic activity, provides cardiovascular and renal protection in young, male, obese ZSF<sub>1</sub> rats. However, these effects were associated with significantly reduced food consumption. Therefore, the objective of this study was to examine the metabolic and renal effects of long-term treatment (32 weeks) with 2HE (10  $\mu$ g/kg/h) and pair-feeding (vs. 2HE) in obese diabetic male and ovariectomized (OVX) female ZSF<sub>1</sub> rats. 2HE improved the oral glucose tolerance test and reduced HbA<sub>1c</sub>, leptin and cholesterol levels and exhibited significant estrogenic effects in male rats. Ovariectomy did not affect glucose homeostasis and, in the face of increased food consumption and body weight, decreased plasma cholesterol and triglycerides levels, proteinuria and renal injury. In OVX rats, 2HE markedly increased triglycerides and cholesterol levels, augmented proteinuria and renal hypertrophy, and increased glomerulosclerosis. This study suggests limited value of estradiol metabolites with estrogenic activity in metabolic syndrome associated renal disease. Further investigation of non-estrogenic metabolites of estradiol (i.e., 2-methoxyestradiol) in diabetic kidney is warranted.

### 10.17

#### DYSREGULATED ESTRADIOL METABOLISM IN PREECLAMPSIA

Stevan Tofovic<sup>1</sup>, Edwin Jackson<sup>2</sup>, Gligor Tofovic<sup>3</sup>

<sup>1</sup>Center for Clinical Pharmacology Department of Medicine, University of Pittsburgh School of Medicine, 100 Technology Drive, Suite 450, Pittsburgh, PA, 15219, <sup>2</sup>Center for Clinical Pharmacology, Department of Pharmacology, University of Pittsburgh School of Medicine, 100 Technology Drive Suite 450, Pittsburgh, PA, 15219, <sup>3</sup>Department of Obstetrics and Gynecology, University St Cyril and Methodius School of Medicine, 50 Divizija bb, Skopje, Macedonia, 1000.

There is a line of evidence suggesting that the cardiovascular protective effects of estradiol (E<sub>2</sub>) are mediated by its non-estrogenic metabolite 2-methoxyestradiol (2ME). 2ME is a product of E<sub>2</sub> 2-hydroxylation and subsequent O-methylation by catechol-O-methyltransferase (COMT). Placental and umbilical cord E<sub>2</sub> levels are similar in preeclamptic (PP) and normal pregnancies (NP). However, induction of E<sub>2</sub> metabolism reduces the risk of pregnancy-induced hypertension and eclampsia, whereas low COMT activity is associated with gestational hypertension and reduced fetal growth. No data are available regarding the presence of E<sub>2</sub> metabolites in humans. Therefore, in the present study, by using a highly sensitive and selective LC-MS/MS method, we measured plasma concentrations of 2ME and 2-methoxyestrone (2ME1) in healthy men and women, and in women with NP and PP. 2ME was detected in 9 of 36 healthy young men ( $361 \pm 73$  pg/ml). In non-pregnant premenopausal women no 2ME1 and  $589 \pm 65$  pg/ml of 2ME were detected. 2ME1 levels tended to increase and 2ME levels tended to decrease in PP group, and a significantly higher 2ME/2ME1 ratio was determined in NP compared to PP group ( $4.4 \pm 1.08$  vs.  $1.32 \pm 0.30$ ;  $p < 0.03$ ). In summary, we report the presence of 2ME in a significant number (25%) of healthy men. Moreover, the present data suggest altered E<sub>2</sub> metabolism and E<sub>2</sub> metabolite disposition in preeclampsia.

### 10.18

#### SLEEP DEPRIVATION AND NOCTURNAL URINE OUTPUT-GENDER DIFFERENCE IN THE EFFECT

B Mahler<sup>1</sup>, K Kamperis<sup>2</sup>, S Hagstroem<sup>1</sup>, E Radvanska<sup>1</sup>, S Rittig<sup>2</sup>, JC Djurhuus<sup>1</sup>

<sup>1</sup>Clinical institute, Aarhus University Hospital, Brendstrupgaardsvej, Aarhus, 8200, Denmark, <sup>2</sup>Pediatric Department, Aarhus University Hospital, Brendstrupgaardsvej, Aarhus, 8200, Denmark.

**Aim:** To investigate the impact of sleep on the nocturnal urine production, salt and water regulating hormones and hemodynamics. **Material:** 20 healthy volunteers (10 males), underwent two 24-hour circadian in-patient studies under standardized conditions regarding diet and fluid intake. Blood samples were drawn every three hours and urine was collected in 3-hour intervals. Blood pressure and heart rate were non-invasively monitored. The participants were randomized to sleep deprivation during one of the two studies. Atrial natriuretic peptide, angiotensin II, aldosterone, and renin were measured in blood. Excretions and clearances were calculated for electrolytes and osmoles. **Results:** During sleep deprivation both genders produced markedly larger amounts of urine, an effect that was more pronounced for males ( $1.05 \pm 0.10$  ml/h/kg vs.  $1.82 \pm 0.22$  ml/h/kg, females  $0.98 \pm 0.09$  ml/h/kg vs.  $1.41 \pm 0.11$  ml/h/kg,  $p < 0.001$ ). An increased urinary excretion of sodium (baseline:  $0.06 \pm 0.01$  mmol/kg/h, sleep deprivation:  $0.12 \pm 0.01$  mmol/kg/h), potassium and osmoles was seen. The night-time dip in blood pressure was less evident during sleep deprivation (baseline:  $76.5 \pm 12.4$  mmHg, sleep deprivation:  $83.2 \pm 11.0$  mmHg,  $p < 0.001$ ) but no effect on heart rate was seen. The compromised fall in mean arterial blood pressure correlated significantly to increase in night time urine volume. Sleep deprivation induced a significant fall in night time plasma renin ( $p < 0.05$ ), angiotensin II ( $p < 0.001$ ) and aldosterone ( $p < 0.05$ ) in both genders. **Conclusion:** Sleep deprivation leads to natriuresis, kaliuresis and osmotic diuresis. The increase in urine output was more evident in males. Altered hemodynamics seems to account for these observations.

### 10.19

#### GENDER BIAS TOWARD A FUNCTIONAL SUBCLASS OF MYELINATED VISCERAL AFFERENT

Baiyan Li<sup>1</sup>, John Schild<sup>2</sup>

<sup>1</sup>Biomedical Engineering, IUPUI, 723 W. Michigan Street, Suite SL174, Indianapolis, Indiana, 46236, <sup>2</sup>Biomedical Engineering, IUPUI, 723 West Michigan Street Suite SL174, Indianapolis, Indiana, 46236.

Compelling evidence exists concerning gender differences across the spectrum of cardiovascular function even extending to the vasoprotective effects of hormone replacement therapy. Estrogen as well as estrogen effected autocrine and paracrine mechanisms (e.g. vasoactive endothelial factors, prostaglandins) can impact arterial blood pressure. Most studies have been conducted from a systemic view point. To date, very little is known concerning any potential for gender differences associated with afferent pathways that contribute to neurocirculatory control, despite the compelling evidence that men and women regulate BP differently and have markedly different experiences and sensation thresholds related to cardiac pain. Our lab has recently established analytical and chemical methods for reliable classification of isolated visceral neurons as either one of two functionally distinct classes of myelinated (A-type and Ah-type) or as unmyelinated (C-type) afferents (Li & Schild, 2007). Here we present data from six separate patch recording sessions using enzymatically dispersed nodose neurons from six day old gender identified neonatal rat pups. The female group (4 pups) and the male group (2 pups) yielded current clamped action potential recordings from 54 and 27 randomly selected cells, respectively. Classification methods revealed the following distributions for female pups: A = 10 (19%); Ah = 11 (20%); C = 33 (61%) and for male pups: A = 8 (30%); Ah = 2 (7%); C = 17 (63 %). These data demonstrate a greater prevalence for Ah-type myelinated afferents in female as compared with age-matched male rat pups. We also show that these Ah-type myelinated afferents express a TTX-resistant Na<sup>+</sup> current that can be markedly up-regulated by prostaglandin E<sub>2</sub> via a cAMP-dependent pathway.

### 10.20

#### ESTROGEN ALTERS MYOSIN HEAVY CHAIN ISOFORM EXPRESSION OF RAT VAGINAL SMOOTH MUSCLE.

M. Basha<sup>1</sup>, Tanchun Wang<sup>1</sup>, J. Lassmann<sup>2</sup>, R. Moreland<sup>1</sup>, A. J. Wein<sup>3</sup>, S. Chacko<sup>2</sup>

<sup>1</sup>Pharmacology & Physiology, Drexel Univ. Col. of Med., 245 N. 15th St., Philadelphia, PA, 19102, <sup>2</sup>Surgery, Univ. of Pennsylvania, 500 S. Ridgeway Ave, Glenolden, PA, 19036.

Although it has been established that estrogen depletion causes general atrophy of vaginal tissue, altered innervation, and reduced blood flow to this organ, little is known of the influence of estrogen on vaginal smooth muscle (VSM). The carboxyl terminal isoforms (SM1 and SM2) of myosin heavy chain (MHC) have been shown to be estrogen dependent in the uterus, bladder and vasculature. The objective of this study was to determine the affect of estrogen status on MHC isoform expression and contractility of VSM. The vagina was harvested from sham operated (S), 3-week ovariectomized (O) and 1-week estrogen-replaced (E) Sprague-Dawley rats. RNA was reverse transcribed and semi-quantitative RT-PCR was performed using primer pairs to amplify SMA/SMB and SM1/SM2 isoforms. MHC and beta-actin protein was detected by Western blotting and SM1/SM2 protein was stained by Coomassie blue. Muscle strip studies were performed to measure maximal velocity of shortening ( $v_{max}$ ) and muscle stress in response to 110 mM KCl. Densitometric analysis of RT-PCR and Coomassie results indicated that ovariectomy increased the % SM2 expression of the vagina, an effect reversed in E rats. No differences were detected in SMA/SMB or MHC expression. There was a decreased  $v_{max}$  and increased muscle stress of vaginal strips from O rats compared to S rats. The results of these studies indicate that estrogen alters molecular and functional characteristics of VSM. Further studies are required to determine whether these changes in smooth muscle contribute to vaginal dysfunction experienced by post-menopausal women. Funding sources: T32-DK007708; P50-DK-052620.

### 10.21

#### SEX HORMONES CONTRIBUTE TO GENDER DIFFERENCES IN PROGRAMMED HYPERTENSION INDUCED BY PLACENTAL INSUFFICIENCY IN THE RAT.

Norma Ojeda<sup>1</sup>, Daniela Grigore<sup>1</sup>, Elliott Robertson<sup>1</sup>, Barbara Alexander<sup>1</sup>

<sup>1</sup>Physiology, University of Mississippi-Medical Center, 2500 North State St., Jackson, Mississippi, 39216.

Gender differences in programmed hypertension are well established in animal models and human epidemiological studies; however, the mechanism(s) involved in mediating sex differences are unclear. Our laboratory uses a rat model of placental insufficiency that results in intrauterine growth restricted offspring (IUGR) that develop hypertension at a pre-pubertal age in both sexes. However, after puberty only male IUGR remain hypertensive whereas female IUGR become normotensive. Thus, the purpose of this study was to determine whether sex hormones contribute to gender differences in this model of IUGR. Castration (CTX) or ovariectomy (OVX) was performed at 10 weeks of age in male or female, respectively; mean arterial pressure (MAP) was measured by radiotelemetry from 12 to 16 weeks; testosterone (T) or estradiol (E<sub>2</sub>) replacement therapy were administered from 14 to 16 weeks in CTX male or OVX female. Gonadectomy abolished hypertension in male IUGR ( $145 \pm 4$  vs.  $104 \pm 2$  mmHg;  $P < 0.05$ , intact vs. CTX, respectively) and induced hypertension in female IUGR ( $122 \pm 2$  vs.  $140 \pm 2$  mmHg;  $P < 0.05$ , intact vs. OVX, respectively). Hormone replacement reinstated hypertension in male CTX-IUGR ( $143 \pm 3$  mmHg;  $P < 0.05$  vs. untreated counterpart) and abolished hypertension in female OVX-IUGR ( $111 \pm 3$  mmHg  $P < 0.05$  vs. untreated counterpart). Therefore, these results suggest that sex hormones contribute to gender differences in programmed hypertension.

### 10.22

#### SEX DIFFERENCES IN RENAL FUNCTION OF BETAMETHASONE-TREATED SHEEP: A MODEL OF FETAL PROGRAMMING

TanYa Gwathmey<sup>1</sup>, Lijuan Tang<sup>2</sup>, Jorge Figueroa<sup>2</sup>, Mark Chappell<sup>1</sup>, James Rose<sup>2</sup>

<sup>1</sup>Hypertension Center, Wake Forest University, Medical Center Blvd, Winston-Salem, NC, 27157-1032, <sup>2</sup>Department of Obstetrics & Gynecology, Wake Forest University, Medical Center Blvd, Winston-Salem, NC, 27157-1032.

The prenatal exposure of the ovine fetus to clinical doses of glucocorticoids during the time of peak nephrogenesis results in a marked reduction in nephron number in adulthood, as well as a sustained 10-15 mmHg increase in blood pressure. In the present study, we examined the gender difference in renal function to betamethasone at day 80 of gestation in 16 ewes and 15 rams at 1.5 years of age. Following recovery from the surgical placement of vascular and bladder catheters, glomerular filtration rate (GFR) - estimated as clearance of inulin and renal plasma flow (RPF) as determined by clearance of p-aminohippuric acid (PAH) were assayed by an initial loading dose followed with constant intravenous (i.v.) infusion of 0.9% sterile saline containing inulin and PAH. An acute sodium load was administered by a continuous infusion of hypertonic NaCl (0.0375 mEq/Kg/min at 0.55 ml/min for 60 minutes) within the period of PAH/inulin infusion. In the male betamethasone sheep, inulin clearance significantly decreased ( $154 \pm 18$  ml/min) as compared with male controls ( $213 \pm 18$  ml/min,  $p < 0.05$ ). The PAH clearance was also significantly lower in the male prenatal betamethasone exposed group ( $883 \pm 65$  ml/min)

# 2007 APS Conference: Sex Steroids and Gender in Cardiovascular-Renal Physiology and Pathophysiology

## ABSTRACTS OF INVITED AND VOLUNTEERED PRESENTATIONS

than controls ( $1079 \pm 30$  ml/min,  $p < 0.05$ ). In contrast, there were no apparent differences of inulin/PAH clearances between the female control and treated groups. These data suggest that prenatal exposure to glucocorticoids alters renal function in adult male sheep by potentially decreasing GFR and RPF, however, the females are protected from this steroid-induced effect. NIH grants HD-47584, HL-68728 & HL-56973.

### 10.23

#### REDUCED UTERINE PERFUSION PRESSURE INCREASES SOLUBLE Flt-1 EXPRESSION IN PREGNANT RATS

Jeffrey Gilbert<sup>1</sup>, Babbette LaMarca<sup>1</sup>, Sara Babcock<sup>1</sup>, Kathy Cockrell<sup>1</sup>, Joey Granger<sup>1</sup>

<sup>1</sup>Physiology, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS, 39216-4505.

In preeclampsia the balance between pro- and anti-angiogenic factors is thought to be altered to favor an anti-angiogenic state that is evidenced by a marked increase in the soluble VEGF receptor fms-like tyrosine kinase-1 (sFlt-1). Recent evidence suggests that placental hypoxia and possibly poor placental perfusion may initiate this imbalance of angiogenic factors. We hypothesized that the hypertension produced by reduced uterine perfusion pressure (RUPP) in the pregnant rat is associated with increases in plasma and amniotic fluid sFlt-1 concentration and increased expression of sFlt-1 in the placenta of the pregnant rat. AP was increased ( $130 \pm 2.8$  v.  $100 \pm 2.4$  mm Hg;  $P < 0.01$ ) and fetal weight ( $1.9 \pm 0.05$  v.  $2.3 \pm 0.05$  g;  $P < 0.01$ ) was decreased in the RUPP compared to the normal pregnant (NP) controls. Plasma sFlt-1 concentration ( $660 \pm 270$  v.  $82 \pm 26$  pg/ml;  $P < 0.05$ ) was increased 8 fold while amniotic sFlt-1 concentration was increased was increased 10% ( $5800 \pm 160$  v.  $5200 \pm 130$  pg/ml;  $P < 0.03$ ) in the RUPP compared to NP dams. Immunoreactive placental sFlt-1, expressed as the ratio of sFlt-1- $\beta$ -actin, was increased 4 fold ( $1.1 \pm 0.1$  v.  $0.3 \pm 0.1$ ;  $P < 0.01$ ) in the RUPP dams versus the normal pregnant (NP) controls. The present findings support our hypothesis that decreased placental perfusion increases the expression of sFlt-1 and may alter the balance of angiogenic factors in the maternal circulation. These data also indicate that the RUPP model of pregnancy induced hypertension may provide an invaluable model for mechanistic studies into the role of sFlt-1 in the pathogenesis of preeclampsia.

### 10.24

#### ELEVATED AGONISTIC AUTOANTIBODIES TO THE ANGIOTENSIN TYPE 1 (AT1-AA) RECEPTOR IN RESPONSE TO PLACENTAL ISCHEMIA AND TNF ALPHA IN PREGNANT RATS

Babbette LaMarca<sup>1</sup>, Ralf Dechend<sup>2</sup>, Gerd Wallukat<sup>2</sup>, Mayte Llinas<sup>1</sup>

<sup>1</sup>Physiology, University of Mississippi Medical Center, 2500 N State St, Jackson, MS, 39216, <sup>2</sup>Oberarzt, HELIOS Clinic, Wiltbergstrasse 50, D-13125, Berlin, Germany.

Circulating factors such as autoantibodies to the angiotensin II type I receptor (AT1-AA) and inflammatory cytokines including TNF alpha may serve as important links between with placental ischemia, maternal endothelial cell dysfunction, and the development of hypertension in women with preeclampsia. Recent studies have demonstrated the presence of AT1-AA in preeclamptic women, factors regulating production of the AT1-AA remain unclear. We report that the increase in mean arterial pressure in response to reductions in uterine perfusion pressure (RUPP) in pregnant rats ( $137 \pm 1$  mmHg, RUPP vs  $101 \pm 1$  mmHg, normal pregnant, NP) is associated with increased circulating levels of TNF alpha ( $48 \pm 13$  pg/ml RUPP vs  $8 \pm 1$  pg/ml NP) and the AT1-AA (RUPP  $15.3 \pm 1.6$  vs NP  $0.6 \pm 0.3$  units). These findings indicate that placental ischemia and inflammatory cytokines may be important stimuli for the production of AT1-AA in preeclampsia. Moreover, TNF alpha induced hypertension ( $97 \pm 2$  to  $112 \pm 2$  mm Hg) in pregnant rats is associated with increased production of the AT1-AA ( $9.2 \pm 2.3$ , TNF rats vs  $1.0 \pm 0.8$  units, NP rats). To determine the importance of AT1 receptor activation in mediating hypertension in RUPP and TNF treated rats, we administered an AT1 receptor antagonist to RUPP, TNF treated and normal pregnant rats. Blood pressure response was attenuated in RUPP rats ( $\Delta 32$  mmHg vs  $\Delta 20$  mmHg, NP) and TNF treated ( $\Delta 10$  mmHg vs  $\Delta 5$  mmHg NP). Collectively, these data indicate activation of the AT1 receptor, possibly via AT1-AA, appears to play an important role in the hypertension produced by placental ischemia and TNF in pregnant rats.

### 10.25

#### ALTERED CEREBRAL VASCULAR FUNCTION IN RESPONSE TO REDUCTIONS IN UTERINE PERFUSION IN PREGNANT RATS

Michael Ryan<sup>1</sup>, Gerald McLemore Jr.<sup>1</sup>, Joey Granger<sup>1</sup>, Babbette LaMarca<sup>1</sup>

<sup>1</sup>Physiology, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS, 39216.

Women with preeclampsia, or hypertension with proteinuria during pregnancy, are at increased risk for ischemic stroke and the development of cerebral edema. The underlying mechanisms leading to this remain unclear; however, some evidence suggests that autoregulation of cerebral blood flow may be impaired. In order to test whether autoregulation is altered, we used pregnant rats with reduced uterine perfusion pressure (RUPP) as a model of preeclampsia and normal pregnant rats as controls. Mean arterial pressure was  $139 \pm 6$  mmHg in RUPP rats compared with  $107 \pm 6$  in controls ( $p < 0.001$ ). Middle cerebral arteries (MCA) were isolated, cannulated, and pressurized to 75 mmHg at 37°C. Basal inner diameter from control and RUPP rats were  $178 \pm 8$   $\mu$ m and  $160 \pm 12$   $\mu$ m, respectively ( $p = 0.25$ ). In order to test MCA autoregulatory function, luminal pressure was increased incrementally from 25 to 150 mmHg under active (with  $Ca^{2+}$ ) and passive (0  $Ca^{2+}$ ) conditions. Tone developed from  $4 \pm 5\%$  at 25 mmHg to a maximum of  $13 \pm 3\%$  at 75 mmHg in MCA from control rats. In RUPP rats, tone was maximal at 25 mmHg ( $21 \pm 3\%$ ) and did not significantly change across the entire range of pressures ( $17 \pm 5\%$  at 150 mmHg). Therefore while RUPP rats have significant cerebral vascular tone; myogenic vasoconstriction in the MCA appears impaired. These data suggest that altered autoregulation may be a contributing mechanism to the cerebral vascular pathophysiology of preeclampsia.

### 10.26

#### VASOMOTOR SYMPATHETIC NEURAL CONTROL IS ENHANCED IN EARLY PREGNANT WOMEN

Qi Fu<sup>1</sup>, Shigeki Shibata<sup>1</sup>, Tiffany VanGundy<sup>1</sup>, Jeffrey Hastings<sup>1</sup>, Benjamin Levine<sup>1</sup>

<sup>1</sup>Exercise Physiology, Institute for Exercise and Environmental Medicine, Presbyterian Hospital of Dallas, 7232 Greenville Ave., Suite 435, Dallas, TX, 75231.

Pregnancy-induced hypertension and preeclampsia have been proposed to be associated with a hyperadrenergic state. However, the state of sympathetic neural regulation of blood pressure in normal pregnancy, especially during early pregnancy, is unclear. We completed a pilot study in a young healthy Caucasian woman during the very early stage (between 4–5 weeks) and the late stage (~35 weeks) of her pregnancy, and 7 weeks after delivery. Muscle sympathetic nerve

activity (MSNA) and hemodynamics were measured in the supine (rotated  $\sim 15^\circ$  into the left lateral) position. We found that during early pregnancy, her supine MSNA was extremely high (43 bursts/min), similar to those of congestive heart failure patients or individuals after chronic severe hypobaric hypoxia exposure. Supine MSNA decreased by  $\sim 28\%$ , while blood pressure increased slightly during late pregnancy, associated with plasma volume expansion and increased cardiac output. Seven weeks after delivery, supine MSNA returned to normal (5 bursts/min). Compared with postpartum, her supine MSNA was approximately 9 fold higher during early pregnancy. Consistent with this observation, marked sympathetic activation was also found in additional two healthy young women during the early stages (5 and 7 weeks) of their pregnancies (32 and 42 bursts/min). Interestingly, despite such a dramatic increase in MSNA, both blood pressure and peripheral vascular resistance decreased during early pregnancy in these three women. Some vasodilator factor(s) associated with early pregnancy must have initiated or countered the marked increase in MSNA in these subjects. Further studies are needed to verify these findings and to identify vasodilator biomarker(s) associated with early pregnancy in healthy humans.

### 10.27

#### ABILITY TO BUFFER CHANGES IN PH DURING ISCHEMIA – ARE THERE SEX DIFFERENCES IN THE NEWBORN HEART?

D. Quaglietta<sup>1,2</sup>, M.P. Belanger<sup>2</sup>, C. Wittnich<sup>1,2,3</sup>

<sup>1</sup>Cardiovascular Sciences Collaborative Program, <sup>2</sup>Department of Surgery, <sup>3</sup>Department of Physiology, University of Toronto, Toronto, Ontario, Canada.

Clinical studies in children have demonstrated that female sex is a risk factor for mortality following cardiac surgery. Sex differences in the development of lactic acidosis and the ability to buffer changes in pH may determine susceptibility to ischemic injury and affect post-ischemic ventricular function. The purpose of this study was to investigate sex differences in  $H^+$  accumulation during ischemia in the newborn heart, and to determine sex differences in the source and ability to buffer  $H^+$  (buffering capacity). Anaesthetized newborn (3–5 day old) female ( $n=13$ ) and male ( $n=7$ ) Yorkshire piglets were intubated, and mechanically ventilated to ensure normal blood gas and pH status. Following left ventricular (LV) *in vivo* biopsies, the heart was excised (onset of ischemia), placed in normothermic Krebs' physiological solution and a biopsy was taken at 60 minutes of ischemia. All tissues were analyzed for adenosine triphosphate (ATP), creatine phosphate (CP), anaerobic end-products lactate ( $\mu$ moles/g dry weight) and hydrogen ion ( $H^+$ ) ( $\times 10^8$  mol/L) and histidine concentration ( $\mu$ moles/g dry weight). Both sexes demonstrated a similar net decrease in ATP levels by 60 minutes of ischemia. Compared to males however, newborn females accumulated a significant 13% more lactate (females:  $221.8 \pm 18.5$ , vs. males:  $196.5 \pm 19.7$ ,  $p=0.016$ ) and a significant 47% greater  $H^+$  (females:  $68.2 \pm 22.4$ , vs. males:  $46.8 \pm 8.9$ ,  $p=0.028$ ). Females also demonstrated 22% lower baseline CP reserves indicating both lower energy levels and a lower buffering capacity. No significant differences in ventricular histidine levels were identified. Thus compared to males, newborn females develop greater lactic acidosis during ischemia that may be due to greater anaerobic glycolysis, ATP depletion, and lower buffering capacity as demonstrated by lower CP levels. These results suggest that the newborn female myocardium is at greater risk of ischemic injury and offers a potential explanation for worse outcome in female children after cardiac surgery. This work was supported by the Heart and Stroke Foundation of Ontario (T-4926).

### 10.28

#### ORTHOTOPIC LIVER TRANSPLANTATION IN NEWBORNS – LOWER SUCCESS RATES FROM FEMALE DONORS AND WHY ISCHEMIC METABOLISM MAY PLAY A ROLE

D. Quaglietta<sup>1,2</sup>, M.P. Belanger<sup>2</sup>, C. Wittnich<sup>1,2,3</sup>

<sup>1</sup>Cardiovascular Sciences Collaborative Program, <sup>2</sup>Department of Surgery, <sup>3</sup>Department of Physiology, University of Toronto, Toronto, Ontario, Canada.

Introduction: Outcome following pediatric orthotopic liver transplantation has been reported to be worse in children receiving organs from female donors. Development of tissue lactate acidosis during prolonged periods of liver ischemia prior to transplantation may result in greater ischemic injury and impair graft and patient survival. The purpose of this study was thus to investigate whether sex differences in anaerobic end-product accumulation exist between newborn male and female livers during no-flow ischemia. Methods: 3 day male ( $n=4$ ) and female ( $n=6$ ) piglets were anesthetized, intubated and mechanically ventilated to maintain normal blood gas and pH status. *In vivo* liver biopsies were taken from the same lobe in all animals, after which a large section of the liver was excised and placed in a solution of Krebs' Henseleit at 37°C and ischemic biopsies were taken at 15, 30, and 45 minutes. All biopsies were analyzed for anaerobic end-products lactate ( $\mu$ moles/g dry weight) and hydrogen ion ( $H^+$ ) ( $\times 10^8$  mol/L) content. Results: No sex differences in baseline lactate and  $H^+$  levels were noted. At 15 minutes of ischemia, livers from newborn females accumulated a significant 15% higher lactate compared to those of males ( $23.6 \pm 4.5$  vs.  $27.6 \pm 2.6$ ,  $p=0.01$ ), and this persisted throughout the remaining ischemic period ( $p < 0.05$ ). A similar profile was noted with  $H^+$  accumulation, where at 15 minutes of ischemia, livers from newborn females accumulated 30% more  $H^+$  compared to males ( $11.5 \pm 1.3$  vs.  $14.9 \pm 2.6$ ,  $p=0.008$ ), which was also sustained throughout the ischemic period ( $p < 0.05$ ). Conclusion: The larger and more rapid development of tissue lactic acidosis in newborn female livers may result in greater metabolic damage during ischemia and offer a potential explanation for pediatric studies reporting worse outcome of recipients receiving livers from female donors. This work was supported by the Heart and Stroke Foundation of Ontario (T-4926).

### 10.29

#### THE ROLE OF SEXUAL PROCESS IN THE REGULATION OF STRESS-INDUCED CARDIOVASCULAR RESPONSES IN RAT

Z. Ghodarzi<sup>1</sup>, N. Hydarieh<sup>1</sup>, A. Khorami<sup>1</sup>, S. Charkh-kar<sup>1</sup>, M. Behnavai<sup>1</sup>, A. Vahabzadeh<sup>1</sup>

<sup>1</sup>Neuroscience Div. (NGO), Iran Univ. of Med. Sci., PO Box 19395-3598, Tehran, Iran.

Stress is one of the main lines of our studies (Vahabzadeh and Fillenz, 1994). We have already investigated the role of sex hormones in both sexes on the stress-induced responses (Dehghani, et al., 2003). Although we focused in the behavioral responses, in the present studies we monitored the heart rate as a cardiovascular index. The present studies aimed to investigate the effect of changes in the balance of the autonomic nervous system within the sexual process on the regulation of stress-induced cardiovascular responses in rat. Sprague-Dawley rats (200–300g) from both sexes divided to 4 groups for each sex. For each groups 15 rats were used ( $n=15$ ). Control groups in both sex were intact; and only used for monitoring of the heart rate in normal condition. Second group were subjected to 5 minute tail pinch stress (Antelman, et al., 1975); and the changes in the heart rate were monitored as a stress response. 3rd group were subjected to normal sexual intercourse; and the 4th groups subjected to atropine as well as epinephrine (ip) in pharmacological doses with time course similar to the process of the normal sexual intercourse. With 6 hours interval, all experimental groups were subjected to 5 minutes tail pinch stress; and the changes in the heart rate were monitored as a stress response. Renal out put also

## 2007 APS Conference: Sex Steroids and Gender in Cardiovascular-Renal Physiology and Pathophysiology ABSTRACTS OF INVITED AND VOLUNTEERED PRESENTATIONS

was monitored for all groups. Variation calculated in the percentage base. All statistical analyses were carried out using absolute data and either student paired t-test (within same group) or ANOVA (within the different groups). The results show that regulation of the balance of parasympathetic and sympathetic systems within the sexual process reduce the activity of cardiovascular system by 27+/-4% (P<0.005, n=15); and the renal system by 33+/-3% (P<0.005, n=15) in the present studies. Similar results were obtained with the use of atropine and epinephrine. Stress-induced changes were similar in both sexes. The present data suggest that sexual process reduce stress-induced cardiovascular as well as renal activity; and enhance the tolerance for tail pinch stress. Although the present data may be considered as a poor physiological finding, on the base of these data as well as the light of the current literature one may come to conclusion that ANS balance as a peripheral index of the limbic system reduce the chance of anxiety-induced renal as well as cardiovascular failure in some cases.

### 10.30

#### AORTIC COARCTATION-INDUCED HYPERTENSION DURING PREGNANCY: A MODEL OF PREECLAMPSIA IN RATS

Ayotunde S. Adeagbo, Nancy L. Alsip, Jurandir J. Dalle Lucca.

Department of Physiology and Biophysics, and OUTCOMES RESEARCH<sup>®</sup> Institute, School of Medicine, University of Louisville, Louisville, KY 40202.

Normal pregnancy entails generalized vasodilatation, increases in blood flow, and decreased responsiveness to vasoconstrictors. Patients with preeclampsia show reduced blood flow to kidneys, uterus and placenta, and increased vasoconstriction leading to maternal hypertension. We induced hypertension in pregnant rats by aortic coarctation (ACOR) and determined hallmarks of human preeclampsia (i.e., proteinuria, decreased fetal viability, and impaired endothelium-dependent relaxations) in such rats. Pregnant (8-day gestation) rats were rendered hypertensive with silver clips (slit opening = 0.279 mm) placed around the aorta immediately distal to the renal arteries; sham rats (SHAM) were surgically opened but without silver clipping of aorta. On gestation days 16 and 17, urinary protein as well as arterial pressures and heart rates were measured. Mesenteric vascular beds were also perfused *ex-vivo* and constricted for acetylcholine-induced relaxation studies. Arterial pressures and urinary protein increased in ACOR (P<0.05) versus SHAM; fetal viability decreased (P<0.05) in ACOR versus SHAM rodents. Cirazoline ( $\alpha_1$ -adrenoreceptor selective agonist) constricted ACOR and SHAM vascular beds similarly but co-infusion of nitro-L-arginine constricted SHAM significantly more than ACOR vessels. Acetylcholine dose-dependently relaxed ACOR and SHAM mesenteric arteries, however, endothelium-derived hyperpolarizing factor (EDHF) via opening of  $K_{Ca}$  channels mediate acetylcholine relaxations in SHAM vessels while nitric oxide and EDHF mediate acetylcholine response in ACOR vessels. CONCLUSIONS: The ACOR rodents exhibited arterial hypertension, proteinuria, reduced fetal viability, and modifications in endothelium function and thus may serve as a suitable rodent model of the disease.

### 10.31

#### IUGR ALTERS COX-2 EXPRESSION THROUGH STEROID SIGNALING AND AFFECTS 11- $\beta$ -HYDROXYSTEROID DEHYDROGENASE TYPE 2 (11 $\beta$ -HSD2) CHROMATIN STRUCTURE IN THE RAT KIDNEY

M. Baserga, M. Hale, X. Yu, Q. Fu, C. Callaway, R. McKnight, and R. Lane.

Div. of Neonatology, Pediatrics, University of Utah SOM, Salt Lake City, UT.

**Background:** Our research goal is to understand the interplay between steroid biology and IUGR renal morbidities. The IUGR rat is characterized by elevated levels of fetal corticosterone, and altered postnatal chromatin structure. The former is associated with decreased renal expression of COX-2, a gene necessary for nephrogenesis. The latter is associated with decreased expression of 11 $\beta$ -HSD2, an enzyme that protects the kidney from the actions of corticosterone. Whether (1) the decreased expression of COX-2 is secondary specifically due to the elevation in corticosterone, or (2) the postnatal decrease in 11 $\beta$ -HSD2 is due to changes in epigenetic determinants of chromatin structure is not known. **Objective:** We hypothesized that (1) in utero administration of RU486 (a steroid antagonist) moderates the decrease in COX-2 expression; and (2) IUGR leads to postnatal changes in 11 $\beta$ -HSD2 chromatin structure. **Design/Methods:** IUGR and controls (CON) were induced at e19. For hypothesis (1), vehicle, 100 mg or 500 mg of RU486 was given at the time of surgery. Kidney was harvested at term (e21.5). For hypothesis (2), pups delivered spontaneously, litters culled to 6, and kidney harvested at d21 of postnatal life. 11 $\beta$ -HSD2 chromatin structure was analyzed using bisulfite modification (DNA methylation) and chromatin immunoprecipitation. **Results:** The 500 mg dose of RU486 increased IUGR e21.5 COX-2 mRNA levels versus vehicle (P = 0.004), in contrast to the 100 mg dose. In the d21 postnatal kidneys, IUGR significantly increased trimethylation of lysine 4 in the IUGR female 11 $\beta$ -HSD2 promoter versus controls, without affecting exon 2 (P = 0.014). In contrast, trimethylation of the 11 $\beta$ -HSD2 exon 2 was significantly increased in the IUGR male kidney (P = 0.006). **Conclusions:** We conclude that (1) a steroid antagonist RU486 moderates the effects of IUGR upon COX-2 mRNA levels and (2) IUGR alters 11 $\beta$ -HSD2 chromatin structure in the postnatal kidney. The link between the two studies is the concept that the IUGR kidney continues to be exposed to increased levels of corticosterone in the fetal and neonatal periods, whether it is secondary to IUGR or through decreased 11 $\beta$ -HSD2. We speculate that these exposures lead to different renal specific morbidities of IUGR, including decreased nephrogenesis and postnatal hypertension (supported by PCMC grant, MB).

## 11.0: SEX STEROIDS, PREGNANCY, PREECLAMPSIA, AND FETAL PROGRAMMING

### 11.1

#### ANG-(1-7) AND ACE2 IN HUMAN PREGNANCY

K. Bridget Brosnihan<sup>1</sup>, Lauren Anton<sup>1</sup>, David C. Merrill<sup>1</sup>

<sup>1</sup>Hypertension and Vascular Research Center, Wake Forest University School of Medicine, Medical Center Blvd, Winston-Salem, NC, 27157-1032.

Pregnancy is a condition in which the renin-angiotensin system (RAS) is elevated. With the discovery of angiotensin-(1-7) [Ang-(1-7)] and ACE2, a novel homologue of ACE, studies have shown that these new components of the RAS are regulated during pregnancy. The chorionic villi (CV) are an essential component of the placenta involved in maternal-fetal oxygen and nutrient transport. The presence and regulation of the RAS, in the CV of normal and preeclamptic placentas have not yet been examined. These studies assessed 1) the immunocytochemical distribution of Ang-(1-7) and ACE2 in the placenta of normal and preeclamptic pregnant subjects; 2) the regulation of the placental chorionic RAS during normal and preeclamptic pregnancies. Ang-(1-7) and ACE2 were found in the syncytiotrophoblast,

cytotrophoblast, endothelium, and vascular smooth muscle of primary and secondary villi. CV Ang II levels were significantly increased in preeclamptic subjects, without any change in Ang I and Ang-(1-7). There was a significant increase in the Ang II/Ang-(1-7). Aogen and AT1 receptor mRNAs were significantly increased in preeclamptic CV. No change was observed for renin, ACE or ACE2 mRNA. The mas receptor mRNA was at the detectable limit, but the AT2 receptor mRNA was not detectable. The AT1 receptor was the predominate receptor subtype with less than 15% of the AT2 and AT1-7 receptors. These results provide evidence for enhanced placental chorionic villous tissue expression of Aogen and AT1 receptor mRNA and Ang II levels in preeclamptic CV. These results indicate that the increased Ang II, resulting from increased Aogen, acting through the AT1 receptor may favor vasoconstriction in the chorionic villous tissue of the placenta leading ultimately to the abnormal regulation of maternal-fetal blood flow and thus development of preeclampsia.

### 11.3

#### PATHOPHYSIOLOGY OF HYPERTENSION DURING PREECLAMPSIA

Joey P. Granger University of Mississippi Med Center, Jackson MS

Despite being one of the leading causes of maternal death and a major contributor of maternal and perinatal morbidity, the mechanisms responsible for the pathogenesis of preeclampsia have not yet been fully elucidated. The initiating event in preeclampsia has been postulated to be reduced uteroplacental perfusion as a result of abnormal cytotrophoblast invasion of spiral arterioles. Placental ischemia is thought to lead to widespread activation/dysfunction of the maternal vascular endothelium which, in turn, causes hypertension by impairing renal function and increasing total peripheral resistance. Inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF) are thought to be important links between placental ischemia and cardiovascular and renal dysfunction. Supporting a potential role of cytokines in preeclampsia are findings that plasma levels of TNF and IL-6 are elevated in women with preeclampsia. Recent studies from our laboratory have indicated that chronic reductions in placental perfusion in pregnant animals are associated with enhanced production of inflammatory cytokines, such as TNF and IL-6. In addition, chronic infusion of either TNF alpha or IL-6 into normal pregnant rats results in significant increases in arterial pressure and a decrease in renal hemodynamics. TNF alpha activates the endothelin system in placenta, renal and vascular tissues whereas IL-6 stimulates the renin-angiotensin system. Collectively, these findings suggest that inflammatory cytokines play a role in causing hypertension in response to chronic reductions in uterine perfusion during pregnancy by activating multiple vasoactive pathways.

### 11.4

#### PREECLAMPSIA AND ANGIOGENIC FACTORS

S. Ananth Karumanchi<sup>1</sup>

<sup>1</sup>Medicine, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, RW 663, Boston, MA, 02215.

Imbalance of angiogenic growth factors in the maternal circulation contributes to the pathogenesis of preeclampsia. Soluble fms-like tyrosine kinase 1 (sFlt1), an endogenous anti-angiogenic protein that antagonizes vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) appears to be a central player in this paradigm. Exogenous gene transfer of sFlt1 into pregnant rats using an adenoviral vector produced hypertension, proteinuria and glomerular endotheliosis, the classical pathological renal lesion of preeclampsia. High serum sFlt1 and low serum free PlGF and free VEGF have been observed in preeclampsia. Abnormalities in these circulating angiogenic proteins also antedate clinical symptoms by several weeks. Another potential soluble factor secreted by the placenta that appears to be elevated in women with preeclampsia is soluble endoglin (sEng). Endoglin (Eng) is an angiogenic receptor expressed mainly on the surface of endothelial cells, but also placental syncytiotrophoblasts. Eng acts as a co-receptor for transforming growth factor-beta (TGF-beta, a potent pro-angiogenic molecule) signaling in endothelial cells. Eng mRNA is up-regulated in the preeclamptic placenta. In addition, the extra-cellular region of endoglin is proteolytically cleaved and that sEng is released in excess quantities into the circulation of preeclamptic patients. Furthermore, sEng appeared to exacerbate the vascular damage mediated by sFlt1 in pregnant rats resulting in severe preeclampsia-like illness including the development of HELLP syndrome and fetal growth restriction. What remains unknown is the etiology of the increased sFlt1 and sEng in preeclamptic patients and whether these markers can be used for the prediction and treatment of preeclampsia.

### 11.5

#### SEX DIFFERENCES IN FETAL PROGRAMMING OF CARDIOVASCULAR DISEASE.

Barbara Alexander<sup>1</sup>

<sup>1</sup>Physiology, Univ Miss Med Ctr, 2500 N Satate St, Jackson, MS, 39216.

Numerous epidemiological studies report an inverse relationship between birth weight and blood pressure suggesting that hypertension may be programmed by factors initiated in utero. Fetal programming occurs in response to an adverse fetal environment and results in permanent adaptive changes that alter organ growth, structure, and physiology leading to increased risk for development of adult cardiovascular disease. Our laboratory utilizes a model of placental insufficiency in the rat that results in intrauterine growth restricted offspring (IUGR) that develop hypertension by 4 weeks of age. However, sex differences are evident as only male IUGR remain hypertensive after passage through puberty. A role for sex hormone involvement is suggested as plasma testosterone levels are two-fold higher in adult male IUGR and castration abolishes hypertension in adult male IUGR. Thus, testosterone appears to contribute to hypertension in adult male IUGR offspring. Ovariectomy leads to hypertension in female IUGR suggesting estrogen provides a protective status in adult female IUGR offspring. Renin angiotensin system (RAS) blockade abolishes hypertension in adult male IUGR and in adult female IUGR that develop hypertension in response to ovariectomy. Thus, sex hormones and the RAS contribute to sex differences in arterial pressure regulation in this model of fetal programmed hypertension induced by placental insufficiency. (NIH HL074927). REFERENCES: Alexander, BT. Fetal Programming of Hypertension, Am J Physiol. 2006;290:R1-R10. Ojeda NB, Grigore D, Yanes LL, Ilescu R, Robertson EB, Zhang H, Alexander BT. Testosterone contributes to marked elevations in mean arterial pressure in adult male intrauterine growth restricted offspring. Am J Physiol. 2007;292:R758-63.

**2007 APS Conference: Sex Steroids and Gender in Cardiovascular-Renal Physiology and Pathophysiology**  
**AUTHOR INDEX**

\*Indicates Invited Speaker

**A**

Abo, J., 5.5  
Adeagbo, A., 10.30  
Alexander, B.\*, 10.21, 11.0, 11.4  
Alsip, N., 10.30  
Anton, L., 11.1  
Arnold, A., 7.3

---

**B**

Babcock, S., 10.23  
Barton, M.\*, 2.2  
Baserga, M., 10.31  
Basha, M., 10.20  
Bastacky, S., 10.16  
Baylis, C.\*, 8.5  
Behnava, M., 10.29  
Belanger, M., 10.27, 10.28  
Berry, C., 10.9  
Bie, P., 5.24  
Bingaman, S., 5.21  
Bobo, J., 8.3  
Bondy, C.\*, 6.3  
Bowles, D.\*, 8.2  
Brokat, S., 5.4  
Brooks, H., 10.11  
Brosnihan, K.\*, 11.1

---

**C**

Cai, Q., 10.11  
Callaway, C., 10.31  
Cantow, K., 5.4  
Cech, J., 5.14  
Chacko, S., 10.20  
Chang, C.\*, 2.3  
Chappell, M.\*, 7.2, 10.3, 10.22  
Charkh-kar, S., 10.29  
Childs, E., 5.16  
Chou, S., 5.15  
Cidlowski, J.\*, 2.6  
Cockrell, K., 10.23  
Cowley, A., 5.27  
Craig, T., 5.26, 10.8  
Cunningham, T., 5.26

---

**D**

D'Angelo, G., 8.3  
Davila, M., 4.15, 10.8  
Dechend, R., 10.24  
DeSouza, C., 5.14  
Djurhuus, J., 10.18  
Dodam, J., 5.9  
Dominguez, J., 4.6, 10.15  
Doperalski, N., 5.7  
Dorsett-Martin, W., 10.10  
Dubey, R., 7.5  
Duckles, S.\*, 3.2, 5.13, 5.19  
Duma, D., 2.6  
Durtschi, A., 4.9, 5.8, 5.9  
Dworatzek, E., 5.11, 9.3

---

**E**

Ecelbarger, C., 10.5  
Ehrenberg, N., 5.4  
Eyster, K., 5.17, 5.20

---

**F**

Figuera, J., 10.22  
Fortepiani, L., 10.14  
Fritschka, S., 5.3, 9.5  
Fu, Q., 5.6, 5.12, 9.6, 10.26, 10.31  
Fuller, D., 5.7

Fulton, R., 5.1

---

**G**

Gaudet, D., 5.27  
Ghodarzi, Z., 10.29  
Gilbert, J., 9.1, 10.23  
Gonzales, R., 4.1, 5.13  
Granger, J.\*, 10.23, 10.25, 11.2  
Grigore, D., 10.21  
Gross, K., 2.6  
Gwathmey, T., 10.22

---

**H**

Hagstroem, S., 10.18  
Hale, M., 10.31  
Hamet, P., 5.27  
Hastings, J., 5.6, 5.12, 10.26  
Hay, M., 10.1  
Hayward, L., 5.7  
Heit, J., 8.6  
Hinojosa-Laborde, C.\*, 5.26, 6.1, 10.8  
Hoetzer, G., 5.14  
Hoyer, P., 10.11  
Hunter, F., 5.16  
Huxley, V., 5.21, 5.23  
Hydarieh, N., 10.29

---

**I**

Iliescu, R., 4.11, 10.4, 10.6, 10.10  
Insel, P., 10.7

---

**J**

Jackson, E.\*, 7.5, 10.16, 10.17  
Jayachandran, M., 8.6  
Jazbutyte, V., 5.2  
Jewell, C., 2.6  
Ji, H., 7.3  
Johnson, A., 10.1

---

**K**

Kamperis, K., 10.18  
Karumanchi, S.\*, 11.3  
Kashimoto, K., 8.6  
Kaufman, S., 5.10  
Kawamoto, M., 5.22  
Keck, M., 4.2, 10.11  
Kelly, K., 10.15  
Khalil, R., 3.3  
Khorami, A., 10.29  
Kikuchi, T., 5.5  
Kotchen, T., 5.27  
Krause, D., 3.2, 5.13, 5.19  
Krontiris-Litowitz, J., 5.1  
Kühne, A., 5.4  
Kushner, E., 5.14

---

**L**

LaMarca, B., 9.4, 10.23, 10.24, 10.25  
Lane, P.\*, 2.1, 2.4  
Lane, R., 10.31  
Lassman, J., 10.20  
Lau, Y., 5.15  
Levine, B., 5.6, 5.12, 10.26  
Li, B., 10.19  
Lin, H., 2.3  
Liu, N., 2.3  
Llinas, M., 10.24  
Loza, P., 10.2  
Lu, N., 2.6  
Lucca, J., 10.30

**M**

Ma, W., 2.3  
MacEneaney, O., 5.14  
Mahler, B., 9.2, 10.18  
Mahmoodzede, S., 5.3, 5.11  
Maric, C., 8.4, 10.13  
Mark, C., 4.10, 5.17, 5.20  
Martin, D., 5.17, 5.20  
Masilamani, S., 10.9  
McKnight, R., 10.31  
McLemore, G., 10.25  
Mehring, C., 5.26  
Merlo, E., 5.27  
Merrill, D., 11.1  
Mifflin, S., 5.26  
Miller, J.\*, 7.4  
Miller, V.\*, 8.6  
Mitchell, A., 4.3, 5.18, 10.12  
Moreland, R., 10.20  
Morimoto, K., 5.22, 5.25  
Mulrone, S., 5.18, 10.12, 10.13  
Musselman, T., 10.9  
Myers, A., 5.18, 10.12

---

**N**

Nikpay, M., 5.27  
Norsk, P., 5.24

---

**O**

Oakley, R., 2.6  
O'Connor, D., 10.7  
Ojeda, N., 10.21  
Ouyang, P.\*, 1.2  
Owen, W., 8.6

---

**P**

Pelzer, T.\*, 2.5, 5.2, 10.2  
Pendergrass, K., 4.8, 10.3  
Piche, C., 7.5  
Pollock, D.\*, 8.1, 8.3  
Pollock, J., 8.3  
Procaccio, V., 3.2, 5.19  
Pump, B., 5.24

---

**Q**

Quaglietta, D., 10.27, 10.28  
Quigley, R.\*, 8.7

---

**R**

Radvanska, E., 10.18  
Rana, B., 4.14, 10.7  
Razmara, A., 3.2, 5.19  
Reckelhoff, J.\*, 1.1, 10.4, 10.6, 10.10  
Redetzke, R., 5.17  
Regitz-Zagrosek, V., 5.3, 5.4, 5.11  
Revollo, J., 2.6  
Reynolds, C., 4.7, 5.7  
Rittig, S., 10.18  
Robertson, E., 10.21  
Roesch, D., 10.5  
Rogers, J., 4.5, 5.18, 10.13  
Romero, D., 10.6  
Romero-Aleshire, M., 10.11  
Rose, J., 10.22  
Rubin, L., 5.8, 5.9  
Ryan, M., 10.25

---

**S**

Sandberg, K.\*, 7.1, 7.3, 10.5, 10.13

Sandgarten, M., 5.12  
Sano, Y., 5.5  
Sartori-Valinotti, J., 10.4, 10.6, 10.10  
Sasaki, R., 4.12, 5.21  
Schild, J., 10.19  
Schoneveld, O., 2.6  
Schork, N., 10.7  
Seda, O., 5.27  
Shi, M., 10.5  
Shibata, S., 5.6, 5.12, 10.26  
Shook, R., 5.6, 5.12  
Sica, D.\*, 6.2  
Silbiger, S.\*, 1.3  
Sparks, J., 2.3  
Stallone, J.\*, 3.1, 3.4  
Stauffer, B., 5.14  
Stirone, C., 5.19  
Sullivan, J., 8.3  
Sunday, L., 5.19

---

**T**

Takamata, A., 5.22, 5.25  
Tang, L., 10.22  
Tatchum-Talom, R., 5.20  
Tawfik, H., 5.10  
Tharakan, B., 5.16  
Thomas, J., 5.4  
Tofovic, G., 10.17  
Tofovic, S., 7.5, 10.16, 10.17  
Torii, K., 5.25  
Tremblay, J., 5.27  
Tsau, S., 5.15

---

**U**

Urushidani, S., 5.5

---

**V**

Vahabzadeh, A., 10.29  
Van Gundy, T., 5.6, 5.12, 10.26  
Vogel, E., 5.17

---

**W**

Wallukat, G., 10.24  
Wang, R., 2.3  
Wang, T., 10.20  
Wang, X., 5.19  
Wang, Y., 5.15  
Ward, C., 5.7  
Watenpaugh, D., 4.4, 5.24  
Wein, A., 10.20  
Westby, C., 5.14  
Westwood, B., 10.3  
Wittnich, C., 10.27, 10.28  
Wu, X., 7.3  
Wyss, J.\*, 7.6

---

**X**

Xue, B., 10.1

---

**Y**

Yanes, L., 4.13, 10.4, 10.6, 10.10  
Yeh, S., 2.3  
Yu, I., 2.3  
Yu, X., 10.31

---

**Z**

Zhang, H., 10.6, 10.14  
Zheng, W., 7.3  
Zhang, Z., 10.9  
Zukowska, Z., 5.18