Cardiovascular disease (CVD) is the leading cause of death worldwide. Pre-menopausal women have a lower incidence and severity of cardiovascular disease (CVD) when compared to age-matched men but the risks for women increase at the onset of menopause. A central feature in patients with CVD is excessive sympathetic stimulation of beta-adrenergic receptors (β-AR’s). Both clinical and animal studies show that estrogen loss and age exacerbate cardiac β-AR signaling and contractile function. Improved changes in cardiac vasculature and function is observed in ovariectomized (OVX) animal models treated with estrogen replacement. However, clinical studies show no benefit of hormone therapy in the heart with confounding factors such as the timing of estrogen therapy and onset of menopause. Such differences highlight the importance of further research on the mechanisms of estrogen loss in the heart. We therefore examined the hypothesis that prolonged estrogen deficiency followed by chronic sympathetic injury worsens left ventricular cardiac function in the aged female heart. Bilateral ovariectomy (OVX) or SHAM surgery was performed in female mice at 2.5 months of age. Mice were infused with Isoproterenol (ISO; 400μg/kg/h) 5 months (5M) or 12 months (12M) post OVX via mini osmotic pumps for 3 days to induce chronic sympathetic stimulation. Transthoracic two-dimensional M-mode echocardiography was used to measure left ventricular (LV) wall thickness, left ventricular end-systolic diameter (LVEDD), percent fractional shortening (%FS), and ejection fraction (EF). Results show that prolonged ovariectomy increased mortality in mice treated with ISO 12-months post-ovariectomy (12M-OVX + ISO) compared to the 12M-SHAM+ISO group and the 5-month OVX+ISO group. Aging alone had no significant change on the %FS and EF between the 5- and 12- month SHAM groups, but significantly increased in the OVX group. However, ovariectomy did result in a significantly higher %FS and EF compared to SHAM at both time points. ISO infusion increased %FS and EF in both SHAM+ISO and OVX+ISO groups compared to SHAM and OVX respectively at each time point. Interestingly, ISO infusion resulted in a 1.8-fold increase in the 5M-OVX+ISO versus the 5M-OVX group and with a 1.2-fold increase in the 5M-SHAM+ISO vs SHAM groups. In the aged groups however, there was a greater fold increase in %FS and EF in the 12M-SHAM+ISO group vs SHAM groups. There was also a significantly higher %FS and EF the aged 12M-SHAM+ISO vs 5M-SHAM+ISO group. There was no difference between the OVX+ISO groups at 5 vs 12 months. LVESD was significantly decreased in the OVX, ISO, ISO+OVX groups at both 5- and 12-months with the largest decrease in the ISO+OVX group. The aged groups however had a greater decrease in LVESD within each time point and compared to the respective 5M groups. There was only a significant decrease in the LVEDD in the SHAM+ISO groups at 12 months. The results presented here show that estrogen loss impairs left ventricular cardiac response to β-AR stimulation, and that prolonged estrogen loss may blunt the sympathetic response in the heart. These results highlight the importance of the long-term effects of estrogen loss during menopause in the treatment and management of heart disease.
established in vivo model capturing man
repeated experimental human endotoxemia, an
tolerance in a large cohort of volunteers undergoing

Objective To determine whether sex affects the innate
development of personalized treat
better understanding of the potential sex
incidence and outcome statistics between the sexes. A
comprise both hyperinflammatory as immunosuppressive
sepsis and COVID
Aron Jansen
The systemic inflammatory response induced by LPS
administration is more pronounced in women than in men
Aron Jansen¹, Niklas Bruse¹, Peter Pickkers¹, Matthijs Kox¹
Intensive Care, Radboud University Medical Center

Introduction Systemic inflammatory diseases such as
sepsis and COVID-19 are highly heterogeneous, and can
comprise both hyperinflammatory as immunosuppressive
phenotypes. Individual characteristics such as sex may
influence the manner in which these syndromes manifest,
which is emphasized by substantial differences in
incidence and outcome statistics between the sexes. A
better understanding of the potential sex-specific
differences in the innate immune response may facilitate
the development of personalized treatment approaches. Objective To determine whether sex affects the innate
immune response and the development of endotoxin
tolerance in a large cohort of volunteers undergoing
repeated experimental human endotoxemia, an
established in vivo model capturing many hallmarks of both
early sepsis and sepsis-induced immunoparalysis. Methods Subjects (54 females and 56 males) were intravenously
challenged with 1 ng/kg bacterial lipopolysaccharide (LPS)
twice: on day 0 to determine the extent of the inflammatory
response and again on day 7 to determine the degree of
endoxoxin tolerance. Blood samples were obtained serially
to construct time-concentration curves of various cytokines.
Areas under the curves (AUCs) were calculated to provide
an integral measure of the cytokine response.

Hemodynamic data were recorded continuously using a
radial artery catheter and tympanic temperature was
measured every 30 minutes. Differences in the immune
response were analysed using unpaired student’s t-tests
on log-transformed AUCs for cytokine data, whereas p-
values for differences in mean arterial pressure (MAP) and
temperature were computed using two-way analysis of
variance (time*sex interaction term). Results Median
[interquartile range] age was 23 [21-25] years for males and
23 [21-24] years for females (p=0.18), whereas BMI was
23.0 [20.8-25.1] and 23.6 [21.9-25.7] kg/m², respectively
(p=0.12). Compared with males upon the first LPS
challenge, females produced significantly higher levels of
tumor necrosis factor (TNF, 41% higher AUC, p<0.01),
interleukin (IL-6 (+50%, p<0.01), interferon gamma induced
protein (IP)-10 (+47%, p<0.001), and IL-1 receptor antagonist
(+112%, p<0.0001), but not IL-10 (-4%, p=0.99). Although
women displayed a more pronounced decrease in MAP
(p<0.0001), the LPS-induced increase in body temperature
was less pronounced and less prolonged in females than in
males (p<0.001). Upon the second endotoxin challenge, a
tolerant response was observed for all measured cytokines
for both sexes, reflected by a significantly lower AUC
compared to the first challenge (all p<0.0001). However, no
difference in the degree of endotoxin tolerance between
the sexes was observed. Conclusion Females mount a
more pronounced inflammatory response to LPS
administration than males, reflected by higher levels of
proinflammatory cytokines and more severe hemodynamic
alterations, while levels of the anti-inflammatory cytokine IL-
10 and the development of endotoxin tolerance were not
different between the sexes. These findings indicate sex-
specific regulation of the innate immune response. Sex
hormone profiles are currently being determined to assess
whether these differences have a hormonal origin.

APSSG21.7
The systemic inflammatory response induced by LPS
administration is more pronounced in women than in men
Aron Jansen¹, Niklas Bruse¹, Peter Pickkers¹, Matthijs Kox¹
Intensive Care, Radboud University Medical Center

Estrogen Regulates Kisspeptins Signaling in Human Airway
Smooth Muscle
Niyati A. Borkar¹, Christina M. Pabelick², Y.S. Prakash²,
Venkatachalem Sathish³
¹Department of Pharmaceutical Sciences, North Dakota
State University, ²Department of Anesthesiology and
Perioperative Medicine, Department of Physiology and
Biomedical Engineering, Mayo Clinic

Rationale: Sex disparity is a recognized aspect of asthma
with epidemiological studies showing a difference in the
prevalence and clinical manifestation (boys>girls and
women>men) in asthma. These data suggest a crucial role
of sex steroids, particularly the female sex steroid,
estrogen in asthma pathophysiology. However, a
dichotomous role of estrogen in asthma, suggests that upstream of estrogen might be altered during asthma. Evidence from central nervous system studies shows kisspeptin (Kp) signaling is regulated by estrogen. Our own recent studies show a lower expression of Kp and its receptor, KISS1R in females compared to males, and the lowest expression of Kp/KISS1R in asthmatics compared to non-asthmatics. Furthermore, Kp plays a role in regulating airway remodeling by inhibiting airway smooth muscle (ASM) cell proliferation via KISS1R activation. These evidences collectively point to mechanisms upstream of sex steroids may be involved in a loss of an intrinsic protective pathway in asthma. However, the role of sex steroids or estrogen per se in regulating Kp is not yet investigated in the disease context of asthma, let alone in the ASM cell. Therefore, in the current study, we hypothesize that estrogen regulates Kp/KISS1R signaling, thereby contributing to the sex-disparity associated with asthma. Methods: Asthmatic and non-asthmatic primary human ASM cells were isolated from human lung tissues (Mayo Clinic IRB-approved) and cultured in DMEM-F12 supplemented with fetal bovine serum and antibiotic-antimycotic. After 24h of serum deprivation, cells were exposed to vehicle, 17β-estradiol (E2; 1nM), in the presence/absence of inflammatory cytokines; TNFα (20ng/mL) or IL-13 (50ng/mL). The modulatory effects of E2 and inflammation on Kp/KISS1R expression were determined using standard procedures for Western blotting and RT-qPCR. The mechanistic basis of E2 and cytokine influence on Kp/KISS1R was evaluated by studying signaling intermediates such as CREB, AP-1, NFκB and STAT6. Results: E2 and TNFα substantially blunted Kp and KISS1R expression in human ASM cells from both males and females, with no significant effect observed with IL-13 exposure. This decrement in Kp and KISS1R expression was more profound in ASM cells from females compared to males and asthmatics compared to non-asthmatics. Simultaneously, the expression of Kp and KISS1R were significantly decreased in asthmatic and non-asthmatic ASM cells, with a more pronounced effect observed in asthmatic ASM when exposed to E2. Furthermore, E2 and TNFα- induced decrease in Kp and KISS1R expression were abolished upon pharmacological inhibition using CREB and NFκB inhibitors respectively. Overall, E2 and TNFα exposures regulate Kp/KISS1R signaling, suggesting importance of future exploration of estrogen on Kp/KISS1R signaling in the airways.

APSSG219
Cardiovascular risk of gender affirming hormone therapy in transgender men
Nina Stachenfeld
Obstetrics, Gynecology and Reproductive Medicine, John B. Pierce Laboratory/Yale School of Medicine

In the US approximately 1.4 million men identify as transgender, a number that is likely to increase with greater recognition of this condition. Gender affirming hormone therapy (GAHT), which attempts to more align the physical appearance with the identified gender, is the primary medical intervention for transgender people and is recognized as medically necessary. GAHT has been associated with increased cardiovascular risk (increased blood pressure, dyslipidemia, and endothelial dysfunction) in transgender men receiving androgens. In trans men, testosterone therapy is associated with increased lipids, triglycerides, LDL-cholesterol and decreased HDL-cholesterol, which are primary risk factors for the development of atherosclerotic cardiovascular disease (CVD). Elevated LDL-C is also associated with impaired endothelial function. Endothelial dysfunction constitutes “the early pivotal event in atherosclerosis”, because it precedes clinically detectable atherosclerotic plaques in the coronary arteries. The impact of endothelial dysfunction on the pathophysiological process leading to CVD is especially apparent in individuals with dyslipidemia. While these two conditions are related, we have generated data in trans men receiving testosterone therapy as well as in women with androgen excess polycystic ovary syndrome (AE-PCOS) demonstrating endothelial dysfunction, independent of metabolic co-morbidities such as obesity and insulin resistance. Thus, we hypothesize that the altered hormonal milieu is the major driver of increased cardiovascular risk in trans men, or when testosterone is exposed to the female vascular system. Further, our data and that of others show increases in systolic blood pressure in young, transgender men receiving testosterone independent of cardiometabolic comorbidities. In addition to effects on lipid profiles, blood pressure and endothelial function, the initiation of androgen exposure within the female vascular system may be associated with sympathetic nervous system dysregulation. While there have been no studies examining changes in sympathetic activity in trans men during androgen treatment as yet, our data show greater resting systolic blood pressure in AE-PCOS women concomitant with altered sympathetic baroreflex control of blood pressure, specifically tied to hormonal milieu. Taken together, these studies suggest that testosterone exposure alters sympathetic control of blood pressure in women, which may be a function of excess testosterone exposure to the female vascular system. In summary, the preponderance of evidence supports that high androgen exposure to a female cardiovascular system is associated with increased LDL-C, increased blood pressure, altered sympathetic control of blood pressure and endothelial dysfunction. Therefore, attention to cardiovascular risk factors should be integral to the care of transgender men.
Sex, Mitochondria and Metabolism
Karthickeyan Chella Krishnan\textsuperscript{1,2}, Laurent Vergnes\textsuperscript{3}, Rebeca Acín-Pérez\textsuperscript{4}, Linsey Stiles\textsuperscript{4}, Michael Shum\textsuperscript{4}, Lijiang Ma\textsuperscript{5}, Casey Romanoski\textsuperscript{6}, Karen Reue\textsuperscript{3}, Marc Liesa\textsuperscript{4}, Johan L.M. Björkegren\textsuperscript{7}, Markku Laakso\textsuperscript{7}, Aldons J. Lu	extit{is}\textsuperscript{2}

\textsuperscript{1}Pharmacology & Systems Physiology, University of Cincinnati College of Medicine, \textsuperscript{2}Medicine/Cardiology, University of California Los Angeles, \textsuperscript{3}Human Genetics, University of California Los Angeles, \textsuperscript{4}Medicine/Endocrinology, University of California Los Angeles, \textsuperscript{5}Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, \textsuperscript{6}Cellular and Molecular Medicine, University of Arizona, \textsuperscript{7}Internal Medicine, University of Eastern Finland

Background and Objectives: Mitochondria plays a major role in the pathophysiology of complex metabolic traits such as obesity, insulin resistance and fatty liver disease. However, the exact causal relationship between mitochondrial function and these traits is not completely understood. Similarly, sex differences in susceptibility to mitochondrial dysfunction has been less studied in mice, humans and other species, with females generally exhibiting a beneficial metabolic profile. Yet, the vast majority of previous studies examined sex differences in phenotypes or gene expression in isolation, generating trait or tissue specific results without putting them in context of genetic variation. Methods: To understand the nature of the sex differences and causal relationships, we examined genetic factors contributing to mitochondrial function using a mouse reference population that were extensively phenotyped called hybrid mouse diversity panel. Results: We identified a genetic locus on mouse chromosome 17 that controls mitochondrial levels and function in adipose tissue in a sex- and tissue-specific manner. It regulates the expression of at least 89 mitochondrial genes, many of them related to oxidative phosphorylation, as well as mitochondrial DNA levels, in female but not male mice. Overexpression studies indicate that the effects of the locus are mediated by the Ndufv2 gene that elevates mitochondrial ROS production, thereby generating a signal to increase mitochondrial biogenesis. The gene is activated by gonadal hormones and is regulated in cis only in females. Conclusion: We report that adipose mitochondria are regulated by both genetic variation and sex hormones and that high levels are an important determinant of metabolic syndrome traits.

Obesity Mediates Cardiovascular Sex Differences in Polycystic Ovary Syndrome for Genetically Predisposed Individuals
Ky'era Actkins\textsuperscript{1}, Lea Davis\textsuperscript{2}

\textsuperscript{1}Department of Microbiology, Immunology, and Physiology, Meharry Medical College, \textsuperscript{2}Department of Medicine, Vanderbilt University Medical Center

Introduction: Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder in reproductive age women with a complex etiology largely characterized by metabolic dysregulation. Despite only being diagnosable in females, males with a family history of PCOS can also exhibit a poorer cardiometabolic profile that can be detected as early as infancy. Therefore, in this study, we aimed to further elucidate the role of sex in the relationship between PCOS and its comorbidities by evaluating their bidirectional genetic pathways. Methods: We first analyzed polygenic risk scores (PRS) for PCOS (PCOSPRS), a measurement of genetic liability, through a phenome-wide association study (PheWAS) to identify comorbid traits for females ($n = 40,802$) and males ($n = 32,022$). Logistic regression models were adjusted for median age, genetic ancestry, and body mass index (BMI) measurements, which captured both genetic and environmental BMI variance. To examine the independent effects of environmental BMI, genetically regulated BMI variance was regressed out. Mediation analyses were then conducted to analyze both

Sex-specific gene expression signature in obese human and rat cardiac hypertrophy
Hangang Yu\textsuperscript{1}, Janelle Striker\textsuperscript{2}, Mackenzie Newman\textsuperscript{1}

\textsuperscript{1}Physiology & Pharmacology, West Virginia University, \textsuperscript{2}Molecular Medicine, California Institute for Biomedical Research

Background: Molecular and genetic biomarkers in cardiac hypertrophy significantly improve early diagnostic and treatment of heart failure. How sex may affect the gene expression profiles of obese human cardiac hypertrophy is not clear. We hypothesized a sex-specific gene expression profiles of hypertrophy in obese human as well as obese rat that may be used to study the mechanisms. Methods: Human heart tissues were grouped according to sex (12 male, 12 female), left ventricular hypertrophy (LVH) and non-LVH non-failed controls (NF). Eight female and eight male obese rat heart tissues were used. Transcriptome sequencing was performed and reads were mapped to human reference genome (hg38) using STAR. Differentially expressed (DE) genes were determined by NOISeq. Research involving human and animal tissues was reviewed and approved by West Virginia University Institutional Review Board and Institutional Animal Care and Use Committee, respectively. Results: We identified 24 DE genes comparing female to male samples. Comparing LVH to NF, there were 1320 female and 1383 significant genes in male subgroup, respectively. Using absolute value of log2 fold-change $> 2$ or extremely small p-value (10–20) as a criterion, we identified 9 significant genes (HBA1, HBB, HIST1H2AC, GSTT1, MYL7, NPPA, NPPB, PDK4, PLA2G2A) in LVH, also found in published dataset for ischemic and dilated cardiomyopathy in heart failure. Five of them (Hbb, Myl7, Nppa, Nppb, Pdk4) were validated by qPCR and protein expression in rat. While Nnpa and Nppb are established biomarkers, new genes (Hbb, Myl7, Pdk4) may provide new insights for sex-dependent obesity-related cardiac LVH. Conclusions: We identified a sex-specific gene expression signature in obesity-related cardiac hypertrophy. Some genes may be developed to be potential sex-specific biomarkers for identifying patient with early heart failure.
types of BMI (total and environmental variance) as mediators for PCOSPRS as the exposure variable and dichotomized clinical diagnoses as the outcome. Results: We found that males with a higher PRS for PCOS were more likely to develop cardiovascular diseases (CVD) compared to females who had higher odds of developing T2D (OR = 1.10, p = 3.91e-07). When BMI genetic risk was unaccounted for in the clinical BMI measurements extracted from electronic health records, significant associations for both sexes were attenuated in the PheWAS analysis. However, when environmental BMI was covaried for instead, T2D reappeared as a significant association in the female analysis (OR = 1.10, p = 2.17e-08) and the CVD associations improved in males. Unsurprisingly, total BMI variance was a strong mediator for cardiometabolic outcomes in both sexes, but environmental BMI alone did not mediate the pathway from PCOSPRS to T2D nor PCOSPRS (p = 0.85) to hypertension (p = 0.83) in males. Conclusions: Our findings show that the genetics of PCOS result in distinct metabolic sex differences with genetically regulated BMI being a larger contributor to predisposed males specifically. BMI can heavily influence associations driven by PCOS genetic risk, therefore, it is possible that the metabolic genetic pathways causal for PCOS are less likely to be solely explained by the etiology of comorbidities than they are by the risk exposures shared between them.

APSSG21.13
The acute effect of the Oral Contraceptive Pill on glycaemic response to an oral glucose load: a randomised crossover study in healthy young women
Julia Cree1, Jennifer Miles-Chan1, Niamh Brennan1
1School of Biological Sciences, University Of Auckland

The oral contraceptive pill (OCP) is widely used by women of childbearing age across the globe yet its influence on carbohydrate metabolism remains under-investigated. Despite observational studies (both cohort and cross-sectional in design) having demonstrated a link between OCP use and glucose metabolism disorders, the effects of modern OCP formulations on postprandial glycaemia and the short-term reversibility of OCP-induced metabolic effects remain under-investigated. Therefore, this cross-over study investigated the effect of combined monophasic OCP phase on glucose homeostasis and metabolic profile in 21 healthy young women who were regular users of OCP formulations containing progestogens classified as either androgenic or anti-androgenic. Testing was conducted once during the “active” (hormone-containing) phase and once during the “inactive” (hormone-free placebo pill) phase of the OCP usage cycle. Following an overnight fast, plasma glycaemic markers were assessed prior to consuming a drink containing 50g glucose, and for a further 4h postprandial. Fasted plasma glucose and insulin did not vary between pill phases for women taking OCP formulations containing either androgenic or anti-androgenic progestogens. For androgenic progestogens during the active phase, the mean postprandial glucose and insulin responses (area-under-the-curve) were ~70% and ~50% greater respectively than when measured during the inactive phase. However, for anti-androgenic progestogens, little change in postprandial glycaemia was observed between phases, with an increase of only ~25% in insulin response during the active phase relative to inactive. Overall the total glucose response above fasted baseline demonstrated a significant interaction between pill phase and pill type (p <0.05). These findings highlight an acute, potentially detrimental influence of the combined OCP on glucose homeostasis, particularly for OCP formulations containing androgenic progestogens, and the need for greater attention to be focused on the metabolic effects of OCP. Given the high prevalence of OCP use and increasingly common prolonged active pill usage (i.e., with no “inactive” phase), that may continue for months, years or even decades, the cumulative effect of such changes in glucose handling may put OCP users at increased metabolic risk. Furthermore, given that the vast majority of the day is spent in the postprandial period, better understanding of mechanisms underlying the influence of the OCP on postprandial glycaemia will greatly assist in the tailoring of dietary recommendations and hormonal contraceptive advice to young women, particularly for individuals with a predisposition for metabolic disease. This research was funded by the Health Research Council. JMEC was supported by Lottery Health Research PhD Scholarship.

APSSG21.14
Sex differences in cerebrovascular reactivity to hypercapnia and isometric handgrip exercise
Stefanie L Ruediger1, Faith K Pizzy1, Jodie L Koep12, Shigehiko Ogo13, Jeff S Coombes1, Tom G Bailey3
1Physiology and Ultrasound Laboratory in Science and Exercise, Centre for Research on Exercise, Physical Activity and Health; School of Human Movement and Nutrition Sciences, The University of Queensland, 2Children’s Health and Exercise Research Centre, Sport and Health Sciences, College of Life and Environmental Sciences, University of Exeter, 3Department of Biomedical Engineering, Toyo University, 4Neurovascular Research Laboratory, Faculty of Life Sciences and Education, University of South Wales, 5School of Nursing, Midwifery and Social Work, The University of Queensland

Background: Cerebral blood flow and reactivity declines with older age, alongside increased risk of cerebrovascular disease and cognitive decline. This decline is slower in females but accelerates around midlife, coinciding with the onset of the menopause and reductions in estrogen. Differences in cerebrovascular reactivity of the middle cerebral artery (MCA CVR) are inconsistent between pre- and post-menopausal females. Recent research shows a gradual lower internal carotid artery (ICA) reactivity in post-compared with pre-menopausal females. Yet, whether differences exist between post-menopausal females and males of similar age is unknown. The aim of this study was to examine whether extracranial and intracranial cerebral blood flow reactivity to 1) hypercapnia and 2) isometric exercise is different in post-menopausal females compared to age-matched males. Methods: 9 early post-menopausal females (F, 55± 3 y) and 7 males (M, 55±7 y) underwent a...
hypercapnic breathing challenge (5% CO2), followed by a 3 min isometric handgrip exercise (HGEx) test at 30%MVC. Intracranial (MCAv) and extracranial (ICA) blood flow were measured using Transcranial Doppler and Duplex ultrasound, respectively. Beat-by-beat blood pressure, heart rate and end-tidal carbon dioxide (PETCO2) were measured throughout. Results: During hypercapnia, the absolute change in MCAv was higher in females compared to males (F: 99.2±17.3 vs M 74.3±12.9 cm/s, p=0.007), with no differences in the relative change (F: 24.2±17.4 vs M: 32.4±13.1 %, p= 0.32). During hypercapnia, there was no difference in ICA peak diameter, blood flow, or shear rate between females and males. During HGEx, the absolute change in MCAv (F: 80.6±17.4; M: 63.8±11.7 cm/s; p=0.046) was higher in females compared with males, with no difference in the relative change. The relative change in ICA velocity (F:15.6±22.3 vs M: 34.6±8.0%; p=0.03) and shear rate (F: 17.9±7.9%; M: 39±20.7%; p=0.014) during HGEx was lower in females compared with males, with no difference in peak diameter. Discussion: The cerebrovascular response to both hypercapnia and isometric exercise was higher in post-menopausal females compared with aged-matched males. ICA reactivity to hypercapnia in post-menopausal females was similar to males. However, postmenopausal females showed a lower ICA velocity and shear rate response to HGEx in the ICA compared with age-matched males. Recent research suggested that reduced circulating estrogen after menopause might play a crucial role in reduced cerebrovascular endothelial function in ageing females. Our early findings suggest that cerebrovascular function in early post-menopausal females is preserved compared with age-matched males.

APSSG21.15
Sex-dependent effects of alcohol on inflammatory pain and limbic system neuroadaptations in the context of pain
Jessica Cucinello-Ragland1, Logan Gold1, Kimberly Edwards1, Scott Edwards1
1Physiology, LSU Health Sciences Center

Although a high percentage of both chronic pain patients and high-risk alcohol drinkers consume alcohol to manage their pain, there is a significant gap in knowledge regarding the mechanisms underlying the anti-nociceptive effects of alcohol. Previous findings from our lab suggest that the anti-nociceptive efficacy of alcohol may change over time in the context of chronic pain. The goals of the current project were to determine the longitudinal effects of alcohol on chronic pain and identify neuroadaptations associated with alcohol-induced anti-nociception. We utilized the complete Freund's adjuvant (CFA) model of inflammatory pain in adult female and male Wistar rats. Both nociceptive and negative motivational aspects of pain were measured using electronic von Frey (mechanical nociception), thermal probe test (thermal nociception), and mechanical conflict avoidance task (pain avoidance-like behavior). All behavioral tests were conducted at baseline and 1 and 3 weeks following intra-planter CFA or saline administration. At both time points post-CFA, animals were treated with each of 3 doses of alcohol (intraperitoneal; 0g/kg, 0.5g/kg, 1g/kg) over separate days in a Latin square design. Alcohol produced dose-dependent analgesia in females but only modest anti-hyperalgesia in males. Consistent with clinical findings, only males displayed increased pain avoidance-like behavior, and this was most effectively attenuated by alcohol 1 week post-CFA. Although alcohol continued to attenuate CFA-induced decreases in both thermal and mechanical nociceptive thresholds in males 1 and 3 weeks post-CFA, females appeared to develop tolerance to these effects 3 weeks post-CFA. A separate cohort of rats was generated using these parameters to determine alterations in endocannabinoid (eCB) and glucocorticoid system-related protein levels in limbic regions, including the basolateral amygdala (BLA), central amygdala (CeA), and cingulate cortex. Western blot analysis revealed that alcohol differentially affects BLA and cingulate levels of the eCB synthetic enzyme diacylglycerol lipase-α (DAGLα) only in females. Conversely, only males displayed an increase in cingulate levels of CB1R in response to CFA. Preliminary data also suggests that alcohol decreases CeA glucocorticoid receptor (GR) phosphorylation only in control females, but not their CFA-treated counterparts. These findings will help elucidate the mechanism of analgesic action of alcohol in the context of chronic inflammatory pain states across sexes.

APSSG21.16
Chronic hyperandrogenemia in female rats affects classical and nonclassical intra-renal Renin-Angiotensin System
Logan Ryals1, Jacob Pruett1, Stephen Everman1, Damien Romero3,4, Licy Cordozo-Yanes1,2,3,4,5
1Cell & Molecular Biology, University of Mississippi Medical Center, 2Mississippi Center for Excellence in Perinatal Research, University of Mississippi Medical Center, 3Women’s Health Research Center, University of Mississippi Medical Center, 4Cardio Renal Research Center, University of Mississippi Medical Center, 5Medicine (Division of Endocrinology, Diabetes and Metabolism), University of Mississippi Medical Center

Polycystic Ovarian Syndrome (PCOS) is the most common endocrine disorder in reproductive aged women, affecting 5-26% of women. PCOS is characterized by androgen excess, ovulatory dysfunction, and polycystic ovaries. PCOS also often includes a cardiometabolic syndrome consisting of insulin resistance, obesity, & increased blood pressure (BP). Androgen excess is an important factor underlying this cardiometabolic syndrome although the etiology is unknown. The Renin-Angiotensin System (RAS), the major effector of BP, is affected by androgens. Dysregulation of this system may be associated with the increased BP in PCOS. To examine if RAS components are dysregulated in PCOS, Western blotting was performed on key components of the RAS pathway in the kidneys of hyperandrogenemic female (HAF) rats. Characterization of the effect of androgens on the RAS may provide a foundation for trial of novel pharmacotherapies to treat high BP in PCOS. Four-week-old female Sprague Dawley rats were randomized to control or dihydrotestosterone exposure (HAF) (7.5mg/90 days) (n= 8/group). At
Reduced Uterine Perfusion Pressure prevents Seizure-Induced reductions in neurotransmitter transporters in pregnant mice

Maria Jones-Muhammad1, Qingmei Shao2, Paula Warrington2
1PhD in Neuroscience program, University of Mississippi Medical Center, 2Neurology, University of Mississippi Medical Center

Preeclampsia, a hypertensive disorder of pregnancy, can advance to eclampsia, if new-onset seizures occur. Previous work showed that the reduced uterine perfusion pressure (RUPP) rat model of preeclampsia has increased susceptibility to chemically-induced seizures; however, the underlying mechanisms are unknown. Because seizures occur due to neurotransmitter activity imbalance, we hypothesized that RUPP mice have elevated excitatory and reduced inhibitory neurotransmitter activity and that seizures exacerbate this imbalance. Timed-pregnant SMA-GFP mice (n=5–6 per group/treatment) were subjected to sham or RUPP surgery on gestational day (GD) 13.5 and seizures were induced using 40mg/kg pentylenetetrazol on GD18.5. Tissues were harvested 30 minutes post-seizure induction. Maximum seizure scores were similar in sham (4.7±0.3) and RUPP (4.5±0.3) mice; p=0.37. Seizures increased [F(1, 16) = 5.99, p=0.03], while RUPP had no effect [F(1, 16) = 1.15, p=0.3] on hippocampal glutamate concentration. Seizures increased [F(1, 16) = 6.96, p=0.02], while RUPP had no effect [F(1, 16) = 0.61, p=0.45] on GABA concentration. No pairwise differences in GABA or glutamate concentration was observed within the sham and RUPP groups exposed to seizures (p>0.05). Western blot analysis showed seizures significantly reduced (Mean±SD) hippocampal NMDAR1 (1.0±0.5 vs 0.6±0.96, p<0.02; 1.0±0.1 vs 0.6±0.0, p<0.04) and GABAAR receptor expression (1.0±0.4 vs 0.35±0.1, p<0.01; 1.05±0.3 vs 0.3±0.1, p<0.05) in sham and RUPP mice. NMDAR2b expression was not changed in sham (1±0.4 vs 0.7±0.2 p=0.4) or RUPP mice (0.9±0.1 vs 1.2±0.2 p=0.4) following seizures. PSD-95 expression was significantly increased in sham (1±0.5 vs 2.0±0.4 p<0.03) and RUPP (0.7±0.3 vs 1.7±0.6 p=0.04) mice following seizure exposure. Vesicular glutamate transporter (VGLUT1: sham: 1.0±0.3 vs 0.5±0.1, p<0.01, RUPP: 0.8±0.2 vs 0.6±0.1, p=0.11), excitatory amino acid transporter 1 (EAAT1: sham: 1.0±0.4 vs 0.6±0.2, p=0.02, RUPP: 0.9±0.1 vs 0.6±0.2, p=0.17) and GABA transporter (GAT1: sham: 1.0±0.5 vs 0.4±0.1, p=0.01; RUPP: 0.8±0.2 vs 0.5±0.1, p<0.12) were reduced in sham, but not RUPP mice, following seizure exposure. Our results indicate that at baseline, RUPP has no effect on the expression of GABA or glutamate, or their major receptors and transporters in the hippocampus. Nevertheless, following seizures, RUPP mice have a different response in hippocampal neurotransmitter transporter expression compared to sham mice. Taken together, our study suggests the RUPP procedure alters seizure responses to neurotransmitter transporter expression, but does not affect the expression of neurotransmitters themselves or corresponding receptors. Ongoing studies assess whether other receptors and transporters are affected or if astrocytic responses to seizures is impaired in RUPP mice. Funding: NIH R00HL129192, R00HL129192-S1, T32HL05324, William Townsend Porter Pre-doctoral Fellowship from the American Physiological Society

Sex differences in metabolic adaptation after weight loss: a secondary analysis of CALERIE studies

Manuel Dote-Montero12, Guillermo Sanchez-Delgado1, Leanne Redman1, Eric Ravussin1
1Clinical Science, Pennington Biomedical Research Center, 2Department of Physical Education and Sports, University of Granada

Context: Whether metabolic adaptation, a greater than expected decrease in energy expenditure in response to weight loss, differs between males and females is unknown. Objective: To investigate sex differences in metabolic adaptation after caloric restriction. Methods: The Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE) phase I and II studies were randomized controlled trials designed to examine the metabolic and psychological impact of caloric restriction in adults without obesity. In CALERIE phase I, 15 males and 20 females (age: 38 ± 6 years; body mass index: 27.6 ± 1.6 kg/m2) were randomized to three different 6-month
interventions: a) 25% caloric restriction; b) 12.5% caloric restriction plus 12.5% increase in energy expenditure by structured exercise; c) low-calorie diet (890 kcal/day) until 15% weight reduction followed by weight maintenance. In CALERIE phase II, 10 men and 24 women (age: 40 ± 6 years; body mass index: 25.5 ± 1.6 kg/m2) were prescribed a 25% caloric restriction for 24 months. Sleeping metabolic rate (SMR) and 24 hours energy expenditure (24hEE) were measured by whole-room indirect calorimetry. Body composition was measured by dual-energy x-ray absorptiometry. Measurements were performed at baseline, after 3 and 6 months of intervention in CALERIE phase I, and after 12 and 24 months of intervention in CALERIE phase II. Metabolic adaptation was defined as the difference between the predicted (by fat free mass, fat mass, age and sex) and measured SMR and 24hEE.

Results: No significant differences in metabolic adaptation in SMR and 24hEE were found between males and females in CALERIE phase I (all p>0.4). Metabolic adaptation in SMR was also similar between men and women in CALERIE phase II (all p>0.4). In contrast, metabolic adaptation in 24hEE was significantly higher in males (-150 ± 103 kcal/day) than in females (-40 ± 104 kcal/day) after 12 months (p=0.01), but not after 24 months (p=0.31) of intervention in CALERIE phase II. Consistent findings were observed after adjusting the analyses by percentage of body weight loss. Conclusion: Over the long term (2 years), metabolic adaptation after weight loss was similar between males and females. However, the higher metabolic adaptation in 24hEE observed in males after 12 months of caloric restriction requires further investigation.

**APSSG21.20**

**Impact of diabetic kidney disease on renal function in female and male ZSF1 rats**

Brandon McFarlin, Donna Ralph, Darren Ha, Timothy Reilly, Alicia McDonough

Physiology and Neuroscience, Keck School of Medicine of USC

Diabetic kidney disease (DKD) is the leading cause of chronic kidney disease and a strong risk factor for cardiovascular related mortality. New treatments that target renal glucose transporters (SGLT2i) blunt progression. While the prevalence of impaired glucose tolerance and obesity are higher in females (F) versus males (M), the prevalence of type 2 diabetes is higher in M versus F. ZSF1 rats are F1 progeny receptor deficient Zucker x Stroke-prone SHR and have high translational value to the progressive human DKD. Animals are either lean (L) - hypertensive without DKD or obese (O) - hypertensive with DKD. AIM 1: Define sex-specific progression of (patho)physiology, fluid and electrolyte handling, and kidney sodium transporter abundance in early stage DKD (age 10-18 wks) in the ZSF1 rat. Glucose handling: OM, not OF, exhibit progressive hyperglycemia and glycosuria with age (P<0.01). Proximal tubule (PT) SGLT1, not SGLT2, increased in both OM and OF (P<0.01). Blood pressure (BP, mmHg) is higher in LM and LF (167±3) vs. OM and OF (155±4), P=0.03. Albuminuria is exacerbated in OM versus OF (P<0.01) and progresses with age in both, despite normal glucose in OF. OM and OF exhibit accumulation of cortical albumin vs LM and LF. Albuminuria and tissue albumin are low in LM and LF rats despite higher BP. Abundance of megalin and cubulin are not different between lean and obese animals suggesting albuminuria is likely due to increased filtration, not reduced reabsorption. Sodium handling: Lithium clearance (CLi+, an estimate of volume flow from the proximal nephron) increases in OM consistent with lower NHE3 and NKCC2p (all P<0.05). Sodium clearance (CNa+, an inverse measure of Na+ reabsorption along the nephron) increases in OM and OF due to higher dietary Na+ intake and lower fractional reabsorption along nephron evident by lower abundance of NHE3, cldn-2, NKCC2p, NCCp, and cldn-7 abundance (all P<0.05). These pronounced transporter differences in obese versus lean rats were the same in OM and OF. Since OM consume 42% more food, thus, 42% more Na+, than age-matched OF, Aim 2 tested the hypothesis that lowering dietary Na+ intake of OM to equivalent levels in OF for 10 wks will improve measures of pathophysiology in early stage DKD. Lowering dietary salt predictably reduced UNaV, UV, and water intake but had no effect on body weight, food intake, UKV, blood glucose, or BP. Lower dietary salt did slightly blunt the rise in albuminuria (P=ns). Summary and Conclusions: Aim 1 sex differences: OM exhibit more rapid diabetic kidney disease progression (albuminuria) than age-matched OF. OF do not exhibit hyperglycemia, glycosuria or DKD at 18 wks despite leptin receptor deficiency and hypertension. However, OF do exhibit obesity, increased SGLT1, increased renal tissue albumin, and rising albuminuria suggesting delayed onset of DKD. Both OM and OF (vs LM and LF) exhibit lower abundance of renal transporters consistent with natriuresis. Transporter reductions likely a consequence of both greater dietary Na+ intake as well as greater peri-renal fat in Obese M,F. Aim 2: Role of sodium intake: Lowering Na+ intake (not calories) in OM did not lower glucose or BP but tended to blunt albuminuria. Overall, OF exhibit a sex-specific protection from early diabetic kidney disease and kidney pathology compared to age-matched OM. Pronounced early hyperglycemia in OM may contribute to sex-specific differences in progression. Factors responsible for the female advantage may include hormonal and inflammatory status. Funding: NIH/NIDDK DK076169 (AMcD) and F31 DK126457 (BM)

**APSSG21.21**

**Sex differences in Gut Microbiome composition and functionality**

José Manuel Fernández-Real, Jordi Mayneris

Diabetes, Endocrinology and Nutrition, Institut d’Investigació Biomèdica de Girona (IDIBGI), Endocrinology, Hospital of Girona

The gut microbiota composition is known to be changed in parallel to a myriad of environmental factors, being diet and antibiotic/drug exposures the main determinants. There is some evidence that sex may influence the diversity, composition, and function of gut bacterial microbiota, although the results are inconsistent. We recently evaluated the possible associations between gut
microbiota composition and the circulating concentrations of the main gonadal steroids. We used O-PLS or binomial distribution by DESeq2 adjusting for age and obesity. Using both approaches, several families from the Bacteroidetes phylum (Sphingobacteriaceae, Prevotellaceae) had positive associations with testosterone levels, whereas several families from the Proteobacteria (Gammaproteobacteria), Firmicutes (Lactobacillaceae) and Actinobacteria phyla had the strongest negative fold change. Fecal microbiota transplantation from human donor to recipient mice resulted in a clear difference in the microbiome composition of male mice that received microbiota from pre-menopausal women and those that were transplanted with microbiota from male donors. Most mice receiving microbiota from obese post-menopausal women had a microbiota profile similar to those that received microbiota from male donors. Interestingly, when evaluating the mice gut microbiota composition 28 days later under a chow diet, we could successfully predict the donor testosterone and progesterone levels from the recipient's mice microbiota by O-PLS modeling. Our results evidenced a clear difference in the gut microbial composition and functionality between men and women, which was influenced by both menopausal and obesity status. Menopause is proposed to induce an androgenization of the microbiome, whereas obesity overrides the sex and menopause differences observed in individuals without obesity. The gut microbiota composition seems to be tightly linked to the circulating levels of gonadal steroids, particularly testosterone.

**APSSG21.22**

**Sex differences in hypertension susceptibility and hypothalamic plasticity**

Teresa Milner1, Natalina Contoreggi1, Fangmin Yu1, Megan Johnson1, Gang Wang2, Clara Woods3, Sanoara Mazid4, Tracey Van Kempen1, Elizabeth Waters2, Bruce McEwen2, Kenneth Korach3, Michael Glass4

1Feil Family Brain and Mind Research Institute, Weill Cornell Medicine, 2Laboratory of Neuroendocrinology, The Rockefeller University, 3Reproductive and Developmental Biology Laboratory, NIH

Hypertension is the leading modifiable risk-factor for cardiovascular disease. Importantly, there are established sex differences in hypertension with men showing a higher incidence of hypertension from early adulthood to mid-life, while women reach rates of hypertension at menopause that equal or even surpass that of men. Notably, hypertension susceptibility in women increases at the transition to menopause, termed perimenopause, a state characterized by erratic estrogen fluctuation and extended hormone cycles. Elucidating the role of estrogen signaling in the emergence of hypertension during perimenopause has been hindered by animal models that are confounded by abrupt estrogen cessation (ovariectomy) or effects of aging. Significantly, a mouse model of accelerated ovarian failure (AOF) induced by 4-vinylcyclohexene diepoxide can recapitulate, even in younger animals, early (i.e., peri-AOF) and late (i.e., post-AOF) stages of human peri- and postmenopause, respectively. The AOF model has proven effective in isolating the role of sex hormones in blood pressure, particularly with respect to models of neurogenic hypertension involving the hypothalamic paraventricular nucleus (PVN), a brain area critical for coordinating sympathetic and neurohumoral processes critical for the regulation of blood pressure. The present study aimed to examine potential sex differences in the role of estrogen receptor beta (ERβ) in blood pressure regulation as well as NMDA receptor-signaling in the PVN. For this, AOF in ERβ reporter mice was induced in young female mice to model peri-AOF characteristic of peri-menopause. We found that administering ERβ agonists suppressed elevated blood pressure in a model of neurogenic hypertension induced by angiotensin II (AngII) in peri-AOF, but not age-matched male mice. We also found that ERβ agonist administration in peri-AOF females, but not males, suppressed the heightened glutamatergic NMDA receptor signaling and reactive oxygen production in ERβ neurons in the PVN. We further demonstrated that deleting ERβ in the PVN of gonadally-intact females produced a phenotype marked by a sensitivity to AngII hypertension. Together, these results suggest that ERβ signaling in the PVN plays an important role in blood pressure regulation in female mice and contributes to hypertension susceptibility in females at an early stage of ovarian failure comparable to human perimenopause.

**APSSG21.23**

**Sex Differences in Dementia**

Michelle Mielke1, Michelle Mielke1

1Quantitative Health Sciences, Mayo Clinic

Although tremendous strides have been made in Alzheimer's disease and related dementias (ADRD) research over the past several years, sex and gender differences have still had limited attention. Several studies, particularly in the United States, do not report large sex differences in the prevalence or incidence of ADRD. However, there is a growing literature on sex differences in risk factors and pathways that differentially contribute to the development and progression of ADRD. This presentation will first identify sex differences in cerebrovascular and Alzheimer's-related pathology and specify risk factors for ADRD that differ by sex or are sex-specific. In addition, the influence of sex and gender differences in the development, detection, and prognostication of ADRD will be discussed.

**APSSG21.24**

**Neurobiological consequences of chronic binge alcohol administration and ovariectomy on markers of hippocampal plasticity in SIV-infected female rhesus macaques**

Taylor Fitzpatrick-Schmidt1, Sophia Marathonitis1, Larry Coleman1, Kimberly Edwards1, Liz Simon1, Patricia Molina1, Scott Edwards1

1Physiology, Louisiana State University Health Sciences Center New Orleans

Human immunodeficiency virus (HIV) infection has profound impacts on the central nervous system, including
HIV-associated neurocognitive disorder (HAND). HIV-associated cognitive deficits can be further exacerbated by chronic unhealthy alcohol consumption. With the rising prevalence of alcohol use disorder (AUD) in females, understanding the neurobiological impact of AUD and HIV infection in this population is increasingly important. The hippocampus is part of the brain's limbic system and plays prominent roles in both cognition and affective regulation. Thus, investigating hippocampal neuroadaptations in the context of comorbid HIV infection and AUD is critical for understanding the mechanisms of neurocognitive and affective impairment in patients. Neurobiological areas of interest in our lab include glucocorticoid, estrogen, and brain-derived neurotrophic factor (BDNF) signaling pathways. Glucocorticoids represent a major stress hormone category and are released as a result of chronic alcohol use and withdrawal, potentially facilitating the progression to AUD as well as alcohol-related cognitive deficits. In contrast, estrogen and BDNF are neuroprotective, and these systems may be compromised as a result of chronic alcohol use. We hypothesized that simian immunodeficiency virus (SIV)-infected, antiretroviral therapy (ART)-treated female rhesus macaques with a history of chronic binge alcohol (CBA) administration and ovariectomy (OVX) (n=7-8 per group) would exhibit decreases in hippocampal BDNF and estrogen signaling, along with increases in glucocorticoid signaling. Preliminary western blot analyses show that CBA administration significantly increased phosphorylation of extracellular signal-regulated kinase (ERK; p=0.0126), a marker of neuronal plasticity associated with BDNF and other signaling pathways. Additionally, data show a trend for increased phosphorylation of the glucocorticoid receptor in the CBA group (p=0.0569), suggesting a potentiation of stress signaling. OVX did not significantly alter any markers of hippocampal signaling pathways studied. These neurobiological data will be integrated with behavioral measures of performance on novel object recognition test, a procedure that serves as a measure of cognitive function. We speculate these preliminary findings may illustrate the therapeutic potential for reducing stress-related glucocorticoid signaling to combat hippocampal deficits produced by chronic alcohol use in women living with HIV.

APSSG21.26
Estrogen Augments the Cardiac Functional Response to β2-Adrenergic Receptor Stimulation in Young Female Rat Hearts
Yuan Liu1, Sushant Ranadive1, Sarah Kuzmiak-Glancy1
1Kinesiology, University of Maryland

Sexual dimorphism exists throughout the cardiovascular system and is likely to play a role in the lower risk of hypertension, heart failure, and cardiovascular disease in pre-menopausal women compared to age-matched men. In young, healthy hearts, β-adrenergic stimulation results in an increase in heart rate and contractility, primarily via classical β-adrenergic receptor signaling through G-coupled proteins. Alterations in β-adrenergic receptor (β-AR) signaling have been implicated in the development of heart failure, with aging associated with blunted cardiac β-adrenergic responsiveness. This is interesting, because many studies report blunted increases in heart rate and contractility upon β-AR stimulation in female compared to male hearts. Further, it has been reported that estrogen signaling can occur via G-coupled proteins, converging with the β-adrenergic signaling pathway. Purpose: Therefore, the purpose of this study is to evaluate the role of estrogen on the responsiveness of male and female rat hearts to β2-adrenergic stimulation. Methods: First, young male and female rats were anesthetized, hearts were excised, and Langendorff-perfused via the aorta at 62 mmHg with a Krebs-Henseleit buffer, pH=7.4, 37°C. Heart rate (HR), coronary flow rate (CFR), and aortic pressure were continually monitored, and, after 5 min functional equilibration, dose-response curves were generated for either 17-β-estradiol or the β2-adrenergic receptor agonist, albuterol. Next, the estradiol dose which consistently resulted in maximal vasodilation was used to evaluate the interaction between estrogen receptor and β2-adrenergic receptor signaling. In a separate group of rats, young (<8 months) and aging (<20 months), male and female hearts, were perfused as described above. 20 μM 17-β-estradiol was added to the perfusate, and after steady state function was established in the presence of estrogen, dose-response curves for albuterol were again generated. Results: Increases in heart rate upon addition of albuterol were blunted in female compared to male rat hearts: HR increased from 244 ± 12 to 298 ± 11 beats/min (CFR increased from 8.0 ± 0.3 to 9.6 ± 0.6 mL/min/g) in male hearts, and HR increased from 236 ± 10 to 252 ± 25 beats/min (CFR increased from 9.1 ± 0.4 to 9.3 ± 0.8 mL/min/g) in female hearts. When estradiol was added to the perfusate prior to albuterol, functional responses were rescued in the female rat hearts. Albuterol induced a HR increase from 225 ± 8 to 278 ± 8 beats/min in male rats, and HR increased from 225 ± 9 to 271 ± 10 beats/min in female rat hearts. Aging male and females both demonstrated lower baseline HRs (207 ± 8 beats/min in males and 210 ± 9 beats/min in females), and, in the presence of estradiol, demonstrated similar increases in HR in response to albuterol (253 ± 5 beats/min in males and 267 ± 6 beats/min in females). Conclusion: Cardiac responses to β-adrenergic stimulation were blunted in young female compared to young male hearts; however, the presence of estrogen rescued the response such that it matched that of male hearts. The findings in this study indicate that the presence of estrogen may play an important role in stimulation of cardiac function via β-AR signaling in female hearts.

APSSG21.27
Sex differences in cardiometabolic complications in intrauterine growth restricted offspring
Barbara Alexander1
1Physiology, University of Mississippi Medical Center

Essential hypertension is a complex condition of unknown pathogeneses. Recent advances in the field of developmental origins of increased blood pressure add another layer of complexity. Complications during
pregnancy that program increased blood pressure in the offspring are varied and can include preeclampsia, parental smoking or alcohol consumption, maternal stress, or poor perinatal nutrition. Low birth weight serves as a crude proxy for impaired fetal growth indicative of intrauterine growth restriction (IUGR) and numerous experimental models of IUGR are utilized to examine the link between adverse events in early life and increased cardiovascular risk. These experimental models provide proof of principle that birth weight is inversely associated with blood pressure and indicate that despite the method of maternal/fetal insult, mutual mechanistic pathways contribute to the etiology of increased blood pressure in IUGR offspring. The renin angiotensin system, the sympathetic nervous system, endothelin, oxidative stress and vascular dysfunction are all implicated as contributors to increased blood pressure that has its origins in early life. Sex and age also effect the long-term consequences of IUGR on blood pressure control. Many models of developmental insult report increased blood pressure in male IUGR offspring in early life relative to their female IUGR counterparts. However, female IUGR offspring do not remain protected and develop an increase in blood pressure with age that involves a shift in the hormonal milieu in addition to a role for the renal nerves and the renin angiotensin system.

APSSG21.29
G Protein-Coupled Estrogen Receptor Involvement in Sex Differences in Pathophysiology in a Preclinical Model of Hyponatremia
Dianna Nguyen1, Joel Little1, John-Bosco Nguyen1, J. Thomas Cunningham1
1Graduate School of Biomedical Sciences, Department of Physiology and Anatomy, UNT Health Science Center, 2Texas College of Osteopathic Medicine, UNT Health Science Center

Hyponatremia is the most common electrolyte disorder and a particular concern in clinical settings. It is also associated with negative outcomes in various acute injuries (e.g., subarachnoid hemorrhage and exercise-induced hyponatremia) and chronic diseases (e.g., cirrhosis and congestive heart failure). Additionally, hyponatremia is an independent risk factor for increased mortality, resulting in a poorer prognosis in patients. Although hyponatremia associated with many of these conditions is related to inappropriate arginine vasopressin (AVP) release, knowledge gaps still exist about AVP release mechanisms and hyponatremia development particularly related to sex differences, which the present study aims to address. Our previous sex differences studies using an animal model of hyponatremia, bile duct ligation (BDL), showed female (intact and ovariectomized) BDL rats did not develop hyponatremia, AVP neuron activation, or increased plasma copeptin (a marker for AVP), compared to sham-ligated females or male groups. We hypothesize estrogen receptors in the hypothalmo-neurohypophyseal system contributes, at least in part, to these observed sex differences. Our intracerebroventricular (ICV) infusion studies of estrogen receptor (ER) antagonist, ICI 182,780 (ICI), in female BDL and sham rats revealed increased plasma copeptin concentration and lowered hematocrit in the BDL ICI group compared to BDL Vehicle and sham controls. These data suggest ER involvement in sex differences observed in pathophysiology of female BDL rats. However, aside from being a non-specific ERα and ERβ antagonist, ICI is also a G protein-coupled estrogen receptor 1 (GPER) agonist. To test GPER involvement, a series of GPER antagonist, G15, and ICI infusion studies were performed using adult female Sprague-Dawley rats. The animals underwent either BDL or sham surgery and 2 weeks of recovery. Sham and BDL rats were further divided into four drug groups: G15+ICI, G15+ICI Vehicle, G15 Vehicle+ICI, and G15 Vehicle+ICI Vehicle. Respective double drug pump implantation surgeries for G15 (40μg/day, subcutaneous pump) and ICI (1.5μg/kg/day, ICV pump) were performed (8 groups total, n= 4-5 rats per group). The animals were then placed into metabolic cages to collect daily food intake, fluid intake, and urine excretion. Animals were sacrificed after 2 weeks when which their brains were harvested and blood collected for subsequent analyses. Results show there is a trend for both lower plasma osmolality and hematocit in the BDL G15 Vehicle +
ICI group compared to the respective sham group; however this trend was not present in the BDL G15+ICI and Sham G15+ICI groups. These data, although still preliminary, suggest the effects of ICI on plasma osmolality and hematocrit in female BDL rats could be due to GPER activation. Future studies will provide further insight about the role of ERs in sex differences in neurohypophyseal function and mortality risk in a preclinical model of hyponatremia. This work is supported by R01 HL142341 and T32 AG020494.

APSSG21.30
High Fat Diet-Induced Obesity in Sex Differences in Neurogenesis
Kristen Zuloaga1
1Neuroscience & Experimental Therapeutics, Albany Medical College

Poor diet and metabolic diseases, including obesity, diabetes or prediabetes, are associated with an increased risk of neurodegenerative and neuropsychiatric disorders, including Alzheimer’s disease, anxiety, and depression. Impaired adult hippocampal neurogenesis may be one mechanism linking these conditions. The goal of this study was to determine if there are sex-specific effects of high fat diet/metabolic disease on neurogenesis, as these could underlie the observed sex difference in these conditions (females more adversely affected than males). Male and female C57BL/6J mice were fed HF or control diet, injected with EdU (to label dividing cells), then euthanized 4 weeks later. Cell proliferation, differentiation/maturation and survival of new neurons in the dentate gyrus were assessed. Females on a control diet had more proliferating cells (Ki67+) and neuroblasts/immature neurons (DCX+) compared to males; however, HF diet reduced these in females to the levels of males. Diet did not affect neurogenesis in males. Further, the numbers of proliferating cells and immature neurons were inversely correlated with both weight gain and glucose intolerance in females only. These effects were robust in the dorsal hippocampus, which supports cognitive processes. Assessment of neuroinflammation in the dentate gyrus using immunofluorescence for Iba1 and CD68 uncovered sex-specific effects of diet, which may contribute to observed differences in neurogenesis. These findings demonstrate sex-specific effects of HF diet/metabolic disease on neurogenesis and highlight the potential for targeting neurogenic deficits to treat cognitive decline and reduce the risk of dementia associated with metabolic disease, particularly in females.

APSSG21.33
Hypertension is Leptin-dependent in Ovariectomized Obese Agouti Yellow Female Mice
Candee Barris1, Taylor Kress1, Jessica Faulkner2, Eric Belin de Chantemele1,2,3
1Vascular Biology Center, Medical College of Georgia at Augusta University, 2Department of Physiology, Medical College of Georgia at Augusta University, 3Department of Medicine (Cardiology), Medical College of Georgia at Augusta University

Obesity, which affects 40% of postmenopausal women, is a major risk factor for hypertension and cardiovascular disease (CVD). While it has been established that obesity abolishes the cardioprotective effects of female sex hormones and predisposes women to hypertension, the specific mechanism by which obesity and menopause interact to elevate blood pressure (BP) remains unknown. Previously, we demonstrated that hypertension is leptin-dependent and aldosterone-mediated in obese female mice of reproductive age. Herein, we hypothesized that loss of female sex hormones with ovariectomy (OVX) increases BP and impairs vascular function in obese mice. Obese agouti yellow mice (Ay) on a KK background and C57BL/6 lean controls underwent OVX or sham surgery at 12 weeks. At 15 weeks, mice were implanted with radiotelemeters to record BP under baseline conditions and in response to leptin blockade with Allo-Aca, a leptin receptor antagonist (0.05mg/kg/day s.c. osmotic minipump). To examine the effects of OVX on autonomic control of BP, we measured BP and HR responses to ganglionic blockade (hexamethonium) as well as to atropine and propranolol. At 18 weeks, mice were euthanized and mesenteric arteries isolated to measure endothelial function via wire myography. Obesity significantly increased mean arterial pressure (MAP) but OVX did not further elevate BP. Unexpectedly, in only Ay mice, OVX significantly reduced MAP (P<0.0413) but preserved HR responses to propranolol and atropine respectively. Allo-Aca treatment significantly decreased MAP similarly in both sham and OVX obese mice, indicating BP elevation is leptin-dependent. Obese mice had significantly impaired relaxation to acetylcholine (2-way ANOVA, P<0.05) but OVX did not further impair relaxation. Compared to lean controls, LNAME trended to impair (2-way ANOVA, P=0.054) relaxation responses to acetylcholine for obese OVX mice indicating endothelial dysfunction could be attributable to impaired endothelial NO bioavailability. Quantitative real-time PCR showed a trend for decreased adrenal aldosterone synthase expression in obese OVX mice despite a trend for increased plasma aldosterone levels, suggesting that a tissue other than the adrenals produces aldosterone in obese OVX mice. All together, these preliminary data suggest obese ovariectomized mice develop hypertension via leptin-induced aldosterone production in response to the absence of female sex hormones with no additional contribution of the autonomic nervous system to BP elevation and potentially have impaired endothelial NO bioavailability. Funding:
APSSG21.14
The hemodynamic response to sympathetic activation differs in women with natural menstrual cycles and women using oral contraceptives
Aaron Voshage¹, Dain Jacob¹, Jennifer Harper¹, Clayton Ivie¹, Jacqueline Limberg¹
¹Nutrition and Exercise Physiology, University of Missouri, Columbia

Objective: Acute increases in sympathetic nervous system activity (SNA) elicit peripheral vasoconstriction and an increase in blood pressure (BP). The vascular response to SNA is termed neurovascular transduction and is greater in young men than young women. Sex-related differences in neurovascular transduction have been attributed to greater β-adrenergic receptor (β-AR) mediated vasodilation in women compared to men. Given β-AR vasodilation is greater in women taking oral contraceptives (OC) compared to women with natural menstrual cycles (NC), we sought to examine the effect of menstrual cycle and oral contraceptive pill phase on the hemodynamic response to acute SNA. We hypothesized acute increases in SNA would elicit greater peripheral vasoconstriction and increases in BP in NC women compared to OC women, independent of cycle/pill phase. Methods: NC (n=11, 25±1 yrs) and OC (n=10, 24±1 yrs) women were studied during the low (early follicular, placebo pill) and high (late follicular, active pill) hormone phases of the menstrual/pill cycle (IRB #2011312). BP (finger photoplethysmography), heart rate (HR, ECG), and forearm blood flow (FBF, venous occlusion plethysmography) were measured and cardiac output (CO) and total peripheral resistance (TPR) were calculated (ModelFlow) at baseline and during a 2-min cold pressor test (CPT). Results: Sympathetic activation via CPT resulted in a time-dependent increase in BP that did not differ between groups and/or phases (p>0.05); however, the mechanisms by which a rise in BP was achieved differed between groups. During the high hormone phase, OC women exhibited greater and sustained increases in HR compared to NC women (Interaction of group and time, p<0.001), resulting in group differences in CO (Interaction, p=0.001). This greater HR was compensatory for lower TPR in the OC women during the CPT (Interaction of group and time, p=0.008). Notably, whereas NC women exhibited vasoconstriction within the forearm vasculature during CPT, OC women exhibited vasodilation (Interaction of group and time, p=0.022). Conclusion: Although the BP response to acute increases in SNA does not differ between NC and OC women, contributing mechanisms are divergent – notably during the high hormone phase of the menstrual/pill cycles. OC women exhibit paradoxical vasodilation and lower TPR during acute SNA compared to NC women, thus requiring a greater increase in HR to maintain BP. These observations may be attributed to β-AR upregulation at the level of the heart and peripheral vasculature with OC use compared to NC, but future work is warranted. Funding: HL153523, HL130199

APSSG21.36
Sex differences in the immune system: placenta and beyond
Lana McClements¹
¹Faculty of Science, University of Technology Sydney

Placenta is a highly vascularized organ that provides oxygen and nutrients to the fetus facilitating its growth. During early pregnancy, fetal extravillous cytotrophoblast cells (EVTs) migrate from the placental villi in order to invade the maternal spiral uterine arteries (SUAs). EVT remodeling of SUAs by replacing the endothelial and smooth muscle cells from these vessels so that they are no longer vasoactive and able to contract. This remodeling results in arteries with large lumens and low-resistance, ensuring consistent blood flow to the placental bed and fetus. Pregancies carrying male and female fetuses do not only differ in pregnancy outcomes and complications including pregnancy loss, preterm birth, intrauterine growth restriction and preeclampsia, but the fetal gender also influences the molecular constitution of the placenta. Therefore when placental research is conducted it is critical to take into the account the fetal gender especially when two or more groups are compared. For example, male fetuses tend to be larger by the second trimester of pregnancy and show a more pro-inflammatory immune response across gestation. The sex differences exist even beyond pregnancy and are reflected in lower incidence of infections in female (including COVID19) and cardiovascular disease, which are likely linked to differences in the steroid hormones. We have been elucidating the role of a novel immunophilin protein, FK506-binding protein like (FKBPL), in placental development, preeclampsia and cardiovascular disease. FKBPL is a chaperon protein that forms a complex with heat shock protein 90 (HSP90) and regulates estrogen, androgen and glucocorticoid receptor signalling. In a complex with androgen receptor, FKBPL has been implicated in male infertility. It was also shown that FKBPL binds to a cell surface receptor, CD44, regulating cell—cell interactions, cell adhesion and migration. Hence, FKBPL has a key role in angiogenesis and inflammation. A recent study identified CD44/FKBPL ratio as a novel predictive and diagnostic biomarker of preeclampsia at 20 weeks’ gestation and following clinically established preeclampsia, respectively. FKBPL and CD44 were also aberrantly expressed in placental tissue and mesenchymal stem cells (MSCs) from women with preeclampsia compared to normotensive controls. Emerging data has demonstrated that MSCs' therapeutic effect on cell migration and tubule formation relevant to placental development and preeclampsia involves FKBPL signalling. Interestingly, the plasma levels of FKBPL are substantially lower in female than male. In summary, as early as in utero fetal sex controls the environment it grows in. The immune system is governed by specific sex chromosome genes and hormones and it plays a key role in placental development and growth, and pregnancy outcomes. Subsequently, adverse pregnancy outcomes including preeclampsia and intrauterine growth restriction where the root cause is impaired placentation, increase the risk of future metabolic, cardiovascular and neurological disorders. Therefore, it is important to understand these differences that fetal sex imposes on
Sex Differences in Vascular Stiffening with Age

Bulouere Wodu1, Maria Bauer2, Logan Smith3, Travis Brady4, Kavitha Nandakumar2, Huilei Wang3, Shivam Rastogi2, Jochen Steppan4, Lakshmi Santhanam2,3
1Biology, Johns Hopkins University, 2Anesthesiology and Critical Care Medicine, Johns Hopkins University, 3Biomedical Engineering, Johns Hopkins University

Introduction: Vascular stiffening is a hallmark of aging and an independent predictor of cardiovascular risk. Multiple studies show that the increase in vascular stiffening with age is steeper in females due to the acceleration in stiffening that occurs after menopause. While this phenomenon has been attributed to estradiol signaling, the role of testosterone is yet to be known. In addition, the cellular and molecular underpinnings of sex-differences in stiffening remain poorly understood as a result of the lack of reliable preclinical models. Thus, targeted therapy remains elusive. So, the primary goal of the study was to use indices of vascular stiffness to evaluate the potential of C57BL/6J mouse as a preclinical model to investigate the mechanisms underlying sex differences in age-induced vascular stiffening. Methods: We used male and female C57BL/6J mice at 3-4 months (young) and 18-20 months (old). In vivo aortic stiffness was assessed by pulse wave velocity; ex vivo passive aortic stiffness was determined by tensile testing. Lastly, wire myography was performed to evaluate the vasocontractile and vasodilatory responses of the isolated aorta, as endothelial dysfunction and vascular smooth muscle cell (VSMC) reactivity evolve with age in both males and females. Results: Active in vivo and passive ex vivo vascular stiffness increased with age markedly more in females than in male counterparts, in good agreement with human and primate studies. Endothelial function and VSMC reactivity decreased with age notably more in females. Conclusions: We show that the age-dependent deterioration of many indices of arterial mechanics and function were more significant in old females when compared with old males which recapitulates changes noted in humans and non-human primates. This suggests that the C57BL/6J mouse model is a robust and reliable pre-clinical model in which to study sex differences in vascular aging.
studies where surgical implantations are performed. We observed a strong effect of host sex on fecal GM structure, further emphasizing the importance of studying male and female animals for externally valid results. Choice of DNA extraction kit influences GM community and planning is needed for a reproducible GM study. Implantation of single-dose antibiotic SQ does not impact GM structure, but antibiotic + surgery decreases alpha diversity in samples using bead beating DNA extraction technology. KAM is supported by intramural research funds from the National Institutes of Health Clinical Center.

**APSSG21.40**

**Sex Differences in Macrophage Migration Inhibitor Factor signaling in COVID-19**

So-Jin Kim, Sydney Bear, Youwei Chen, Emeka Ifedigbo, Elias Coutavas, Patty J. Lee

1Pulmonary, Allergy, and Critical Care Medicine, School of Medicine, Duke University

Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine that maintains homeostasis by regulating physiological signaling pathways. Interestingly, there is accumulating evidence that sex steroid hormones are related to MIF release – MIF is positively correlated with testosterone and negatively with estradiol. Recent COVID-19 studies have demonstrated gender-based differences in response severity and higher mortality in male patients. This study aims to investigate the sex difference in MIF signaling in SARS-CoV-2 (CoV2) infected mice and post-COVID-19 human subjects. The animal studies protocol was approved by the the Duke University School of Medicine Institutional Animal Care and Use Committee (assurance number: A160-19-07). Humanized ACE2 mice of both genders (K18-hACE2) were purchased from Jackson Laboratory. Mice were infected by 104 plaque-forming units of CoV2 (USA-WA1/2020) under anesthesia with isoflurane at the Duke Regional Biocontainment Laboratory. MIF20, a MIF agonist, was orally administered to mice 10 min before exposure to the virus and every 24 h up to 5 days post-infection (DPI). The baseline of body weight and temperature were determined before infection and monitored for up to 6 DPI. Mice were sacrificed, and organs were collected at 6 DPI. Copies of CoV2 Nucleocapsid RNA in lung tissues were determined. The institutional review board approved the human studies of Duke University (Pro00105518). Participants had blood samples collected at the initial study visit, 3 months after COVID infection. All individuals provided written informed consent before study activities. CoV2-infected mice showed marked body weight loss beginning at 4 DPI, and most animals had lost approximately 20% of their body weight by 6 DPI in both gender. After infection, body temperature was increased and peaked at 2 DPI (CoV2 group: 36.6 ± 0.18°C vs. Control group: 32.8 ± 0.13°C), a sign that the mice were infected, also found in people. At 6 DPI, body temperature was dropped to 30.5 ± 0.86°C, a sign of septic shock in mice and people. There was a trend of MIF20 attenuating the hypothermia in male mice. CoV2-infected mice showed significantly increased viral nucleocapsid gene expression levels, and MIF20 augmented this gene expression only in female mice. Male mice showed increased MIF gene expression in lung tissues after CoV2 infection, but there was no change in female mice. In addition, CoV2 increased gene expression of Cd74, a MIF receptor, in male mice but decreased in female mice. This pattern was further supported by post-COVID-19 people, with increased serum CD74 levels in men compared to healthy controls, but no changes in women at 3 months after recovery from COVID-19. Plasma MIF levels were decreased 3 months after recovery compared to 6 weeks after recovery, and there was no gender difference. We successfully established a mouse model of COVID-19 that mirrors key physiological features of human COVID-19. Here, we discovered MIF20 augmented CoV2-induced increased viral gene expression only in female mice. Also, male mice showed increased MIF and CD74 gene expression by CoV2, indicating the gender-based differences in MIF signaling response to CoV2.

Interestingly, a higher correlation between MIF and CD74 expression was observed in CoV2-infected male mice (R2 = 0.4491, p value <0.05). The post-COVID sample analysis confirmed increased plasma CD74 again only in men. In addition, CoV2-infected mice showed increased MIF levels while COVID-19 patients showed decreased MIF levels after recovery, indicating the need for disease phase targeting therapy. These findings collectively suggest the involvement of MIF signaling in the sex differences observed in COVID-19 and the implementation of sex- and disease phase-specific therapeutic strategies against COVID-19. Further studies will investigate the role of MIF signaling in COVID-19 and test MIF agonist (MIF20) and MIF antagonist (MIF98) in CoV2-infected mice and people during acute COVID-19 as well as during prolonged recovery from COVID-19.

**APSSG21.41**

**Inflammatory and microbial-associated metabolic signatures are correlated with depression during pregnancy**

Beatriz Penalver Bernabe, Andrea Ohl, Elizabeth Wenzel, Unnathi Nagelli, Mohit Jain, Lisa Tussing-Humphreys, Pauline Mak, Jack Gilbert

1Department of Biomedical Engineering, University of Illinois at Chicago, 2Department of Psychology, University of Illinois at Chicago, 3Biomedical Engineering, University of Illinois at Chicago, 4Department of Pharmacology, University of California San Diego, 5Department of Kinesiology and Nutrition, University of Illinois at Chicago, 6Department of Psychiatry, University of Illinois at Chicago, 7Department of Obstetrics and Gynecology, University of Illinois at Chicago, 8Department of Pediatrics, University of California San Diego, 9Scripps Institution of Oceanography, University of California San Diego

Prenatal depression (PND), depression during pregnancy, is common (10-20%) and have negative obstetric consequences, including preterm birth and low-birth weight. An under-explored mechanism that could contribute to the onset of PND is the microbiome-gut-brain axis (MGBA). Identification the associations between elevated symptoms of depression during pregnancy and
quantifiable characteristics of the MGBA, including fecal microbiota and serum metabolites and cytokines, could increase our limited understanding of the pathobiology of PND. For that, we recruited 65 pregnant women at less than 16 gestational weeks from an outpatient obstetric clinic at the University of Illinois at Chicago, and we followed them longitudinally at each trimester during pregnancy. At each visit, participants answered a battery of mental health questionnaires (e.g., PHQ-9) and provided fecal samples; blood samples at the first two visits. Gut microbial composition, immune activity and plasma metabolomics were characterized with 16S rRNA sequencing, Luminex and LC/MS/MS respectively. Participants were 55% Black and 30% Latina with a 22% rate of PND (national average is 12%). Our results indicated that the MGBA was dynamic and distinct in women with PND versus those without. MGBA dysregulation was manifested in the gut microbiota composition, host metabolism and immune system. For instance, Lactobacillus (a probiotic with antidepressant potential) presented lower abundance in the first trimester and F. prausnitzii species, producers of anti-inflammatory metabolites, in both first and second trimesters in women with PND. A parallel analysis of the host’s inflammatory and systemic metabolism showed that women who developed PND had higher serum concentrations of proinflammatory cytokines, including IL-17A and IFN-γ; amino acids and their derivatives (e.g., proline, hypoxantine, histidine); saturated fatty acids (e.g., myristic and palmitic acids); unsaturated fatty acids (e.g., arachidonic and oleic acid); and secondary bile acids (e.g., glycodeoxycholic acid). Hippurate, which is associated with increased microbial diversity and inversely correlated with metabolic syndrome, was depleted in women with PND. In conclusion, MGBA is dynamically altered during pregnancy and distinct in women with PND versus those without. Our results showed the potential of the MGBA to diagnose and predict the onset of PND and increase our understanding to the pathobiology of PND. Funding sources: Arnold O. Beckman Postdoctoral Award (BPB), BIRCWH K12 HD101373 (BPB) and NICDH R03 HD095056

APSSG21.42
Maternal B Cell Depletion Decreases Blood Pressure and Improves Fetal Weights in Offspring of a Rat Model of Preeclampsia
Nathan Campbell1, Owen Herrock1, Dylan Solise2, Sarah Fitzgerald1, Evangeline Deer1, Ty Turner1, Kathy Cockrell2, Tarek Ibrahim1, Morgan McCray1, Kyberlee Evans1, Nicole Ingram1, Lorenza Amari1, Babette Lamarca1
1Pharmacology and Toxicology, University of Mississippi Medical Center, 2Obstetrics and Gynecology, University of Mississippi Medical Center

Preeclampsia (PE), new onset hypertension during pregnancy, is the leading cause of death and morbidity for the mother and low birth weight in offspring. PE women have activated B cells producing agonistic autoantibodies to the angiotensin II type I receptor (AT1-AA). We have shown Rituximab (R), used clinically for B cell depleton, lowers mean arterial pressure (MAP), B cells, and AT1-AA in the RUPP rat model of PE. Clinical studies show no untoward effects on offspring of pregnant women maintained on R for treating lymphoma. R is not used during PE, therefore, effects of maternal B cell depletion on offspring survival and growth in response to placental ischemia is unknown. We hypothesize that R will deplete maternal B cells in RUPP rats without worsening the effect of placental ischemia on pup growth and survival. To test this hypothesis, R (250 mcg/kg) was given on gestation day (GD) 14 via mini-osmotic pump. On GD 19 B cells were measured by flow cytometry, and MAP and pup weights were recorded. A separate group of dams were allowed to deliver, pup weights were recorded within 12 hours and weekly until 16 weeks, and B cells were analyzed. A one-way ANOVA was used for statistical analysis. MAP increased in RUPP 12±2 % (n=19, p<0.05) compared to NP controls 101±1 % (n=18) and was 106±3 mmHg in RUPP+R (n=8, p<0.05). On GD19, maternal circulating B cells were 16±2 % (n=14) in RUPPs, 8±2 % in NP rats, (n=7, p<0.05), and 5.5±1 % gate in RUPP+R (n=5, p<0.05); RUPP male and female offspring were smaller (5.1±0.23 g, 5.19±0.14 g; n=4, n=4) at birth than NP offspring (6.09±0.15 g, 5.87±0.12 g; n=6, p<0.05; n=6, p<0.05) or RUPP+R offspring (5.75±0.24 g, 5.36±0.28 g; n=6, p=0.11; n=6, p=0.67). At 12 weeks, male and female RUPP offspring had elevated circulating B cells (21±3, 20±1 %; n=6; n=9) compared to NP (1±0.23, 1±0.06 %; n=4, p<0.05; n=3, p=0.04) which was normalized in RUPP+R offspring (0.4±0.1, 0.3±0.03 % gate; n=3, p<0.05; n=8, p<0.05). At 16 weeks, B cells were comparable in male and female offspring from NP (0.78±0.09 %, n=10; 1.06±0.21 % gate, n=6) and RUPP+R rats (0.80±0.04 % gate, n=3; 1.88±1.00 % gate, n=2). Our findings indicate that R lowers maternal circulating B cells and MAP in RUPP rats and improves fetal weight and circulating B cells, indicating that R does not worsen adverse fetal outcomes in response to placental ischemia.

APSSG21.43
Serum Testosterone and Cardiovascular Risk in Transgender Men: A Protocol for Systematic Review and Meta-analysis
Keila Turino Miranda1, Chantal Rytz1, Nathalie Saad1, Sandra Dumaniski1, Sofia Ahmed1
1Nephrology, University of Calgary

Background Transgender men (individuals assigned female at birth who identify as men) have poorer cardiovascular health compared to cisgender men. Elevated testosterone levels are associated with increased cardiovascular risk in cisgender women (individuals assigned female sex at birth who identify as women), though whether this applies to transgender men is yet unknown. Objective To determine the association between serum testosterone levels and cardiovascular morbidity and mortality in transgender men on gender-affirming testosterone hormone therapy. Methods Electronic bibliographic databases (MEDLINE, EMBASE, and PsycINFO) from inception to July 30, 2021 will be searched for studies examining cardiovascular outcomes in transgender men on testosterone therapy. The systematic review protocol has been registered in PROSPERO. Two investigators will independently screen
identified publications for inclusion into the review. Studies will be eligible for inclusion if they include: 1) post-pubertal transgender men; 2) gender-affirming testosterone therapy; 3) serum testosterone levels; and 4) cardiovascular-related morbidity and/or mortality (events [e.g., myocardial infarction], mortality, surrogate measures of cardiovascular risk [e.g., blood pressure]). Eligible study designs will include randomized controlled trials and observational studies. Data on study design characteristics, population, gender-affirming testosterone therapy, comorbidities and cardiovascular outcomes will be independently extracted by each investigator, and quality and risk of bias will be assessed. All discrepancies between reviewers were resolved by discussion or with the involvement of a third reviewer who served as the final adjudicator. Where possible, these data will be summarized using pooled or combined estimates for the risk ratio or hazard ratio of cardiovascular mortality, cardiovascular outcomes, and surrogate markers of cardiovascular risk (e.g., blood pressure). A random effects model will be used to explore potential sources of heterogeneity. Subgroup analyses assessing route of administration, dose, duration and frequency of testosterone exposure will be completed. Conclusion Improved understanding of the association between serum testosterone levels and cardiovascular morbidity and mortality in transgender men will help to guide future clinical practice and allow for further informed decision-making in the use of gender-affirming testosterone therapy.

APSSG21.46
Questioning the sex-specific differences in the association of smoking on the survival rate of hospitalized COVID-19 patients
Athar Khalil1,2, Radhika Dhingra 3, Jida Al-Mulki4, Mahmoud Hassoun4, Neil Alexis5
1Department of Genetics & Genome Sciences, case western reserve university, 2Clinical Research Unit, Rafik Hariri University Hospital, 3Department of Environmental Sciences and Engineering, University of North Carolina, 4Department of Pulmonary and Intensive Care Unit, Rafik Hariri University Hospital, 5Center for Environmental Medicine Asthma and Lung Biology, University of North Carolina

Introduction: In the absence of a universally accepted association between smoking and COVID-19 health outcomes, we investigated this relationship in a representative cohort from one of the world’s highest tobacco consuming regions. This is the first report from the Middle East and North Africa that tackles specifically the association of smoking and COVID-19 mortality while demonstrating a novel sex-discrepancy in the survival rates among patients. Methods: Clinical data for 743 hospitalized COVID-19 patients was retrospectively collected from the leading centre for COVID-19 testing and treatment in Lebanon. Logistic regression, Kaplan-Meier survival curves and Cox proportional hazards model adjusted for age and stratified by sex were used to assess the association between the current cigarette smoking status of patients and COVID-19 outcomes. Results: In addition to the high smoking prevalence among our hospitalized COVID-19 patients (42.3%), enrolled smokers tended to have higher reported ICU admissions (28.3% vs 16.6%, p<0.001), longer length of stay in the hospital (12.0 ± 7.8 vs 10.8 days, p<0.001) and higher death incidences as compared to non-smokers (60.5% vs 39.5%, p<0.001). Smokers had an elevated odds ratio for death (OR=2.3, p<0.001) and for ICU admission (OR=2.0, p<0.001) which remained significant in a multivariate regression model. Once adjusted for age and stratified by sex, our data revealed that current smoking status reduces survival rate in male patients ([HR]=1.9 [95% CI]=1.689; p= 0.041) but it does not affect survival outcomes among hospitalized female patients([HR]=0.79 [95% CI]= 0.374-1.689); p= 0.551). Conclusion: A high smoking prevalence was detected in our hospitalized COVID-19 cohort combined with worse prognosis and higher mortality rate in smoking patients. Our study was the first to highlight potential sex-specific consequences for smoking on COVID-19 outcomes that might further explain the higher vulnerability to death from this disease among men.

APSSG21.47
Prenatal Exposure to BPA Substitutes and Vascular Endothelial Function: Role of Estrogen Signalling
Liam Connors1, Emma Walsh1, Hai-Lei Zhu1, Radha Singh1, Jennifer Thompson1
1Physiology and Pharmacology, University of Calgary

Background: Bisphenol A (BPA) is among the world’s most ubiquitous industrial chemicals, used as a plasticizer in the manufacture of plastics and resins. Bisphenols interfere with estrogen receptor (ER) signalling, which has been linked to several health conditions, including cardiovascular disease. These effects are sex dependent due to variations in estrogen levels (1). After regulatory agencies declared BPA to be a toxic substance in 2010, manufacturers turned to substitutes such as bisphenol S (BPS). BPS exposure is on the rise; a recent study revealed that BPS was detectable in 81% of adult urine samples in the United States (2). BPS can cross into the placenta, and it accumulates in the fetal compartment to a greater extent than BPA (3). BPS may also disrupt estrogen-dependent activation of nitric oxide (NO) production, a vasodilatory agent that plays an important role in maintaining vascular health (4). This project focuses on understanding the sex-dependent risks of developmental exposure to BPS on later-life vascular function. Objective: To determine if prenatal BPS exposure will influence adult vascular health by modulating blood vessel regulation in a sex-dependent manner. Methods: Mesenteric arteries were excised from male and female C57BL/6 mice prenatally exposed to 250 nM BPS and mounted on a pressure myograph. After precontraction with phenylephrine, cumulative doses of acetylcholine were added to assess dilation. Vessel stiffness of the aorta was assessed with a wire myograph. Results: Male derived vessels did not exhibit changes in their dilatory or contractile response after prenatal BPS exposure. Increased dilation was observed in female BPS-exposed mice, and sensitivity to contractile agonists was

APSSG21.48
Microbiome in Obese Pregnancy and Preeclampsia
Jenny Sones1, Kalie Beckers2
1Veterinary Clinical Sciences, Louisiana State University School of Veterinary Medicines, 2Veterinary Clinical Sciences, Louisiana State University School of Veterinary Medicine

Preeclampsia (PE) is a devastating pregnancy-specific disorder that affects over 8 million pregnancies globally with an estimated 76,000 women dying annually. PE is diagnosed after 20 weeks of gestation with the onset of hypertension (≥140mmHg systolic blood pressure (BP) or ≥90mmHg diastolic BP), and either proteinuria or another accompanying sign/symptom, such as thrombocytopenia, or neurological symptoms, including headaches or visual impairment. There is no known cure for PE and the only effective treatment is delivery of the fetus (and placenta), which is often preterm. There is strong evidence to support abnormal placentaion playing a causal role in the development of the maternal PE syndrome. Unfortunately, the exact mechanism(s) is unknown. A number of pre-conception maternal conditions have been labeled as risk factors, such as pre-gestational diabetes, chronic hypertension, and obesity, but we are still not able to positively predict which mothers will develop PE. Women with a body mass index (>35 kg/m2) prior to pregnancy have a 30% increased risk of developing PE compared to lean counterparts. Pre-conception obesity, with increased white adipose tissue (WAT), is proposed to interfere with the establishment of adequate blood flow to the placenta due to heightened systemic inflammation. Although the association between maternal obesity and PE is well documented, there is limited knowledge about the mechanism whereby obesity contributes to PE. A common sequela of obesity is gut dysbiosis and “leaky gut syndrome”. Together, this contributes to a heightened state of inflammation, which in pregnancy may contribute to altered placental development and downstream PE syndrome. Obesity alters the gut microbiota through proliferation of pro-inflammatory bacterial byproducts. Thus, the gut microbiome of obese women with large amounts of WAT preconditions them to be in a pro-inflammatory state prior to pregnancy that can significantly influence outcomes. Existing data supports that maternal obesity impairs proper placental formation and contributes to adverse pregnancy outcomes, but the interaction of the gut-placental axis has not been fully explored. This is due in part to the novelty of this emerging field, but also to limitations in testing weight loss in pregnant women and sampling placental tissue. The mouse with its similarities to human placentation and cost effectiveness as a laboratory animal has many advantages as a model of pregnancy-related disorders. We hypothesize that gut dysbiosis due to obesity pre-conception contributes to the maternal microbiome in BPH/5 mice, which is altered compared to control normotensive mice, and can be restored with maternal adiposity reduction prior to pregnancy. Utilizing the BPH/5 mouse, which spontaneously develops a PE-like phenotype, allows us to investigate events before, during, and after pregnancy that may contribute to PE in women. By identifying key dysregulated factors in maternal WAT during pregnancy we can more fully understand which pregnant women may go on to develop PE. Obese women who alter their metabolic phenotypes through diet, exercise, and reduction in adiposity prior to pregnancy may have more successful outcomes.

APSSG21.50
Divergent renal bioenergetic and oxidative stress related pathways in healthy male and female rats
Morgan J. Spicer1,2, Ryan Schibalski2, Regina Sultanova2, Mark Domondon2, Thelma Amoah1, Courtney J. Christopher3, Hector F. Castro3, Kerin Cahill4, Allison McCreimmon4, Kristzian Stadler4, Shawn R. Campagna3, Daria Ilatovskaya1,2
1Physiology, Augusta University, 2Medicine, Medical University of South Carolina, 3Chemistry, University of Tennessee, Knoxville, 4Oxidative Stress and Disease, Pennington Biomedical Research Center

Introduction: It is established that sex plays a role in the development and severity of renal and vascular disease; many renovascular diseases are closely linked with mitochondrial dysfunction, oxidative stress, and inflammation. Discrepancies in male and female mitochondrial function prior to the onset of disease could be contributing to the observed sex-specific trends. Here, we hypothesize that female mitochondria have higher sensitivity to oxidative stress, resulting in earlier opening of the mitochondrial permeability transition pore (mPTP), which translates into metabolic changes that contribute to renoprotective mechanisms pre-menopause. Methods: Isolated renal mitochondria were obtained from 11-weeks-old Sprague Dawley (SD) rats, and Seahorse XF assay was
performed to measure OCR. CaGreen, TMRM and Amplex Red were used to measure mitochondrial Ca2+ uptake, membrane potential and H2O2 levels, respectively. Electron spin resonance spectroscopy (EPR) was employed to detect lipid peroxide radicals. Metabolomic profiles of renal cortices and medullae were generated using UHPLC-HRMS, and metabolites were identified by retention time exact mass using MAVEN and MetaboAnalyser software. Results: Spectrofluorimetry revealed higher mitochondrial membrane potential in female rat (SDF) medulla as compared to male rat (SDM) medulla (p<0.001); the SDF group exhibited higher overall H2O2 production (p<0.001 in cortex and medulla). Similar lipid peroxide radical levels were observed in all groups. Seahorse assay indicated that SDF mitochondria displayed decreased OCR compared to SDM rats, and that medullary OCR was lower across all parameters, regardless of sex. Interestingly, calcium uptake analysis showed that the opening of the mitochondrial permeability transition pore (mPTP) occurred earlier in SDF rats than in SDM. Relative abundances of metabolites associated with inflammation, such as UDP-glucose and S-methyl-S'-thioadenosine, were different in mes female kidneys independent of the region (p<0.01). Furthermore, the bile salt cholate and its derivatives were more abundant in females (p<0.001). Interestingly, acetylyasine and N-acetylglutamine, both recently suggested as markers of CKD, were also significantly higher in females (p<0.001). Conclusions: Renal mitochondria displayed sexual dimorphisms in bioenergetics, primarily in OCR, H2O2 production, and mPTP opening, suggesting higher mitochondrial sensitivity to ROS in females. We report differential profiles of pro- and anti-inflammatory metabolites in males and female kidneys, indicative of unique pathways by which each sex mediates inflammation. These data offer a scaffold for further exploration of oxidative stress-related inflammatory pathways which likely diverge in males and females.

Funding: R01 HL148114 (to DVI), and R01 DK115749 (to KS)

APSSG21.51
Guanylate cyclase-C and anxiety-like behavior: gender and estrus cycle differences
Martina Ratko1,2, Nikola Habek1,2,3, Aleksandra Dugandzic1,2,3
1Croatian institute for brain research, School of Medicine, University of Zagreb, 2Centre of Excellence for Basic, Clinical and Translational Neuroscience, School of Medicine, University of Zagreb, 3Department of Physiology, School of Medicine, University of Zagreb

Anxiety-like disorders are the most common mental disorders in the modern world with an incidence two times higher in women than in men. Amygdala, the brain region involved in emotional processing and fear conditioning, shows distinctive structural and physiological sexual dimorphism. Agonists of membrane-bound guanylate cyclase (GC) A and B have been shown to possess dose-dependent anxiolytic properties. Therefore, the aim of this study is to determine if activation of guanylate cyclase C (GC-C) in amygdala could affect anxiety-like behaviour differently in female than in male mice. In this study we used immunohistochemical staining in male and female wild-type (WT) animals, with GC-C knock-out animals (GC-C KO) as controls. GC-C mRNA levels in amygdala and hypothalamus were evaluated using qPCR. Anxiety levels were tested with two behavioural tests (home cage escape, elevated plus maze). Vaginal swabs were stained with 0.1% cresyl violet stain and analysed using a stereomicroscope to determine the phase of the oestrus cycle. GC-C is expressed in the neurons of basolateral nucleus and cortical area of amygdala. During the oestrus cycle, GC-C expression changes differently in amygdala compared to hypothalamus. Therefore, only female mice in diestrus showed different anxiety levels compared to male mice, which is even more pronounced in GC-C KO mice. As expected, no difference in anxiety levels between genotype was present in male animals. Female mice demonstrate different anxiety levels during the diestrus phase compared to male animals. GC-C is present in amygdala, and its inhibition during diestrus could be responsible for the difference in anxiety levels between genders and during different phases of the oestrous cycle. Our results indicate that GC-C activation may have anxiolytic properties similar to activation of other membrane-bound GCs. FUNDING: This work has been supported by Croatian Science Foundation under the project FURNACE (IP-2018-01-7416) and co-financed by the European Union through the European Regional Development Fund, Operational Programme Competitiveness and Cohesion, grant Agreement No. KK.01.1.1.01.0007, CoRE - Neuro.

APSSG21.52
Regulation of brown adipose tissue activity by brain uroguanylin is dependent on gender and phase of estrous cycle
Aleksandra Dugandzic1,2, Nikola Habek1,2,3, Martina Ratko1,2, Milan Kordić4, Aleksandra Dugandzic1,2,3
1Croatian Institute for Brain Research, University of Zagreb, 2Centre of Excellence for Basic, Clinical and Translational Neuroscience, School of Medicine, University of Zagreb, 3Department of Physiology, School of Medicine, University of Zagreb, 4MKP Ltd., MKP Ltd.

Postprandial activation of brown adipose tissue (BAT) is gender- and age-dependent. Since uroguanylin (UGN), as an agonist of guanylate cyclase C (GC-C), leads to browning after prolonged i.c.v. application and is released from the gut after a meal, our aim was to determine the acute activation of BAT by UGN. In this study, male and female C57Bl/6NCrl mice were used. The activity of BAT was determined by infrared thermography (FLIR T1020). The expression of UGN in hypothalamus upon insulin or GLP-1 stimulation was determined by GUCA2B ELISA Kit. GC-C was localized in the Arcuate nucleus of hypothalamus by immunohistochemistry. In older animals, i.n. application of doses five times smaller led to a significant increase in BAT activity compared to i.p. application. This activation was smaller in female animals in diestrus and not present in estrus. Differences in BAT activation due to estrous cycle could be explained by increased and different pattern of expression of GC-C in
hypothalamus in female mice in diestrus. The increase in BAT activity upon insulin and GLP-1 application is again gender-dependent. UGN KO female mice showed no increase in BAT activity upon GLP-1 application when compared to WT female mice (all in diestrus). GLP-1, 2h after i.n. application, decreased pro-UGN expression in hypothalamus with no similar changes in plasma and CSF concentrations. When analogues of GLP-1 are used in treatment of diabetic patients, the changes in BAT activity and glucose expenditure by BAT could be expected. This study could lead to development of medication for activation of BAT for treatment of hyperglycaemia in diabetic patients, which will improve glucose metabolism and postpone insulin application. Funding: This study is financed by the Croatian science foundation research grant (IP-2018-01-7416).

APSSG21.53
Sex-specific bone matrix signatures divergently influence endothelial cell survival.
Aikta Sharma1, Roger JH Emery2, Andrew A Pitsillides1, Richard OC Oreffo4, Sumeet Mahajan2, Claire E Clarkin1
1Biological Sciences, University of Southampton, 2Surgery and Cancer, Imperial College London, 3Comparative Biomedical Sciences, Royal Veterinary College, 4Institute of Developmental Sciences, University of Southampton, 5Chemistry, University of Southampton

Background. Physiological bone formation is regulated by osteoblast (OB)-derived vascular endothelial growth factor (VEGF) during development and repair. We have reported that the vasculature of the skeletal system is sexually dimorphic [1,2] and now hypothesise that this dimorphism is driven by sex-differences in the composition of the OB-extracellular matrix (ECM). Herein, we have investigated whether the ECM profiles of male and female OBs are distinct and if this leads to divergence in vascular cell behaviour. Materials and Methods. Primary long bone-derived OBs were isolated from 4-day old male and female C57BL/6 mice and cultured for 7 days before the addition of labelled bone marrow-derived endothelial cells (BMECs). BMEC survival on the OB ECM was assessed by fluorescent cell staining and automated cell counts using fluorescence microscopy. As a control, the impact of soluble factors on BMEC number was also assessed by treatment with male and female OB-derived conditioned media (CM). Raman spectroscopy of individual male and female OBs (N=25 cells) was performed to characterise matrix composition and the extent of mineralisation. Results. Followiing 24 hours of direct-contact co-culture with male OBs, BMEC numbers were 1.39-fold higher than in co-cultures with female OBs (P<0.005). Raman spectroscopy of OBs revealed divergence in amorphous calcium phosphate and carbonated apatite precursors of hydroxyapatite mineral, with males producing higher levels (3.22 and 1.33-fold, respectively) than female OBs. Collagen-specific proline and hydroxyproline levels in comparison were 1.52 and 2.12-fold higher in female versus male OB cultures, respectively. This correlated with sex-specific changes in the stability of the collagen helices, which were 1.41-fold higher in female versus male cultures, suggesting the male OBs are able to advance into the mineralisation phase while female OBs are primarily synthesising the collagenous matrix. Male and female OB-derived CM did not divergently affect BMEC number (P=0.53). Conclusions. Sex-differences in OB pro-angiogenic potential are associated with divergence in ECM composition, with BMEC survival promoted on more mature, mineralised collagen matrices. Defining the mechanisms regulating sex-specific OB ECM production could offer a new therapeutic route to effectively control pathological skeletal angiogenesis distinctively in men and women. References. [1] Goring, A., Sharma, A., Javaheri, B., Smith, R.C., Kanczler, J.M., Boyde, A., Hesse, E., Mahajan, S., Olsen, B.R., Pitsillides, A.A., Schneider, P., Oreffo, R.O., Clarkin, C.E., 2019. Regulation of the Bone Vascular Network is Sexually Dimorphic. J. Bone Miner. Res. 34, 2117-2132. [2] Sharma, A., Goring, A., Johnson, P.B., Emery, R.J.H., Hesse, E., Boyde, A., Olsen, B.R., Pitsillides, A.A., Oreffo, R.O.C., Mahajan, S., Clarkin, C.E., 2021. Multiscale molecular profiling of pathological bone resolves sexually dimorphic control of extracellular matrix composition. Disease Models & Mechanisms, dmm.048116.

APSSG21.55
Cluster of Differentiation 14 (CD14) Attenuates Salt Sensitive Hypertension and Renal Injury In Females but not Males
David Mattson1, John Henry Dasinger1, Justine Abais-Battad1, Daniel Fehrenbach1
1Physiology, Medical College of Georgia at Augusta University

Genomic sequence and gene expression association studies in animals and humans have nominated genes that may be integral in the pathogenesis of various diseases. The gene encoding Cluster of Differentiation 14 (CD14), a co-receptor with Toll Like Receptor 4 (TLR4), is associated with cardiovascular disease and hypertension in humans. We have shown that CD14 and TLR4 are upregulated in renal macrophages of Dahl Salt-Sensitive animals when on a high-salt diet and are testing the hypothesis that CD14 contributes to the elevated pressure and renal injury observed in salt-sensitive hypertension. Using CRISPR/Cas9, we created a targeted mutation in the CD14 gene on the Dahl SS background and validated the absence of CD14 peptides via mass spectrometry. Radiotelemetry was used to monitor blood pressure throughout a high salt challenge of wild-type and SSCD14/- animals. Transplant of SSCD14+/+ or SSCD14/- bone marrow was used to isolate the effects of CD14 knockout to hematopoietic cells and ovariectomy was used to remove the influence of female sex hormones. Infiltrating renal immune cells were identified using flow cytometry. Initial in vitro studies demonstrated that CD14 signaling opposes the pro-inflammatory effects of TLR4 in freshly isolated peritoneal macrophages. Germline knockout of CD14 was subsequently shown to exacerbate salt-sensitive hypertension and renal injury in female animals but not males. SSCD14/- females demonstrated increased infiltrating macrophages but no difference in infiltrating lymphocytes in the kidney following high salt feeding. Bone
There are sexual dimorphisms in renal ammonia metabolism and structure, many of which are mediated by testosterone. The androgen receptor (AR) is present in the proximal tubule (PT) in both the male and female kidney, and not detectable in other renal epithelial cells. This study’s objective was to determine the role of renal AR in these sex differences. To avoid known systemic effects from AR blockade/deletion, we generated mice with kidney-specific AR deletion (KS-AR-KO) using Cre/loxP techniques (AR floxed mice and Pax8-Cre mice); control mice were Cre-negative littermates (WT). In male, but not female, mice KS-AR-KO increased ammonia excretion (M-WT, 44±14 μmol/day; M-KS-AR-KO, 92±74; P < 0.05; N= 8-11 in each group), which eliminated the sex difference.

Although renal structural size typically parallel ammonia excretion, KS-AR-KO decreased kidney size (M-WT, 222±27 mg; M-KS-AR-KO, 175±23; P<0.05; N= 5-6 in each group), cortical proximal tubule volume density (M-WT, 61±2%; M-KS-AR-KO, 42±2; P<0.05; N= 5-6 in each group) and cortical proximal tubule cell height in males; neither were altered in females and collecting duct volume density was unaltered in both sexes. Expression of phosphoenolpyruvate carboxykinase (PEPCK), a major PT ammonia generating protein, and NKCC2, the major mechanism of TAL ammonia reabsorption, were increased significantly by KS-AR-KO in male mice, but not in female mice. KS-AR-KO decreased expression of NHE-3, the major mechanism of PT ammonia secretion, and NBCe1-A, a basolateral PT transporter that regulates PT ammonia metabolism, in male mice, but not in female mice, and did not alter the sex-specific difference in collecting duct RhbG and Rhcg expression in either sex. These effects occurred even though KS-AR-KO did not alter plasma testosterone (M-WT, 67±74 ng/dl; M-KS-AR-KO, 239±471; F-WT, 27±10; F-KS-AR-KO, 29±11; P=NS; N= 8-11 in each group), food intake or serum Na+, K+, or HCO3- significantly in either sex. We conclude: 1) AR-dependent signaling pathways in male, but not female, kidney regulate PEPCK and NKCC2 expression and lead to the sexual differences in ammonia excretion; 2) opposing effects on NHE-3 and NBCe1-A expression likely limit the magnitude of ammonia excretion changes; 3) since AR is not present in the TAL, the effect of KS-AR-KO on NKCC2 expression is indirect; and, 4) AR mediates the greater kidney size and PT volume density in male than in female mice.

APSSG21.57
Sex Differences in Dysregulated Lipolysis and Lipogenesis in the Offspring of Metabolically Dysfunctional Pregnancies.
Taylor Sheidli, Larissa Baker, Anna Mikolajczak, Nada Salam, Radha Singh, Emma Walsh, Jennifer Thompson.
Physiology and Pharmacology, University of Calgary. 

Background: Offspring of pregnancies confounded by metabolic abnormalities such as gestational diabetes or obesity are prone to the development of cardio-metabolic disease. Our lab has shown that these offspring develop adipose tissue dysfunction, characterized by insulin resistance (IR), increased lipolysis and consequent spillover of free fatty acids (FFA) into the circulation. We observed sex differences in this phenotype, with males more vulnerable to insulin resistance and FFA spillover. In control diet (CD)-fed animals, males displayed increased circulating FFA (p=0.0026), while females showed a significant decrease (p=0.0025). Male, but not female, offspring of metabolically abnormal pregnancies demonstrated severe diet-induced hyperinsulinemia, a key marker of IR, when compared to controls (p=0.0009). IR in the adipose tissue, measured by ADIPO-IR, was also shown to deviate based on sex. Male offspring of metabolically abnormal pregnancies trended toward higher ADIPO-IR relative to controls. Further, male offspring from both normal and metabolically abnormal pregnancies demonstrated diet-induced IR (p=0.0038, p=0.0023, respectively). Females, however, were found to be protected from these diet-induced effects. Only female offspring of metabolically abnormal pregnancies showed a significant increase in ADIPO-IR in response to diet (p=0.014), with a significant difference (p=0.027) between high fat/fructose (HFF)-fed females born from metabolically abnormal vs. normal pregnancies. Together, our data suggest that male offspring are more vulnerable to developmental and diet-induced metabolic effects. We will further explore these sex differences by examining signaling pathways involved in insulin-mediated inhibition of lipolysis and lipogenesis in male and female offspring to advance our understanding of the mechanisms involved in FFA spillover. Methods: Dams heterozygous for the leptin receptor mutation (HetDB) will be used to model maternal metabolic dysfunction. HetDB females or wild type (Wt) females are mated with C57BL/6J males at 12 weeks of age, and only Wt offspring examined. At 7 weeks of age, offspring will be randomized to control diet (10% kCal fat) or high fat/high fructose diet (45% kCal fat, 35% kCal sucrose). At 22 weeks of age, offspring will be subjected to an intra-peritoneal injection of insulin 10 minutes prior to being sacrificed, after which liver and inguinal subcutaneous adipose tissue (SAT) will be collected and flash frozen. Western blot analysis of protein isolated from the liver or SAT will be used to mark the markers of insulin regulated lipolysis and lipogenesis, such as SREBP1c, PI3K, SCD1, and AKT/pAKT in the liver and pHSL/HSL, AKT/pAKT, perilipin, and insulin receptor in the
These results highlight GPER1 as a potential therapeutic target for salt-sensitive hypertension in postmenopausal women.

APSSG21.59
Sex and Stress: The Sex-Specific Impact of Early Life Stress on Adult Behaviour and the Microbiome in Rodents
Annie Cuskelly1,2,3, Emily Hoedt1,4, Lauren Harms2, Melissa Tadros3,4, Simon Keely3,4, Deborah M. Hodgson2,3
1Psychology, University of Newcastle, 2School of Psychology, University of Newcastle, 3HMRRI, Hunter Medical Research Institute, 4College of Health, Medicine and Wellbeing, University of Newcastle

Anxiety and gastrointestinal (GI) disorders demonstrate comorbidity with each other and both disorders present with a higher prevalence in women. While sex differences in anxiety disorders are well-established, it remains unclear whether these differences have a biological basis in the gut. Both disorders share common pathologies that have been shown to be linked to early life stress (i.e. infection). The early life environment is critical to the establishment of sex differences in anxiety, which links the gut with neuroimmune pathways. This axis is highly plastic throughout the perinatal period and is a possible mediator of sex-based disparities in psychological and GI disorders. Using a well characterised model of neonatal stress (neonatal immune activation), we investigated the role of sex on the gut-brain-axis. We hypothesised that early life stress in rodents would induce anxiety-like behaviour, GI inflammation (with an increase in pro-inflammatory cytokines) and microbiome community disruption in adulthood and that these changes would be sex specific. Male and female Wistar rats were injected with 0.05mg/kg of LPS to induce neonatal immune activation, or saline, on postnatal days 3 and 5. In adulthood, behavioural tests were performed to assess anxiety-like behaviour, including elevated plus maze, open field and social interaction. qPCR was performed on inflammatory markers (IL1b, TNF, IL6, IL17) in the colon. Additionally, we assessed CRHR1, a hormone receptor which plays a role in stress and anxiety, to ascertain the role of stress hormones in the gut. Microbiome analyses of faecal samples was carried out using 16S sequencing. Neonatal immune activation induced sex-specific changes in behaviour, GI inflammation and microbiota composition. There were distinct phenotypes for LPS treated males and females. LPS treated males displayed typical anxiety behaviours with decreased social interaction, and increased defecation relative to controls. LPS treated females displayed a behavioural phenotype characterised by increased social interaction and exploration compared to controls. Microbiota profiling revealed a significant increase in the Bacteroidota phylum in LPS treated females while Proteobacteria was decreased in LPS rats for both sexes. Beta diversity of the microbiome composition demonstrates distinct bacterial community differences between treatment and sex. Neonatal immune activation induced sex-specific changes in GI inflammation with LPS treated males displaying decreased inflammatory cytokines, including IL1b, TNF. With regard to CRHR1, we found a decrease in LPS-exposed males and LPS-exposed females conversely displayed an increase. Our study
showed mixed findings, with LPS treated females displaying a more hypervigilant behavioural phenotype and LPS treated males a more typical anxiety phenotype. The defection findings corroborate previous reports that irritable bowel syndrome diarrhoea subtype is more prevalent in males. We also show that early life stress alters the adult rat colon inflammation and microbiome communities in sex specific ways. These findings highlight the importance of sex in determining the impact of early life stress in anxiety and GI disorders.

APSSG21.60
Sex Differences in the Role of Endothelial Cell Mineralocorticoid Receptors in Cardiovascular Disease
Iris Jaffe
Molecular Cardiology Research Institute, Tufts Medical Center

There are well known sex differences in the incidence and outcomes of cardiovascular disease. Young women are protected from heart attack and cardiovascular death relative to men yet a decade after menopause, women catch up and ultimately exceed men in the incidence of cardiovascular disease (CVD). Obesity is a worldwide epidemic that disproportionately affects women and when combined with metabolic dysfunction, obesity mitigates the cardiovascular protection in young women. Understanding the molecular mechanisms for these observations could allow for precision medicine strategies to alter the trajectory of CVD risk for men and women. My lab studies the mineralocorticoid receptor (MR), the terminal step in the renin-angiotensin-aldosterone system and a critical regulator of blood pressure. This presentation will summarize novel insights into sex-specific roles of MR in endothelial cells (EC) that sheds light on these important clinical observations. First, in a mouse atherosclerosis model, we demonstrate that female mice have larger plaques but with less plaque inflammation. Inflamed plaques are more likely to rupture and cause heart attack. By intravital microscopy, we show that in males, EC-MR contributes to leukocyte trafficking into the vasculature by regulating expression of ICAM1 and E-selectin and that this is suppressed in females by estrogen receptor (ER) inhibition of MR transcriptional activity. In a mouse model of obesity with metabolic dysfunction, we explored the impact on microvascular EC function. Obesity impairs microvascular dilation to acetylcholine and this is reversed specifically in female EC-MR KO mice. We show that in females, deletion of MR leads to increased production of nitric oxide (NO). Estrogen is known to induce NO production by activation of ER alpha to the protein striatin in the plasma membrane. We recently demonstrated that EC-MR blocks ER-alpha activation of eNOS and competes with ER-alpha to bind to striatin. Thus, ER-alpha and MR interact in endothelial cells in a genomic fashion to regulate cell adhesion molecules involved in vascular inflammation and in a non-genomic fashion to regulate NO production in response to obesity. We believe that these mechanisms help explain the sex differences in incidence of MI in premenopausal women and the sexually dimorphic impact of obesity on microvascular function. Understanding the mechanism nominates novel sex-specific therapies, specifically MR antagonists, to mitigate CVD risk in men, post menopausal women, and young women with obesity and metabolic syndrome.

APSSG21.61
Smooth muscle mineralocorticoid receptor mediates the exacerbated cardiovascular response to hypertensive stimuli after sFlt-1 induced preeclampsia
Lauren Biwer, Qing Lu, Jaime Ibarrolla, Joshua Man, Brigett Carvajal, Zsuzsanna Zsengeller, Ellen Seely, S. Ananth Karumanchi, Iris Jaffe

1Molecular Cardiology Research Institute, Tufts Medical Center, 2Pathology, Beth Israel Deaconess Medical Center, 3Endocrinology, Brigham and Women’s Hospital, 4Nephrology, Cedars-Sinai Medical Center

Background: Preeclampsia (PE), a syndrome of high blood pressure (BP) and renal damage in late pregnancy, is associated with increased soluble VEGF receptor (sFlt1) and survivors have increased risk of future hypertension with increased angiotensin II (AngII) and salt sensitivity. Hypothesis: Since smooth muscle cell mineralocorticoid receptor (SMC-MR) contributes to AngII sensitivity and BP control, we hypothesized that high sFlt1 exposure during pregnancy may induce a post-partum state of enhanced vascular sensitivity via SMC-MR activation.

Methods/Results: A PE model was induced by transient viral expression of sFlt1 in pregnant C57Bl6 mice. Elevated serum sFlt1, BP and glomerular endotheliosis was confirmed, which all resolve post-partum. Two months later, resistance arteries from post-PE mice show no change in vasoconstriction to AngII and equivalent aldosterone levels and vascular MR expression compared to control mice. In a small cohort of women we confirmed that prior PE enhances salt sensitivity of BP thus, postpartum mice were implanted with telemetric BP monitors and exposed to high salt or AngII (600mg/kg/day) infusion. Mice with prior PE had a significantly increased BP response to both hypertensive stimuli. Microvessels from mice after PE and hypertensive stimuli had enhanced ex vivo myogenic tone and AngII vasoconstriction. To test the direct impact of SFlt1 on SMC-MR function, MR-driven luciferase reporter assays were performed in cultured SMC transiently exposed to SFlt1 (24 hours). SMC-MR transcriptional activity in response to aldosterone or AngII were significantly increased after SFlt1 exposure. Aldosterone induction of SMC-MR target gene expression was also enhanced after SFlt1 exposure. Finally, the role of SMC-MR in the post-PE phenotype was tested in vivo by PE induction in SMC-MR-KO vs MR-intact littermates. Plasma sFlt1 and glomerular endotheliosis were increased in sFlt1 injected mice and SMC-MR-KO did not exacerbate or diminish these signs. Post partum, SMC-MR KO mice were protected from the PE-induced increase in systolic blood pressure, aortic stiffness, microvascular myogenic tone and AngII vasoconstriction. Conclusion: sFlt1-induced PE produces a state of enhanced SMC-MR sensitivity to AngII that persists post partum and contributes to hypertension and vascular stiffness. These data support testing of MR antagonists or MR downstream
Sex Differences in HPA Axis Dynamics in the Rat: Interaction of Opioid Abstinence and Sleep Restriction

Hershel Raff, Christopher Olsen, Carol Everson

1 Medicine, Medical College of Wisconsin, 2 Endocrine Research Laboratory, Aurora St. Luke's Medical Center/Advocate Aurora Research Institute, 3 Neuroscience Res Inst/Pharmacology & Toxicology, Medical College of Wisconsin

The hypothalamic-pituitary-adrenal (HPA) axis is disrupted by exposure to and withdrawal from opioids. There is evidence that endogenous glucocorticoids modulate drug seeking during abstinence. Chronic co-morbid sleep disruption may influence HPA axis abnormalities during abstinence and increase the vulnerability to relapse. While the problems of opioid addiction and relapse affect both men and women, females may be more vulnerable; this may align with sex differences in HPA axis dynamics. We hypothesize that chronic sleep restriction interacts with opioid abstinence to alter the HPA axis response to acute stressors in a sexually dimorphic manner. We developed a rat model to evaluate the interaction of opioid abstinence and persistent sleep loss on HPA axis dynamics in male and female adult rats. Plasma ACTH and corticosterone were measured diurnally (at 1600 h [PM] and 0800 h [AM]) and in response to acute restraint stress in male and female adult rats. Then, rats self-administered oxycodone iv (0.1 mg/kg) for 10 days. During subsequent abstinence, sleep restriction [SR] was induced (vs. ambulatory control [AC]). SR was produced by brief and intermittent forced ambulation resulting in sleep fragmentation and a validated and standardized 35% reduction of total sleep amount. AC conditions were similar except that the accommodation requirements were consolidated to permit longer opportunities to obtain uninterrupted sleep. At 22-23 days of abstinence and AC or SR, diurnal and restraint-stress induced plasma ACTH and corticosterone were reassessed. All blood samples (processed to EDTA plasma) were obtained by tail clip. There was no effect of opioid abstinence on diurnal plasma ACTH and corticosterone in AC rats. However, PM (not AM) plasma ACTH, but not corticosterone, was increased in male, but not female SR rats during abstinence suggesting an interactive, sexually dimorphic effect at the circadian HPA axis peak. Interestingly, the corticosterone, but not the ACTH response to restraint in the AM was reduced in male, but not female SR rats. There was no effect on any of the treatments or interventions on adrenal weight normalized to body weight. Our findings suggest an unexpected, sexually dimorphic interactive effect of opioid abstinence and sleep restriction on the HPA axis acting directly at the level of the adrenal cortex. It is also possible that this interactive effect led to a decrease in sensitivity to glucocorticoid negative feedback at the circadian peak in males. Profound sleep disturbances during abstinence from opioid addiction have long been suspected of perpetuating vulnerability to relapse. These results show a sexual dimorphic interaction of abstinence and chronic sleep disturbance resulting in the dysregulation of the HPA axis. Persistent sleep disruption may cause or perpetuate HPA axis abnormalities during oxycodone abstinence,
androgen excess also decreased NRF1 expression, pointing to decreased mitochondrial biomass. SGLT2 decreased fat mass in HAF rats, which was accompanied by increased expression of SODs and NRF1. Our data suggest that SGLT2 improves adiposity in females with androgen excess by alleviating MD in WAT. Supported by NIH grants: NIGMS P20GM121334 (LLYC and DGR), NIDDK R21DK113500 (DGR), NIDDK F30DK127527 (JEP), NIGMS P20GM104357, NHLBI P01HL51971.

**APSSG21.65**

**Sexual dimorphism of diurnal Nile grass rats in response to a high fat diet and time restricted feeding**

Melissa Puppa1, Hayden Johnson2, Wangkuk Son1, Suman Sharma1, Richard Bloomer1, Chidambaram Ramanathan1, Aaryani Tipirneni-Sajja2, Maie van der Merwe1

1College of Health Sciences, University of Memphis, 2Department of Biomedical Engineering, University of Memphis

The response of individuals to a high fat diet and fasting interventions may be sex dependent. To date, the majority of studies utilizing fasting do not consider sex-dependent differences despite substantial evidence that there is sexual dimorphism in the response to both obesity as well as caloric restriction. To our knowledge no one has examined if this sexual dimorphic response persists with other types of fasting including time restricted feeding (TRF). Therefore, we examined sexually dimorphic responses to time restricted feeding and the timing of the feeding window during the development of high fat diet induced obesity and insulin resistance in the diurnal Nile Grass Rat (NGR) model. Adult male and female NGR, aged 12-18 months, were randomly assigned to one of three groups: animals had access to a 60% high-fat (HF) diet ad-libitum (HF-AD), animals had time-restricted access to the HF diet for the first 6 hours of the 12 hour light/active phase (HF-AM) or the second 6 hours of the 12 hour light/active phase (HF-PM). All animals remained on their respective protocols for six weeks. Regardless of diet, females displayed an increase in hepatic lipid storage compared to males and lower visceral fat stores. Morning feeding was associated with decreases in hepatic saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids in females, but not males. Hepatic glucose levels were lower in females compared to males and increased with TRF in females, while there were no sex differences in serum glucose levels. Interestingly, HF diet decreased liver succinate levels in females, but not males. In the soleus muscle mRNA expression of IGF-1 increased with time restricted feeding in males, but decreased in females and GLUT4 mRNA levels decreased with HF-AD which was partially attenuated with TRF in females. Overall, these results point toward dimorphic hepatic and muscle responses, which may contribute to the development of metabolic syndrome. More work is needed to better understand the mechanisms behind the dimorphic regulation of lipid and carbohydrate metabolism in this diurnal model of metabolic syndrome.
APSSG21.66
Angiotensin II-induced hypertension and kidney injury: lack of significant sexual dimorphism in PT-Agtr1a-/− mice
Ana Paula Oliveira Leite1,2, Rumana Hassan1,2, Xiaol C Li1,2, Jia L. Zhuo1,2
1Physiology, Tulane University, 2Tulane Hypertension & Renal Center of Excellence, Tulane University

Recently, there has been an explosion of interests in studying the important roles of sexual dimorphism in the regulation of blood pressure and the development of hypertension. The objective of the present study was to test the hypothesis that there are significant sex differences in angiotensin II (Ang II)-induced hypertension and kidney injury using male and female wild-type and proximal tubule-specific AT1a receptor knockout mice (PT-Agtr1a-/-). Twelve groups (n=8-12 per group) of adult male and female wild-type and PT-Agtr1a-/− mice were infuse with a pressor dose of Ang II via osmotic pump for 2 weeks (1.5 mg/kg/day, i.p.) and simultaneously treated with or without losartan (20 mg/kg/day, p.o.) to determine the respective roles of AT1a receptors in the proximal tubules versus systemic tissues. Basal systolic, diastolic, and mean arterial pressure were approximately 13 ± 3 mmHg lower (P<0.01), while basal 24 h urinary Na+, K+, and Cl-excretion were significantly higher in both male and female PT-Agtr1a-/− mice than wild-type controls (P<0.01) without significant sex differences between different strains. Both male and female wild-type and PT-Agtr1a-/− mice developed hypertension (P<0.01), and the magnitudes of the pressor responses to Ang II were similar between male and female wild-type and PT-Agtr1a-/− mice (n.s.). Likewise, Ang II-induced hypertension was significantly attenuated in both male and female PT-Agtr1a-/− mice (P<0.01). Furthermore, losartan attenuated the hypertensive responses to Ang II to similar extents in both male and female wild-type and PT-Agtr1a-/− mice. Finally, Ang II-induced kidney injury was attenuated in PT-Agtr1a-/− mice (P<0.01). In conclusion, the present study demonstrates that deletion of AT1a receptors in the proximal tubules of the kidney attenuates Ang II-induced hypertension and kidney injury without revealing significant sex differences. Supported by NIH/NIDDK grants (1R01DK123144-01, 2R01DK067299-10A1, and 2R01DK102429-03A1).

APSSG21.68
Exploring sex differences in renal sodium transporters with Four Core Genotype (FCG) model
Alicia McDonough1, Donna Ralph1, Joanne Soong2, Rolando Carrizo-Gaytan2, Thomas Kleyman3, Lisa Satlin2
1Physiology and Neuroscience, Keck School of Medicine of USC, 2Pediatrics, Icahn School of Medicine at Mount Sinai, 3Renal-Electrolyte Division, Department of Medicine, University of Pittsburgh

In the kidney, sex-specific differences in Na+ transporter profiles along the nephron are now evident (“transporters” = co-transporters, channels, claudins and pumps, their phosphorylation (p)); females (F) exhibit variable differences vs. males (M) along the proximal tubule (PT), and higher Na+ transporter abundance and activity vs. M along the distal (DT) and collecting duct (CD). Circulating levels of gonadal hormones determine sex differences in many physiologic traits; however, recent studies indicate that sex differences may also be due to the sex chromosome complement (SCC; XX vs. XY). The novel FCG C57BL/6 mouse model dissociates gonadal sex (ovaries (F) or testes (M)) from sex chromosomal complement (SCC; XX, XY). That is, traits influenced by gonadal hormones are similar between MXX and MXY (testes) vs. FX and FXY (ovaries); whereas, traits influenced by SCC are similar between MXX and FX vs. MXY and FXY (independent of gonads). Aim: Determine the contributions of gonadal hormones vs. SCC to the sexual dimorphisms in transporter protein abundance along the nephron. Method: Implement the 4CG model along with transporter profiling (semi-quantitative immunoblotting; n = 4–5 mice/genotype). Results (all P<0.05). Along the PT: NHE3 and OAT1 were lower and AQP2 and SGLT2 higher in abundance in gonadal F (FXX) vs M (MXY), but SCC also contributed to differences: NHE3, SGLT2, AQP1 and AQP2p were lower by 15-30% in MXX vs. MXY, and SGLT2 was 15% lower in FXY vs FXX. In the medullary thick ascending limb (TAL): α and β NKATPase were 60% higher in FXX vs. MXX and 25-30% lower in FXY vs. FXX; α was 15% lower in MXX vs. MXY. Along the cortical TAL and DT: NKCC2, NKCC2p, NCC, NCCp, α and β NKATPase, claudin 7, SPAK and SPAPK kinase abundance were 1.4- to 2.6-fold higher in FXX vs. MXY; SCC differences were limited to 15, 30, and 20% lower NCC, NCCp, and claudin 7 in MXX vs. MXY. Along the CD: ENaC subunits were 30-50% higher, claudin-8 2-fold higher, and pendrin and UMOD 20% higher in FXX vs. MXY; SCC differences included 15-20% lower ROMK, Kir 4.1, and ENaC β and γ subunits in MXX vs. MXY. Conclusion: Results provide evidence for impact of both gonadal hormones and SCC on kidney transporter abundance, in some cases offsetting the effects of the other. Further progress will come from defining whether X vs Y genes contribute to the differences. These findings have potential impact on electrolyte and blood pressure (patho)physiology in F vs. M. *(DR and JS contributed equally). Support: R01 DK038470 and P30 DK079307 to TK and LS, R01 DK083785 to AM.

APSSG21.69
Adoptive transfer of T regulatory cells prevents Ang II-induced postmenopausal hypertension
Megan Sylvester1,2, Keila Espinoza3, Emma Louise1, Josh Uhln1, Heddwen Brooks1,2,3
1Physiological Sciences, University of Arizona, 2College of Medicine, University of Arizona, 3Sarver Heart Center, University of Arizona

Premenopausal females are protected from the development of Ang II-induced hypertension; however, this protection is lost following menopause. In postmenopausal females we have shown that hypertension is associated with a reduction in splenic and renal T regulatory cell populations. In premenopausal females we demonstrated that T regulatory cell depletion, via anti-CD25 antibody infusion, increased susceptibility to Ang II induced...
Research and Training. Erectile function was impaired in type 2 diabetic mice model were sacrificed in accordance to APS (WT) and hetero(db/+), which hypothesized that in type 2 diabetes, cavernosum diminishes erectile function. Aim: We aimed to clarify the role of vascular relaxation. Our previous studies have shown decreased endothelium-dependent relaxations in corpus cavernosum only in db/+ mice, with no effect for db/db animals. Conclusions: Our results suggest that despite increased genetic expression of KCa2.3 and KCa1.1 channels in erectile tissue, the decreased protein expression of KCa2.3 and KCa1.1 channels compared to WT mice may underpin the decreased endothelium-dependent relaxation and erectile dysfunction in both db/+ and diabetic db/db mice. The impaired KCa2.3 channel function may contribute to the increased noradrenaline contraction with KCa1.1 channel deficiency and furtherly impair erectile function in diabetes.

**APSSG21.71**

Erectile dysfunction and decreased contribution of KCa1.1 and KCa2.3 channels in penile tissue of type-2 diabetic db/db mice

Simon Gabriel Comerma Steffensen\(^1\), Judit Prat Duran\(^1\), Susie Mogensen\(^1\), Rafael Fais\(^2\), Estefano Pinilla\(^1\), Ulf Simonsen\(^1\)

\(^1\)Pharmacology/Biomedicine/Health Faculty, Aarhus University, \(^2\)Animal Physiology/Biomedical Sciences/Veterinary Faculty, Central University of Venezuela, \(^3\)Pharmacology Department/Health Faculty, Sao Paulo University

Hypercapnic chemosensitivity is the ventilatory response to increased partial pressure of CO\(_2\) and is the result of central and peripheral chemoreceptors stimulation. Previous research has primarily focused on the response of the central chemoreceptors at rest and has not examined potential sex-differences in peripheral chemosensitivity during exercise. We sought to measure the hypercapnic chemosensitivity of the peripheral chemoreceptors during moderate exercise in males and females. We hypothesized that females would have a reduced ventilatory response compared to males. Twenty-five healthy subjects (n=11 females) participated in one test day involving transient hypercapnic chemosensitivity testing during rest and moderate exercise, and a maximal exercise test. Female
subjects were tested during the active phase of their birth control (n=6) or self-reported low hormone phase (n=5) of their menstrual cycle if they were not using birth control. The hypercapnic chemosensitivty test involved two breaths of 10% CO2 repeated 5 times at rest and the first two exercise stages. Between each set of two breaths there was 30-45 seconds where the subject breathed room air. Exercise started at 60W and 80W for females and males respectively, and both increased by 20W for stage 2. After stage 2, subjects progressed into the maximal exercise test with intensity increasing 20W every 1.5 minutes for both sexes. Compared to females, males had a higher relative (F: 36.7±7.1 mL/kg/min, M: 44.6±8.7 mL/kg/min) and absolute (F: 2.2±0.5 mL/min, M: 3.8±0.8 mL/min) VO2max (both p<0.05), but there were no differences in end-exercise heart rate or RER (p>0.05). Maximal ventilation was higher in the male subjects (16±3 L/min) vs female subjects (10±2 L/min) (p<0.05); however, the metabolic equivalents, VE/VO2 (F: 46±5.4, M: 42±3.5) and VE/VCO2 (F: 39±4.2, M: 37±2.3), were not different (p>0.05). Peripheral chemosensitivity to hypercapnia was not significantly different between males (rest: 0.85±0.57 L/min/mmHg, stage 1: 1.18±0.52 L/min/mmHg, stage 2: 1.06±0.46 L/min/mmHg) and females (rest: 0.69±0.40 L/min/mmHg, stage 1: 0.94±0.38 L/min/mmHg, stage 2: 0.78±0.31 L/min/mmHg) (p>0.05).

There was a significant effect of exercise intensity with stage 1 (1.075±0.47 L/min/mmHg) being increased from rest (0.78±0.5 L/min/mmHg) (p<0.05); however, this did not differ based on sex. These results suggest that the response of peripheral chemosensors to hypercapnia is not impacted by sex with both sexes experiencing an increase in chemosensitivity from rest to mild exercise. Funding: NSERC

APSSG21.73

Gender differences in kinetics of visceral adipose tissue immune cells in the mouse model of high fat diet-induced obesity.

Natsumi Imano1,2, Kayoko Tamaki2, Ken Shinmura2
1Department of Bioscience, Kwansei Gakuin University, 2General internal medicine, Hyogo College of Medicine

[Purpose] The accumulation pattern of adipose tissue associated with obesity is different between men and women. In young, visceral adipose tissue (VAT) accumulates in men, whereas subcutaneous adipose tissue mainly accumulates in women. VAT gradually accumulates in woman from menopause. Accumulation of VAT induces the imbalance between pro-inflammatory and anti-inflammatory immune cells. This causes chronic inflammation in VAT, leading to systemic metabolic disorders. However, it is unclear whether gender differences exist in the development of adipose tissue inflammation associated with obesity. [Methods] Phase 1: C57BL/6 mice were fed with either a control diet (HFC) or a high-fat diet (HFD) from 5 weeks of age. Oral glucose tolerance test and insulin tolerance test were performed at 4, 10, 16, and 22 weeks after starting either HFC or HFD. Stromal vascular cells were isolated from VAT and flow-cytometry analysis was performed at 17, 23, and 29 weeks of age. Phase 2: At 6 weeks of age, female mice were randomly divided into two groups and performed either Sham-operation or bilateral ovariectomy (OVX). They were fed either HFC or HFD from 7 weeks of age. Stromal vascular cells were isolated from VAT and flow-cytometry analysis was performed at 19 weeks of age. [Result] Phase 1: Impaired glucose tolerance was observed in both sexes with HFD at 9 weeks of age. The area under curve was maximal at 15 weeks of age in HFD-fed male mice, whereas that in HFD-fed female mice increased over time. However, VAT weight was almost the same between both sexes with HFC or HFD at 15 weeks of age. Thus, we focused on VAT immune cells obtained from 15-week-old mice. The number of total and CD11c+ inflammatory type (M1) macrophages in VAT was significantly higher in HFC- and HFD-fed males than females, but the degree of increase in total and M1 macrophages with HFD was much higher in corresponding female mice. CD4+ T cells were predominantly observed in VAT of both sexes fed with HFC. CD8+ T cells mainly increased in HFD-fed male mice, while only CD4+ T cells increased in HFD-fed female mice. Senescence-related T cells (PD-1+CD44hiCD4+) increased markedly in HFD-fed male mice, whereas they did not change in female mice. The number of regulatory T cells in VAT was significantly higher in males than in females with HFC, and it substantially decreased in HFD-fed male mice. Phase 2: HFC-fed OVX mice did not show glucose intolerance at 11 weeks of age but showed glucose intolerance at 17 weeks of age. HFC-fed OVX mice exhibited the increase CD4+ and CD8+ T cells and senescence-related T cells in VAT. Furthermore, HFD-fed OVX mice had a similar pattern in kinetics of adipose immune cells to that observed in HFD-fed male mice. [Discussion] These results demonstrated gender differences in kinetics of VAT immune cells during the progression of HFD-induced obesity. The inflammatory environment in VAT might develop earlier during HFD-induced obesity in males than in females. In addition, the results obtained from OVX mice suggested that estrogen plays a key role in gender differences in VAT immune cell kinetics.

APSSG21.74

Sex Different Responses to Hypoxia in Male and Female Human Pulmonary Microvascular Endothelial Cells According to Proteomics Analysis

Daria S. Kostyunina1, Eugene Dillon2, Keith D. Rochfort3, Philip M. Cummins3, Paul McLoughlin1
1School of Medicine, University College Dublin, 2Conway Institute, University College Dublin, 3School of Biotechnology and National Institute for Cellular Biotechnology, Dublin City University

Pulmonary arterial hypertension (PAH) is a severe pulmonary disease, that frequently leads to right heart failure and death. PAH is more common in females than in males (female to male ratios 2:1-4:1). Despite female predisposition to PAH, females have better survival with PAH and other forms of pulmonary hypertension (PH) than males. This discrepancy has been called “oestrogen” paradox. However, oestrogen and other sex hormones
cannot fully explain sex differences in PAH. Sex hormone independent mechanisms contribute to the sex differences in a mouse model of PH but the specific mechanisms and their role in humans are unknown (1). Pulmonary endothelial cells are central in the development of PH and hypoxia is one of the stimuli that alters endothelial cell function during the development of PH. The aim of this study was to identify differences in the changes in protein expression in response to hypoxia in male and female pulmonary endothelial cells, cultured in the absence of sex hormones. Human pulmonary microvascular endothelial cells (HPMEC) from three male (60, 65, 69 years old) and three female (53, 56, 70 years old) non-PH non-smoking donors were cultured in hypoxic conditions (1% O\(_2\)) for either 24 or 48 hours under physiological shear stress. Label-free quantitative proteomics and RNA sequencing were performed to reveal sex differences between males and females in response to hypoxia (normoxia as a control group). According to Gene Set Enrichment Analysis (GSEA (2)) of proteomics data, 24 hours of hypoxia induced “HYPOXIA” and “GLYCOLYSIS” gene sets (Hallmark gene set collection (3)) enrichment (FDR<0.25) in both male and female hypoxic HPMEC. Eight gene sets were enriched in one sex only in response to 24 hours of hypoxia and, hence, could be considered as sex different. “WNT BETA CATENIN SIGNALING” was enriched in female hypoxic HPMEC only. In male hypoxic HPMEC the following gene sets were enriched exclusively: “TGF BETA SIGNALING”, “IL2 STAT5 SIGNALING”, “TNFA SIGNALING VIA NFkB”, “ANDROGEN RESPONSE”, “UV RESPONSE DN”, “EPITHELIAL MESENCHYMAL TRANSITION”. In normoxia “SPERMATOGENESIS” was enriched in male hypoxic HPMEC, and “ESTROGEN RESPONSE LATE” was enriched in female HPMEC. Proteomics analysis revealed sex differences in gene set enrichment in male and female HPMEC in response to 24 hours of hypoxia. Some of the sex different pathways are crucial in PAH development, including “TGF BETA SIGNALING”, “TNFA SIGNALING VIA NFkB”, “WNT BETA CATENIN SIGNALING”. Moreover, HPMEC were cultured in the absence of sex hormones, hence, sex differences could be induced by sex hormone independent mechanisms, i.e. sex chromosomes. Further research is needed to explore the contribution of these pathways to sex differences in PAH. (1) Umar et al. (2018). Am Journal Respir Crit Care Med, 197(7), 952–955.(2) Subramaniana et al.(2005). PNAS, 102(43), 15545–15550.(3) Liberzon et al. (2015). Cell Syst, 1(6), 417–425.

APSSG2175
Maternal plasma proteome profiling of biomarkers for stratification of early-onset and late-onset preeclampsia
Hao Chen1,2, Ingrid Aneman1, Valentina Nikolic2, Natasa Orlic4,5, Zeljko Mikovic4,5, Milan Stefanovic3,6, Zoran Cakic7, Hristina Jovanovic3, Stephanie Town1, Matthew Padula1, Lana McClements1
1School of life sciences, University of Technology Sydney, 2Centre for Inflammation, Centenary Institute, 3Department of Pharmacology and Toxicology & Department of Internal Medicine - Gynaecology, Medical Faculty, University of Nis, 4Department of Gynaecology and Obstetrics, Narodni Front, 5Medical Faculty, University of Belgrade, 6Department of Gynaecology and Obstetrics, Clinical Centre Nis, 7Department of Gynaecology and Obstetrics, General Hospital of Leskovac

Background Preeclampsia is a cardiovascular disorder in pregnancy characterized by new onset of hypertension and organ damage. It is a multifactorial disease and a leading cause of mortality and morbidity in pregnancy. There are different phenotypes of preeclampsia based on the time of onset during gestation including early-onset preeclampsia (EOPE) and late-onset preeclampsia (LOPE), diagnosed before or after 34 weeks’ of gestation, respectively. Generally, EOPE is associated with more severe complications in pregnancy than LOPE. Although, there are some overlapping features between EOPE and LOPE, molecular differences driving the distinct outcomes between EOPE and LOPE are yet to be elucidated. Methods and Results We conducted a comprehensive and unbiased proteomic profiling of the maternal plasma samples collected from patients with EOPE (n =17) or LOPE (n =11), and healthy pregnancies as controls (n =18). In total, there were 26 and 20 differentially abundant proteins between EOPE or LOPE, and normotensive controls, respectively. Notably, inter-alpha-trypsin inhibitor heavy chain 3 (ITIH3) was increased in EOPE (fold change (FC) =1.60, false discovery rate (FDR) =1.18 x 10-2), and ITIH2 was increased in LOPE (FC =1.29, FDR =3.30 x 10-2), compared to healthy controls. Insulin-like growth factor-binding protein 4 (IGFBP4) was dramatically elevated in both EOPE (FC=4.25, FDR =0.30 x 10-3) and LOPE (FC=4.91, FDR =3.96 x 10-3). We also identified substantial differences in terms of signalling pathways between EOPE and LOPE. EOPE phenotype was characterized by perturbed homeostasis-related pathway including platelet activation, signalling and aggregation, whereas LOPE showed aberrant complement activity of the immune system. A protein-protein interaction (PPI) networks highlighted that proteins associated with lipid metabolism were dysregulated in EOPE, however ECM proteins had a more pronounced role in LOPE. Conclusions Collectively, a comprehensive proteomic profiling of EOPE and LOPE suggested distinct pathogenic mechanisms between EOPE and LOPE. This data-enriched resource provides insights into the utility of new biomarkers for the personalized management of preeclampsia that could be utilized clinically in the future.
Membrane androgen receptor-induced neurodegeneration
Rebecca L. Cunningham1
1Pharmaceutical Sciences in the School of Pharmacy, University of North Texas Health Science Center

Sex differences are present in several neurodegenerative disorders associated with aging, such as Alzheimer’s disease (AD) and Parkinson’s disease (PD). Aging dramatically affects the endocrine system, especially in women. Increased incidence of AD and PD is observed following menopause in women. Women typically experience menopause in their 50’s. During menopause, ovarian estradiol rapidly decreases while ovarian androgens are relatively unaffected, resulting in an androgen sex hormone profile in post-menopausal women. The role of androgens in post-menopausal women is poorly understood. Our laboratory has found that androgens can be neuroprotective or neurodamaging, depending on the physiological environment, such as the oxidative stress load. Under conditions of elevated oxidative stress, androgens can further exacerbate oxidative stress generation through membrane androgen receptor (mAR) activation of the brain angiotensin system. Thus, androgens could exacerbate oxidative stress associated neurodegenerative diseases, such as AD and PD. Therefore, increased activation of brain mAR– angiotensin mediated oxidative stress could mediate the increased incidence of neurodegeneration in post-menopausal women compared to men.

Interaction of Sex, Chronic Pain and Obesity on Cortisol in Adolescent Humans
Hershel Raff2, Jonathan Phillips2, Steven Weisman3, Keri Hainsworth3
2Medicine, Medical College of Wisconsin, 3Endocrine Research Laboratory, Aurora St. Luke’s Medical Center/Advocate Aurora Research Institute, 3Anesthesiology, Medical College of Wisconsin

Adolescent obesity, the prevalence of which has tripled in the past few decades, augments the development and sequelae of chronic pain. Furthermore, obesity impedes the treatment of chronic pain in adolescents. The exacerbation of chronic pain by obesity in adolescents is associated with increased systemic inflammation. In addition, pain and obesity each independently affect the hypothalamic-pituitary-adrenal (HPA) axis. However, the interaction of pain and obesity on the HPA axis and the potential for sexual dimorphism in this phenomenon is not established, particularly in adolescents. Furthermore, cortisol is anti-inflammatory, even at physiological concentrations, and there are many sites of interactions between inflammatory mediators and HPA axis control. We hypothesized that dysregulation of the hypothalamic-pituitary-adrenal axis occurs in a sexually dimorphic manner in human adolescents with chronic pain, obesity, or the combination of the two. We measured serum cortisol in 13-17-year-old adolescents (N=144; 79 females) during the daytime (0830-1730 h). They were categorized as healthy weight/no pain (controls; BMI=56th percentile [37-71]), healthy weight with chronic pain (pain duration ≥3 months), obese without pain (BMI=97th percentile [95-99]), or the combination of obesity and chronic pain. Serum cortisol in female controls (26.6±3.5 μg/dL [N=20]) was significantly higher than male controls (14.9±1.9 μg/dL [N=20]; P=0.006), as expected. Females with chronic pain alone, obesity, or pain+obesity had dramatically decreased serum cortisol (17.1±2.0 μg/dL [N=20]; 13.8±2.0 μg/dL [N=20] and 12.4±1.4 μg/dL [N=19], respectively) compared to female controls (P<0.001). Notice the trend for cortisol in females with obesity or pain+obesity to be lower compared to chronic pain alone. Remarkably, there was no effect of chronic pain alone in males compared to male controls. Males with chronic pain+obesity had lower serum cortisol (10.5±2.2 μg/dL [N=8]) compared to males with obesity alone (17.0±1.7 μg/dL [N=20]; P=0.033). There were no systematic effects of the time of day the sample was drawn on the cortisol findings. Chronic pain, obesity and their combination all dramatically decreased serum cortisol in female adolescents. This could be due to input from pain pathways on the hypothalamic-pituitary-adrenal axis and/or a decrease in estrogen-mediated plasma cortisol binding globulin concentrations. The decrease in the anti-inflammatory effects of cortisol may contribute to chronic pain and its resistance to treatment with concurrent obesity in female adolescents. Funding: Advancing a Healthier Wisconsin Endowment and the Advocate Aurora Research Institute

Effect of Gonadal Hormones on Autoimmunity, Pathology and Behaviour in the 3xTg-AD Mouse Model of Alzheimer’s Disease
Margaret Fahnestock1, Wei Song1, Samantha Creighton2, Bernadeta Michalski2, Donglai Ma2, Boris Sakic1, Iva Zovkic2
1Dept. of Psychiatry & Behavioural Neurosciences, McMaster University, 2Dept. of Psychology, University of Toronto Mississauga, 3Dept. of Pathology & Molecular Medicine, McMaster University

Sex-dependent discrepancies in prevalence and autoimmune indicators are characteristics of Alzheimer’s disease (AD). Using the 3xTg-AD mouse model, we previously reported that adult males show early manifestations of systemic autoimmunity along with early onset of behavioural dysfunction, altered epigenetic factors (enhanced expression of the histone variant macroH2A1), and loss of plaque/tangle pathology. Conversely, adult females display less severe autoimmunity and retain AD-like pathology. The present study examines whether gonadal hormones play a role in the etiology and/or maintenance of these traits in current cohorts of 3xTg-AD mice. 3xTg-AD and wild-type mice were gonadectomized or sham-operated at 3 months of age. After behavioural phenotyping at 6 months of age, the animals were assessed for organ mass, serologic markers of autoimmunity, molecular markers of early AD pathology and expression of genes and histone variants associated with neurodegeneration. We show that in female transgenic mice, gonadectomy results in reduced levels of
circulating anti-nucleosome antibodies and poorer spatial learning and memory performance. In contrast, in transgenic male animals, gonadectomy improved spatial memory but had no significant impact on autoimmunity. Analysis of AD neuropathology and epigenetic factors further support such sex hormone-related differential effects on behaviour, as only gonadectomized AD females exhibited enhanced expression of mouse (m) Mapt and reduced binding activity of the histone variant macroH2A1 at the mMapt gene body compared to their sham counterparts. AD females showed higher levels of cortical Ab42 than AD males irrespective of gonadal hormones, whereas gonadectomized AD males showed significantly reduced cortical soluble Ab42 levels and reduced histone variant H2afy levels compared to sham-operated AD males. Our work suggests that adult gonadal hormones contribute to sex differences in autoimmunity, AD pathology and behaviour. Female sex hormones may enhance autoimmunity and spatial memory, whereas male sex hormones may be detrimental to spatial memory but have no effect on autoimmunity. Female sex hormones appear to decrease expression of the tau gene and increase macroH2A1 binding at the mMapt promoter in 3xTg-AD mice, consistent with a repressive effect of macroH2A1 on transcription. Additionally, these hormones have no effect on Ab42 levels in females, whereas male sex hormones increase expression of Ab42 and H2afy. We conclude that gonadal hormones play a role in the etiology of AD. The actions of these hormones involve sex-specific effects on autoimmunity, AD pathology, and cognitive function, possibly via epigenetic mechanisms. Funded by grant #SVB-158618 from the Canadian Institutes of Health Research to MF.

APSSG21.79
The Effect of Inorganic Nitrate on Arterial Stiffness and Blood Pressure Across the Menstrual Cycle in Healthy Subjects
Austin Hogwood¹, Joaquin Ortiz de Zevallos¹, Kae'oe Kruse¹, Meredith Buckley¹, Arthur Weltman¹, Jason Allen¹
¹Department of Kinesiology, University of Virginia, ²School of Medicine, University of Virginia

Introduction: Estrogen endogenously increases NO bioavailability via the eNOS pathway. However, estrogen fluctuates throughout the menstrual cycle (MC), causing associated changes in NO bioavailability and potentially effecting hemodynamic parameters like arterial stiffness and blood pressures (BP). Exogenous supplementation of inorganic nitrate (NO3⁻) has been shown to increase NO bioavailability and improve arterial hemodynamics, especially in individuals with hypertension, but it is unknown if NO3⁻ impacts hemodynamics differentially throughout the follicular phases of the MC, where estrogen fluctuates the most. Thus, the purpose of this study was to examine differences in pulse wave velocity (PWV), augmentation index (Aix), and central and peripheral BP across the early (EF) and late follicular (LF) phase of the MC after either beetroot juice (BR; ~13 mmol NO3⁻) or identical placebo (PL) supplementation. Methods: Seven recreationally active women (age: 24.7 ± 4 yrs, VO2peak: 34.4 ± 8 mL/kg/min) with normal MC who were not using contraceptives were recruited in this double-blinded crossover study. Subjects were randomized to consume BR or PL for 5 days prior to testing that was conducted during EF and again during LF (1-5 and 11-14 days after menses onset, respectively). Following a washout period (14 days) testing was repeated the following month with the opposite supplement. A linear mixed effects model was used to determine differences between menstrual cycle phase, across supplements, and any interactions. Data are mean ± SD and significance was determined at p < 0.05. Results: Outcome measures included PWV, Aix, Alx normalized to heart rate, systolic BP, diastolic BP, mean arterial pressure (MAP), pulse pressure (PP), aortic SBP, aortic DBP, aortic PP, and pulse transit time. Mixed effects models revealed that measures were not different across the MC or between the PL and BR supplementations (all p > 0.05). Additionally, testing revealed no significant interaction between MC phase and supplementation for any measures including PWV (BR EF: 4.7 ± 0.7; PL EF: 4.7 ± 0.4; BR LF: 4.8 ± 0.7; PL LF: 4.3 ± 0.5 m/s; p = 0.3), Alx (BR EF: 2.9 ± 14; PL EF: 8.0 ± 16; BR LF: 4.7 ± 16; PL LF: 10 ± 21 %; p = 0.9), or MAP (BR EF: 77 ± 7; PL EF: 79 ± 12; BR LF: 80 ± 12; PL LF: 80 ± 8 mmHg; p = 0.8). Conclusion: This preliminary data suggests that the follicular phase of the menstrual cycle does not influence arterial hemodynamics in young, healthy adult women and that NO3⁻ does not affect these outcomes. Thus, the utility of NO3⁻ to impact arterial hemodynamics in healthy young women appears limited. This approach should be extended to the luteal phase as well as to older women with hypertension and other risk factors for CVD.

APSSG21.80
Sex Differences in Dental-Associated Cardiovascular Disease
Kristine DeLeon-Pennell¹,²
¹Medicine-Cardiology, Medical University of South Carolina, ²Research Service, Ralph H Johnson VA Medical Center

Oral and gum health has been associated with incidence and outcomes of cardiovascular disease for years. Regression analysis has revealed that periodontal disease increases myocardial infarction (MI) mortality by seven-fold however, the mechanisms are not fully understood. We and others have shown that chronic infusion of periodontal pathogens alters the post-MI remodeling response leading to an acceleration of the macrophage timeline and decreased fibrosis. Interestingly, male mice were effected to a greater extent than female mice with almost two-fold higher numbers of macrophages present with the LV at post-MI day 1. We hypothesized that this may be due to increased activation of the adaptive immune response. Chronic exposure to the periodontal pathogen Porphyromonas gingivalis lipopolysaccharide increased activation of CD8+ T-cells in the left ventricle at post-MI day 1. While no differences were observed in total CD8+ T-cell numbers between male and female mice, there was an increase in a activation marker, CD44 in the male pre-exposed to LPS before MI. In contrast, CD8+ T-cells
isolated from females had a more robust response with increased CD44 expression and proliferation when exposed to cardiac damage associated molecular patterns (DAMPs) in vitro. Estrogen was able to inhibit DAMP-induced proliferation but had no effect on activation. Our data indicates chronic inflammation due to periodontal pathogens activated CD8+ T-cells to a higher extent in males than in females. Estrogen may play a role in dampening this response in females leading to an improvement in LV function post-MI.

APSSG21.81
Long-term cardiovascular impact of maternal depression with perinatal onset: gender-based differences in the adult offspring
Jagoda Kruszewska1, Dorota Sztechman1, Agnieszka Segiet-Święcicka2, Katarzyna Czarzasta1, Elżbieta Sajdel-Sulkowska2
1Department of Experimental and Clinical Physiology, Laboratory of Centre for Preclinical Research, Medical University of Warsaw, 2Department of Psychiatry, Harvard Medical School

We have previously developed a rat model of perinatal maternal depression and examined its impact on the cardiovascular system in the adolescent offspring. The present project aimed to extend previous studies and examine sex-specific differences in cardiovascular system of the adult offspring, which was prenatally exposed to the maternal stress. Sprague Dawley rat females were randomized into two groups: seven stress-exposed dams (SD) subjected to chronic pregestational mild stress with repeated restraint (CMS) and seven control dams (CD) handled daily. Exposure to stress was associated with the decreased body weight in SD, but increased plasma corticosterone level and weight of adrenal glands. Blood pressure and heart functions (ECHO) were assessed in the offspring, derived from CD (control offspring; CO, six females, six males) and SD dams (stressed offspring; SO, six females, eight males). Exposure to maternal pregestational stress was associated with significantly higher values of both systolic (SBP) and diastolic (DBP) blood pressure in SO compared to CO. The increase in SBP was detected both in male SO (p=0.001) and female SO (p=0.01) compared to male CO and female CO, respectively. However, the increase in DBP was only observed in male SO compared to male CO (p=0.004). ECHO analysis revealed mild left ventricular (LV) hypertrophy and an increase in the interventricular septum thickness at end-diastole (IVSd) and LV posterior wall thickness at end-diastole (LVPWd) in SO compared to CO (IVSd, p=0.018; LVPWd, p=0.016). Furthermore, the increase in LVPWd and IVSd was significantly higher in female SO (LVPWd, p=0.015; IVSd, p=0.009), while the effect in male SO was not statistically significant (LVPWd, p=0.082; IVSd (p=0.081). Additionally, ECHO analysis showed the development of LV diastolic dysfunction in SO, as evidenced by a decreased mean value of early diastolic lateral and medial mitral ring velocity (e′; p=0.001) and the increased ratio of early diastolic mitral inflow velocity (E/e′ (p=0.001). The e′ values were significantly lower in female SO (p=0.01) and male SO (p=0.019) compared to corresponding controls. However, E/e′ ratio was significantly increased only in male SO (p=0.002). These data suggest that maternal perinatal depression may have a long-term sex-dependent impact on cardiovascular health in adulthood. The study was approved by the Local Animal Ethics Committee in Warsaw (WAW2/022/2019 and WAW2/090/2019), according to Directive 2010/63/EU of the European Parliament. This project was supported by the Internal Grants of the Medical University of Warsaw (G/M/8/8/20(t) and 1MA/PM2/18).

APSSG21.82
Sex differences in alcohol consumption and alcohol-associated liver disease
Vijay Shah, Camille Kezer
1Gastroenterology & Hepatology, Mayo Clinic, 2Gastroenterology and Hepatology, Mayo Clinic

Alcohol-associated liver disease is becoming increasingly prevalent throughout the United States. While previously alcohol-associated liver disease was known to affect men more often than women, this gap between the sexes is narrowing. Studies show that women develop liver disease with lesser alcohol exposure and suffer worse disease as compared to men. This review article explores the increasing prevalence of alcohol-associated liver disease in women, reasons for changing patterns in alcohol consumption and liver disease development including obesity and bariatric surgery, proposed mechanisms of sex-specific differences in alcohol metabolism that may account for this discrepancy between men and women, and sex differences in treatment enrollment and response. Studies were identified by performing a literature search of PubMed and Google Scholar and through review of the references in retrieved articles. Search terms included alcohol-associated liver disease, alcoholic hepatitis, alcoholic cirrhosis, sex, gender, female, epidemiology, bariatric surgery, obesity, treatment. Due to the paucity of literature on some of the relevant subject matter and inclusion of landmark studies, no date range was selected. Studies were included if their methods were sufficiently robust and they made a comparison between the sexes that is clinically relevant. Understanding of the changing epidemiology and mechanisms of liver disease development unique to women are paramount in creating appropriate and effective interventions for women who represent a rapidly growing subset of patients with alcohol-associated liver disease.

APSSG21.83
New studies in mechanisms of polycystic ovary syndrome
Elisabet Stener-Victorin
1Department of Physiology and Pharmacology, Karolinska Institutet

We know that hyperandrogenism plays a key pathogenic role and that polycystic ovary syndrome (PCOS) runs in families, with an estimated heritability of 70%. Indeed, in a register-based study of nearly 30,000 daughters of women
with or without PCOS, we recently found that daughters of women with PCOS have a five-fold increased risk of being diagnosed with the syndrome. But how PCOS is inherited is unclear as PCOS loci identified by genome-wide association studies account for only 10% of the heritability. Growing evidence suggests that epigenetic and developmental programming contributes significantly to the inheritance of PCOS. Women with PCOS have abnormally high levels of circulating androgens throughout pregnancy, thereby increasing the supply of androgen to the foetus. Exposing pregnant mice to the non-aromatizable androgen dihydrotestosterone (DHT) triggers the development of PCOS-like traits in first-generation (F1) female offspring, suggesting that androgen-receptor pathways are molecular gateways to PCOS transmission. Moreover, we recently demonstrated that PCOS-like traits induced by DHT exposure during pregnancy in mice can be passed on from mothers (F0) to daughters (F1), granddaughters (F2), and even great-granddaughters (F3), and that transcriptional and mitochondrial perturbations of oocytes accompany this transmission. Recent studies indicate that not only PCOS daughters but also their sons also have an increased risk of developing disorders associated with PCOS. However, to what extent male offspring are affected by prenatal DHT exposure and maternal obesity is not known, nor is it known how such transmission might occur. Our preliminary data show that male offspring (F1–F3) of obese and androgen-exposed mothers (F0) develop aberrant reproductive and metabolic traits in adulthood (unpublished). Moreover, small-noncoding RNA sequences carried by the sperm contribute to a transgenerational epigenetic inheritance of phenotypic traits. Although the clinical diagnostic features of a male PCOS counterpart remain to be defined, these preliminary findings suggest that maternal obesity and prenatal androgen exposure is a previously unrecognized factor influencing lifelong male health. References 1. Risal, S., et al. Prenatal androgen exposure and transgenerational susceptibility to polycystic ovary syndrome. Nature medicine 25, 1894-1904 (2019). 2. Maliqueo, M., et al. Placental STAT3 signaling is activated in women with polycystic ovary syndrome. Hum Reprod 30, 692-700 (2015). 3. Stener-Victorin, E., et al. Animal models to understand the etiology and pathophysiology of polycystic ovary syndrome. Endocr Rev (2020). 4. Crisosto, N., et al. Reproductive and metabolic features during puberty in sons of women with polycystic ovary syndrome. Endocr Connect 6, 607-613 (2017). 5. Crisosto, N., et al. Higher luteinizing hormone levels associated with antimullerian hormone in postmenarchal daughters of women with polycystic ovary syndrome. Fertil Steril 111, 381-388 (2019). 6. Cesta, C.E., et al. Maternal polycystic ovary syndrome and risk of neuropsychiatric disorders in offspring: prenatal androgen exposure or genetic confounding? Psychol Med, 1-9 (2019). 7. Stener-Victorin, E and Deng, Q. Epigenetic inheritance of polycystic ovary syndrome - challenges and opportunities for treatment Nat Rev Endocrinol. 2021 Jul 7. doi: 10.1038/s41574-021-00517-x. Online ahead of print.PMID: 34234312 8. Stener-Victorin E, Deng Q. Transmission of Polycystic Ovary Syndrome via Epigenetic Inheritance. Trends Mol Med. 2021 Jun 11;S1471-
asthma (8.4% versus 13.8%; p<0.001). Fewer men lived under the poverty line and more of them than women had active internet subscriptions. Other socioeconomic variables were similar between the sexes. Unadjusted logistic regression showed a higher odds of death in hospitalized men during both the pre-COVID and COVID periods (pre-COVID OR for men versus women: 1.66 and COVID OR for men versus women: 1.98). After adjustment for relevant clinical and demographic factors, the higher risk of male death attenuated towards the null in the pre-COVID period (OR: 1.27; 95% CI 0.97–1.66; p= 0.08) but remained significantly higher in the COVID period (OR: 1.98; 95% CI 1.70–2.31; p<0.001). · Conclusion- The higher risk of death for men during the COVID time period despite adjustment for multiple sociodemographic and clinical factors supports the hypothesis that physiological sex differences, such as a difference in immune responses, expression levels of proteins that determine viral entry such as angiotensin-converting enzyme 2 (ACE2) and Transmembrane Serine Protease 2, and androgen-mediated regulation of those proteins may be responsible for the higher risk for male mortality with COVID.

APSSG21.86
The Many Menopauses: Cognitive effects of early life ovarian removal
Gillian Einstein1,2,3
1Psychology, University of Toronto, 2Rotman Research Institute, Baycrest Hospital, 3Tema Genus, Linköping University

In this talk I will discuss the many types of menopause, their characteristics, the lack of differentiation in the literature, and the resulting contradictions in cognitive findings that can undermine clinical usefulness. Menopause and the menopausal transition have become key timepoints for studying women’s increased risk of Alzheimer’s disease. However, when it comes to symptoms as well as brain and behavior, all menopauses are not created equal. There are multiple pathways to the end of menses. For most women, menopause occurs at an average age of 51 (spontaneous menopause). However, for a small but significant number of women, ovarian cessation occurs earlier and for a myriad of reasons. There is premature (younger than 40 y), early (between 40 and 45 y), and induced menopauses (oophorectomy with or without hysterectomy, bilateral salpingo-oophorectomy [BSO], the removal of ovaries and fallopian tubes, or ovarian ablation through radiation). Thus, using menopause as a blanket term to describe any “cessation of menses” does a disservice to understanding the physiology, etiology, and brain health outcomes of each type of menopause (Edwards et al., 2018). In fact, there are many menopauses. One such menopause, ovarian removal prior to the age of 48, has been documented to lead to a higher risk of late-life dementia (Rocca et al., 2007); a steeper decline in a global cognitive score (Bove et al., 2014), and within 6 months of removal, decreased performance on episodic and associative memory if not estrogen-replaced (Phillips & Sherwin, 1992). As well, in women carrying the breast cancer gene mutation who have BSO as prophylaxis for breast and ovarian cancers at an average age of 42 y, these changes are longer lasting with verbal episodic and spatial working memory still affected at an average of five years later (Gervais et al., 2020). Decrements in spatial working memory continue over time (Gravelsins et al., in preparation). Preliminary data suggest that frontal and hippocampal cortical changes as well as associative memory and their correspondence to changes in sleep physiology are also affected adversely. Thus, if a ‘menopausal’ cohort with an average age of 51 or older includes women with premature or early menopause, included are women with behavioural and brain changes occurring 10 years earlier than likely, most of the cohort. In addition, while some of the changes may resemble those in spontaneous menopause, others, may differ. Thus, to really understand the role that spontaneous menopause (at av. 51 y) plays in women’s late-life brain health, understanding clearly who is in the cohort and the reasons for their menopause is critical for understanding the contributing role of ‘menopause’ in women’s late life brain health. Bove et al., 2014. Age at surgical menopause influences cognitive decline and Alzheimer pathology in older women. Neurolog. 82, 222–229. Edwards et al., 2019. The many menopauses: Searching the cognitive research literature for menopause types. Menopause. 26, 1–21. Gervais et al., 2020, Cognitive markers of dementia risk in middle-aged women with bilateral salpingo-oophorectomy prior to menopause. Neurobiol. Aging. 94, 1-6. Phillips, S.M., Sherwin, B.B., 1992. Effects of estrogen on memory function in surgically menopausal women. Psychoneuroendocrinol. 17, 485–495. Rocca et al., Increased risk of cognitive impairment or dementia in women who underwent oophorectomy before menopause. Neurology. 69, 1074–1083.

APSSG21.87
Establishment of novel placenta-on-a-chip model
Sahar Masoumeh Ghorbanpour1, Claire Richards1
1Life science, University of Technology Sydney

Background: Preeclampsia is a cardiovascular disorder diagnosed post 20 weeks of gestation. It is the leading cause of morbidity and mortality in pregnancy. Inappropriate placentalion due to aberrant angiogenesis and inflammation are the root causes of preeclampsia. However, difficulties in obtaining early pregnancy placental tissue, has impeded the progress in understanding the molecular mechanisms regulating placental development and growth. In this study, we investigated the role of important vascular and inflammatory proteins, FKBPL and Gal-3, in preeclampsia using human plasma/placental samples and developed a new 3D microfluidics model of placental tissue. Methods: ELISA or Western blotting were utilised to determine FKBPL and Gal-3 concentrations or expression in plasma (n=17 controls; n=30 preeclampsia) and placental samples (n=6 per group), respectively. Vascularisation and remodelling of human umbilical vein endothelial cell (HUVEC) in a co-culture with extravillous trophoblast cells (ACH-3Ps) within a 3D microfluidics chip was determined following exposure to inflammatory tumour necrosis factor (TNF)-α. Immunofluorescent staining,
Western blotting and ELISA were used to determine the expression of FKBPL and Gal-3 in this novel placenta-on-a-chip model. Results and discussion: FKBPL and Gal-3 protein expression was increased in plasma (FKBPL; p<0.0001, Gal-3; p<0.05) and placental (FKBPL; p<0.05, Gal-3; p<0.05) samples from women with preeclampsia compared to healthy controls. Inflammation in 3D vascularised microfluidics placental models also led to an increase in FKBPL and Gal-3 protein expressions (FKBPL; p<0.05, Gal-3; p<0.05), in conjunction with changes in vascular pattern (branching pattern) and reduced vasculo-genesis potential (CD31; p<0.005). Conclusions: Our novel 3D microfluidics model of human placental tissue can recapitulate aberrant placenta in preeclampsia. Upregulation of FKBPL and Gal-3 indicative of restricted angiogenesis, appear to be key mechanisms involved in inappropriate placental development and vascular dysfunction in preeclampsia.

APSSG21.90
Quantifying dynamic cerebral autoregulation during repeated squat-stand maneuvers with 20% resistance above body weight
Kailey Newel1, Joel S. Burma1, Joseph Carere1, Jonathan Smirl1
1Faculty of Kinesiology, University of Calgary

Background: Repeated squat-stand maneuvers (SSM) have been shown to produce ~30-50 mmHg swings in mean arterial pressure (MAP) which are buffered in the brain through dynamic cerebral autoregulation. To further challenge this system, the current study employed 20% resistance above body weight, as this is expected to further augment the alterations in MAP while still enabling beat-to-beat blood pressure (BP) to be assessed. Furthermore, females often display a greater risk of cerebrovascular accidents in their lifetime, however the underlying mechanisms for this biological sex difference has yet to be fully elucidated. Therefore, the current study aimed to examine the regulatory mechanisms of the brain during normal and resistance exercise during SSM and sought to examine the extent healthy females and males differ with respect to cerebrovascular regulation. Methods: A total of 12 female and 12 male participants completed two bouts of 5-minute SSM for both body weight and resistance conditions (10% body weight in each arm). These included frequencies of 0.05Hz (10-seconds squat/stand) and 0.10Hz (5-seconds squat/stand), which have widely been utilized to examine cerebrovascular regulation. Cerebral blood velocity was indexed in the middle cerebral artery (MCA) and posterior cerebral artery (PCA) with transcranial Doppler (TCD) ultrasound. Additionally, heart rate, BP and end-tidal values of carbon dioxide were recorded. The linearity between blood pressure fluctuations and CBV in both arteries were computed using transfer function analysis in diastolic, mean, and systolic aspects of the cardiac cycle. A 2x2 analysis of variance was utilized to determine differences between resistance vs. body weight squats and between biological sexes. Results: As expected, mean and systolic BP power density spectrums were elevated with resistance squats compared to body weight squats at both frequencies (all p<0.03). Despite this, no differences were noted in the associated phase and gain metrics used to assess the brain’s ability to regulate BP perturbations between squat conditions (all p>0.067; negligible/small effect sizes). Interestingly, males showed augmented systolic regulatory mechanisms compared to females (all p<0.045; small to large effect sizes). No differences were noted between sex in the diastolic and mean components of the cardiac cycle (all p>0.102; negligible/small effect sizes). Conclusion: The absence of strong CBV increases in the MCA and PCA despite the systemic BP demonstrating increases between normal and resistance squats suggests dynamic cerebral autoregulation is actively minimizing these systemic effects from occurring in the cerebrovasculature. This indicates the brain is autoregulating to prevent under and over perfusion to the brain when it is being challenged. Additionally, the lower levels of autoregulation found during the systolic aspect of the cardiac cycle in females is very interesting and warrants further research especially with respect to sex-related cerebrovascular event risk factors and post-concussion symptom differences.

APSSG21.90
Academic Twitter: A New Skill in Your Trainee Tool Belt
John Henry Dasinger1
1Physiology, Augusta University

Graduate training focuses on the skills necessary to be a successful researcher, and one of the most important skills to develop is how to be an effective communicator. While it is vital to perfect written and oral communication skills for the more traditional types of publications and presentations, it is important to expand upon communication skill sets as technology evolves. Social media is an ever-growing occurrence in the science community that allows scientists to connect on a new level. Academic twitter allows for a unique opportunity for self-promotion about recent publications or upcoming presentations that can highlight the work occurring in the laboratory as a marketing tool. This could serve as an advertisement for future career opportunities. It allows scientists to connect with their science idols or peers from institutions around the world in a low-pressure situation. It also a great way to support each other in a career that can have challenging times. With the ongoing COVID-19 pandemic, it provides the scientific community an atypical method to stay connected during a challenging time. There are many benefits of an academic social media presence, and it can be a useful tool for scientists of all academic backgrounds.

APSSG21.91
Musculoskeletal alterations in male and female rats exposed to in micro- and partial gravity environments
Megan Rosa-Caldwell1, Marie Mortreux1, Dong-Min Sung2, Ann-Sofie Schreurs3, Mary Bouxsein1, Ursula Kaiser4, Seward Rutkove1
1Department of Neurology, Beth Israel Deaconess Medical Center and Harvard Medical School, 2Neurology, Beth
Decades of research on the physiological implications of spaceflight have revealed significant muscle and bone loss with long duration spaceflight. However, comparatively little is known about musculoskeletal alterations or protections during partial gravity environments such as those noted on Mars (0.4g). Moreover, how biological sex may interact with micro- or partial gravity environments and subsequent musculoskeletal outcomes remains inconclusive. Purpose: to investigate the influence of biological sex on musculoskeletal outcomes after exposure to simulated micro- and partial gravity environments.

Methods: Male and female Fischer rats were divided into either simulated microgravity (0g, n=5-7), Martian gravity (0.4g, n=5-7), or earth gravity (tg, n=5-7). Microgravity was induced using the well characterized hindlimb unloading model. Martian gravity was completed using a specialized harness system that allowed for 40% of weight bearing. Control animals maintained full weight bearing. Animals underwent designated interventions for 28 days. Before and after interventions, bodyweight, grip strength, maximal plantarflexion, and trabecular bone density were quantified. Data were analyzed by percent change from baseline with a covariate of baseline values. Results: Both 0g and 0.4g rats lost bodyweight (~10% and ~5% decreased bodyweight respectively), with no differences between sexes (p=0.759 and p=0.760 respectively). Male and female rats had ~40% lower grip strength after 28 days at 0g or 0.4g without any differences between sexes (p=1.000, and p=0.999 respectively). Female 0g rats had ~20% greater decline in maximal plantarflexion force compared to 0g males (p=0.025). Similarly, female 0.4g rats had ~30% greater decline in maximal plantarflexion force compared to 0.4g males (p<0.001). Finally, both 0g and 0.4g rats lost ~20% of trabecular bone density without any differences between sexes (p=0.999 and p=1.000 respectively). Conclusions: For many outcomes, exposure to either partial gravity or microgravity elicited similar decrements in males and females. Both males and females had substantial alterations to musculoskeletal function; however, where sex differences occurred, females had exacerbated deteriorations compared to males. More work is necessary to confirm sex differences in muscle deconditioning and, if confirmed, to explore mechanisms. Acknowledgements: This study was supported by NASA Awards: 80NSSC10K0311 and 80NSSC19K9518.

**APSSG21.92**

**Squat-stand maneuvers alter cardiovascular and autonomic recovery in females**

Joseph Carere1, Joel Burma1, Kailey Newel1, Courtney Kennedy1, Jonathan Smirl1

1Kinesiology, University of Calgary

Cerebrovascular Concussion Lab, Faculty of Kinesiology, University of Calgary, Alberta, Canada

Background: The autonomic nervous system (ANS) is a key regulator of cardiovascular activity and systemic blood pressure (BP). Resistance exercise, through the form of repetitive squat-stand maneuvers (SSM), is known to cause large fluctuations in BP. However, it is unknown the extent to which these SSM cause alterations to ANS function, both during and following activity, especially with respect to biological sex. Therefore, this study will examine ANS and cardiovascular recovery following bouts of prolonged body weight (BW) and resistance SSM at controlled frequencies, with particular attention paid to whether recovery differs between males and females. Methods: 12 males and 13 females (all individuals identified gender as the same as their biological sex) performed four 5-minute bouts of SSM: two bouts of BW SSM, and two bouts of resistance SSM (10% BW). Each were followed by quiet-rest performed as 5-minutes seated and 5-minutes standing. The SSM frequencies were controlled such that a bout of each BW and resistance SSM were done at 0.05 Hz (10 second squat / 10 second stand) and 0.10 Hz (5秒 squat / 5 second stand). These frequencies were selected as they have been widely used to assess cerebrovascular autoregulation. A Finapres® NOVA monitoring system quantified beat-to-beat BP, and heart activity was measured using a 3-lead electrocardiogram (ECG). Heart rate, heart rate variability (HRV), and baroreceptor sensitivity metrics were extracted from the ECG and BP recordings in both time- and frequency-domains. To assess the differences between type of squats and between biological sexes, a 2x2 Analysis of Variance was utilized. Results: No differences in ANS recovery were noted following both the BW and resistance SSM. However, the BP power spectrum density (PSD) were augmented, and the RRI-intervals were reduced during the resistance SSM (p<0.001; moderate/large effect sizes). Of interest, despite females having a higher heart rate compared to males during recovery (p=0.017; small effect size), females also had greater level of HRV in both time-domain (p<0.047; small effect size), and high frequency domain HRV measures (p=0.002; moderate effect size), while low-frequency HRV parameters were reduced (p=0.002; moderate effect size). Conclusion: Interestingly, the current data suggests there is a decoupling of the BP and heart rate responses present during the resistance SSM compared to the body-weight SSM. Furthermore, following both forms of SSM, females demonstrated paradoxical findings, as this group showed both an elevated heart rate and displayed greater disparities in HRV metrics. Typically, when heart rate is elevated, there is a reduction in HRV. It is speculated the SSM resulted in augmentations to the naturally occurring Mayer waves that persisted throughout the recovery period. As the baroreflex is maintained (the same level of HR change per mmHg change in BP: ms2/mmHg), these enhanced SSM induced Mayer wave BP fluctuations result in females experiencing a greater level of HRV when the cardiovascular system is challenged in this manner. This study received NSERC and Integrated Concussion Research Program funding.
APSSG21.94
Estrogen regulates miR-17~92 to alter sodium transport in the kidney distal nephron
Corinne Farrell1, Neja Ozbaki-Yagan1, Xiaoning Liu1, Andrew J. Bodnar2, Jacqueline Ho2, Michael Butterworth1
1Department of Cell Biology, University of Pittsburgh, 2Department of Pediatrics, UPMC Children’s Hospital of Pittsburgh

Background: Hypertension affects more than one billion people worldwide. One regulator of blood pressure homeostasis is the mineralocorticoid hormone aldosterone. Aldosterone increases sodium (Na+) transport in the kidney distal nephron to regulate blood volume. Premenopausal women are less likely to develop hypertension than age-matched men, due to both lower aldosterone levels and estrogen signaling. We previously demonstrated that aldosterone alters the expression of microRNAs (miRs) in collecting duct epithelial cells to modulate the Na+ transport response to aldosterone. However, the sex-specific regulation of miRs and role of estrogen to alter miR expression in the kidney distal nephron has not been explored. This study investigated the hypothesis that estrogen alters aldosterone signaling in the distal nephron by regulating the expression of miRs in collecting duct epithelia. Methods: Primary cortical collecting duct (CCD) cells derived from male and female mice as well as cultured mCCD-clf cells were grown on permeable filter supports to measure Na+ transport by short-circuit current recordings in Ussing chambers. Cells were incubated with estrogen for time and dose responses to determine the impact on aldosterone stimulation and Na+ transport. RT-qPCR quantified miR and mRNA expression and western blot analysis quantified protein expression after aldosterone and estrogen stimulation. In vivo regulation of miRs by aldosterone was tested in CCD cells isolated from male and female mice placed on Na+ deficient diets. Inducible, nephron-specific miR knockout and gain-of-function mice were used to examine the impact of altering miR expression on target protein expression. Results: A sex-specific upregulation of the miR-17~92 cluster was observed in female mice placed on a low-Na+ diet to stimulate aldosterone release. MiRs-19 a & b were upregulated in mCCD cells stimulated with estrogen. Estrogen pretreatment blunted aldosterone stimulation in CCD epithelia. CCD cells pre-stimulated with aldosterone exhibited a time-dependent decrease in Na+ transport over 6 hours with estrogen exposure. Luciferase assays demonstrated that miRs-19a&b bind to the 3’-UTR of the serum and glucocorticoid induced kinase (SGK1). Overexpression of miR-19 in mCCD cells using miR mimics significantly inhibited aldosterone stimulation of Na+ transport. Conversely, aldosterone stimulation was greater in mCCD cells transfected with a miR-19 inhibitor (antagomir). SGK1 expression in CCD cells from miR-17~92 KO mice was increased compared to littermate controls, while gain-of-function miR-17~92 mice had lower SGK1 expression. Conclusion: The miR-17~92 cluster is regulated by aldosterone and estrogen. Mir-19 targets SGK1 and alters Na+ transport in CCD cells. This may account for part of the sex-specific differences in aldosterone signaling observed in vivo. Ongoing studies aim to determine if altering the expression of miR-17~92 disrupts aldosterone signaling in the kidney and alters blood pressure in vivo.

APSSG21.95
Menopause diminishes protection of young female mice against metabolic and cognitive impairments in a vascular dementia model
Olivia Gannon1, Janvie Naik1, Febronia Mansour1, David Riccio1, Charly Abi-Ghanem1, Richard Daniel Kelly1, Abigail Salinero1, Kristen Zuloaga1
1Department of neuroscience and experimental therapeutics, Albany Medical College

Without adequate blood supply, the brain accumulates damage which is the source of vascular contributions to cognitive impairment and dementia (VCID). VCID is the second leading cause of dementia. Risk factors for VCID include stroke, hypertension, obesity, and diabetes. These conditions all present with sex differences: women are protected compared to men- at least before menopause. After menopause, ovarian production of estrogen ceases, withdrawing the protection estrogen provides against VCID risk factors. Estrogen induces vasodilation to increase cerebral blood flow and reduces risk of stroke, diabetes, and obesity. Even though the vast majority of women with dementia are post-menopausal, exactly how menopause impacts dementia is unclear. Based on the known protective effects of estrogen, we hypothesized that menopause would exacerbate cognitive impairment in a mouse model of VCID. To address this question, we utilized the 4-vinylcyclohexene diepoxy menopause model which is an ovary-intact model that closely matches estrogen fluctuations seen in human menopause. To model VCID, we performed a unilateral common carotid artery occlusion surgery, which leads to prolonged cerebral hypoperfusion. Because we have previously found correlations between visceral adiposity and spatial memory in female mice and because menopause in humans leads to metabolic changes, we also examined metabolic factors in these mice. We found that menopause led to more dramatic weight gain, increased visceral adiposity, and impaired glucose tolerance. To test for cognitive impairments, we performed behavior tests including the novel object recognition test (episodic-like memory) and nest building test (activities of daily living). We found that menopause impaired episodic-like memory and activities of daily living only in VCID mice. There was a significant correlation between the degree of blood flow deficit and activities of daily living. In contrast to post-menopausal mice, pre-menopausal mice did not suffer significant episodic memory impairments from the VCID surgery and thus were relatively protected. To assess white matter damage- a hallmark of vascular damage- we used luxol fast blue staining and found no significant effect of VCID or menopause at this timepoint. This may be due to the protection that young female mice have against vascular damage. Further investigation into pathological features (brain inflammation, white matter damage, changes in estrogen receptor expression, and vascular damage) may reveal the mechanisms through which menopause impacts cognitive impairment. Overall, we have found that young
female mice lose their protection against metabolic and cognitive impairments in post-menopausal VCID.

**APSSG21.96**  
Comparison of the Phenotypic Development of C26 Colorectal Cancer-Induced Cachexia Between Biological Sexes  
Francielly Morena da Silva¹, J. William Deaver², Seongkyun Lim¹, Ana Regina Cabrera¹, Eleanor Schrems¹, Landen Saling¹, Tyrone Washigton¹, Nicholas P. Greene¹  
¹Health, Human Performance, and Recreation, University of Arkansas, ²Molecular Physiology and Biophysics, Vanderbilt University Medical Center

Introduction: Cachexia is a multifactorial syndrome commonly experienced by cancer patients. Cachexia is clinically defined by involuntary weight loss greater than 5% in six-months and is generally not responsive to nutritional interventions alone. Cancer cachexia (CC) is associated with resistance to anti-cancer treatment and is responsible for 20-40% of cancer-associated deaths. Atrophy, muscle weakness, and fatigue are the primary hallmarks of CC. Mechanisms of cachexia are not fully understood, and current interventions lack efficacy. Studies from our group and others suggest differences in CC between biological sexes. However, direct comparisons of the phenotypic development of cachexia between biological sexes are scarce. Purpose: Therefore, the purpose of this study was to characterize phenotypic differences between biological sexes during the development of CC in a time-course manner in a preclinical C26 colorectal cancer model. Methods: A total of 129 (69 males and 60 female) 8-week old BALB/c mice were separated into PBS control, 10-, 15-, 20- and 25-day of tumor-bearing group (*10-12 animals/group). Cancer groups were injected with a total 1 million C26 cells bilaterally to the hind flanks, while equal volume of PBS was injected in PBS control (age-matched with 25-day animals). Tissue collection was performed at each designated time point represented by each group; Gonadal-Fat, Liver, Spleen, Heart, Soleus, Plantaris, Gastrocnemius, EDL and TA muscles were weighed and snap frozen in liquid nitrogen. All tissue weights were normalized to tibia length to account for differences in body size. A one way-ANOVA across timepoints within each sex was performed as the global analysis with α=0.05. RESULTS: Tumor free body weight was significantly lower in male 25-day mice by 15% (p=0.0001) when compared to PBS control, while no differences in body weight were noted in female mice across groups. For muscle weights: soleus, gastrocnemius, and TA muscle weights were 9.4%, 16.8%, and 19.4% (p<0.0001) lower in male 25-day mice compared to PBS group, respectively. No statistical differences in muscle weights were observed in female mice. In males, 25- and 20-day groups showed 49% (p<0.0001) and 23% (p=0.0165) lower fat content compared to PBS control. Accordingly, female 25-day fat content was reduced by 55% compared to PBS control (p=0.0136). Spleen weight was significantly greater in 20- (87%, p<0.0001) and 25-day (155%, p<0.0001) groups when compared to PBS control in males. In females, a similar pattern was noted, 20- and 25-day had a significantly heavier spleen by 61% and 118% respectively, compared to PBS control (p<0.0001). Conclusion: Despite demonstrating classic cachexia phenotypes regarding splenomegaly and losses in fat mass, female mice appear to protect skeletal muscle wet weights relative to males during development of cancer cachexia. Acknowledgements: This study was funded by the National Institutes of Health, Award: 5 R01 AR075794-02.

**APSSG21.97**  
Circulating cell-free mitochondrial DNA in preeclampsia  
Styliani Goulopoulou¹  
¹Physiology and Anatomy, University of North Texas Health Science Center

Circulating cell-free mitochondrial DNA (ccf-mtDNA) is a marker of inflammation and mitochondrial dysfunction that has been implicated in various disease processes and pathologies such as autoimmune disorders, trauma and sepsis, and cardiovascular disease. Changes in ccf-mtDNA levels reflect cellular stress and death or impaired cellular respiratory capacity and thus, it may be an easily accessible proxy of tissue health. Our work focuses on the role of ccf-mtDNA in pregnancies with preeclampsia, a hypertensive disorder of pregnancy with severe maternal cardiovascular features and adverse perinatal outcomes. Although preeclampsia is a leading cause of maternal deaths worldwide, there is neither cure for preeclampsia nor effective treatments, with delivery of the placenta being the most used clinical management in severe cases. Here, we address the presence of various biological forms of ccf-mtDNA in pregnancies with preeclampsia, the potential downstream signaling of these forms, as well as methodological considerations to produce robust and reliable ccf-mtDNA outcomes. Data from women with healthy pregnancies and pregnancies with preeclampsia as well as evidence from experimental studies with animal and cell culture models will be presented. Furthermore, we will present results from a penalized logistic regression model that addresses the association between maternal ccf-mtDNA and clinical diagnosis of preeclampsia. Collectively, our data suggest that aberrance of ccf-mtDNA dynamics is associated with preeclampsia.

**APSSG21.98**  
Fluid Balance in Long Term Hormonal Oral Contraceptive Users  
Whitley C. Atkins¹, Emily R. Nelson¹, Brendon P. McDermott¹  
¹Exercise Science Research Center, University of Arkansas

The benefits of optimal hydration in resting conditions include enhanced mood maintenance, cognitive function, blood sugar balance and prevention of chronic disease. In order to meet hydration needs, the importance of individualized hydration plans has been proposed. While there has been extensive research on fluid balance, the female population is largely excluded due to mechanisms by which female sex hormones, estrogen and progesterone, may impact fluid regulation. Complicating the understanding of hydration regulation in women, the
use of oral contraceptives has increased in recent decades. The exogenous hormones used to turn-off the hypothalamic-pituitary-gonadal axis are administered in 3-10 times the amount of endogenous hormones, potentially impacting fluid balance. There has been little research to show the effects of oral contraception use on water turnover (retention and excretion). Purpose: To assess hydration status variables, 24-hr urine volume, and 3-hr water turnover in females who use oral contraceptives (OCs). Methods: Methods were approved by the university’s institutional review board before data collection. Fourteen female volunteers (25 ± 5y, 60.2 ± 7.1kg, 38.7 ± 3.2kg/LBM) provided consent and enrolled in our study. All were long-term oral contraceptive users (> 6 months) and participated in two trials, one during the active pill (High Hormone, HH) dose of their prescribed OC and one during the sham pill (Low Hormone, LH) dose. Participants collected their urine for 24-hrs prior to trials. Diet and fluid intake was matched between trials. On trial days, participants reported to the laboratory euhydrated, fed a standard breakfast (bagels and cream cheese or peanut butter) and remained seated for 60 min. Participants were then provided a bolus of room temperature water in the amount of 12mL/kg/LBM. The bolus was divided into four equal amounts to be consumed over 20 min. Urine output over 180 min following bolus consumption was measured. Nude body weight (NBW) was measured pre- and post-trial. Urine specific gravity (USG) was analyzed via refractometry and urine osmolality was analyzed via freezing point depression. Dependent t-tests were used to assess mean differences. Results: There was no significance difference in 24-hr urine volume between HH (1382 ± 451mL) and LH (1468 ± 550mL), p = .567). Nor were there significant differences in 24-hr USG (HH1:010 ± .007, LH1:012 ± .009; p = .542) or 24-hr osmolality (HH388 ± 272, LH: 421 ± 331mOsm/kg; p = .673). 3-hr urine volume was not different between HH (767 ± 201mL) or LH (748 ± 202mL; p = .780). There were no differences between 3-hr USG (HH1:005 ± .001, LH1:005 ± .001; p = .656) or 3-hr osmolality (HH193 ± 40, LH203 ± 60mOsm/kg; p = .485). NBW change did not differ between trials (HH0:29 ± 0.36, LW0:44 ± 0.29kg, p = .151) Conclusions: In our preliminary data, we did not identify differences between the active pill dose versus sham pill dose in USG, urine osmolality, or urine volume in 24-hr samples and 3-hr samples. Despite fluctuations in exogenous hormone concentrations, fluid balance seems unaffected in females who have taken OCs for > 6 months. This study highlights that the exclusion of females using hormonal oral contraceptives in hydration studies may not be warranted.

Cerebral injury produces reactive oxygen species which cause cell damage and death. We investigated the effects of sex and advanced age on vascular cell resilience to acute oxidative stress imposed by hydrogen peroxide (H2O2) in posterior cerebral arteries (PCAs) from young (4-6 mo) and old (22-26 mo) male and female C57BL/6 mice (n=4-7 per group). PCAs were isolated, pressurized (90 cm H2O) and exposed to H2O2 (200 μM) for 50 min at 37°C; cell death was quantified using Hoechst 33342 dye (1 μM; stains nuclei of all cells) and propidium iodide (2 μM; stains nuclei of dead cells). SMC death was greater (P<0.05) in young vs. old males (21±4% vs. 10±2%) and lower in females regardless of age (<5%). For ECs, H2O2 killed ~10% in each group. Consistent with elevated [Ca2+]i initiating cell death, peak [Ca2+]i responses (Fura-2 fluorescence) to H2O2 were greater in PCAs of young (ΔF340/F380=0.5±0.03) vs. old (ΔF340/F380=0.20±0.03) males and consistently lower in PCAs of females (young: ΔF340/F380=0.11±0.02; old: 0.09±0.02). Selective inhibition of transient receptor potential vaniloid 4 (TRPV4) channels (HC-067047, 1 μM) attenuated the rise of [Ca2+]i and reduced SMC death in response to H2O2 most effectively in young males (3%±1%) but had negligible effect in PCAs from old males or females. Activating TRPV4 channels (GS-K1016790A, 50 nM) during H2O2 increased EC death to >80% in all groups but did not alter SMC death; the TRPV4 agonist by itself did not induce cell death. Depolarization of mitochondrial membrane potential (ΔΨm) can lead to cell death. To evaluate ΔΨm during H2O2 exposure, PCAs were equilibrated with tetramethylrhodamine methyl ester (TMRM; 10 nM). Over 30 min, H2O2 induced greater depolarization of ΔΨm (loss of TMRM fluorescence) in PCAs from young (F/F0=0.40±0.04) vs. old males (F/F0=0.58±0.06) and was reduced in PCAs from young females (F/F0=0.63±0.03) compared to males with no further effect in PCAs from old females (F/F0=0.56±0.05). Removal of extracellular Ca2+ attenuated ΔΨm depolarization in young males (F/F0=0.74±0.04), as did nonselective (ruthenium red, 5 μM; F/F0=0.66±0.06) and selective (HC-067047; F/F0=0.76±0.11) inhibition of TRPV4 channels. In the absence of H2O2, ΔΨm was well preserved (F/F0=0.94±0.02). We conclude that SMCs in cerebral arteries of females are inherently more resilient to acute oxidative stress than males and that advanced age promotes resilience most effectively in males. Increased susceptibility to H2O2 in young males is associated with greater TRPV4-dependent Ca2+ entry and ΔΨm depolarization. Funding source: AHA 10TPA34850102, R01HL136292.
Sex bias in murine bone studies
Lysanne Michels1, Aikta Sharma1, Andrew Pitsillides2, Julie Greeves3, Valentina Cardo4, Claire Clarkin1
1School of Biological Sciences, University of Southampton, UK, 2Comparative Biomedical Sciences, Royal Veterinary College, UK, 3Army Health and Performance Research, Army Headquarters, UK, 4Winchester School of Art, University of Southampton, UK

Bone as an organ is sexually dimorphic throughout life and thus physiologically distinct in men and women. Degenerative bone loss or osteoporosis is often perceived as a female condition linked to frailty, the menopause and ageing. Today, many preclinical skeletal studies focus on only one sex or use mixed sexes, consequently important sex-specific regulators of skeletal homeostasis may be overlooked. Further, clear reporting of sex specific phenotypes in murine models in the literature is inconsistent. Herein, we aimed to assess whether any sex bias has developed in murine skeletal studies bone using the PubMed database. We grouped research articles published between 2010-2020 by inclusion of key words ‘male(s)’/‘female(s)’, ‘mouse’/‘mice’/‘murine’, and ‘bone’/‘skeletal’/‘skeleton’ in the paper title and/or abstract. Of the 52,690 articles identified, only 3.9% explicitly mentioned the mice’s sex (2,077 articles); there were more male (50.4%, 1,047 articles) than female studies reported (49.6%, 1,020 articles), with a smaller number including both (11.8%, 245 articles). Manual analysis of the first 100 papers sorted by “best match” where the terms ‘male(s)’ and ‘female(s)’ were not included in the title and/or abstract (96.1% of all studies, 50,613 articles), revealed that only 60% reported sex within the main body of the article. When a single sex was assessed (24 articles), a bias towards males (62.5%, 15 articles) over females (37.5%, 9 articles) was present. When both sexes were assessed (36 articles), over half separated males from females for analyses (58.3%, 21 articles), while the remainder pooled sexes together (41.7%, 15 articles). Why this male bias exists is unclear but it is of importance given that women are more susceptible to osteoporosis and present a larger clinical need. Improvements in the reporting of sex in the literature is urgently required to i) better inform our understanding of mechanisms driving sexual dimorphism of the skeletal system and ii) facilitate our understanding of any sex bias in preclinical experimental design as it emerges.

Linking maternal obesity and fecal microbiome in hypertensive offspring of preeclamptic-like mice
Kalie Beckers1, Juliet Flanagan1, Vivane Gomes1, Chin-Chi Liu1, Christopher Schulz2, Jenny Sones1, Gary Childers2
1Veterinary Clinical Sciences, Louisiana State University School of Veterinary Medicine, 2Microbiology, Southeastern Louisiana University

Preeclampsia (PE) is a pregnancy specific hypertensive disorder that affects up to 10% of women worldwide. It is characterized by new onset hypertension during the second half of pregnancy and signs such as proteinuria. It is a leading cause of maternal and fetal morbidity/mortality. Long-term health consequences for the offspring of PE mothers are cardiovascular and metabolic disease. Maternal obesity increases risk of PE and has been found to be a key predictor of childhood obesity and metabolic complications of the offspring. This contributes to a heightened state of inflammation, which may contribute to abnormal placental development and PE. This vicious cycle
of obesity and increased inflammation may play a role in the pathogenesis of PE and the generational consequences. The maternal gut microbiome may contribute to the development of PE by exaggerating the inflammatory response. Our BPH/5 female model spontaneously develop obesity, increased blood pressure, hyperleptinemia, and then PE, which may be passed down from mother to offspring. The BPH/5 male do not demonstrate obesity or hyperleptinemia. Therefore, we hypothesized that differences in the fecal microbiome exist between BPH/5 offspring and control normotensive mice, and pair-feeding of the dams will alter the offspring fecal microbiome in a sex dependent manner. PERMANOVA of the Bray-Curtis dissimilarity in the vegan R package was used to investigate differences in the fecal microbiome of the offspring. Differences were found in the male and female BPH/5 offspring compared to the C57 controls (p=0.001) at the community level. Additional community differences were found in offspring born to BPH5 pair-fed dams when compared to BPH5 offspring born to ad libitum dams (p=0.003). Further analyzes using phylofactor R package using a generalized linear model to evaluate strain specific differences, determined the BPH5 male and female offspring showed a decrease of Fircmicutes, Tenericutes, and Actinobacteria when compared to C57 controls. Male offspring of both strains showed a decrease in Lachnospiraceae. Obese BPH5 female showed an increase of Ruminococcaceae, UCG_014 compared to offspring born to pair-fed dams. These community differences may suggest that an unhealthy maternal obesogenic environment may interrupt important regulators in development of the fecal microbiome and have a role in sex-dependent cardiometabolic outcomes.

**APSSG21.104**

**CD4+ T-Cells from Preeclamptic patients Promote B-Cell Survival and result in AT1-AA, Fetal Growth Restriction, and hypertension in recipient rats during pregnancy**

Owen Herrock1, Darby Whitney2, Kristin Reeve3, Lorena Amaral1, Babbette LaMarca4

1Pharmacology & Toxicology, University of Mississippi Medical Center, 2Toxicology & Pharmacology Department, University of Mississippi Medical Center, 3SOM-OB/GYN, University of Mississippi Medical Center, 4Pharmacology and Toxicology, University of Mississippi Medical Center

Introduction: Preeclampsia (PE), new-onset hypertension during pregnancy, is associated with activated T and B lymphocytes compared to Normal Pregnancy (NP). We previously established adoptive transfer of T-cells from PE women causes a PE-like phenotype in pregnant rats; and, inhibition of T cell-B cell communication via CD40L-CD40 prevents hypertension in recipient rats of PE CD4+ T cells, suggesting an important role for B cells in mediating hypertension during preeclampsia. We hypothesize APRIL, cytokine mediatior of long-term B cell survival, and AT1-AA are increased in response to PE CD4+ T cells into pregnant recipient rats. Methods: Placentas were collected immediately after delivery and CD4+ T cells were isolated by negative selection and validated by flow cytometry. NP or PE CD4+ T Cells were then adoptively transferred into pregnant immunodeficient rats on Gestational Day (GD) 12. In both groups, mean arterial pressure (MAP) was measured and blood was collected on GD19 for ELISAs and AT1-AA cardiomyocyte assay. Results: Immunodeficient pregnant recipient rats of PE CD4+ T cells had elevated MAP (114±1mmHg)(p<0.05) compared to recipients of NP CD4+ T cells (97±3mmHg). Recipients of PE CD4+ T cells had smaller pups (1.23±0.075g) compared to recipients of NP CD4+ T Cells (1.507±0.139g)(p=0.07)(ns). Agnostic autoantibodies to the angiotensin II type 1 receptor (AT1-AA) expression is increased in rats receiving PE CD4+ T cells compared to rats receiving NP CD4+ T cells (19.8±0.9 bpm vs 13±0.9 bpm, p<0.05). Immunodeficient recipient rats of PE CD4+ T cells had elevated APRIL (1.276±0.0.13ng/mL) compared to recipients of NP CD4+T cells (0.434±0.11ng/mL)(p<0.01). Conclusions: These data support our hypothesis that activated CD4+ T cells from PE patients stimulate pathways of B cell survival and thus AT1-AA secretion which is associated with hypertension during pregnancy.

**APSSG21.105**

**Sex-specific modulation of kinases activity and lipidomic profile in visceral adipose tissue from obese mice exposed to early life stress**

Jacqueline Leachman1, Justin Creeden1, Carolina Dalmasso1, Andrew Morris1, Terry Hinds1, Analia Loria1

1Pharmacology and Nutritional Sciences, U of Kentucky

Early life stress is an established independent risk factor for chronic disease development including obesity and hypertension. Previously, our lab has reported that maternal separation and early weaning (MSEW), a mouse model of early life stress, induces greater blood pressure response to chronic high fat diet (HF) in a sex-specific manner. In this sense, female MSEW mice fed a HF display exacerbated perigonadal white adipose tissue (pgWAT) expansion and a metabolic syndrome phenotype compared to controls. Contrary to females, male MSEW mice display similar levels of adiposity with increased neurogenic hypertension compared to controls. Thus, the aim of this study was to determine a pgWAT sex-specific signature of lipids and kinase pathways associated with early life stress in combination with HF. pgWAT was collected from MSEW and Control, male and female mice fed a HF to assess serine/threonine kinase activity using the PamGene microarray kinome technology. We used the equipment to measure kinase activity, and along with bioinformatics, generated a kinome pathway that indicated hyper- and hypo-active kinases. In a separate set of samples, lipidomic analysis was performed by HPLC at the small molecule Mass Spectrometry Core Laboratory at the University of Kentucky. The results show that MSEW induces significant changes (mostly decreased phosphorylation) of 7 phosphokinases (|Z| >=1.5) in females (Glik, MLK, PKCH, MST, STE7, PEP, FRA1) and 5 in males (AKT, SGK, PKB, MARK, CDK). While 15 were affected in both sexes (DMPK, PMA, PKG, RSK, PKL, DYRK, NMO, CAMK1, JNK, PAKA, RAD53, ERK, PAKB, PKD, PI3, AMPK). This data provides new insights into the dysregulation of adipose tissue expansion in females, by identifying...
Background: Neurovascular coupling (NVC) describes the parallel increase in cerebral blood flow to active neural tissue engaged in a given task. Previous research has highlighted “Where’s Waldo?” produces a more robust NVC response similarly between males and females. The current study aimed to elucidate how task complexity impacts the neurovascular coupling response. Previous studies quantifying the NVC response with males should engendering. To determine task and biological sex differences, a 4x2 Analysis of Variance was utilized. Additionally, Spearman’s rank correlation coefficients (p) were computed to assess correlations between ratings of task engagement and NVC metrics. These correlations were stratified between female and male participants to understand potential sex differences in task engagement. Results: The “Where’s Waldo?” task evoked greater PCA percent increase (all p<0.001; all Cohen’s d>1.21 [large]) and area-under-the-curve of the task (all p<0.001; all Cohen’s d>1.12 [large]) compared to all simple shapes tasks. Females displayed greater baseline and peak PCA and MCA velocities across all tasks (all p<0.001; Cohen’s d>0.80 [large]); no differences were noted within the NVC response itself (all p>0.116; Cohen’s d<0.20 [small/negligible]). Subjective task engagement displayed moderate levels of correlation with the relative PCA percent increase (p=0.58) and PCA total activation (p=0.60) metrics in males, whereas these had weak correlations for females (p=0.43 and p=0.38, respectively). Conclusions: The “Where’s Waldo?” task greatly augmented the signal-to-noise ratio compared to various simple shape durations, including one designed to induce similar eye movements with “Where’s Waldo?”. Interestingly, while both males and females displayed a similar NVC response, males had greater correlation with task engagement. Therefore, future studies quantifying the NVC response with males should use the maximally engaging task to ensure a robust response is produced. However, it appears a similar response can be elicited within females, with a moderately to maximally engaging task. This study received funding from the Natural Sciences and Engineering Research Council of Canada.

APSSG21.106
Does task complexity impact the neurovascular coupling response similarly between males and females?
Joel Burma1, Rebecca Wassmuth1, Courtney Kennedy1, Lauren Miutz2, Kailey Newel1, Joseph Carere1, Jonathan Smirl1
1Faculty of Kinesiology, University of Calgary

Background: Neurovascular coupling (NVC) describes the parallel increase in cerebral blood flow to active neural tissue engaged in a given task. Previous research has highlighted “Where’s Waldo?” produces a more robust NVC response within the posterior cerebral arteries (PCA) that supply the visual processing centers in the brain, compared to a task where simple shapes are presented every ~2-seconds. However, it is unknown how altering the duration of the simple shape presentation affects the NVC response. Furthermore, the NVC response has yet to be investigated for differences between biological sexes. Therefore, the current study aimed to elucidate how potential biological sex differences and subjective ratings of engagement may correlate with NVC responses.

Methods: A total of 39 participants (female=22) completed four randomized visual paradigms. Three involved participants viewing simple geometric shapes that moved around the screen at 0.5-seconds, 2-seconds, and 4-seconds. The color, shape, and location of these shapes were randomized to elicit activation within both the ventral (“what”) and dorsal (“where”) visual perception streams. The fourth paradigm was “Where’s Waldo?”. Each task consisted of eight cycles of ~20-seconds eyes-closed followed by 40-seconds eyes-open. Cerebral blood velocity was quantified in both the middle cerebral artery (MCA) and PCA using transcranial Doppler ultrasound. Following the completion of each task, participants self-reported task engagement (f: minimally to 10: maximally engaging). To determine task and biological sex differences, a 4x2 Analysis of Variance was utilized. Additionally, Spearman’s rank correlation coefficients (p) were computed to assess correlations between ratings of task engagement and NVC metrics. These correlations were stratified between female and male participants to understand potential sex differences in task engagement. Results: The “Where’s Waldo?” task evoked greater PCA percent increase (all p<0.001; all Cohen’s d>1.21 [large]) and area-under-the-curve of the task (all p<0.001; all Cohen’s d>1.12 [large]) compared to all simple shapes tasks. Females displayed greater baseline and peak PCA and MCA velocities across all tasks (all p<0.001; Cohen’s d>0.80 [large]); no differences were noted within the NVC response itself (all p>0.116; Cohen’s d<0.20 [small/negligible]). Subjective task engagement displayed moderate levels of correlation with the relative PCA percent increase (p=0.58) and PCA total activation (p=0.60) metrics in males, whereas these had weak correlations for females (p=0.43 and p=0.38, respectively). Conclusions: The “Where’s Waldo?” task greatly augmented the signal-to-noise ratio compared to various simple shape durations, including one designed to induce similar eye movements with “Where’s Waldo?”. Interestingly, while both males and females displayed a similar NVC response, males had greater correlation with task engagement. Therefore, future studies quantifying the NVC response with males should use the maximally engaging task to ensure a robust response is produced. However, it appears a similar response can be elicited within females, with a moderately to maximally engaging task. This study received funding from the Natural Sciences and Engineering Research Council of Canada.

APSSG21.107
Sex Differences in the Formation and Composition of Colonic Tertiary Lymphoid Tissues: the role of the Aryl Hydrocarbon Receptor Activity in Intestinal Epithelial Cells
Erika L. Garcia-Villatoro1, Evelyn S. Callaway2, Kimberly F. Allred3, Stephen H. Safe4, Robert S. Chapkin1, Arul Jayaraman2, Clinton D. Allred2,3
1Department of Nutrition, Texas A&M University, 2Department of Chemical Engineering, Texas A&M University, 3Department of Nutrition, University of North Carolina Greensboro, 4Department of Veterinary Physiology and Pharmacology, Texas A&M University

Background: After birth, the development of secondary lymphoid tissues (SLOs) in the colon is dependent on the expression of the Aryl Hydrocarbon Receptor (AhR) in immune cells as a response to the availability of AhR ligands. As organized structures that develop at sites of inflammation or infection in the colon during adulthood, tertiary lymphoid tissues (TLTs) serve as localized centers of adaptive immune responses, and their presence has been associated with the resolution of inflammation and tumorigenesis in the large intestine. TLT formation follows a sequential pattern involving an inflammatory event, initiation signaling, recruitment of chemokine-secreting stromal cells, and the organization and activation of the TLT in the mucosa. Preliminary data from our laboratory have
shown that intestinal epithelial cell (IEC)-specific AhR knockout mice exposed to azoxymethane, a chemical carcinogen, developed significantly fewer TLTs, while expression of II-22 and other chemokines involved in TLT formation were also significantly downregulated. Hence, we hypothesized that the conditional loss of AhR activity in IECs would reduce the formation of and change the immune cell composition of TLTs by reducing the production of lymphoid chemokines in a model of acute inflammation. Methods: IEC-specific knockout of AhR was induced in sexually mature male and female mice (CDX2PCreT2 x AhRfl/fl-IahRKO). To induce intestinal inflammation, we then administered 2.5% DSS for 5 days and assessed disease activity index (DAI), intestinal permeability (FITC-Dextran), expression of functional-IEC genes (real-time qPCR), and TLT formation/composition (H&E staining/Immunofluorescence) after a 10-day recovery period. Throughout the experiment, mice received a semi-purified diet supplemented with or without 3,3'-diindolylmethane (DIM), a known AhR ligand. Results: Summary: In females, loss of AhR activity in IECs reduced the formation of TLTs without significant changes in disease outcomes nor immune cell composition within the TLTs. In males lacking AhR expression in IECs, increased DAI, lower expression of functional-IEC genes (Ocln, Il-22), increased number of TLTs, increased T-cell density, and lower B: T cell ratio was observed; these findings may represent an unfavorable prognosis when exposed to DSS-induced epithelial damage compared to females. Equally important, AhR activation by DIM promoted intestinal barrier integrity through the upregulation of various tight junction genes at a basal state and genes involved in the signaling that allows the formation of TLT after an inflammatory event in a sex-dependent manner. Conclusion: These data suggest that the formation of TLT in the colon is influenced by sex and AhR expression in IECs.

**APSSG21.109**

**Sex differences in offspring of maternal methamphetamine use**

Daniela Rüedi-Betttschen

1Psychiatry and Human Behavior, University of Mississippi Medical Center

Methamphetamine (METH) abuse during pregnancy is an urgent public health concern, but knowledge on the effects of the developing fetus is limited. In the present rat study, we investigated the effects of daily high-dose METH self-administration throughout pregnancy on pregnancy outcome, offspring development, physiology, and behavior in exposed pups relative to control offspring. In addition to identifying overall effects of prenatal METH abuse, the current study investigated sex-specific effects of in utero METH exposure. In utero high-dose METH-exposure was achieved via daily 6-hr access to METH self-administration by the dam throughout pregnancy. Maternal health and weight gain was assessed daily. Pregnancy success, litter size and composition, as well as pup weight and postnatal development was determined daily until weaning. Offspring behavior and physiological measures were assessed during adulthood at approximately 8 months (i.e., “adult”) and 24 months (i.e., “aged”) of age. Spontaneous locomotor activity, as well as METH-induced hyperlocomotion was assessed in male and female prenatally METH−exposed and control offspring. As physiological measure, basal blood pressure was determined and in adult and aged male and female offspring of both exposure groups. In utero METH-exposure did not affect pregnancy outcome or litter size.
However METH-exposed offspring tended to be smaller and were significantly slower in reaching species-specific developmental milestones than control offspring. In addition, the observed impairment was more pronounced in male offspring relative to female littermates. In locomotor studies in adult animals, METH-exposed offspring significantly demonstrated greater locomotor activity after a high-dose METH challenge compared to controls. No differences were evident in baseline locomotor activity between the exposure groups. However, while the significantly increased activity compared to controls was evident in males at 1.0 mg/kg METH challenge, their female siblings demonstrated the same increased activity after a 3.2 mg/kg METH dose. In aged animals, locomotor activity in response to a 1.0 mg/kg METH challenge was reduced in prenatally METH-exposed males compared to control males, whereas in females there was no difference between the exposure groups. When basal blood pressure in adult animals was determined, there was no difference in blood pressure between the exposure groups in both sexes, although a trend for METH-exposed males to show increased blood pressure compared to controls was revealed. In aged animals, METH-exposed offspring of both sexes showed increased blood pressure compared to controls. Again, the effect was more pronounced in male METH-exposed offspring relative to their female siblings. These results show that in utero exposure to high METH doses has sex-dependent effects on development, behavior, and blood pressure compared to controls. Moreover, these effects are long-lasting, suggesting that METH use during pregnancy may negatively impact exposed offspring throughout the lifespan, with more robust effects occurring in male offspring. This research was supported by the following grants: NIGMS P30GM103328 and P20GM121334

**APSSG21.110**

**CD4+T Cells cause mitochondrial dysfunction, an increase in glucose, and hypertension in a novel Rat Model of Gestational Diabetes Mellitus**

Evangeline Deer1, Jan Michael Williams1, Lorena Amaral1, Sarah Fitzgerald1, Owen Herrock1, Ty Turner1, Nathan Campbell1, Babbette LaMarca1

1Pharmacology & Toxicology, University of Mississippi Medical Center

Hypertensive (HTN) disorders of pregnancy complicate approximately 10% of all pregnancies worldwide and contribute to maternal and fetal morbidity and mortality. HTN increases risks for greater pathophysiology in gestational diabetes mellitus (GDM) pregnant women, thereby supporting a need for controlling maternal glucose levels and blood pressure. GDM is characterized by hyperglycemia and β-cell dysfunction associated with increased oxidative stress, inflammatory cytokines, and activated CD4+ T cells. Streptozotocin (STZ) is used in non-pregnant rats to induce β-cell destruction causing features of diabetes. However, STZ is not ideal for pregnancy and leads to unsuccessful pregnancy outcomes, therefore other ways to establish animal models of GDM must be pursued. Previously, we showed CD4+T cells from a rat model of preeclampsia causes HTN and mitochondrial (mt) dysfunction/ROS compared to normal pregnant (NP) rats. Therefore, we hypothesize CD4+ T cells from a diabetic rat model could cause mt dysfunction/ROS and pancreatic β-cell destruction and lead to increased glucose and HTN during pregnancy. To examine our hypothesis, we adoptively transferred CD4+ T cells from STZ Dahl diabetic rats into pregnant Sprague Dawley (SD) rats and measured GDM features. Circulating CD4+ T cells were isolated from STZ induced diabetic Dahl virgin female rats and injected into pregnant SD rats on gestational day (GD) 12. On GD19, blood pressure (MAP) and tissues were collected and glucose levels were measured after 2h fasting in STZ CD4+ T cell recipients (GDM) and NP controls. Mt respiration and mtROS was measured in isolated mitochondria. On GD19, MAP increased to 105±0.5 mmHg (n=4, p<0.05) in GDM pregnant rats compared to control NP rats 91±2.1 mmHg (n=3). Blood glucose levels were elevated in GDM rats (139 ± 7 mg/dl, n=4, p<0.05) compared to NP controls (94 ± 1 mg/dl, n=3). Placental state 3 (26.4±5.9 vs 53.9±1.7 pmol/sec/mg, p<0.05) respiration rates, indicative of ATP production, was reduced in GDM rats (n=4) compared to NP controls (n=3). Placental mtROS was significantly increased in GDM rats (190 ± 27.1 % gated, n=3, p<0.05) compared to NP rats (100 ± 2.7 % gated, n=3). Collectively, the data indicate adoptive transfer of STZ CD4+ T cells causes increased circulating glucose, placental mt dysfunction and mtROS and HTN during pregnancy. These data demonstrate the importance of CD4+T cells in mechanisms causing the pathophysiology of GDM, and also introduces a potential novel rodent model of GDM.

**APSSG21.111**

**IL-17 Blockade Attenuates RUPP TH17 Cell Induced Hypertension and Mitochondrial Dysfunction**

Sarah Fitzgerald1, Evangeline Deer1, James Hogg2, Nathan Campbell1, Owen Herrock1, James Lemon1, Lorena Amaral1, Denise Cornelius1, Ty Turner1, Kathy Cockrell1, Tarek Ibrahim1, Babbette Lamarca1

1Experimental Therapeutics and Pharmacology, University of Mississippi Medical Center, 2Maternal and Fetal Medicine, University of Mississippi Medical Center, 3Emergency Medicine, University of Mississippi Medical Center

Preeclampsia (PE) is characterized as new onset hypertension (HTN), intrauterine growth restriction (IUGR), multi-organ dysfunction, and is associated with an increase in inflammatory immune profiles, including increased IL-17 and T helper 17 (Th17) and Natural Killer (NK) cells. Recently, a role for mitochondrial (mt) dysfunction/ROS has been shown to play a role in the pathogenesis of PE. However, the causative factors have yet to be fully identified. Although we have shown a role for in Th17 cells, NK cells, and mt dysfunction to contribute to the HTN in a rat model of PE (RUPP), we don’t know the role of Th17 cells or IL-17 to cause mt dysfunction. Therefore, we hypothesize Th17 cells cause HTN and mt dysfunction which is alleviated with IL-17RC. To test our hypothesis, 1 million RUPP Th17 cells were adoptively transferred into normal pregnant Sprague Dawley (NPSD) rats on gestational day 12 (GD12). One
group received IL-17RC (100pg/day) on GD14-19. Blood pressure (MAP), NK cells, and mt function were measured on GD19 in all groups. MAP increased to 112±1 mmHg (n=12) (p<0.0001) in response to RUPP Th17 compared to 92±3 mmHg (n=12) in NPSD and was lowered to 98.2±1.9 mmHg (n=12) (p<0.0001) with IL-17RC in NP+RUPPTh17. Circulating NK cells increased in from 0.09±0.08 % lymphocytes in NPSD to 2.7±0.6 %lymphocytes (p<0.005) in NP+RUPPTh17 and was lowered 0.3±0.2 % lymphocytes (p<0.005) with IL-17RC in NP+RUPPTh17. Similarly, placental NK cells increased from 0.1±0.08 % lymphocytes in NPSD to 2±0.5 % lymphocytes (p<0.0005) in NP+RUPP Th17, and was normalized (0.14±0.08 % lymphocytes (p<0.005)) with IL-17RC. Renal MtROS increased from 100±12% (n=6) in NPSD to 143±3 %fold change (n=11) (p<0.0001) in NP+RUPP Th17, and was normalized (68±3 %fold change (p<0.0001)) with IL-17RC. Placental Mt ROS decreased to 50±2.6 %fold change (n=6) (p=0.0001) compared to NPSD (100±6 %fold change (n=6)) and was normalized to 66±3.4 % fold change (n=5) (p<0.05) with IL-17RC. IL-17RC inhibition normalizes HTN, NK cell activation, and multi-organ mt dysfunction caused by Th17 cells stimulated in response to placental ischemia.

APSSG21.113
Role of two X sex chromosomes in the development of atherosclerosis
Yasir Alsiraj1, Heba M Ali1, Lisa Cassis1
1Pharmacology and Nutritional Sciences, University of Kentucky

Background: Circulating lipids and atherosclerosis are different between women and men. Estrogens promote favorable lipids, but hormone replacement therapy in postmenopausal women increased risk of heart disease. We recently demonstrated that sex chromosomes, namely an XX sex chromosome complement, promoted levels of circulating proatherogenic lipids and the development of atherosclerosis in mice. In this study, we hypothesized that XX female (XXF) mice will have higher levels of circulating pro-atherogenic lipids and atherosclerosis than XO females (XOF) through a gene dosage mechanism. Methods and Results: We bred male low-density lipoprotein receptor deficient (Ldlr/-) mice with a structurally re-arranged Y chromosome to female XX Ldlr/- mice to generate female mice with one or two X chromosomes. Mice were fed a Western diet (42% kcal as fat; 0.15% cholesterol, Teklad TD88137) for 3 months. At study endpoint, body weight was not significantly different between XXF and XOF mice (XXF: 24.5±1.2; XOF: 22.6±0.6 g; P>0.05). Similarly, total serum cholesterol concentrations were not significantly different between XXF and XOF mice (XXF: 1,746±93; XOF: 1,702±61 mg/dL; P=0.05). In contrast, the percentage of atherosclerotic lesions in the aortic arch was significantly lower in XOF than XXF mice (XXF, 20.6±10.3; XOF, 8.1±4.4 % lesion surface area; P<0.05). To investigate gene dosage effects from two X chromosomes, we quantified mRNA abundance of genes known to escape X-inactivation in thoracic aortas (the site for atherosclerotic lesion formation) from XXF and XOF mice. Two genes known to escape X-inactivation (Kdm5c and Kdm6a), lysine histone demethylases) exhibited higher mRNA abundance in thoracic aortas of XXF than XOF mice (Kdm5c: XXF, 1.09 ± 0.19; XOF, 0.33 ± 0.21; P<0.05; Kdm6a: XXF, 1.05 ± 0.15; XOF, 0.43 ± 0.15; P<0.05). Conclusion: These data indicate that two X chromosomes in female mice augment the development of atherosclerosis compared to females with one X chromosome. Higher atherosclerosis of XXF than XOF mice was not accompanied by increased levels of circulating cholesterol. Rather, genes escaping X-inactivation in thoracic aortas, such as Kdm5c and Kdm6a, may contribute to gene dosage influences from two X chromosomes.

APSSG21.115
Simulated Microgravity Alters Cardiovascular Function in the Female Sprague Dawley Rats
Omar Shaltout1, Liliya M. Yamaleyeva1, Ebrahim Elsangeedy1, Nildris Cruz-Diaz1, Jeffrey S. Willey2, Victor M. Pulgar1
1Surgery/Hypertension, Wake Forest School of Medicine, 2Radiation Oncology, Wake Forest School of Medicine

The adaptations of cardiovascular system to spaceflight are complex and not well understood. Our previous studies reported sex differences in arterial stiffening in response to simulated microgravity in middle-aged Sprague-Dawley (SD) rats. Compared with males, the female rats develop aortic stiffness and increased intima-media thickness 14 days after the exposure to hindlimb unloading (HLU). However, the mechanisms of cardiovascular dysfunction following the exposure to simulated microgravity are unknown. In this study we investigated whether ovariectomy (OVX) modifies the response of cardiovascular system after 14 days of HLU. Female rats were either OVX or undergone sham surgery 2 weeks prior the exposure to HLU. At 20 weeks of age, intact and OVX females were either tail suspended via HLU or remained full weight bearing in similar cages for 14 days. At the end of the study, all rats underwent echocardiography and aortic and carotid pulse-wave velocity (PWV) assessments to determine the effect of HLU, OVX, or HLU+OVX on cardiac function and arterial stiffness using high resolution ultrasound imaging system Vevo LAZR (FujiFilm, VisualSonics). There were no significant changes in body weight or cardiac-to-body weight ratio in rats exposed to OVX, HLU, or OVX- HLU. Aortic arch PWV was greater in rats exposed to HLU (1.3-fold vs. intact females, p<0.05, n=4) or OVX (1.6-fold vs. intact females, p<0.05, n=4 in each group). However, OVX+HLU tended to decrease aortic PWV (p=0.06). Carotid PWV was greater by HLU (1.5 fold, p<0.05, n=4 in each group) or OVX (1.7 fold, p<0.05, n=4 in each group), but not affected by OVX+HLU. Cardiac systolic function was not altered by OVX, HLU, or OVX+HLU. However, the OVX+HLU prevented the increase in E/e` ratio and increased e` (n=3-4). In summary, our preliminary studies show that the exposure to simulated microgravity or ovariectomy increases aortic and carotid arterial stiffening and alters cardiac diastolic function. We conclude that the loss of ovarian hormones modifies cardiovascular response of the female rats to microgravity.
**APSSG21116**

**Sex Differences in Response to Sepsis**

Denise Cornelius1,2, Ann Tardo, Olivia Travis3, Christopher Nutter1, Chelsea Giachelli4, Hannah Glenn1, Jan Williams2

1Emergency Medicine, University of Mississippi Medical Center, 2Pharmacology and Toxicology, University of Mississippi Medical Center, 3Developmental Neurobiology, St. Jude Children's Research Hospital

Sepsis, the body’s exaggerated immune response to infection that leads to life-threatening organ dysfunction, is a major cause of acute lung injury and acute kidney injury. We have previously determined that NLRP3 activation is increased in the modified cecal ligation and puncture (CLP) rat model of abdominal polymicrobial sepsis. NLRP3 is a cytoplasmic protein complex that mediates inflammation and immune activation. Recent studies have determined that sepsis mortality is increased in males compared to female patients. We set out to investigate sex differences in NLRP3 expression, plasma cytokines, pulmonary edema, and renal function in male and female CLP rats. Sham or CLP was performed in male and female rats (n=5-7/group). At 24 hrs post-CLP, the necrotic cecum was removed and abdominal cavity rinsed with warm saline. At 72 hrs post-CLP, animals were sacrificed and blood and tissues were collected for analysis. Expression of NLRP3 in the kidney and lungs was assessed by qPCR, wet/dry ratio was determined for lungs, renal function was assessed by the clearance of FITC-sinistrin, and plasma IL-1β and IL-18 were quantified using multiplex bead analysis. Compared to female Sham rats, renal NLRP3 expression was not changed in female CLP rats (1.0±0.2 vs 1.2±0.3; n.s.). Alternatively compared to male Sham, renal NLRP3 expression was greater than 3-fold higher in male CLP rats (p<0.05). A similar trend was observed in pulmonary expression of NLRP3 mRNA. Lung wet/dry ratio was similar in Sham vs CLP rats in both sexes. GFR was unchanged between Sham and CLP female rats. However, polymicrobial sepsis significantly reduced GFR in male CLP rats compared to Sham male rats (3.9±0.2 mL/min vs 5.6±0.4 mL/min; p<0.05). The NLRP3 associated inflammatory cytokines, IL-1β and IL-18, were significantly higher in female and male CLP rats compared to their gender-specific Sham controls. These data demonstrate sex differences in expression of NLRP3 in the lung and kidney in response to polymicrobial sepsis. Sex differences are also observed in sepsis-induced renal dysfunction. Future studies will investigate pyroptosis and vascular activation by NLRP3 as potential mechanisms that mediate sex differences in sepsis-induced pathophysiology.

**APSSG21117**

**Battle of the Sexes: Androgens and Estrogens in Control of White Adipose Tissue Metabolism**

Annie Newell-Fugate

Veterinary Physiology and Pharmacology, Texas A&M University

Men have twice the odds of developing type 2 diabetes mellitus (T2DM) and are more prone to visceral (v) white adipose tissue (WAT) accumulation than women. However, menopause increases the risk for T2DM and increases abdominal WAT accumulation in women. Estrogens are well-established as regulators of adipocyte insulin sensitivity, nutrient uptake, and mitochondrial function via estrogen receptor alpha (ERα) and beta (ERβ). Interestingly, androgen effects on adipocyte nutrient turnover and mitochondrial function are dependent on sex, age, and tissue type. Variation in the response of adipocyte nutrient turnover to androgens could be driven by WAT interconversion of androgens to estrogens and androgen metabolites, some of which have estrogenic activity. We hypothesized that the potent androgen, dihydrotestosterone (DHT), would be converted to androgen metabolites in subcutaneous (sc), but not visceral, WAT which would yield increased nutrient turnover and improved mitochondrial function in scWAT via ERβ. vWAT and abdominal scWAT explants were collected from male (n=6) and female (n=8) pigs and treated in vitro with no steroid or 1nM of: DHT, flutamide (FLUT), enzalutamide (ENZ), fulvestrant (FULV), DHT+FLUT, DHT+ENZ, DHT+FULV, DHT+FLUT+FULV, or DHT+ENZ+FULV. WAT explants were assessed for: 1) basal and stimulated (10nM insulin) BODIPY C12 fatty acid uptake; 2) basal and stimulated (10 nM isoproterenol) glycerol release; and 3) mitochondrial membrane potential (MMP) via MitoTracker CMXRos. Explants treated with BODIPY C12 and MitoTracker were fixed and imaged with confocal microscopy. Explants for assessment of MMP were probed with ERβ-Alexa Fluor 405 antibody prior to imaging. Media was assessed for glycerol (Sigma kit) and steroids (LC-MS/MS). Total fluorescent stain uptake per 20?m WAT z stack was quantified with ImageJ (NIH). Normality of the data was checked prior to analysis with PROC MIXED (SAS, Inc.). Components in the statistical model were: sex, tissue, insulin, and steroid treatment. Irrespective of sex, media from DHT-treated vWAT explants had ~ 520 pM DHT, whereas media from DHT-treated scWAT explants contained undetectable levels of DHT but ~ 20 nM 3-beta-androstenediol (3-β-diol). Media from DHT-treated vWAT and scWAT from females had ~ 175 pM androstenedione which was undetectable in media from female explants not treated with DHT. In males, DHT did not affect glycerol release but did suppress basal fatty acid uptake in scWAT under androgen receptor (AR) and/or ER? control. In females, DHT increased basal glycerol release in scWAT which was abrogated by both AR and ER? blockers and had no effect on fatty acid uptake. Interestingly, in both sexes DHT-treatment of scWAT increased MMP and mitochondrial ERβ abundance. By contrast, DHT-treatment of vWAT in males and females had the opposite mitochondrial effects. In conclusion, DHT exerts opposing effects on adipocyte nutrient turnover in the scWAT of males and females via androgenic and estrogenic mechanisms. Importantly, irrespective of sex DHT has beneficial effects on scWAT mitochondria, possibly via 3-β-diol activity at ERβ, and deleterious effects on vWAT mitochondria.
Sex-dependent impact of postnatal BPS on vascular and cardiometabolic health
Sarah Easson¹, Emma Walsh¹, Hai-Lei Zhu¹, Liam Connors¹, Radha Singh¹, Jennifer Thompson¹
¹Physiology & Pharmacology, University of Calgary

Background: Bisphenols are among the world’s most produced industrial chemicals, commonly used as plasticizers in household plastics such as food containers, water bottles, and fluid pipes. Largely due to their endocrine disrupting properties, bisphenols have been linked to reproductive, developmental, and cardiometabolic disorders. Bisphenol A was the primary analogue used in North America prior to its classification as a toxic substance by Health Canada and the FDA in 2010. As a result, manufacturers have turned to alternatives such as Bisphenol S (BPS), despite little being understood of the health risks posed by these analogues. Exposure to BPS has exponentially increased, with daily intake measured at 0.009µg/kg/day in a 2012 biometric study across the US and Asia. Infants and children have been shown to have the highest daily intake of bisphenols per body weight in the population and are therefore at greater risk to adverse health effects. This study explores the effects of postnatal exposure to BPS on vascular and metabolic health.

Methods: On postnatal day 21, male and female C57BL/J6 mice were exposed to BPS (250nM) or vehicle through drinking water in glass bottles. At 12 weeks, the glucose responses to an intraperitoneal delivery of glucose or insulin were measured to examine glucose tolerance and insulin sensitivity, respectively. Body composition was determined using TD-NMR. Serum and mesenteric arteries were collected for use in measuring markers of oxidative stress. Mesenteric arteries were dissected and mounted on a pressurized myograph setup. Endothelial-dependent and independent vasodilation was assessed by measuring dose responses to methacholine and sodium nitroprusside, respectively. All results were stratified by sex. Results: While female (p=0.0878) BPS-treated mice displayed impaired glucose tolerance relative to sex-matched vehicle-treated mice, BPS treatment had no significant impact on glucose uptake in male mice. TD-NMR showed an increase in % fat mass in males (V: 7.787 ± 1.189 vs. BPS: 12.37 ± 1.207, p= 0.046); BPS had no effect on females. Vessels extracted from BPS-treated males displayed impaired endothelial-dependent vasodilation in response to methacholine administration (p = 0.0002); whereas endothelial function was unchanged by BPS exposure in females. Significance: The findings of this study suggest that early-life exposure to a BPA analogue has sex-dependent effects on metabolic function and endothelial health. Impaired glucose tolerance was shown in BPS-exposed females, while impaired endothelial function manifested in BPS-exposed males. The sex-dependent effects of bisphenols like BPS are likely due to their interaction with endocrine receptors, such as estrogen receptors. Our understanding of the potential health outcomes following exposure to bisphenol analogues such as BPS is lacking, yet these chemical analogues are marketed as safer alternatives to BPA.

Funding sources: Heart and Stroke Foundation, Libin Cardiovascular Institute

Sex differences in atherosclerosis
Hester den Ruijter¹
¹Experimental Cardiology, University Medical Center Utrecht

Although sex differences in coronary artery disease are widely accepted with women developing more stable atherosclerosis than men, the underlying mechanisms of such differences remains largely unknown. Underrepresentation of women in studies on coronary artery disease in combination with a lack of sex stratification has caused knowledge gaps on atherosclerotic mechanisms in women. While integrative systems biological studies have shown involvement of gene regulatory networks and key driver genes in atherosclerosis, data for males and females separately remain uncovered. In this presentation, the consequence of pooling data on gene regulatory networks of atherosclerosis between sexes is shown as well as separate analyses for male and female. The female biology of atherosclerosis is highlighted which points to smooth muscle cell switching as a predominant mechanism. In order to improve on health equity between women and men with atherosclerotic disease, an increased emphasis on sex-stratified approaches in the analysis of multi-omics data sets of atherosclerosis is warranted.

How to convince your grandmothers and government agencies that research is important. Speak up to make a difference. Advocacy for science.
Mila Becker¹, Rebecca Osthus²
¹Government and Public Affairs, Endocrine Society, ²Government Relations and Science Policy, American Physiological Society

Scientists from all disciplines share a common goal of advancing knowledge and answering questions about the world around us. This has never been more important than it is right now as the world faces unprecedented challenges that need science-based solutions. This session will address why it is important for you as a scientist to engage in advocacy; how to tell your story and make your case effectively; and where you can find opportunities to take action.

Low energy expenditure during adolescence/adulthood predicts diet-induced weight gain more than energy expenditure during breeding/rearing
Michael Ponte¹, Matthew Morris²
¹Physiology, University of Kansas Medical Center, ²Molecular Integrative Physiology, University of Kansas Medical Center
Background: Parental high-fat diet and exercise have been shown to have opposite impacts on offspring metabolic health. The metabolic benefits of exercise or increased physical activity are hypothetically due to increased energy expenditure (EE). However, whether parental energy expenditure (EE) independent of physical activity, impacts offspring susceptibility to diet-induced metabolic disease phenotypes is not known. Previously, we have shown total EE is ~40% lower (resting EE ~ 60% less) in male and female mice housed at thermoneutral temperatures (~28-30°C) compared to normal vivarium (~20-22°C). As such, we assessed the impact of divergent breeding and adolescent/adult housing EE on offspring metabolic phenotypes by determining diet-induced weight gain during one-week HFHS feeding in male and female mice.

Methods: C57Bl6/J breeding pairs were housed at 20°C or 30°C. F1 offspring from litters 2 – 4 were individually housed at 6 weeks of age, with half of each litter switched and housed at the other temperature (e.g. -20°C to 30°C, or 30°C to 20°C). At 10 weeks of age, offspring were placed on a high-fat, high-sucrose (HFHS) diet for 1 week. At the start and end of HFHS feeding, food intake, body weight, and body composition data were collected. Results: No difference was observed in HFHS-induced weight gain between breeding temperature within housing temperature for both male and female mice. However, male and female mice housed at 30°C had greater HFHS-induced weight gain than 20°C, regardless of breeding temperature. Specifically, male 30°C bred and housed (30/30) mice gained 39% more body weight compared to 30°C bred and 20°C housed (30/20), and 20°C bred and 30°C housed males (20/30) had 56% greater weight gain than 20°C bred and housed (20/20). Similar findings were observed in female mice, with 30/30 and 20/30 mice gained 28% and 50% more weight than 30/20 and 20/20, respectively. Similar to weight gain, male and female mice housed at 30°C gained more fat mass than those housed at 20°C. Also, mice housed at 20°C had greater energy intake than mice housed at 30°C, regardless of breeding temperature or sex. The percent metabolic efficiency (ME) was calculated as the energy content of the gained fat and lean mass divided by energy intake to assess the allocation of stored energy. ME was similar between male and female mice during the HFHS, with 30°C housed mice having a larger %ME than 20°C housed. Female 30/30 mice showed 49% greater %ME than 30/20, and 20/30 had 53% greater %ME than 20/20. Male 30/30 mice had 33% greater %ME than 30/20, and 20/30 had 43% greater %ME than 20/20. Conclusions: As previously observed, 20°C housing uncoupled energy intake from weight gain, regardless of sex. Additionally, mice adolescent/adult housed at 30°C gained more weight and fat mass, regardless of sex or breeding temperature. Our data suggests that adolescent/adult EE is a more powerful predictor of diet-induced weight gain than breeding EE, regardless of sex.
Integrin’s are heterodimeric receptor consists of non-covalently associated α and β subunits. These cell surface receptor play a pivotal role in kidney functions and fibrosis. There are significant variations in kidney disease among men and women. Very little is known about gender dependency in the expression of integrin in the kidney. The objective of this study is to study gender dependence in the expression pattern of integrin β1 and associated proteins in the kidneys. In addition, the role of integrin β1 in kidney damage and fibrosis has been studied. Kidney cortex from 2 months old C57BL/6 mice (both male and female) was obtained. mRNA was isolated using Trizol-chloroform methods and expression level were determined using qRT-PCR. Western blotting was used to confirm the protein expression. Integrin β1 KO mice were generated using ggt cre mice and subjected to acute kidney injury. There is a significantly lower level of integrin β1 (n=6, p<0.05) in 2 months old female mice compared to 2 months old male mice kidneys. qRT-PCR shows lower integrin β1 mRNA in female compared to male (Male: 1.097 ± 0.08, Female: 0.693± 0.11). Immunoblotting also shows about 20% decrease of integrin β1 protein in female compared to male. Integrin β1 WT and KO mice were subjected to acute kidney injury and injury parameters were measured. 3 days after acute kidney injury, there was no significant variation in the BUN values between the integrin β1 WT and KO mice (58 mg/dL and 62mg/dL). After 14 days, integrin β1 KO mice showed higher fibrosis compared to WT type mice. The expression of integrin adaptor protein talin1 was also investigated. qRT-PCR shows lower talin1 gene expression in female compared to males (Male: 1.101 ± 0.02, Female: 0.664± 0.05) (n=5, p<0.05). Western blotting also supports, that there is a 22% decrease talin1 expression in female compared to male. There was no significant difference in the expression of talin1 between integrin β1 WT and KO mice. In the kidney, the level of expression of integrin β1 and talin1 shows a gender dependence. Integrin β1 exert protective functions in kidney from acute injuries. Gender-specific evaluation and the role of other integrin-dependent proteins in renal function and kidney fibrosis are progressing. The expression of the integrin adaptor protein talin1 has not been altered in integrin β1 KO mice, suggesting a lack of reciprocal regulation.
interacting protein 1 (TNIP1) mediated increase of NF-κB signaling that results in increased TNF superfamily member 15 (TNFSF15) Flt1 splicing. These studies have the potential to significantly impact human health by identifying the CB as a new component of a regulatory miRNA network in PE that leads to disruption of sFlt1. Providing a novel insight into sFlt1 dysregulation can lead to a better understanding of PE and new therapies for maternal hypertension.

**APSSG21.129**

Sex-specific regulation of mitochondrial function and lifespan in Drosophila melanogaster

Christopher Axelrod, Elizabeth Zunica, Analisa Taylor, Alyssa Johnson, John Kirwan

1Integrative Physiology and Molecular Medicine, Pennington Biomedical Research Center, 2Biological Sciences, Louisiana State University

Background: The common fruit fly, Drosophila melanogaster, is a widely employed model organism for a range of human diseases and conditions in biomedical research. Importantly, D. melanogaster serve as a primary model system for the study of aging due to simplicity of genetic alterations, responsiveness to pharmacotherapies and environmental manipulation, and relatively short lifespan. Sexual dimorphism for lifespan is a widely observed but poorly understood phenomenon across species. However, little is known as to whether sex contributes to susceptibility of age-related mitochondrial decline. The purpose of this study was to identify sex-specific regulation of mitochondrial function in aged D. melanogaster.

Methods: Two days after hatching, Canton S flies were housed according to sex at 22°C on a 12-hour light/dark cycle for the duration of lifespan. At 35-40 days of age, thoraxes were dissected to permeabilize indirect flight muscles (IFM), including the dorsal longitudinal and dorsoventral muscles. Oxidative phosphorylation (OXPHOS) and electron transfer (ET) capacity supported by pyruvate and malate, succinate, proline, glycerol-3-phosphate, and ascorbate/TMPD was determined by high-resolution respirometry and normalized to thorax mass.

Results: Females exhibited a 10% extension of lifespan compared to male flies (P<0.0001). Female IFM mass was significantly greater than male flies (P<0.0001). OXPHOS supported by NADH-linked substrates (P=0.002) and proline (P=0.010) were lower in females compared to male flies whereas succinate and glycerol-3-phosphate were comparable. Maximal electron flow in the presence of glycerol phosphate or ascorbate/TMPD was also comparable between female and male flies. Conclusions: Our findings indicate that lifespan and respiratory function in D. melanogaster are differentially regulated in a sex-specific manner. These data emphasize the need to perform lifespan and bioenergetic evaluation discretely according to sex.

**APSSG21.130**

The Loss of Peroxidasin Leads to a Sex-Dependent Susceptibility to Vascular Injury

Selene Colon, Cameron Meyer, Gautam Bhave

1Division of Nephrology and Hypertension, Vanderbilt University Medical Center, 2Vanderbilt Center for Kidney Disease, Vanderbilt University Medical Center, 3Vanderbilt Center for Matrix Biology, Vanderbilt University Medical Center

Millions die each year from complications associated with CKD and its transition to end stage renal disease (ESRD). Although women tend to present with CKD with higher rates than men, their progression to ESRD is dramatically slower than men. While premenopausal women are generally known to be somewhat protected from the disease processes associated with progression, the underlying mechanism of this protection has yet to be determined. Our lab recently discovered that peroxidasin (Pxdn), an animal heme peroxidase found within the extracellular matrix (ECM), generates HObR to form novel sulfilimine cross-links in collagen IV. Collagen IV is a prominent constituent of basement membranes (BM), a specialized sheet-like form of ECM that underlies cell layers in all tissues such as the glomerular BM (GBM) of the kidney glomerulus. The loss of Pxdn and sulfilimine cross-links in Pxdn knock-out (KO) mice leads to reduced sulfilimine cross-links and BM strength. Using the unilateral nephrectomy with angiotensin II infusion (Unx + Ang II) model of kidney injury, we discovered that the loss of Pxdn in our mice exacerbated injury in female KO mice compared to all other mice, including male KO mice. In these experiments, injured female Pxdn KO mice presented with a significant decrease in survival. To compensate for this, we conducted the experiment with two separate endpoints at 2 weeks and 4 weeks of injury. Pxdn KO females trended towards a decrease in renal function and an increase in both F4/80+ macrophage accumulation and renal fibrosis after 2 weeks when compared to wildtype females. Wildtype female mice had a significantly higher number of F4/80+ macrophages when compared to both wildtype and Pxdn KO males after 4 weeks suggesting a sex dependent effect on inflammation in this model. In this work we found that the loss of Pxdn disproportionately affects females more than males in the Unx + Ang II model of kidney injury. These data suggest that both loss of Pxdn and sex contribute to renal fibrosis and vascular inflammation in response to vascular mechanical injury. This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants R01-DK-116964 (G. Bhave), R01-DK-116964-01S1 (S. Colon), a Burroughs Wellcome Fund Career Award for Medical Scientists (13030995) to G. Bhave, and developmental funds from the Vanderbilt University Medical Center Division of Nephrology (G. Bhave).
Polycystic Ovary Syndrome and COVID-19

Damian Romero

Cell and Molecular Biology, University of Mississippi Medical Center

SARS-CoV-2, the causative agent of COVID-19, infects host cells using the angiotensin I converting enzyme 2 (ACE2) as its receptor after priming by host cell proteases. COVID-19 affects multiple organ systems, and male patients suffer increased severity and mortality. Polycystic Ovary Syndrome (PCOS) is the most common endocrine disorder in reproductive-age women and is characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology. PCOS is associated with obesity and cardiometabolic comorbidities, both being risk factors associated with severe COVID-19 pathology. We hypothesize that elevated androgens in PCOS regulate SARS-CoV-2 viral entry proteins in multiple tissues and that such regulation is modulated by an obesogenic diet. Female mice were treated with dihydrotestosterone (DHT) for 90 days and maintained in regular chow diet, high-fat diet (HFD) or low-fat diet (LFD). Body composition was measured by EchoMRI. Fasting glucose was determined by an enzymatic method. mRNA and protein levels of ACE2, Tmprss2, Cathepsin L, Furin, Tmprss4, and Adam17 were quantified by RT-qPCR, Western-blot, or ELISA in tissues, serum, and urine. In animals maintained in regular chow diet, DHT treatment increased body weight, fat and lean mass, and fasting glucose. Ace2 mRNA was upregulated in the lung, cecum, heart, and kidney, while downregulated in the brain by DHT. ACE2 protein was upregulated by DHT in the small intestine, heart, and kidney. The SARS-CoV-2 priming proteases Tmprss2, Cathepsin L, and Furin mRNA were upregulated by DHT in the kidney. ACE2 shedding Adam17 mRNA was upregulated by DHT in the kidney, which aligned with increased urinary ACE2 in DHT treated mice. When animals were challenged with an obesogenic diet, HFD exacerbated DHT-induced increase in body weight, fat mass, and cardiac and renal hypertrophy. In the heart, DHT upregulated AR protein in both LFD and HFD, ACE2 in HFD, and ADAM17 in LFD. In the kidney, AR protein expression was upregulated by both DHT and HFD. Moreover, ACE2 and ADAM17 were upregulated by DHT in both diets. Renal Tmprss2, furin, and cathepsin L were upregulated by DHT and differentially modulated by the diet. DHT upregulated urinary ACE2 in both diets, while neither treatment modified serum ACE2. Moreover, renal AR mRNA expression positively correlated with Ace2, Tmprss2, furin, cathepsin L, and Adam17. Our results highlight the potential for increased cardiac, renal, and gastrointestinal dysfunction in women with PCOS and COVID-19. Furthermore, our study suggests that weight loss by lifestyle modifications (i.e., diet) could potentially mitigate COVID-19-associated deleterious cardiorenal outcomes in women with PCOS. (Supported by NIH grants R01GM121334 to LLYC and DGR, and NIH NIDDK R21DK113500 to DGR.)
protein was downregulated indicating unhealthy adipose expansion. Gene and protein expression analysis showed a significant downregulation in Cox7a, Cidea, and Elov3 browning markers in DHT-treated miR21KO mice which was not observed in WT mice. Conclusion and significance: These findings suggest that BAT miR-21 may have a protective role in PCOS and ameliorate the DHT-mediated molecular changes and altered thermogenic responses. Adipose tissue-specific modulation of miR-21 levels could be a novel therapeutic approach for the treatment of PCOS-associated metabolic derangements. (Supported by NIH grants NIGMS P20GM121334 to LLYC and DGR, NIDDK R21DK113500 to DGR, NIGMS P20GM104357 and NHLBI P01HL51971)

APSSG21.133
Polarity and Diversity in Gender Expression: A novel measurement for sex and gender-based analysis conducted in clinical research with cisgender female participants
Shannon Cummings1, Kaylee Ramage1, Natalie Scime2, Sofia Ahmed3, Erin Brennand1
1Obstetrics and Gynecology, University of Calgary, 2Community Health Sciences, University of Calgary, 3Medicine, University of Calgary

Objective: Explore the utility of self-reported gender scores and the concept of gender polarity for sex and gender-based analysis in clinical research conducted in cisgender female populations. Methods: A self-reported gender expression tool was incorporated into a questionnaire administered to individuals seeking care for pelvic organ prolapse (POP). The tool was used to classify patients as gender polar (i.e., reporting only feminine traits) or with diverse gender scores (i.e., reporting both feminine and masculine traits). Association of gender scores and gender polarity with traditional socio-demographic variables of self-identified gender, sexual orientation, age, education, ethnicity, income, marital status, rural vs. urban, and income were explored by multivariable modelling. Descriptive statistics of socio-demographic variables for the gender polar and diversity in gender scores groups were reported by frequency, proportion and mean (SD) as appropriate. Association of gender expression with selection of hysterectomy-based or uterine-preserving POP surgery for POP was explored with multivariable modelling. Results: As part of a larger longitudinal study on women’s experiences with prolapse and their outcomes after surgery, we analyzed 198 participants, 89% (n=177) completed the gender score questionnaire and 83.3% (n=165) underwent surgical correction of POP. Median feminine gender score was 5 (IQR 4-6) and masculine gender score was 0 (IQR 0-1), indicating the sample had a more feminine gender expression. Majority of respondents were classified as gender polar (67.23%, n=119). The only sociodemographic variable directly associated with women with diverse gender scores was younger age. The group with diverse gender scores was significantly associated with increased odds of selecting a uterine-preservation based surgery for POP (OR=2.64 [95%CI 1.03 – 5.96]). Conclusion: Gender scores and gender expression are novel measurements in research with cisgender women. Gender polarity appears to be associated with women’s choice to undergo hysterectomy. Further research is required to understand this relationship and implications in clinical outcomes. Funding: This work was supported through a CIHR Women’s Health Clinical Mentorship Grant and MSI Foundation Grant. Shannon Cummings was supported by an Alberta Innovates Summer Research Studentship. Natalie Scime is supported by a Canadian Institutes of Health Research Doctoral Award.

APSSG21.134
High Plasma Soluble Prorenin Receptor is Associated With Elevated Systolic Blood Pressure In Aged And Super-aged Males But Not In Female Mice
Bruna Vilniauskas1, Christopher TY Wong1, Stephanie L. Crabbtree1, Jennifer Hong1, Virginia Reverte1, Carla B. Rosales1, Hernan Mejia-Gomez2, Ricardo Mostany3, Sarah H. Lindsey4, Minofa C. Prieto1,3
1Physiology, Tulane University, 2Pharmacology, Tulane University, 3Hypertension and Renal Center of Excellence, Tulane University

The protective cardiovascular (CV) effects of estrogen are evident by the lower incidence of CV diseases in pre-menopausal women. Elderly subjects are susceptible to disruptions in the activation of the intrarenal renin-angiotensin-aldosterone system (RAAS), which raises the risk for hypertension and renal dysfunction. Prorenin receptor (PRR), a RAAS component, contributes to blood pressure regulation and Na+ reabsorption. The soluble PRR (sPRR) is elevated in patients with essential hypertension, preeclampsia, chronic kidney disease and diabetes mellitus. However, whether aging contributes to changes in plasma sPRR levels and is associated hypertension and renal dysfunction in a sex dimorphic fashion is unknown. We hypothesize that increases in plasma sPRR contribute to the development of hypertension and renal dysfunction during aging. Male and female C57Bl/6J mice were randomly distributed in 4 groups: 1) young adult (4–6 months of age, mo.), N=5-8; 2) middle-aged (10-14 mo. N=7-8; 3) aged (15–19 mo.), N=7-12; and 4) and super-aged (<20 mo.), N=7-9. Systolic blood pressure (SBP, mmHg) was measured by tail-cuff method after two weeks of training. Levels of sPRR (ELISA, IBL America, Inc), sex hormones (testosterone, 17β-estradiol, progesterone; ELISA, R&D Systems) were measured in plasma. Renal function was evaluated by volume urine, urinary sodium, creatinine, BUN, and eGFR. SBP were increased in aged (118±2) and super-aged (129±2) male compared to young (103±1) and middle-aged (105±1); P<0.001) mice. No significant changes in SBP were found in females. In young mice, plasma sPRR did not differ between males and females (young or middle-aged). However, plasma sPRR was significantly higher in aged male mice (3.8±0.2 ng/ml) and even greater in super-aged (4.9±0.4 ng/ml), compared to young (1.8±0.2 ng/ml) and middle-aged (1.8±0.1 ng/ml) (P<0.001) mice. In contrast, only super-aged female mice showed significantly increased plasma sPRR (4.3±0.1 ng/ml) compared to younger mice (P=0.03). Interestingly, plasma sPRR was positively correlated with age (R=0.856, P<0.001) and SBP.
The dopamine D2 receptor (D2R) plays a significant role in preventing renal inflammation and injury. Knockdown of D2R gene (Drd2) in the mouse kidney promotes renal inflammation. We studied the effects of D2R in the mouse renal proximal tubule by generating Drd2 f/f, PSGLT2::Cre+ mice (D2R PT-/-) that lack D2R only in the renal proximal tubule and Drd2 f/f, PSGLT2::Cre- (D2R PT+/+) mice that do not have the deletion. We studied male and female mice (n=5/group). Renal mRNA expressions of TNF-α, TGFβ1, Fn1 and Col1a1 were higher (P<0.01) in female D2R PT+/+ than in male D2R PT+/+ mice. By contrast, the expression of the kidney injury marker, Kim-1, was higher (P<0.01) in male D2R PT+/+ than in female D2R PT+/+ mice. Male D2R PT-/- mice expressed less (P<0.01) renal TNF-α, TGFβ1, Col1a1, Fn1 and cell proliferation marker Mki-67 than female D2R PT-/- mice. However, the expression of Kim-1 was less (P<0.01) in female than in male D2R PT-/- mice. A high salt intake has deleterious effects in the kidney. In mice on a high salt diet (4%, 7 days) renal mRNA expressions of TNF-α, TGFβ1 and Fn1 were similar in male and female D2R PT+/+ and D2R PT-/- mice but the high salt diet increased (P<0.05) the renal mRNA expressions of Col1a1 and Kim-1 in male D2R PT+/+ and D2R PT-/- but not in females of the two genotypes. High salt diet also increased the renal mRNA expression of Mki-67 in D2R PT+/+ males. Some common single nucleotide polymorphisms (SNPs; rs6276 and 6277) in the human DRD2 are associated with high blood pressure and result in decreased D2R expression and function. We determined the influence of DRD2 SNPs in the response to the nephrotoxic aristolochic acid (AA, 5 µg/ml, 24 h) in immortalized human renal proximal tubule cells (RPTCs) from male and female humans. D2R protein expression was higher in males than in females with DRD2 wild-type (WT) but lower in males and females with DRD2SNPs (23±2%, P<0.05), relative to their WT counterparts. The renal TNFα mRNA was higher in males than females with DRD2 WT and DRD2 SNPs; AA increased 9-10-fold in male and female WT but only 2-3-fold in both sexes with SNPs. Renal TGFβ mRNA was similar in male and female WT and increased to the same extent in those with DRD2 SNPs. Col1a1 mRNA was higher (30%) in male and female WT than in those with DRD2 SNPs; AA decreased TGFβ mRNA in all groups. FN1 mRNA was higher (30-40%) in males and females with DRD2 SNPs than in those with DRD2 WT; AA increased renal FN1 mRNA only in males and females with DRD2 SNPs. The proliferation marker Mki-67 mRNA was higher in females than in males with DRD2 WT (1.5-2 fold) and in both sexes with DRD2 SNPs; AA increased renal Mki-67 mRNA to a greater extent in males than in females with DRD2 WT and DRD2 SNPs while renal Kim-1 mRNA was higher in males than in females and AA only increased renal Kim-1 mRNA in males, regardless of DRD2 genotype. Our data show striking differences in the mRNA of genes related to inflammatory response, cell proliferation and kidney injury between males and females with females expressing more inflammatory and proliferation markers but less injury than males.

APSSG21.135
The Inflammatory Response to Downregulation of Dopamine D2 receptor in the Renal Proximal Tubule is Sex-dependent.
Shaun Moore, Megha Kumar, Daniel Yaqub, Laureano Asico, John Gildea, Robin Felder, Pedro Jose, Ines Armando
1Medicine, George Washington University, 2Pathology, University of Virginia

The dopamine D2 receptor (D2R) plays a significant role in preventing renal inflammation and injury. Knockdown of D2R gene (Drd2) in the mouse kidney promotes renal inflammation. We studied the effects of D2R in the mouse renal proximal tubule by generating Drd2 f/f, PSGLT2::Cre+ mice (D2R PT-/-) that lack D2R only in the renal proximal tubule and Drd2 f/f, PSGLT2::Cre- (D2R PT+/+) mice that do not have the deletion. We studied male and female mice (n=5/group). Renal mRNA expressions of TNF-α, TGFβ1, Fn1 and Col1a1 were higher (P<0.01) in female D2R PT+/+ than in male D2R PT+/+ mice. By contrast, the expression of the kidney injury marker, Kim-1, was higher (P<0.01) in male D2R PT+/+ than in female D2R PT+/+ mice. Male D2R PT-/- mice expressed less (P<0.01) renal TNF-α, TGFβ1, Col1a1, Fn1 and cell proliferation marker Mki-67 than female D2R PT-/- mice. However, the expression of Kim-1 was less (P<0.01) in female than in male D2R PT-/- mice. A high salt intake has deleterious effects in the kidney. In mice on a high salt diet (4%, 7 days) renal mRNA expressions of TNF-α, TGFβ1 and Fn1 were similar in male and female D2R PT+/+ and D2R PT-/- mice but the high salt diet increased (P<0.05) the renal mRNA expressions of Col1a1 and Kim-1 in male D2R PT+/+ and D2R PT-/- but not in females of the two genotypes. High salt diet also increased the renal mRNA expression of Mki-67 in D2R PT+/+ males. Some common single nucleotide polymorphisms (SNPs; rs6276 and 6277) in the human DRD2 are associated with high blood pressure and result in decreased D2R expression and function. We determined the influence of DRD2 SNPs in the response to the nephrotoxic aristolochic acid (AA, 5 µg/ml, 24 h) in immortalized human renal proximal tubule cells (RPTCs) from male and female humans. D2R protein expression was higher in males than in females with DRD2 wild-type (WT) but lower in males and females with DRD2SNPs (23±2%, P<0.05), relative to their WT counterparts. The renal TNFα mRNA was higher in males than females with DRD2 WT and DRD2 SNPs; AA increased 9-10-fold in male and female WT but only 2-3-fold in both sexes with SNPs. Renal TGFβ mRNA was similar in male and female WT and increased to the same extent in those with DRD2 SNPs. Col1a1 mRNA was higher (30%) in male and female WT than in those with DRD2 SNPs; AA decreased TGFβ mRNA in all groups. FN1 mRNA was higher (30-40%) in males and females with DRD2 SNPs than in those with DRD2 WT; AA increased renal FN1 mRNA only in males and females with DRD2 SNPs. The proliferation marker Mki-67 mRNA was higher in females than in males with DRD2 WT (1.5-2 fold) and in both sexes with DRD2 SNPs; AA increased renal Mki-67 mRNA to a greater extent in males than in females with DRD2 WT and DRD2 SNPs while renal Kim-1 mRNA was higher in males than in females and AA only increased renal Kim-1 mRNA in males, regardless of DRD2 genotype. Our data show striking differences in the mRNA of genes related to inflammatory response, cell proliferation and kidney injury between males and females with females expressing more inflammatory and proliferation markers but less injury than males.

APSSG21.136
Sex Differences in Irritable Bowel Syndrome
Adil Bharucha
1Gastroenterology, Mayo Clinic

Functional gastrointestinal disorders (FGIDs) result from central and peripheral mechanisms. They typically cause chronic remitting-relapsing symptoms, are associated with comorbid conditions (e.g., fibromyalgia, anxiety and depression) and impaired quality-of-life. This talk will review sex- and gender-based differences in the prevalence, pathophysiology, clinical features, and management of non-ulcer dyspepsia (NUD) and irritable bowel syndrome (IBS), which are the two most common FGIDs, and together affect approximately 1 in 4 people in the United States. These diseases are more common in women. Women are also more likely to have severe symptoms, and coexistent anxiety or depression. Diarrhea is more common in men while constipation and bloating are more common in women, perhaps partly because defecatory disorders, which cause constipation are more common in women. Current concepts suggest that biological disturbances (e.g., persistent mucosal inflammation after acute gastroenteritis) interact with other environmental factors (e.g., abuse) and psychological stressors, which influence the brain and the gut to alter gastrointestinal motility and/or sensation, causing symptoms. Our understanding of sex-based differences in the pathogenesis of FGIDs lags our understanding of these mechanisms in animal models. Slow gastric emptying and colon transit are more common in healthy women than men but the effects of gonadal hormones on transit are less significant than in rodents. Likewise, while increased visceral sensation partly explain symptoms, the effects of sex on visceral sensation, colonic permeability and the gut microbiome are less prominent in...
humans than rodents. There is limited evidence that sex- or gender affect the response to medications or behavioral therapy for NUD or IBS, perhaps partly because most studies have enrolled a majority of women.

APSSG21.137
Sex Differences in Developmental Origins of Adult Cardiovascular Disease
Sandra Davidge¹
¹Obstetrics and Gynecology, University of Alberta

The developmental origins of health and disease (DOHaD) theory posits that sub-optimal environments in utero and/or early postnatal life can cause structural and functional changes in key organ systems, including the cardiovascular system, thereby predisposing the individual to chronic disease in later life. Indeed, there is now a substantial body of evidence showing that offspring born from complicated pregnancies are at greater risk of cardiovascular morbidities in adult life. Reduced delivery of oxygen to the fetus is one of the most common complications of pregnancy. My laboratory has observed both direct and latent sex differences in adult rat offspring exposed to prenatal hypoxia. We have shown that prenatal hypoxia impairs capacity for adult hearts to recover from cardiac ischemia reperfusion injury in both sexes but the mechanistic pathways regarding the levels and phosphorylation of cardiac proteins involved in calcium cycling are unique to each sex. Moreover, our treatment strategy of placental-targeted treatment has demonstrated interesting sex-specific effects on both the placenta and the mechanism of improved cardiac recovery from ischemic reperfusion injury (1). In our model system, we have further assessed the endothelin-1 (ET-1) pathway. Cardiac ischemia reperfusion upregulates the ET-1 system leading to elevated levels of ET-1 (a peptide with potent physiological and pathophysiological effects on the cardiovascular system) that through activation of the endothelin-A receptors (ETAR) results in impaired cardiac recovery. We found that exposure to prenatal hypoxia alters the cardiac ET-1 system in a sex-specific manner in adult offspring. As expected, Inhibition of ETAR improved cardiac recovery after ischemia reperfusion in normoxic control animals. Interestingly, ETAR inhibition also improved cardiac recovery in prenatal-exposed female offspring; however, surprisingly, it prevented cardiac recovery in prenatal hypoxic-exposed males. Thus, activation of ETAR contributes to the development of cardiac dysfunction in normoxic males but both normoxic and prenatal hypoxic-exposed females; while in males exposed to prenatal hypoxia, activation of ETAR may be a compensatory mechanism that is essential for cardiac tolerance to ischemia reperfusion. These data are critically important as studies have shown ETAR inhibition improves cardiovascular function for some conditions; however our data indicate that prenatal environment exposure is important to understand for precision medicine. Overall, our data indicate that sex-specific determination of mechanisms and susceptibility to developmental stressors must be taken into consideration. Moreover, from a biological perspective, treatment strategies (either early intervention such as placental targeted or adult interventi) also must take into account the intersection of sex and perinatal history when developing therapeutic strategies to improve life-long cardiovascular health. Reference cited 1. Hula N, Spaans F, Vu J, Quon A, Kirschman R, Cooke C, Phillips TJ, Case CP, Davidge ST. Pharmacological Research, 165:105461, Jan 26, 2021

Acknowledgement This research is supported by The Stollery Children’s Hospital Foundation and the Alberta Women’s Health Foundation through the Women and Children’s Health Research Institute

APSSG21.139
Women, Opioids and Addiction in the Time of COVID-19
Carolyn Mazure¹
¹Psychiatry, Yale School of Medicine

As the COVID-19 pandemic continues to rage in this country, the opioid epidemic also endures (Goetz et al, 2021). This presentation will highlight how the ongoing opioid epidemic evolved in relation to the treatment of pain, that women have been and continue to be the majority of those who are prescribed opioids, and the use of these prescribed medications became the primary pathway to misuse and addiction for women. Mitigation in opioid prescribing has been followed by increases in the use of other synthetic opioids, such as heroin and fentanyl, in both women and men. However, reduction in opioid prescriptions has been transient and is now three times higher than in 1999, with women continuing to receive the majority of these medications. This higher rate is found for women of all ages and reported identities as non-Hispanic White, non-Hispanic Black and Hispanic. Although the rate of opioid use and overdose remains greater in men than women, we have witnessed a greater change in the rate of overdose death in women. How opioid use and addiction affect the health outcomes of women and men differently has important implications for addressing the epidemic effectively. Examples of how recognition of the extent of women’s exposure to opioids and its consequences can inform research, treatment and health policy will be offered. Goetz TG, Becker JB, Mazure CM. Women, opioid use, and addiction. The FASEB Journal, 35(2), DOI: 10.1096/fj.202002125R, 2021.

APSSG21.140
Intestinal Epithelial Axin1 Drives Sex Differences in the Gut Microbiome and Obesity
Shari Garrett¹, Yongguo Zhang¹, Yinglin Xia¹, Jun Sun¹,²,³,⁴
¹Obstetrics and Gynecology, University of Illinois Chicago, ²Microbiology and Immunology, University of Illinois Chicago, ³Cancer Center, University of Illinois Chicago, ⁴Jesse Brown VA Medical Center, University of Illinois Chicago

Background: The incidence of obesity and metabolic syndrome have increased in the last few decades and is characterized as low-grade chronic inflammation. Men and women differ in the degree of diet induced obesity and
Axin1 mutations in obesity related inflammation and the development of Axin1 dysfunction in obesity and metabolic syndrome in males and females. Previous studies have shown the importance of the Axin1/Wnt/β-catenin signaling pathway in intestinal epithelial inflammation and infection. The role of Axin1 in obesity and gender-difference has not been studied. Hypothesis: The current study examines the relationship between the gut microbiota, intestinal Axin1 and the development of metabolic syndrome in males and females. We hypothesize that sex differences in obesity and metabolic disorders are due to gender variations in the microbiota induced by intestinal Axin1 status. Method: To explore the novel role of intestinal epithelial Axin1 in regulating sex dependent obesity, we generated a unique Axin1 conditional knockout model in intestinal epithelial cells (Axin1ΔIEC). Colonic fecal samples were collected and analyzed for 16S metagenomic sequencing. These mice, including their Axin1loxP controls were fed a 60% high fat diet (HFD) for 13 weeks. Serum was collected to measure glucose and insulin tolerance. Results: We found that loss of intestinal Axin1 lead to sex-specific dysbiosis. Specifically, Axin1loxP female mice and Axin1ΔIEC male mice had increased diversity compared to their gendered counterparts. This difference in the microbiota was also associated with genera specific differences. Most notably female Axin1ΔIEC mice have blooms in Odoribacter and Clostridiales genera while Axin1loxP males had enriched abundances in Akkermansia. After high fat diet challenge, Axin1loxP female mice and Axin1ΔIEC male mice gained more weight compared to their Axin1loxP mates. Conversely, Axin1ΔIEC male mice gained less weight compared to Axin1loxP males. Despite this, high fat diet fed Axin1loxP male mice were more susceptible to glucose intolerance. Conclusion: Our study demonstrates a novel role of intestinal epithelial Axin1 in mediating the microbiome and obesity in a sex-dependent manner. Intestinal epithelial dysfunction of Axin1 leads to risk of diet induced obesity specifically in female mice, which were colonized with commensal populations associated with obesity and inflammation. Further studies are needed to elucidate mechanisms of Axin1 dysfunction in obesity and Axin1 mutations in obesity related inflammation and metabolic disorders.

APSSG21.142
Androgen Effects on Baroreflex Sensitivity in Women with Androgen-Excess Polycystic Ovary Syndrome

Tori Stone1,2, Mari Chiles1, Cheryl Leone1, Lubna Pal3, Nina Stachenfeld1,2
1Integrative Environmental Physiology, The John B. Pierce Laboratory, 2Obstetrics, Gynecology & Reproductive Sciences, Yale School of Medicine

Polycystic ovary syndrome (PCOS) is a reproductive endocrinopathy affecting ~10% of reproductive-age women and is commonly associated with increased androgens. Hyperandrogenism may increase sympathetic activity and blood pressure (BP) in women with the Androgen-Excess PCOS phenotype (AE-PCOS). We hypothesized that androgen exposure impairs BP regulation and baroreflex sensitivity (BRS) in insulin resistant (IR) AE-PCOS compared to IR control women (IRCON). Subjects were pre-screened for IR by a 3-hr OGTT and HOMA-IR method (≥2=IR). Data are from 6 AE-PCOS (age=25±5 y, BMI=40±2 kg/m2, HOMA-IR=4.3±1.4 units) and 3 IR CON (age=32±8 y, BMI=35±7 kg/m2, HOMA-IR=2.9±0.7 units). BRS was measured at 3 separate visits: Baseline (BSL); hormone suppression with a gonadotropin-releasing hormone antagonist (ANT, 250 μg/day, 4 days); hormone suppression+methyltestosterone (T, 5 mg/day, 4 days). Muscle sympathetic nerve activity [MSNA] burst frequency], systolic and diastolic BP [(SBP, DBP) mm Hg] and R–R intervals [ECG, seconds (s)] were recorded at rest and during the modified Oxford protocol to assess the cardiovagal and sympathetic BR. Resting sympathetic activity was recorded for 2 min and expressed as Total MSNA in AU/min, and gain during the modified Oxford was used as the index of BRS for cardiovagal (CVBR) and sympathetic BRS. Resting BP in AE-PCOS was higher at BSL versus IR-CON (148±15 vs. 128±15 mm Hg, respectively, P=0.06). In AE-PCOS, ANT decreased resting SBP (136±11 mm Hg, P=0.05 vs. BSL), but T did not restore SBP to BSL (139±17 mm Hg, P=0.18 vs. BSL). The effects of hormone intervention on resting SBP in IR-CON were trivial (ANT=131±16, T=134±21 mm Hg). Total MSNA in AE-PCOS was 194.7±92.0 AU/min at BSL and was reduced by ANT (150.3±44.6 AU/min), but T partially restored MSNA to BSL (170.3±65.2 AU/min). These levels were higher than resting MSNA in IR-CON (BSL=152.8±37.4, ANT=127.1±21.2, T=167.5±45.9 AU/min), but hormone intervention had a lesser effect in IR-CON. In AE-PCOS, ANT improved sympathetic BRS at BSL (BSL=0.479±0.1764, ANT=0.616±0.205 bursts/100 Hz/mmHg, P=0.03), and T attenuated the improved BRS (T=0.488±0.178 bursts/100 Hz/mmHg, P=0.11, ANT vs. T). Hormone intervention did not affect sympathetic BR gain in IR-CON. ANT did not impact CVBR gain in AE-PCOS (BSL=0.223±0.013 vs. ANT=0.27±0.027 s/mmHg) or IR-CON (BSL=0.014±0.014 vs. ANT =0.011±0.009 s/mmHg). However, T reduced CVBR gain in both AE-PCOS and IR-CON (0.015±0.009 and 0.009±0.005 s/mmHg for AE-PCOS and IR-CON, respectively) compared to BSL. Suppressing testosterone and estrogen in women with AE-PCOS lowered resting SBP and MSNA and increased sympathetic BRS, suggesting hormone suppression improves BP regulation in AE-PCOS. Reintroducing androgens attenuated this improved response.
Acute kidney injury prior to pregnancy decreases late-gestation urine volume and increases plasma aldosterone levels

Desmond Moronge, Riyaz Mohamed, Ellen Gillis, Jennifer Sullivan, Jessica Faulkner

Physiology, Augusta University

Clinical data report a three-to-five fold increase in adverse pregnancy outcomes among women with a medical history of acute kidney injury (AKI), despite recovery of renal function before pregnancy. Our group recently developed a rat model of pregnancy post-AKI that mimics this increased risk. We showed that female Sprague-Dawley (SD) rats fully recover renal function as measured by creatinine clearance 30 days post bilateral renal ischemia reperfusion as an experimental model of AKI. However, these rats exhibited decreased creatinine clearance, increased blood pressure, increased uterine artery resistance and decreased fetal growth during pregnancy compared to pregnant rats who underwent sham surgery. Our work also shows that high aldosterone levels in female rodent models induce adverse cardiovascular outcomes, however a role for aldosterone in post-AKI pregnancy is not known. The current study tested the hypothesis that AKI prior to pregnancy induces heightened renin-angiotensin aldosterone system activation (RAAS). Female SD rats were randomized to 45-minute warm, bilateral renal ischemia followed by reperfusion or sham surgery (N=4 Sham, N=6 AKI). All rats were allowed 1 month for recovery prior to mating. Creatinine clearance of the female rats subjected to AKI was comparable to that of sham controls prior mating. Gestational day 1 (GD1) was identified through vaginal smearing. Rats were placed in metabolic cages on GD19 for 24 hour urine collection. Rats were euthanized on GD20 and plasma and tissues collected. Kidney to body weight ratio significantly increased in post-AKI pregnant rats (0.003±0.00017) compared to shams (0.002±0.00019, P<0.05). However, post-AKI pregnant rats excreted a significantly lower urinary volume (14±5 pg/ml) compared to the sham pregnant rats (26±11 pg/ml, P<0.05), indicating renal insufficiency. Plasma aldosterone levels, measured via ELISA, were greater in post-AKI pregnant rats (342±143 pg/ml) compared to sham (144±131 pg/ml, P=0.058). RT-PCR showed no increase in renal inner medullary (IM) mRNA expression of mineralocorticoid receptor (MR) (0.18 ± 0.4-fold-change from sham, P=0.65) or α-epithelial sodium channel (α-ENaC) (0.006 ± 0.3-fold change from sham, P=0.98) in AKI rats compared to sham, indicating no increase in aldosterone-sensing sensitivity with AKI prior to pregnancy. In addition, mRNA expression of renin (0.27±2.7 fold-change from sham, P=0.92) and angiotensin converting enzyme 1 (ACE1) (6.6±7.2-fold-change from sham, P=0.3) were not increased in post-AKI and sham pregnant rats. Therefore, at GD20 pre-pregnancy AKI induces markers of renal deficiency in reduced urine volume despite increased kidney weight in AKI rats in association with increased plasma aldosterone levels. However, markers of renal RAAS did not increase in the IM of pregnant rats post-AKI. These data implicate renal cortical or adrenal RAAS activation in post-AKI pregnant rats which may mediate adverse maternal and fetal effects of pre-pregnancy AKI, a notion that warrants further investigation. Funding sources: 4 R00 HL146948-03 and AHA858380 to JLF and 17EIA33410565 to JS

Sodium-Glucose Cotransporter-2 Inhibition Decreases Visceral but Not Subcutaneous Adipocyte Size in Hyperandrogenemic Female Rats

Faridah Salau, Lucy Taylor, Jacob Pruett, Steven Everman, Damian Romero, Licy Yanes Cardozo

Women’s Health Research Center, UMMC, Cardio Renal Research Center, UMMC, Department of Medicine, UMMC

Polycystic Ovary Syndrome (PCOS) is the most common endocrinopathy in premenopausal women. Androgen excess and ovulatory dysfunction characterize PCOS; obesity affects 80% of this population. Sodium-glucose cotransporter-2 inhibitors (SGLT2i) decrease fat mass (FM) in PCOS women. Previously, we reported increased body weight, FM, and insulin resistance (IR) in hyperandrogenemic female (HAF) rats, with SGLT2i treatment decreasing FM without lowering food intake or IR. We hypothesized that androgens increase adipocyte size in white adipose tissue (WAT) depots and that SGLT2i treatment decrease adipocyte size. At 4-weeks-old, 40 female SD rats were randomized to a placebo (PBO) group or continuous dihydrotestosterone (7.5 mg/90 days) exposure (HAF) group. After 10 weeks of exposure, rats were given drinking water alone or with the SGLT2i emagliflozin (10 mg/kg/day) for 3 weeks. Subcutaneous WAT (sWAT) and visceral WAT (vWAT) were collected. Images were acquired at 40x magnification and the adipocyte area of at least 100 cells per rat and WAT depot were quantified using the Adiposoft software by investigators blinded to the sample identity. GraphPad Prism was used to calculate relative frequency of adipocyte area (bins of 200 um2 for vWAT and of 300 um2 for sWAT), and data were analyzed by 2-way ANOVA. In the vWAT, HAF had lower frequency of small adipocytes around 200 um2 compared to PBO (12.5 ± 1.1 vs 18.2 ± 2.1 %, P<0.001). This trend continued up to 800 um2 when HAF began having larger adipocytes than PBO (NS). In HAF, SGLT2i increased relative frequency of adipocytes at 400 um2 (27.5 ± 1.6 vs 21.7 ± 1.9 %, P<0.001); this trend continued until 800 um2. In PBO, SGLT2i increased relatively
frequency of adipocytes at 400 um2 (30.2 ± 2.0 vs 24.0 ± 1.6 %; P<0.001) with this trend continuing until 600 um2. In the sWAT, compared to PBO, HAF had a higher frequency of adipocytes at 400 um2 (15.5 ± 2.0 vs 7.1 ± 1.7 %; P<0.0001) with lower frequency at 1300 um2. HAF appeared to have more large adipocytes compared to PBO from 2800 um2 to 3400 um2 (NS). SGLT2i had no significant impact on sWAT in HAF. Meanwhile, in PBO, SGLT2i increased frequencies of adipocytes at 400 um2 (17.8 ± 2.8 vs 7.1 ± 1.7 %; P<0.0001) and 700 um2 while decreasing frequency at 1,900 um2. In conclusion, HAF rats had a lower frequency of small adipocytes compared to PBO in sWAT, and SGLT2i increased frequency of small adipocytes in both PBO and HAF rats. Meanwhile, in sWAT, HAF rats had higher frequency of small adipocytes and decreased frequency of medium adipocytes compared to PBO. In sWAT, SGLT2i only decreased adipocyte size in PBO rats while this effect was blunted in HAF rats. These data suggest that androgens and SGLT2i treatment have differential effects on adipocytes depending on WAT depots. This information may help customize therapies aimed at obesity-associated complications in PCOS.

Supported by NIH grants: NIGMS P20GM121334 & P20GM104357, NIDDK R21DK113500 & F30DK127527, NHLBI P01HL51971

APSSG21.147

Sex differences in the expression of the renin angiotensin system (RAS) components in aging offspring of polycystic ovary syndrome (PCOS) rat model

Skylarr Beerman1, Jane Reckelhoff2, Noha Shawky1

1Cell and Molecular Biology, University of Mississippi Medical Center, 2Cell and Molecular Biology, Mississippi Center of Excellence in Perinatal Research, Women's Health Research Center, University of Mississippi Medical Center, University of Mississippi Medical Center

Background: PCOS is characterized by hyperandrogenemia and elevated blood pressure (BP). Due to exposure to prenatal hyperandrogenemia, male and female offspring of hyperandrogenic female (HAF) dams (rat model of PCOS) are born with low birth weight. Upon aging (>16 mos-old), despite the exaggerated pressor response to angiotensin (Ang) II in aging male offspring of HAF dams (F1HAF), and the attenuated pressor response to Ang II in aging female FIHAF, HAF offspring maintained a normal baseline BP compared to age and sex-matched F1Contr. Yet, both male FIHAF and male offspring of control dams (F1Contr) have higher baseline BP compared to age-matched female FIHAF and F1Contr. The present study tested the hypothesis that aging female FIHAF have an upregulation of the vasodilator arm of RAS (AT2R and ACE2) that prevents them from developing hypertension at baseline or after Ang II infusion. Methods: Hyperandrogenemia was induced in female SD rats (5α-dihydrotestosterone pellets 7.5 mg/90 d, s.c. at 4 wks. of age and throughout life). HAF and controls (10-12 wks. of age) were mated, allowed to deliver and lactate. Male and female F1 HAF and F1 Contr. were left untreated until 16-20 mos-old. Offspring (n = 6-8, 1 rat/litter/group) were euthanized, kidneys were collected, and the cortices were separated. Western blot was used to measure angiotensinogen, ACE1, ACE2, AT1R and AT2R protein expression. Results: Renal cortical angiotensinogen was higher in male F1contr. compared to female F1contr. (2.1 ± 0.3 AU vs 1.0 ± 0.1 AU, p<0.05) and in male FIHAF compared to both female F1Contr. and female FIHAF (2.5 ± 0.4 AU vs 1.4 ± 0.3 AU and 1.0 ± 0.1 AU, respectively, p<0.05), but was similar between the 2 male groups. Renal cortical ACE2 was higher in male FIHAF compared to male F1contr., female FIHAF, and female F1Contr. (3.1 ± 0.6 AU vs 1.7 ± 0.3 AU, 0.7 ± 0.1 AU, and 1.0 ± 0.2 AU, respectively, p<0.05). Renal cortical AT2R was higher in male F1Contr. and male FIHAF compared to both female F1Contr. and female FIHAF (3.5 ± 0.9 AU and 3.3 ± 1.0 AU vs 1.0 ± 0.2 AU and 1.1 ± 0.2 AU, respectively, p<0.05), but was similar between both male groups. No significant differences in angiotensinogen, ACE2 and AT2R were observed between both female groups. In addition, no significant differences were observed in ACE1 or AT1R expression between the 4 groups. Conclusion: Aging male FIHAF and F1contr. have an increase in their renal cortical angiotensinogen compared to females, which could partly explain their increased BP at this age. On the contrary to our hypothesis, upregulation of AT2R in aging male FIHAF and F1contr. could be a compensatory mechanism against further elevated BP. Upregulation of ACE2 in aging male FIHAF at baseline could be protecting them from developing exaggerated hypertension at baseline compared to male F1Contr. Future studies should test the expression of RAS components in Ang II-treated aging male and female FIHAF in order to better understand the sex differences in their response to Ang II. Funding Sources: R01HL135089, P01HL051971, P20GM121334, P20GM104357

APSSG21.148

Tissue Specific Androgen Receptor Expression in Hyperandrogenemic Female Mice: Implications for Women with Polycystic Ovary Syndrome

Alexandra Huffman1, Samar Rezq1, Jelina Basnet1, Licy Yanes Cardozo2, Damian Romero1

1Cell and Molecular Biology, University of Mississippi Medical Center, 2Medicine, University of Mississippi Medical Center

Introduction and Purpose: Polycystic Ovary Syndrome (PCOS) is recognized as the most common endocrine disorder in women of reproductive age. Notably, a common diagnostic feature of PCOS women is hyperandrogenism, which is associated in severity with several comorbidities including obesity, infertility, and insulin resistance. In order to explore the molecular mechanisms by which elevated androgens can influence tissue-specific pathophysiology, we explore Androgen Receptor (AR) expression across multiple tissues in a hyperandrogenemic mouse model of PCOS. Methods: Four-week-old C57BL/6N female mice were implanted subcutaneously with dihydrotestosterone (DHT, 8.0 mg) or vehicle Silastic tubes (n=8/group). Animals were euthanized after 90 days of treatment. AR mRNA expression and protein levels were assessed using RT-qPCR and Western blotting in twelve tissues including the left ventricle, kidney, lung, brain, tibialis anterior muscle,
small intestine, cecum, colon, liver, subcutaneous fat, ovary and uterus. Results are considered significant p<0.05.

Results: Serum DHT concentration was 3.56-fold higher in DHT-treated female mice than their vehicle counterparts (Vehicle: 0.57nM; DHT: 2.03nM) by LC-MS/MS. Of all tissues under study, AR mRNA expression was the highest in the kidney followed by the ovary in female control animals. Analysis of AR mRNA expression in individual tissues in DHT animals showed decreased AR expression in the left ventricle (0.70-fold), the tibialis anterior muscle (0.83-fold), the colon (0.47-fold), the liver (0.51-fold), and the ovary (0.86-fold) compared to their vehicle counterparts. Additionally, our results indicate an upregulation of AR mRNA in the kidney (1.22-fold) and the uterus (1.56-fold) in DHT-treated animals compared to vehicles. We observed the highest AR protein expression upregulation in the small intestine (23.67-fold) in DHT-treated animals compared to controls. Other tissues with increased AR protein levels in DHT-treated animals include the left ventricle (2.53-fold), the kidney (1.78-fold), the lung (3.90-fold), the brain (5.27-fold), the tibialis anterior (1.80-fold), the cecum (4.61-fold), the colon (3.78-fold), the subcutaneous fat (1.58-fold), the ovary (1.48-fold), and the uterus (2.0-fold). Only the liver had a decrease in AR protein levels in DHT-treated animals compared to vehicles (0.43-fold). Conclusions: Our results indicate that higher circulating androgen levels result in upregulation of the AR protein in most tissues in hyperandrogenemic female mice. Given our findings, hyperandrogenemia in PCOS may generate a positive feedback in AR protein expression in multiple tissues. Effective, safe, and specific antiandrogen therapies are not available to treat women with PCOS. These results strongly suggest that AR blockade may be imperative to prevent the cascade of deleterious effects triggered by elevated androgens in PCOS. Funding: (Supported by NIH grants NIGMS P20GM-121334 to LLYC and DGR, and NIH NIDDK R21DK-113500 to DGR.)

APSSG21.149
Cardiovascular disease in transgender and gender diverse adults taking feminizing gender-affirming hormone therapy
Sean J. Iwamoto

Medical/Endocrinology, Metabolism & Diabetes, University of Colorado School of Medicine & Rocky Mountain Regional VA Medical Center

An estimated 1.4 million U.S. adults identified as transgender in 2016 [1]. Transgender and gender diverse (TGD) persons have a gender identity that does not align with their sex assigned at birth (gender incongruence—e.g., transgender women were assigned male at birth but have a female gender identity). Gender dysphoria, the distress associated with gender incongruence, can lead to increased depression, anxiety, suicidality, and other mental health and medical disparities [2, 3]. Gender-affirming hormone therapy (GAHT—e.g., estrogen +/- antiandrogen for feminization) significantly improves mental health outcomes and quality of life while alleviating gender dysphoria [3, 4]. GAHT initiation includes informed consent discussions between TGD patients and their prescribers about the benefits and risks associated with pharmacologic exogenous sex hormones. While feminizing GAHT has desired effects (e.g., body shape changes, breast growth, decreased facial/body hair) [4], data are concerning for its association with increased venous thromboembolism, stroke, and cardiovascular disease (CVD) risk compared to the general population [3-5]. The underlying mechanisms for these risks remain unknown but may be related to age, duration and type of estrogen +/- antiandrogens, body composition and weight changes, history of orchietomy, lifestyle (e.g., physical activity, nutrition, smoking), or other factors. This talk will summarize existing data on increased CVD risk associated with feminizing GAHT. Data from novel pilot studies will also highlight research underway to better elucidate potential mechanisms for increased CVD risk, including vascular and metabolic parameters, in transgender women taking feminizing GAHT. Funding: University of Colorado Building Interdisciplinary Research Careers in Women’s Health-BIRCH (K12 HD057022; PI: Regensteiner JG and Santoro NF), World Professional Association for Transgender Health, Colorado Nutrition Obesity Research Center (P30 DK048520; PI: MacLean P), Colorado Clinical and Translational Sciences Institute Clinical & Translational Research Centers (UL1 TR002535; PI: Sokol RJ), Ludeman Family Center for Women’s Health Research at the University of Colorado Anschutz Medical Campus. References: 1. Flores, A.R., Herman, J.L., Gates, G.J., Brown, T.N.T. How many adults identify as transgender in the United States? 2016. Accessed: August 14, 2021; Available from: https://williamsinstitute.law.ucla.edu/wp-content/uploads/How-Many-Adults-Identify-as-Transgender-in-the-United-States.pdf. 2. Brown, G.R. and K.T. Jones. Mental Health and Medical Health Disparities in Transgender Veterans Receiving Healthcare in the Veterans Health Administration: A Case-Control Study. LGBT Health, 2016. 3(2):122-31. 3. Iwamoto, S.J., et al. Health considerations for transgender women and remaining unknowns: a narrative review. Ther Adv Endocrinol Metab, 2019. 10:2042018819871166. 4. Hembree, W.C., et al. Endocrine Treatment of Gender-Dysphoric/Gender-Incongruent Persons: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab, 2017. 102(11):3869-3903. 5. Getahun, D., et al. Cross-sex Hormones and Acute Cardiovascular Events in Transgender Persons: A Cohort Study. Ann Intern Med, 2018. 169(4):205-213.

APSSG21.150
Sex differences in lung inflammation in asthmatic mice exposed to ambient ozone
Keishla Colon Montanez, Patricia Silveyra

Environmental and Occupational Health, Indiana University Bloomington

Exposure to air pollution is a major health risk, as it can worsen lung disease symptoms. Ambient ozone, a product of photochemical reactions between volatile organic compounds and nitrogen oxides, is known to be one of the most dangerous air pollutants. Ozone inhalation can aggravate inflammatory lung diseases such as asthma, which is more frequently diagnosed in females than males.
Despite this, the molecular mechanisms underlying the effects of ozone in the male and female lung have yet to be discovered. We hypothesized that exposure to ozone exerts differential inflammatory responses in the male and female asthmatic lung. To test it, we treated adult male and female C57BL/6J mice with an allergen (house dust mite extract) intranasally for 5 weeks to trigger asthma phenotypes. We then exposed mice to 2 ppm of ozone or filtered air (FA) for 3 hours, and collected lung tissue 24 hours later. We assessed histological changes by microscopy and extracted lung RNA with Trizol, to measure the expression of 92 immune response associated genes by PCR with the TaqMan® Array 96-well Mouse Immune Response Plate (ThermoFisher). Our preliminary results show that females exposed to ozone had increased peribronchial inflammation and hyperplasia when compared to males. Males, on the other hand, displayed higher perivascular inflammation. Male mice also had higher lung expression of immune response genes whereas asthmatic females had higher expression of pro-inflammatory cytokines, transcription factors, and regulators of immunity. We conclude that ozone exposure triggers differential inflammatory mechanisms in the male and female lungs of asthmatic mice.

APSSG21.151
Endothelin-A Receptor Antagonism Improves Nitric Oxide-Dependent Vasodilation in Women during the Low Hormone, but not High Hormone, Phase of Oral Contraceptives and in Men
Casey Turner1, Megan Wenner2, Brett Wong1
1Department of Kinesiology and Health, Georgia State University, 2Department of Kinesiology and Applied Physiology, University of Delaware

The effect of combined oral contraceptives on cardiovascular risk and outcomes in women remains controversial. Endothelin-1 is implicated in the pathogenesis of hypertension and endothelial dysfunction, but the effect of COC use on endothelin-1 signaling is not well understood. The purpose of this study was to investigate the contribution of endothelin-A receptors (ETAR) to nitric oxide (NO)-dependent vasodilation in women during the low hormone and high hormone phases of combined oral contraceptives and in men. Young, healthy premenopausal women taking combined oral contraceptives of any generation (low hormone phase, n=4; high hormone phase, n=4) and age-matched men (n=5) participated in this study. Participants were instrumented with two microdialysis fibers, and each site was randomized as control (lactated Ringer’s) or ETAR antagonism (500 mM BQ-123). Laser-Doppler flowmetry (LDF) and local heaters were used to measure skin blood flow and induce localthermal hyperemia, respectively. Each site was heated from 33°C to 39°C at a rate of 0.1°C/sec. Once a plateau to local heating was established, 20 mM L-NAME, a non-specific NO synthase inhibitor, was infused at each site to quantify NO-dependent vasodilation. Maximal vasodilation was induced by heating the skin to 43°C and infusing 54 mM sodium nitroprusside. Data are shown as mean %NO ± SD. At control sites, NO-dependent vasodilation was greater in women during the high hormone phase (76 ± 14 %NO) compared with women during the low hormone phase (39 ± 19 %NO, p = 0.01) and men (49 ± 13 %NO, p = 0.04). Compared with respective control sites, BQ-123 increased NO-dependent vasodilation in women during the low hormone phase (69 ± 8 %NO; p = 0.03) and in men (73 ± 8 %NO, p = 0.06, Cohen’s d effect size 2.19) but not in women during the high hormone phase (72 ± 9 %NO, p > 0.99). There were no observed statistical differences in NO-dependent vasodilation between groups at BQ-123 sites. Our preliminary data indicate women in the high hormone phase of combined oral contraceptives have greater NO-dependent microvascular vasodilation than women in the low hormone phase and men. Further, ETAR appear to contribute to attenuated NO-dependent vasodilation in women during the low hormone phase of combined oral contraceptives and in men. These preliminary data further suggest an effect of exogenous hormone exposure on the balance between vasoconstrictor and vasodilator mechanisms in the cutaneous microvasculature. This work is supported by NIH grant HL141205 to Brett Wong.

APSSG21.152
Sex differences in the cardiorespiratory response to isometric exercise or passive movement of the lower limb
Tania Pereira1, Heather Edgell1
1Kinesiology & Health Sci, York University

Dynamic exercise evokes both the mechanoreflex through physical deformation of a muscle and the metaboreflex through the accumulation of metabolites. Many exercise-based studies do not account for the contributions of each isolated reflex; thus, this study will use post-exercise circulatory occlusion (PECO) to isolate the metaboreflex and use passive leg movement (PLM) to isolate the mechanoreflex. Further, previous studies have found sex differences in the response to PECO of the forearm where men have an enhanced blood pressure response and women do not increase ventilation at all. It was previously hypothesized that a smaller muscle size in women could account for the observed sex differences in the metaboreflex. Therefore, in the current study, reflex function of the lower leg is used to determine if a larger muscle mass will abolish these sex differences in metaboreflex function. Further, due to the absence of VE (ventilation) observed previously in women during forearm PECO, yet known increases of VE during exercise in women, we hypothesize that the ventilatory stimulus to exercise is driven by the mechanoreflex in women. Heathy participants (n=19; Men (M): n=10 age 23±2, BMI 27±5kg/m2; Women (W): n=9, age 23±2, BMI 27±5kg/m2) were recruited to perform 2 minutes of isometric plantarflexion (80%MVC) followed by 3 minutes of PECO, or 3 minutes of PLM (trials randomized). Heart rate (HR), ventilation (VE), and mean arterial pressure (MAP) were continuously measured (ECG, pneumotach, Nexfin). Despite men being larger in stature than women (height p<0.01; weight p<0.001), there was no significant difference in estimated calf volume based on girth measurements (M: 1.57±0.28 vs. W: 1.37±0.17 L, p=0.08). In response to
isometric exercise, VE (M: 12.7±0.5 vs. 14.3±0.7 L/min; W: 10.3±1.0 vs. 11.5±1.3 L/min), HR (M: 68±3 vs. 78±5 bpm; W: 68±3 vs. 86±5 bpm), and MAP (M: 87±2 vs. 93±3 mmHg; W: 80±2 vs. 92±4 mmHg) increased from baseline in both men and women (Main effects of time: p<0.01, p<0.001, and p<0.001, respectively), yet the responses between sexes were not different (all p>0.05). HR and MAP remained significantly elevated during PECO compared to baseline (HR, M: 68±3 vs. 72±4 bpm; W: 68±3 vs. 73±3 bpm, Main effect of time p<0.001; MAP, M: 87±2 vs. 91±2 mmHg; W: 80±1 vs. 90±3 mmHg, Main effect of time p<0.001). During PECO in men and women, VE (M: 11.8±0.6 vs. 13.7±0.8 L/min; W: 10.8±0.7 vs. 12.2±0.8 L/min; main effect of time p<0.001) and HR (M: 68±3 vs. 70±4 bpm; W: 67±3 vs. 71±3 bpm; main effect of time p<0.05) increased within the first 30 seconds of PECO compared to baseline and remained elevated at the end of PECO (VE, M: 13.6±0.4 L/min; W: 12.0±0.7 L/min, main effect of time vs baseline, p<0.001; HR, M: 70±4 bpm; W: 75±4 bpm; main effect of time vs. baseline p<0.001). There was no change in the pressor response to PECO nor any sex differences (all p>0.1). Our study demonstrates that there are no observed sex differences in the ventilatory, cardiovascular or pressor response to leg mechanoreflex or metaboreflex activation. The current study suggests that individuals with similar limb volume should have similar exercise pressor reflex responses. Additionally, women were tested during the low hormone phase of the menstrual cycle, which may have attenuated any potential sex differences due to the low level of estrogen and progesterone.

APSSG21.154

Biological sex influences glomerular podocyte endowment in rats
Sarah Walton1, Debra Fong1, Reetu Singh1, Rebecca Flower1, John Bertram2, Kate Denton1
1Department of Physiology and Monash Biomedicine Discovery Institute, Monash University, 2Department of Anatomy & Developmental Biology and Monash Biomedicine Discovery Institute, Monash University

Sex differences in kidney function and susceptibility to injury may be underpinned by fundamental structural differences. Podocytes, the epithelial cells that wrap around the glomerular capillaries, have key roles in renal filtration capacity. Given podocyte depletion is implicated in renal patholgy and disease, we have examined whether podocyte endowment is influenced by biological sex. Accordingly, kidneys were collected from male and female Sprague-Dawley rats and at 3 and 10 weeks of age. Podocytes were immunostained using specific podocyte markers and imaged with confocal microscopy. Individual podocyte number, individual glomerular volumes and podocyte density were determined via the Weibel-Gomez method. At three weeks of age, podocyte number and glomerular volume and podocyte density were similar between males and females. Surprisingly, in males, podocyte number was ~30% greater at 10 weeks of age compared to 3 weeks of age. Podocyte number was unaffected by age in females, indicating podocyte endowment is complete prior to weaning in females but not males. Glomerular volume increased with age in both sexes, although this occurred to a lesser extent in females compared to males. Podocyte density declined with age, but did not differ significantly according to sex. We have identified podocyte endowment is influenced by sex in rats. Remarkably, postnatal podocyte gain was detected in males but not females, indicating sex differences in the regulation and cessation of podocyte generation. Whether
these sex differences in podocyte endowment translate to differences in kidney function in this model requires further investigation.

**APSSG21.155**

Methods of prescribing exercise intensity and heterogeneity peak VO2 gain in response to aerobic training

Laurence Poirier¹², Hugo Parent-Roberge¹², Eleonor Riesco¹²

¹Department of Anthropokinetics, University of Sherbrooke, ²Department of chronic diseases, Research Center on Aging

Introduction: The heterogeneity of aerobic training-induced adaptations is still controversial, and many studies suggested that the method of prescription is an important factor. Few studies have compared the impact of exercise prescription method on the proportion of exercise non-responders and the magnitude of change of peak oxygen uptake (ΔVO2Peak) (1-3), an independent predictor of numerous chronic diseases. After 3 months of aerobic training, they all reported a greater ΔVO2Peak and less non-responders when exercise was prescribed according to the ventilatory thresholds (VT), compared to % of heart rate reserve (%HRres). However, while the proportion of exercise non-responders was lower in the VT groups, the authors failed to demonstrate homogeneity of the response. Objective: To examine the heterogeneity of the ΔVO2Peak in response to aerobic training according to the method of prescription. Methods: Data from 91 individuals (women n=54, 59%), from three different studies (1-3) from the same research group, were extracted. Coefficient of variation (CV), median and interquartile range (median [IQR]) of ΔVO2Peak were calculated for both %HRres and VT groups. Raw ΔVO2Peak data, available in the published articles, were extracted by using the Plot Digitizer software (Version 4.4, Plot Digitizer, CA, USA). Results: In the %HRres groups, ΔVO2Peak CV was 127% (ΔVO2Peak: +4.7 [12.0]) in young adults (33.0 ± 9.8 years), 109% (ΔVO2Peak: +9.2 [10.2]) in middle-aged adults (51.2 ± 12.5 years), and 120% (ΔVO2Peak: 12.9 [13.9]) in older adults (67.4 ± 8.3 years). In the VT groups, ΔVO2Peak CV was 41% (ΔVO2Peak: +11.2 [1.6]) in young adults (31.7 ± 9.6 years), 33% (ΔVO2Peak: +10.1 [13.9]) in middle-aged adults (44.9 ± 11.4 years), and 56% (ΔVO2Peak: +11.2 [1.6]) in older adults (64.9 ± 10.0 years). Discussion/Conclusion: These results suggest that exercise intensity prescription based on VT results in training adaptations that are less heterogeneous than those based on %HRres, regardless of the age group. However, there is still a marked inter-individual variability in the elderly following a personalized training (VT). This heterogeneity results in VO2Peak gain ranging from 6.5% to 31.6%, which may represent a difference of up to 8 ml/kg/min between two older adults after a personalized aerobic training. To conclude, even if personalized exercise intensity prescription provides greater and less heterogeneous cardiorespiratory fitness gain, there is still a great variability (>50%) in older adults that require attention as this population is at higher risk of chronic disease. Future studies should focus on this variability as well as being carried out in clinical populations, with other routine clinical measures, such as ambulatory blood pressure which is a strong independent predictor of cardiovascular diseases. References 1- Wolperrn et al. BMC Sports Science, Medicine, and Rehabilitation, 2015; 7:16; 2- Weatherx, R. M (2019). Medicine & Science in Sports & Exercise, 5(4), 681-691; 3- Dalleck, L., et al. (2016). Journal of Fitness Research, 5(3),15-27.

**APSSG21.156**

Care of the transgender adolescent

Natalie Clericuzio¹

¹Ob-Gyn, University of Mississippi

In recent decades, the field of research about transgender healthcare has expanded dramatically and demonstrated that adolescence is a critical time for intervention in transgender patients. As society as a whole becomes more welcoming of trans people, more trans people will be comfortable self-identifying, and the proportion of the population who identifies as trans is expected to grow in coming years. For this reason, healthcare providers have an even greater need to be prepared to treat them appropriately and according to standards of care. The Ob-Gyn is bound to encounter transgender adolescents in practice, thus emphasizing the need to be versed in the basics of their care and the appropriate timeline for intervention. This article offers a review of recent literature with regards to the benefits to a patient’s well-being of early intervention, including both mental health and hormonal benefits, as well as the importance of consideration of fertility preservation prior to gender-affirming hormone therapy.

**APSSG21.157**

Sex Differences on Protein Expression of NOX5 and Endogenous Antioxidant Enzymes in Human Aortic Endothelial Cells Under Basal and Inflammatory Conditions

Rami Najjar¹, Brett Wong², Rafaela Feresin¹

¹Nutrition, Georgia State University, ²Kinesiology, Georgia State University

Background: NADPH-oxidase (NOX) is a major source of reactive oxygen species and contributes to oxidative stress, while antioxidant enzymes such as superoxide dismutase (SOD), and catalase counteract these effects. Under inflammatory conditions, such as increased circulating concentrations of tumor necrosis factor (TNF)-α, oxidative stress is exacerbated which can contribute to endothelial dysfunction. Men may have higher levels of oxidative stress, and this potentially contributes to the observed increase in cardiovascular disease risk. Differences in response to inflammatory insult could contribute to these dissimilarities. Thus, we sought to examine sex differences in the expression of pro- and antioxidant proteins in human aortic endothelial cells (HAECs) under basal and inflammatory conditions. Methods: HAECs (Cell Applications, San Diego, CA) derived from healthy, young male and female were treated with or without 20 ng/mL of TNF-α for 24 h. Cells were then
collected, and protein expression was assessed using western blot. Proteins were normalized to β-actin. Data were analyzed utilizing one-way ANOVA followed by Tukey-Kramer post-hoc test (P ≤ 0.05). Results: Under basal conditions, NOX5 protein expression was not different between male and female-derived cells (P = 0.7). However, NOX5 was increased in TNF-α-treated male-derived cells compared with male cells at basal conditions (P = 0.0005). In contrast, TNF-α-treated female-derived cells expressed lower NOX5 compared with basal female (P = 0.009) cells. SOD1 was not different between male and female-derived cells under basal conditions (P = 0.65). However, with TNF-α insult, SOD1 was greater in female compared to male-derived cells (P = 0.04). Mitochondrial SOD2 expression was greater under basal (P = 0.02) and inflammatory conditions (P < 0.0001) in male compared to female-derived cells. TNF-α treatment elicited a substantial increase in SOD2 cell in male (P < 0.0001) and female (P < 0.0001) HAECs compared to basal conditions. Catalase expression was greater in male-derived cells at basal (P = 0.01) conditions and with TNF-α treatment (P = 0.0003) compared with female-derived cells. Conclusion: Male-derived HAECs had increased SOD2 and catalase expression, irrespective of TNF-α treatment. Female-derived HAECs appear better able to tolerate inflammatory insult elicited by TNF-α due to reduced NOX5 and increased SOD1 expression. These findings warrant further investigation into the transcriptional mechanisms accounting for these differences.

APSSG21.158
Sex differences in willingness to participate in exercise physiology experiments
James Nuzzo1,2, Robert Deane3
1Exercise Science Laboratory, Vitruvian, 2Adjunct lecturer, School of Medical and Health Sciences, Edith Cowan University, 3Psychology Department, Grand Valley State University

Different proportions of male and female participants in exercise physiology and sports science experiments might be attributed, in part, to sex differences in interests and willingness to participate. Here, we tested the hypothesis that men and women are not equally willing to undergo certain procedures and that men and women consider different factors when deciding to participate in research. An online survey was promoted on social media and survey-sharing websites and asked men (n = 147) and women (n = 251) about their interest to learn about specific health and fitness outcomes, their willingness to undergo specific research procedures, and the importance of certain factors when deciding to participate in research. Survey responses were measured using 5-point Likert scales. Men were more interested than women to participate in exercise research (d = 0.24, p = 0.03). Men were more interested than women to learn their muscle mass amount, running speed, jump height, and ball throwing ability (all d ≥ 0.25, p ≤ 0.03). Men were more willing than women to receive strong electrical shocks of nerves or muscles, stay awake for 48 hours, cycle or run until exhaustion, compete against others in an obstacle course, complete strength training exercise that causes muscle soreness and stiffness, and take muscle-building supplements (all d ≥ 0.24, p ≤ 0.03). Women were more interested than men to learn their flexibility or joint range of motion (d = -0.26, p = 0.02). Women were more willing than men to complete online surveys about exercise experiences (d = -0.31, p = 0.01). Women were more willing than men to participate in stretching interventions and group aerobics interventions (both d ≥ -0.33, p ≤ 0.001). Compared to men, women rated the following items as more important when deciding to participate in exercise research: invasiveness of study procedures, possible side effects of study procedures, pain or discomfort associated with study procedures, amount of time required to complete study procedures, type of facility where the research is conducted, qualifications of the researchers and trust in them, confidence in their own abilities, mental and physical health status, and potential anxiety during testing (all d = -0.26, p ≤ 0.02). The results, which are consistent with previously published data on sex differences in preferences and dispositions, suggest sex differences in interests and willingness to participate in research might contribute to different proportions of male and female participants in exercise physiology and sports science research.

APSSG21.159
Alterations in the stool microbiome with polycystic ovary syndrome
Melanie Cree-Green1
1Pediatrics, University of Colorado

Polycystic ovary syndrome (PCOS) is a condition of excess testosterone in females leading to menstrual irregularities and increased risk of metabolic disease. Recent evidence indicates that the stool microbiome is different in women with PCOS. Microbiota differences may relate to testosterone concentrations and persist regardless of obesity. The role of dietary and medical therapies to shift the microbiome to decrease PCOS symptoms or metabolic disease is a potential therapeutic option.

APSSG21.160
Sex differences in body mass index associated with hypertension in chronic kidney disease patients under hemodialysis
Rodrigo Maranon1,2,3, Susana Lossi4, Juan Carlos Santos4,5
1Physiology, Faculty of Medicine - INSIBIO, National University of Tucuman, CONICET, 2Women’s Health Research Center, University of Mississippi Medical Center, 3Physiology, University of Mississippi Medical Center, 4Nephrology, Fresenius Medical Care Tucuman, 5Medicine, Faculty of Medicine, National University of Tucuman

Chronic kidney disease (CKD) patients under hemodialysis treatment have high cardiovascular complications associated with hypertension (HTN). Pieces of evidence of risk estimates indicate that at least two-thirds of the prevalence of hypertension is directly attributed to obesity.
However, the study of obesity and hypertension in this particular population has been little explored. Aims. a) to establish the prevalence of obesity and HTN in patients undergoing hemodialysis treatment, b) to identify a sex difference in the prevalence of HTN and obesity in CKD patients. Methods. From three hundred patients in HD of Fresenius Medical Care Tucumán, sixty-five CKD patients undergoing hemodialysis treatment (men=73, n=31, 63±2 years old vs. women=27, n=34, 61±2 years old) were selected according to exclusion criteria: diabetes, uncontrolled hypertension, and peripheral vascular disease. The study was reviewed and approved by the institutional Ethical Committee of the Ministry of Health of Tucuman. After a complete explanation of the study, written informed consent was obtained from all participants. We measured systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP). Also, we evaluated body weight, height, body mass index (BMI), and lean tissue (LTI), and fat tissue (FTI) indexes using the Body Composition Monitor (BCM) analysis system. Results. We found that SBP was higher in men than women (M-SBP: 149±2 mmHg vs. W-SBP: 141±3 mmHg, p<0.05). 77% of men and 60% of women had a BMI higher than 25 (overweight=OW). Furthermore, while 60% of men had overweight plus HTN, in women, overweight plus HTN were present in 26%. Interestingly, 29% of women had a normal BMI plus HTN, 34% had overweight plus normal BP, and 11% showed normal BMI plus normal BP. Contrary, in men, 17% had high BMI plus normal BP, and 9% in normal BMI plus HTN, and normal BMI plus normal BP, respectively (men vs. women, p=0.0137). The LTI was higher in men than women (W-LTI:10.7±0.3, n=34 vs. M-LTI: 13.7±0.6, n=31; p<0.05). Also, despite a higher percentage of overweight men vs. women, the values of FTI were similar (p:NS). However, when we analyze the FTI in patients according to the relationship between BMI and BP values, we observed a higher FTI in overweight women than men (W-OW+NSBP: FTI=20.5±2.3, n=12 vs. M-OW+NSBP: FTI=13.5±1.6, n=5; p<0.05) and W-OW+HTN: FTI=18.3±1.4, n=9 vs. M-OW+HTN: FTI=14.8±0.9, n=20; p<0.05). Conclusions. This data suggests that the pathophysiological mechanisms of HTN could be different in CKD men and women patients under hemodialysis treatment. While overweight women present a similar percentage between normal and high blood pressure, overweight men plus HTN were higher than those with normal blood pressure. Further investigations are necessary to determine the role of obesity on blood pressure regulation in CKD men and women in hemodialysis.

APSSG21.161

MicroRNA-21 Overexpression Ameliorates Cardiometabolic Outcomes In A Mouse Model of Polycystic Ovary Syndrome

Macy Cummings1, Alexandra Huffman1, Samar Rezzq1, Jelina Basnet1, Maryam Syed1, Jane Reckelhoff1, Licy Yanes Cardozo1,2, Damian Romero1
1Cell and Molecular Biology, University of Mississippi Medical Center, 2Medicine, University of Mississippi Medical Center

Purpose of Study: Polycystic Ovarian Syndrome (PCOS) is the most common endocrine disorder in women of reproductive age. PCOS is characterized by excess androgen production, ovulatory dysfunction, polycystic ovaries, and increased rates of obesity. Additionally, women with PCOS have an increased risk for cardiometabolic comorbidities. MicroRNAs, and microRNA-21 specifically, have been found to have dynamic expression in tissues involving PCOS pathophysiology. One of the key tissues involved includes the adipose tissue. Adipose expansion via hyperplastic growth instead of hypertrophic has been associated with improved cardiometabolic outcomes in both human and animal models. This study aims to determine the role of microRNA-21 overexpression on cardiometabolic outcomes in a mouse model of PCOS by comparison of frequency distributions of adipocyte size in three key fat depots. Methods Used: Three-week-old microRNA-21 overexpression (miR21OE) or wild-type (WT) C57BL/6J female mice were implanted subcutaneously with Silastic tubes containing the non-aromatizable androgen dihydrotestosterone (DHT, 8.0 mg) or placebo for 90 days. Weekly weights were taken and body composition was assessed via Echo-MRI after 90 days of treatment. Animals were sacrificed and white adipose tissue samples were then harvested for histological analysis. Adipocyte sizes were determined in hematoxylin and eosin stained adipose tissue (including mesenteric, retroperitoneal, and subcutaneous fat depots) sections using ImageJ software with the Adiposoft plugin. Two-way ANOVA and Kolmogorov-Smirnov statistical analyses were performed using GraphPad Prism 8. Summary of Results: Our results revealed that DHT significantly increased body weight in both WT and miR21OE animals mice compared to their controls. There was a significant increase in the Fat/Lean mass ratio in DHT-treated WT mice but this was not found in DHT-treated miR21OE mice. DHT treatment was shown to decrease the frequency of small adipocytes and increase the frequency of large adipocytes in all three fat depots analyzed. When comparing DHT-treated groups, the miR21OE animals showed an attenuation in the decrease of small adipocyte and in the increase of large adipocyte frequencies in all fat depots analyzed. Conclusions: In summary, miR21OE mice had an ameliorated response to the deleterious cardiometabolic outcomes associated with the hyperandrogenism observed in PCOS. This pronounced change in hyperplastic adipose expansion of the three fat depots of DHT-treated miR21OE animals is an indication to the potential mechanism by which these animals may have increased protection. Further studies will include investigating the molecular and...
cellular level differences in the modulation of adipogenesis and inflammation markers of the white adipose tissue.

APSSG21.163

Sympathetic transduction during hypoxia and hypercapnia in healthy young women and men
Ana Luiza Sayegh¹, Jui-Lin Fan², Lauro Vianna², Mathew Dawes³, Julian Paton⁴, James Fisher⁵
¹Manaaki Mānawa - The Centre for Heart Research, Department of Physiology, University of Auckland, ²NeuroVASQ - Integrative Physiology Laboratory, University of Brasilia, ³Department of Medicine, University of Auckland

The rhythmic discharge of the sympathetic nerves plays a key role in both the moment-to-moment and longer-term regulation of vasomotor tone and blood pressure (sympathetic transduction). Notable sex-differences exist in the sympathetic regulation of blood pressure at rest and in response to physiological stressors. Hyperoxic and hypercapnic conditions may occur in clinical situations (e.g., lung diseases) and are used experimentally to assess the peripheral and central chemoreflexes, respectively. We sought to explore whether there are sex-differences in sympathetic transduction during hypoxia and hypercapnia. Ten women (29±5yr, 22.8±2.4kg/m²) and ten men (30±7yr, 24.8±3.2kg/m², P=0.62, P=0.13) undertook randomized 5-min breathing trials of room-air (eucapnia), isocapnic hypoxia [10% inspired oxygen (O2)], hypercapnic hyperoxia [7% inspired carbon dioxide (CO2), 50% O2] and hypercapnic hypoxia (7% CO2, 10% O2). Muscle sympathetic nerve activity (MSNA; microneurography), blood pressure and cardiac output were continuously measured. Total peripheral resistance was calculated as mean blood pressure / cardiac output and expressed relative to body surface area (TPR). Sympathetic transduction was determined first, as the quotient of TPRi and MSNA (TPRi/MSNA) and second, as the peak diastolic blood pressure (DBP) response occurring ~5 s following a spontaneous MSNA burst using a signal-averaging technique. DBP responses to MSNA bursts were separately characterized following both single bursts (occurring in isolation) and multiple bursts (adjacent to at least one other burst). Women were studied during the first five days of their menstrual cycle (early follicular phase). Compared to eucapnia (0.095±0.025 and 0.079±0.021au), TPRi/MSNA was blunted during isocapnic hypoxia (0.070±0.028 and 0.057±0.016au), hypercapnic hyperoxia (0.034±0.014 and 0.036±0.016au) and hypercapnic hypoxia (0.019±0.007 and 0.034±0.016au, P=0.001) in women and men, respectively. Similarly, the magnitude of the peak rise in DBP following multiple MSNA bursts was also blunted during isocapnic hypoxia (3.87±1.88 and 4.42±2.00mmHg), hypercapnic hyperoxia (3.63±2.66 and 4.96±1.78mmHg) and hypercapnic hypoxia (4.73±2.79 and 4.59±1.61mmHg) compared to eucapnia (6.32±2.51 and 6.58±2.78mmHg; P=0.002) in women and men, respectively. The peak DBP following single MSNA bursts was not different between trials (P=0.16). Importantly, neither TPRi/MSNA (P=0.57) nor the peak DBP response to single (P=0.68) and multiple MSNA bursts (P=0.46) were different in women and men. In summary, sympathetic transduction is similarly blunted in women and men during isocapnic hypoxia, hypercapnic hyperoxia and hypercapnic hypoxia. Whether this remains the case following menopause, when ovarian hormone concentrations are attenuated, should be determined.

Support was provided by Auckland Medical Research Foundation, Health Research Council of New Zealand, The Sydney Taylor Trust and Auckland District Health Board.

APSSG21.164

Sex-based Differences in Immunological and Physiological Responses to Systemic Inflammation in Rodents
Caitlyn Clifford¹, Cara Campanaro¹, Shiloh Tackett¹, Zezhong He¹, Kofi-Kermit Horton¹, Dave Nethery¹, Yee-Hsee Hsieh¹, Frank Jacono¹,², Thomas Dick¹,³
¹Pulmonary, Critical Care and Sleep Medicine, Case Western Reserve University, ²Pulmonary, Critical Care and Sleep Medicine, Louis Stokes VA Medical Center, ³Department of Neurosciences, Case Western Reserve University

The physiologic and immunologic responses to systemic infection may differ between sexes. To test this possibility, we induced systemic infection in male (?) and female (?) Sprague Dawley rats (n=24? and 42?). We compared proinflammatory cytokine expression within body compartments (central and peripheral), and examined predictability of the ventilatory waveform, i.e. Ventilatory Pattern Variability (VPV), 12 hours after intraperitoneal implantation of fibrin pellets containing 0 (D0) or 100 x 106 (D100) E. coli colony forming units (cfu). Inoculated animals of each sex were compared to naive groups that did not receive intervention. At 12 hours, we euthanized rodents and collected aliquots of serum and tissue from caudal medulla, rostral medulla, pons, lungs, and liver to measure levels of IL-1β, IL-6, IL-17, KC and TNFα via LUMINEX assay. Whole body plethysmography was used to acquire physiological data of the ventilatory cycle for analysis before and 12 hours after inoculation. At baseline, the respiratory rate in naive rats did not vary with sex, but 12 hours after inoculation, frequency (fR) was greater in males (779.57±11.45 vs ?122.38±31.16 breaths/minute; p=0.005) and was greater for both sexes after receiving D100 compared to D0 (?64.46±6.17 and ?64.69±7.29 breaths/minute). VPV, assessed using the Non-linear Complexity Index (NLCI), has been shown to increase in certain disease states, indicating greater predictability in the respiratory pattern. For E. coli inoculated rats, NLCI was less in females than males (70.19±0.12 vs 70.28±0.10; p=0.009) 12 hours post-implantation. In response to E. coli infection, both male and female rats showed increased cytokine expression in different compartments. For example, a dose dependent response was evident in the expression of IL-1β in female lung tissue (?D0: 65.54±29.27 vs ?D100: 384.31±218.94 pg/mg, p=0.0001) and IL-6 expression in male lung tissue (?D0: 54.10±44.65 vs ?D100: 1819.64±394.21 pg/mg, p<0.0001). To assess if physiological differences in the ventilatory pattern were associated with the immunological response, we correlated changes in NLCI to changes in cytokine expression. We found that correlations between
cytokine expression and NLCI were stronger in peripheral than central tissue in male rats and that correlations were stronger in males than females. For example, in the liver, male IL-6 expression increased compared to females (2.39, 3.71), 2.37 [1.03, 3.71] increase in odds ratio for preeclampsia, respectively. Out of these three oxidative stress biomarkers, IMA shows the most promising diagnostic potential with sensitivity of 0.838 (95% CI: 0.739, 0.905) and specificity of 0.827 (95% CI: 0.671, 0.918). No heterogeneity was reported between the studies for any of the biomarkers (Higgins’ I2 = 0%). Conclusion This systematic review and meta-analysis identified IMA, UA and MDA as the most promising oxidative stress biomarkers associated with established preeclampsia. IMA as a biomarker of tissue damage exhibited the best diagnostic test accuracy. These oxidative stress biomarkers should be explored in future studies for their diagnostic utility in preeclampsia and even their potential in earlier pregnancy to determine risk.

APSSG21.165
The diagnostic potential of oxidative stress biomarkers for preeclampsia: systematic review and meta-analysis
Dinara Afrose1, Hao Chen1,2, Amal Ranasinghe3, Chia-Chi Liu4,5,6,7, Annemarie Henessy4,7, Philip Hansbro4,7, Lana McClements5
1School of Life Science, University of Technology Sydney, 2Centre for Inflammation, University of Technology Sydney, 3Faculty of Science, University of Colombo, 4The Heart Research Institute, University of Sydney, 5Kolling Medical Research Institute, University of Sydney, 6School of Medicine, Western Sydney University, 7School of Medicine, Western Sydney University, 8Campbelltown Hospital, Campbelltown Hospital, 9School of Life Sciences, University of Technology Sydney

Introduction Preeclampsia is multifactorial cardiovascular disorder of pregnancy that is lacking effective monitoring and treatment strategies. Adequate management of preeclampsia has been impeded due to its poorly understood pathogenesis. Mitochondrial dysfunction in placental cells leading to the generation of reactive oxygen species (ROS) and subsequent oxidative stress, have been implicated as one of the key pathogenic mechanisms. Several oxidative stress biomarkers have already demonstrated to be associated with the onset of preeclampsia. Objective In this study, we conducted a systematic review and meta-analysis to determine the most promising oxidative stress biomarkers associated with established preeclampsia. Materials and methods The following databases were searched systematically to identify studies assessing the diagnostic potential of oxidative stress markers in preeclampsia: PubMed, ScienceDirect, ResearchGate and PLOS (1900 to March 2021). The included studies were evaluated for the quality utilising Quality Assessment for diagnostic Accuracy Studies-2 (QUADAS-2) tool. Random-effects model forest plots and hierarchical summary of receiver operating characteristic (HSROC) curves were generated to determine diagnostic test accuracy (DTA) in R (4.03) using the ‘mada’ package. Heterogeneity of Higgins’ I2 and Cochran’s Q were reported measuring the robustness of identified biomarkers. Diagnostic biomarkers were evaluated from three or more independent studies. Result Based on our search, 9 studies with 343 preeclampsia cases and 354 normotensive controls were included in the meta-analyses. Three oxidative stress biomarkers including ischemia modified albumin (IMA), uric acid (UA) and malondialdehyde (MDA) were identified as the most promising diagnostic biomarkers of preeclampsia. IMA, UA and MDA were associated with 3.38 [2.23, 4.53], 3.05 [2.39, 3.71], 2.37 [1.03, 3.71] increase in odds ratio for preeclampsia, respectively. Out of these three oxidative stress biomarkers, IMA shows the most promising diagnostic potential with sensitivity of 0.838 (95% CI: 0.739, 0.905) and specificity of 0.827 (95% CI: 0.671, 0.918). No heterogeneity was reported between the studies for any of the biomarkers (Higgins’ I2 = 0%). Conclusion This systematic review and meta-analysis identified IMA, UA and MDA as the most promising oxidative stress biomarkers associated with established preeclampsia. IMA as a biomarker of tissue damage exhibited the best diagnostic test accuracy. These oxidative stress biomarkers should be explored in future studies for their diagnostic utility in preeclampsia and even their potential in earlier pregnancy to determine risk.

APSSG21.166
Impact of Maternal Alcohol Intake on Development and Long-term Health of Offspring
Kate Denton1
1Physiology and Biomedical Discovery Institute, Monash University

Medical guidelines around the world generally recommend abstinence from alcohol while pregnant. Alcohol (ethanol) is a known teratogen and when pregnant, or planning a pregnancy, not drinking is the safest option. Concerningly however, many women still expose their unborn child to the adverse effects of alcohol during pregnancy. A staggering 50-60% of women consume alcohol around the time of conception. Studies also indicate that ~25% of women drink in the first month of gestation, but not later in pregnancy. However due to associated stigma this is thought to be an underestimate. The detrimental effects of high levels of alcohol intake during pregnancy on infants, known as fetal alcohol syndrome are serious and include marked neurodevelopmental disorders. However, less well known is the impact of even mild to moderate of alcohol consumption on fetal development and the longterm risk of disease in adulthood. This review will summarise our work in animal models examining the impact of fetal alcohol exposure on renal and cardiovascular development and function. Our findings show that a single ‘binge’ or a glass of alcohol a day throughout pregnancy alters fetal development with longterm consequences for cardiovascular and renal disease, with differential effects in male and female offspring. In conclusion, no level of
alcohol intake during pregnancy has been shown to be safe.

APSSG21.168
ACE2 contributes the normal regulation of arterial pressure and immunity in females of reproductive age
Katrina Mirabito Colafella1, Lucinda Hilliard Krause1, Chris Tikellis2, Robert Widdop3, Antony Vinh4, Kate Denton1
1Physiology, Monash University, 2Baker, Baker Heart and Diabetes Institute, 3Pharmacology, Monash University, 4Physiology, Anatomy and Microbiology, La Trobe University

Hypertension and cardiovascular disease are age and sex dependent. These differences may, in part, be mediated by the depressor/pressor balance of the renin angiotensin system. Here we determined the role of angiotensin converting enzyme 2 (ACE2) in the regulation of arterial pressure in females of reproductive age and investigated whether targeting deficits in ACE2-generated angiotensin (Ang)-(1-7) restores the normal regulation of arterial pressure during pregnancy. Mean arterial pressure (MAP) was measured via telemetry in 14 week old wild-type (WT) and ACE2 knockout (ACE2-KO) male mice and WT and ACE2-KO female mice receiving vehicle or the MasR agonist, AVE-0991 (24 μg/kg/min s.c) prior to and during pregnancy. FACS analysis was used to determine circulating immune cell activation and infiltration into kidneys (baseline and Gd18) and placentae (Gd18). Basal MAP was lower in WT females than ACE2-KO females, WT males and ACE2-KO males (91±2 vs 100±1, 98±1 and 102±2 mmHg, respectively; all P<0.05 vs WT female). In ACE2-KO females, AVE-0991 lowered basal MAP by 5±1 mmHg (P=0.03). In WT females, MAP decreased during pregnancy reaching a nadir at Gd9 before returning to pre-conception levels during late gestation. In contrast, in ACE2-KO mice, MAP increased significantly during late gestation (P<0.0001 vs WT) and this effect was prevented by AVE-0991 (P>0.05 vs vehicle). This effect of AVE-0991 on MAP was due to changes in diastolic rather than systolic arterial pressure. ACE2-KO mice had smaller litters but greater birth/pup weight than WT mice. AVE-0991 normalised litter size and birth/pup weight in ACE2-KO mice to that observed in WT mice. Circulating and renal T- regulatory cells were lower in non-pregnant and pregnant female and male ACE2-KO mice than their WT counterparts. Treatment with AVE-0991 did not alter the proportion of T-regulatory cells. These data indicate that ACE2 plays an important role in the regulation of arterial pressure and immunity in females of reproductive age. A corollary of this is that deficits in ACE2-generated Ang-(1-7) may contribute to an increased risk of hypertension in non-pregnant and pregnant premenopausal females and therefore may be a novel therapeutic target.

APSSG21.169
Orchietomy exacerbates lung mechanical consequences of intermittent hypoxia in C57BL/6J mice
Gauthier Ganouna-Cohen1,2, Fatemeh Khadangi1,2, François Marcouiller1, Ynuk Bosse1,2, Vincent Joseph1
1Département de Pédiatrie, Centre de recherche de l’Institut Universitaire de Cardiologie et Pneumologie de Québec, 2Faculté de Médecine, Université Laval

Sleep apnea (SA) is characterized by airway obstruction and intermittent hypoxia (IH). There are growing concerns that SA worsens pulmonary pathologies and IH induces oxidative stress and inflammation associated with an increase in airway resistance. Male SA patients have low circulating testosterone levels and the severity of SA in overweight patient is negatively correlated with testosterone levels, but the interactions between testosterone and IH are unknown. Since testosterone has been reported as an antioxidant and anti-inflammatory hormone, we tested the hypothesis that low testosterone aggravates the pulmonary responses to IH. For this, we used intact (Sham) or orchiectomized (ORX) male mice (C57BL/6J) exposed to IH (14 days, 12h/day, 10 cycles/h, 6% oxygen nadir) or to normoxia (Nx : 14 days). Mice were used to measure tidal volume using whole body plethysmography, then anesthetized, tracheotomized and paralyzed for measurements of respiratory mechanics with the flexiVent system (SCIREQ). On 2 occasions (n = 5/group and n = 7/group) we measured several mechanical parameters, including respiratory system resistance (Rrs), respiratory system elastance (Ers), Newtonian resistance (Rn: which is a surrogate for the resistance of the large airways), tissue resistance (G) and tissue elastance (H), which were expressed as normalized to the Sham Nx group. On the first occasion (n=5/group) we also used the partial pressure-volume maneuver to evaluate quasi-static compliance (Cst) and inspiratory capacity (IC). On the second occasion (n=7/group), we also measured: 1- the degree of airway responsiveness by monitoring the changes in several mechanical parameters caused by incremental doses of methacholine (0 to 100 mg/ml in PBS); 2- total and differential cell counts in broncho-alveolar lavages (BAL); and 3- lung volume by water displacement. Orchietomy increases Rrs in mice exposed to Nx, which is mostly driven by an increase in Rn. ORX mice also have a lower response to methacholine compared to Sham mice (for matched IH or Nx exposure) for all mechanical parameters. IH decreases the changes in Ers and H in response to methacholine in both Sham and ORX mice. It also reverses the increases in Rrs and Rn caused by orchietomy. ORX IH mice also demonstrate greater tidal volume, inspiratory capacity, quasi-static compliance and lung volume compared to other groups, as well as a higher number of inflammatory cells in BAL via an increase of lymphocytes. In conclusion, while orchietomy increases large airway resistance in mice exposed to Nx, it decreases airway responsiveness to methacholine in mice exposed to both Nx and IH. IH in Sham mice also decreases airway responsiveness, at least when the changes in Ers and H are used to monitor the response. The greatest alterations arises when orchietomy and IH are combined. More specifically, although IH reverses the increase in large
airway resistance caused by orchiectomy, it seems to amplify airway inflammation and lung enlargement induced by intermittent hypoxia, funded by CIHR and Réseau en Santé Respiratoire du Québec.

**APSSG21.170**

**Orchidectomy exacerbates breathing instability induced by intermittent hypoxia on C57BL/6J mice**

Gauthier Ganouna-Cohen1, François Marcouiller1,2, Vincent Joseph1,2

1Département de Pédiatrie, Centre de recherche de l’Institut Universitaire de Cardiologie et Pneumologie de Québec, 2Faculté de Médecine, Université Laval

Sleep apnea (SA) is characterized by airway obstructions leading to intermittent hypoxia (IH). In rodents, IH exposures induce a strong oxidative stress in the peripheral chemoreceptors (the main oxygen sensors), which increase their activity and lead to instability of the respiratory control system, ultimately increasing the frequency of apneas during sleep. In men, SA patients have low circulating testosterone levels and the severity of SA in overweight patients is negatively correlated with testosterone levels. Because testosterone has been shown to reduce oxidative stress in some animal and clinical models, we tested the hypothesis that testosterone modulates the breathing instability responses to IH. For this, we used intact (Sham) or orchiectomized (ORX) male mice (C57BL/6J) exposed to IH (14 days, 12h/day, 10cycles/h, 6% oxygen nadir) or to normoxia (Nx : 14 days in room air). We then used whole body plethysmography on freely behaving and non-anesthetized mice to evaluate the stability of the respiratory control system by measuring the frequency of sighs (deep inspiration followed by rapid expiration), the frequency of spontaneous and post-sigh apneas (at least 2 missed breaths), the length of apneas and whether several apneas are repeated after a sigh. ORX increases the frequency of sighs (Sham Nx 19.4 ± 4.4 vs ORX Nx 26.6 ± 3.7; p-value = 0.0003) and this is abrogated when the ORX mice is exposed in IH (20.1 ± 3.6). IH increases the proportion of sighs inducing an apnea (Sham IH 55 ± 13% vs Sham Nx 22 ± 12%; p-value < 0.0001), the frequency of post-sigh apneas (Sham IH 18.2 ± 11.0 vs Sham Nx 7.5 ± 5.3; p-value = 0.0040) and the mean apnea length (Sham IH 1.39 ± 0.20 seconds vs Sham Nx 0.89 ± 0.17; p-value < 0.0001). IH exposures in ORX mice exacerbate the effects of IH by increasing the proportion of sighs inducing an apnea (Sham IH 55.0 ± 12.6% vs ORX IH 66.2 ± 9.5%; p-value = 0.043) and by increasing the mean apnea length (Sham IH 1.39 ± 0.20 s vs ORX IH 1.56 ± 0.20 s; p-value = 0.039). ORX IH mice also have a significantly higher proportion of sighs leading to several apneas (ORX IH 52 ± 19 % vs Sham IH 26 ± 14 %; p-value = 0.0005 // vs ORX Nx 17 ± 17%; p-value < 0.0001 // vs Sham Nx 19 ± 17%; p-value < 0.0001). We conclude that ORX exacerbates the effects of IH on the respiratory control system and could indicate that in Sham mice testosterone reduces oxidative stress in peripheral chemoreceptors, contributing to reduce their activity therefore lowering respiratory instabilities recorded during sleep.

**APSSG21.172**

**Sex-specific effects of indomethacin-induced inflammatory bowel disease on mitochondrial function**

Ngoc Hoang1, Karen Brook1, Kristin Edwards1

1Cell and Molecular Biology, University of Mississippi Medical Center

Introduction: Inflammatory bowel disease (IBD) is a term used to describe disorders that involve chronic inflammation of the digestive tract, such as Crohn’s Disease and Ulcerative Colitis. IBD currently effects three million people in the United States, with many going undiagnosed. Women appear to have more severe and recurring symptoms of IBD compared to men, most likely due to hormonal fluctuations. A few IBD patient studies have shown alterations in mitochondrial function. Our goal is to determine the role mitochondrial dysfunction and mitochondrial reactive oxygen species (mtROS) in the development of IBD in males and females. Methods: Male and female rats 8-10 weeks of age received two injections of indomethacin (7.5 mg/kg) exactly 24 hours apart. The peak of the disease is between day 2 and 3 post-injection. A tissue homogenate containing colon mitochondria was prepared from isolated, washed colons. Mitochondrial respiration was measured using glutamate/malate, succinate, oleate, or octanoate as substrates. Mitochondrial reactive oxygen species (mtROS) was measured simultaneously with mitochondrial respiration using an Oroboros Fluorespirometer. Activities of individual mitochondrial electron transport complexes were also measured. Citrate synthase activity was measured as a marker for mitochondrial content. Results: In the indomethacin-induced Crohn’s Disease group (CD), rats showed a significant decrease in body weight compared to controls. Females show a 12% loss (p=0.0002) and males a 6% loss (p=0.0324). Female CD rats showed a significant decrease in mitochondrial respiration compared to controls using glutamate/malate (p=0.0014), succinate (p=0.0002), oleate (p=0.0112), or octanoate (p=0.0018). Male CD rats only showed a significant decrease using glutamate/malate (p=0.0392) and succinate (p=0.0162). CD rats showed a significant increase in mtROS production compared to controls. Female CD rats showed a 4-fold increase (p<0.0001) while males showed a 2.5 fold increase (p=0.0008). Female CD rats showed a significant decrease in each of the individual mitochondrial electron transport complex activities (I p=0.0002, II p=0.0002, III p=0.004, and IV p<0.0001) while males only showed a significant decrease in complex II (p=0.0249), III (p=0.0431), and IV (p=0.0054) activities. Both male and female CD rats showed a significant decrease in mitochondrial content compared to controls (females p=0.0051, males p=0.0297). Conclusion: Alterations to mitochondrial function, mtROS production, and mitochondrial content were observed in the indomethacin-induced rat model of Crohn’s Disease suggesting a link between mitochondrial dysfunction and Crohn’s Disease. Females showed more significant changes in body weight and mitochondrial dysfunction compared to males. This may explain the observed differences in symptom severity between men and women. Further research is needed in this area. This study provides a better understanding of the role mitochondria in the
development of IBD and an avenue for the development of strategies to re-establish normal mitochondrial function that could provide more options for preventive and therapeutic interventions for IBD. Supported by NIH grants: P20PGM121334

APSSG21.173  
Sex Differences in Transplantation  
Joel Neugarten1  
1Nephrology, Montefiore Medical Center

Donor and recipient sex influence many aspects of transplantation. However, the precise nature of these interactions and the underlying pathogenic mechanisms underlying them remain unclear. Hormonal and chromosomal differences between the sexes influence immunologic responsiveness as well as the transport and metabolism of immunosuppressive drugs, which in turn may influence allograft survival. The role of H-Y allograft immunity remains controversial but may play a significant role in the outcome of stem cell, corneal and renal transplantation. In renal transplantation, size mismatch between donor organ nephron supply and recipient metabolic demand may lead to nephron underdosing with adverse effects on allograft survival. A similar phenomenon may influence survival after cardiac and liver transplantation. Moreover, recent investigations point to significant interactions between donor and recipient sex mismatch, organ size mismatch and donor and recipient age in influencing graft and patient survival. The complexity of these interactions may explain disparities in reported data. In addition, compliance with immunosuppressive agents differs between the sexes, which may also impact outcome. Lastly, disparities in access to transplantation between the sexes reflect psychosocial and economic factors.

APSSG21.174  
Obesity, Pregnancy, and Hypertension  
Joey Granger1  
1Physiology, University of Mississippi Medical Center

Preeclampsia (PE) is estimated to affect 5-7% of all pregnancies in the U.S. and approaches rates of 15% in African-Americans. Despite its position as a leading cause of maternal death and major contributor to maternal and perinatal morbidity, the only effective treatment for PE is early delivery (removal of the placenta). Furthermore, the incidence of PE has increased by 40% over the last several decades as a result of a significant increase in risk factors such as obesity. Obesity is a major epidemic in developed countries and in the U.S. the percentage of women who are obese or overweight has increased almost 60% in the last 30 years. While the relationship of obesity to increase Type 2 diabetes and cardiovascular disease is well recognized, it also has important implications for pregnancy outcomes. There is compelling evidence that obesity markedly increases the risk of developing PE. Indeed, the rate of PE is 4 to 5 times higher in severely obese pregnant women. Despite the fact that obesity is the leading attributable risk for PE in developed countries, the pathophysiological mechanisms whereby obesity increases the risk for developing PE are unclear. Several lines of evidence indicate that obesity may lead to PE by impacting many sites in the pathway that links placental ischemia and hypertension. The overall goal of my presentation will be to discuss how various obesity related metabolic factors may impact spiral artery remodeling, angiogenic balance, and endothelial and vascular function in pregnancy. The full elucidation of these mechanisms will hopefully lead to a more complete understanding of the etiology of preeclampsia and lead to successful therapeutic intervention through the targeted disruption of novel pathways.

APSSG21.176  
Sex-dependent Effects of Immunosuppressants on Hypertension  
Rodrigo Maranon1,2,3, Jane Reckelhoff4,5, Mohadetheh Moulanan,6  
1Physiology, Faculty of Medicine, INSIBIO, National University of Tucuman, CONICET, 2Physiology, University of Mississippi Medical Center, 3Women’s Health Research Center, University of Mississippi Medical Center, 4Cell and Molecular Biology, University of Mississippi Medical Center, 5Mississippi Center of Excellence in Perinatal Research, University of Mississippi Medical Center, 6Department of Psychiatry and Human Behavior, University of Mississippi Medical Center

In the last 20 years, the role of the immune system and immunosuppression causing hypertension have been studied. Chronic inhibition of the immune system attenuates hypertension and renal damage in several animal models of hypertension. However, whether there is a sex-dependent response to immunosuppressants has not received much attention. The present study tested the hypothesis that there is a sex-dependent difference in mean arterial pressure (MAP) and renal injury responses to different immunosuppressants [tacrolimus (FK-506) and mycophenolate mofetil (MMF)] in young male and female spontaneously hypertensive rats (SHR). Young male (YM) and female (YF) SHR, 3 months of age (n= 4/group) received tacrolimus (0.25 mg/kg/day i.p.), MMF (20 mg/kg/day, i.p.), or placebo (P) for 14 days. MAP (by radiotelemetry), renal injury (albuminuria and proteinuria), and urinary nitrate/nitrite (NOx), index of total body nitric oxide, were assessed. Tacrolimus increased MAP in males (YM-P: 143±3 vs. YM-T: 163±4 mmHg, p<0.05) but had no effect in females (YF-P: 132±3 mmHg vs. YF-T: 133±2 mmHg, p=NS). In contrast, MMF significantly reduced MAP in both males (YM-P: 153±2 mmHg vs. YM-MMF: 140±2 mmHg, p <0.05) and females (YF-P: 128±2 mmHg vs. YF-MMF: 113±2 mmHg, p<0.05). Albuminuria and proteinuria were significantly increased in males (YM-P: 1.49±0.08 mg/24h vs. YM-T: 0.7±0.04 mg/24h and YM-P: 2.08±0.3 mg/24h vs. YM-T: 3.3±0.1 mg/24h, respectively) after tacrolimus administration but without significant changes in females (YF-P: 0.28±0.07 mg/24h vs. YF-T: 0.3±0.05 mg/24h and YF-P: 1.03±0.3 mg/24h vs. YF-T: 1.5±0.4 mg/24h, p=NS). In contrast, MMF significantly improved...
albuminuria and proteinuria in males (YM-P: 1.51±0.06 mg/24h vs. YM-MMF: 0.93±0.03 mg/24h and YM-P: 2.67±0.1 mg/24h vs. YM-MMF: 1.14±0.4 mg/24h, p<0.05, respectively) and females (YP-F: 0.27±0.02 mg/24h vs. YF-MMF: 0.1±0.04 mg/24h and YP-F: 1.15±0.1 mg/24h vs. YF-MMF: 0.81±0.1 mg/24h, respectively, p<0.05). Interestingly, male SHR excreted higher baseline NOx compared to females (YM-P: 3.6±0.14 μmol/24h/kg body weight vs. YF-P: 2±0.5 μmol/24h/kg, p<0.05), and NOx levels were further increased after Tacrolimus in males (p<0.05) and after MMF in both males and females (p<0.05). These data suggest a sex difference in responses to tacrolimus and MMF in young male and female SHR. Further investigations are required to examine the contribution of specific immune cell types to the hypertension in young male and female SHR. Sullivan and colleagues showed previously that hypertension in male and female SHR is inversely correlated with intrarenal regulatory T cells. Moreover, our data propose that various immunosuppressants may have differential effects on men and women; and management of hypertension by immunosuppressive therapy and post-transplant hypertension should be specified by gender.

APSSG21.177
Sex-dependent role of adipose tissue HDAC9 in diet-induced obesity and metabolic dysfunction.
Samah Ahmadieh1, Abdellrahman Zarzour2, Brandee Goo1, David Kim2, Mourad Ogbi1, Ha Won Kim2, Neal Weintraub2
1Department of Medicine and Vascular Biology Center, Medical College of Georgia at Augusta University, 2Division of Cardiology, and Vascular Biology Center, Medical College of Georgia at Augusta University

Objective Obesity is a major risk factor for both metabolic and cardiovascular disease. We reported that histone deacetylase 9 (HDAC9) is upregulated in adipose tissues of mice during diet-induced obesity (DIO), and global deletion of HDAC9 protected mice against DIO-associated metabolic dysfunction. Here, we compared adipose tissue expression of HDAC9 in male versus female mice and tested the impact of adipose-specific HDAC9 gene deletion on DIO. Methods We crossed HDAC9 floxed mice with adiponectin-cre mice to generate adipose-specific HDAC9 knockout mice (AdipCre-HDAC9), which exhibited selective downregulation of HDAC9 expression in mature adipocytes. Male and female mice fed high fat diet (HFD) or standard chow diet (CD) from 8-12 weeks of age were housed in thermoneutral housing (28-30°C) environment. Mice underwent whole body animal calorimeter and metabolic testing, HDAC9 gene and protein expression was measured by Western blot and qRT-PCR. Adipose tissues were fractionated to quantify HDAC9 gene expression in mature adipocytes (MA) and stromal vascular (SV) fraction. Adaptive thermogenesis was tested by placing mice at 4°C for three hours and measuring core body temperature. Results Adipose tissue HDAC9 protein expression was significantly higher in males than in females fed both CD and HFD. Furthermore, HDAC9 expression was preferentially expressed in the SV fraction, as opposed to MA, in male mice. Consistent with this finding, female, but not male AdipCre-HDAC9 mice exhibited reduced body weight, improved insulin sensitivity and glucose tolerance on HFD. Female mice also had less visceral adipose tissue weight and adipocyte hypertrophy on HFD, whereas no difference was observed in liver weight. Furthermore, adipcre-HDAC9 female mice had significantly higher energy expenditure and oxygen consumption as assessed by calorimetry testing despite similar food intake and activity. 4°C cold challenge for up to three hours demonstrated that Adipcre-HDAC9 female mice maintained core body temperature significantly more efficiently as compared to wild-type mice. Conclusion Adipose-specific HDAC9 gene deletion protected female, but not male, mice against DIO-associated metabolic dysfunction by improving insulin sensitivity, energy expenditure, and adaptive thermogenic capacity. The protective effects of adipocyte HDAC9 deletion seen only in female mice may be explained by preferential expression of HDAC9 in the adipose tissue SV fraction of male mice. This study was funded by grants HL124097, HL126949, HL134354, AR070029 and AG064895 (Neal L. Weintraub) from the National Institutes of Health.

APSSG21.178
Chest Pain in a Young Transgender Woman: A Case Report
Paul Connelly1, Paul Rocchioccioli2, Christian Delles1
1Institute of Cardiovascular and Medical Sciences, University of Glasgow, 2Golden Jubilee National Hospital, NHS

Case Presentation A transgender woman in her mid-twenties was admitted to hospital with dull, central chest pain without radiation. The pain settled with no intervention and was not associated with any shortness of breath or autonomic features. Current prescriptions included estradiol valerate 5 mg daily and triptorelin 11.25 mg every four months. On examination lungs were clear to auscultation, heart sounds were pure and there was no sign of peripheral oedema. Investigations demonstrated an elevated high sensitivity troponin of 46 ng/L that increased to 48 ng/L (≤16 ng/L [females]; ≤34 ng/L [males]). D-dimer assay was negative and inflammatory markers were satisfactory (C-reactive protein 6 mg/L). Echocardiogram demonstrated normal chamber sizes, good ventricular function, and no valvular abnormalities. A cardiac MRI was performed that demonstrated no evidence of myocarditis. A similar episode consisting of chest pain and troponemia occurred two years prior to this. Coronary angiography at this time demonstrated normal caliber coronary vessels with no evidence of calcified or non-calcified plaque. Although a degree of diagnostic uncertainty persists, these episodes likely represent the diagnosis of myocardial infarction with nonobstructive coronary arteries (MINOCA). Discussion The effect of gender-affirming hormone therapy on long-term cardiovascular health in people who are transgender is broadly unknown. Limited data suggests that transgender women who use estradiol may be at higher risk of venous thromboembolism, ischemic stroke and potentially myocardial infarction. There is an absence of guidelines for
the investigation or management of people who are transgender than develop cardiovascular diseases. Indeed, it is uncertain what sex-specific diagnostic troponin threshold should be used for the diagnosis of myocardial injury in people who are transgender. Research is urgently required to clarify the role of gender-affirming hormone therapy in cardiovascular conditions, such as MINOCA, to facilitate the development of evidence-based guidance and equitable health care for transgender individuals.

APSSG21.179
Estrogen Receptor α modulates the action of the lupus susceptibility locus Sleiβ
Jared Graham1; Karen Gould1
1Genetics, Cell Biology, and Anatomy, University of Nebraska Medical Center

Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by immune cell hyperactivation, loss of immune tolerance, and the production of anti-nuclear autoantibodies. 90% of lupus patients are women, and this sex bias is due, at least in part, to estrogens, which promote lupus pathogenesis. Our lab has shown that estrogen receptor alpha (ERα) mediates the effect of estrogens in lupus and that ERα acts in a B cell intrinsic manner to promote SLE pathogenesis. Genetic factors also contribute to lupus. One major lupus susceptibility locus, Sleiβ, controls immune cell hyperactivation, loss of tolerance, and autoantibody development. The impact of Sleiβ is much stronger in females than males, and we showed that this female sex bias is abrogated by ERα deficiency. The mechanism by which ERα modulates the action of Sleiβ is not known. Sleiβ enhances lupus by dampening BCR signaling and thereby allowing autoreactive B cells to evade tolerance induction mechanisms. Sleiβ consists of linked polymorphisms in the SLAM gene cluster resulting in differential splicing of the Ly108 (Slamf6) gene. Slamf6 interacts with phosphatases that negatively regulate B cell receptor (BCR) signaling, and this activity is amplified in mice carrying Sleiβ. In some cells, ERα interacts with SHP phosphatases and modulates their activity, but these effects have not been shown in B cells. Furthermore, in other cells, ERα is known to interact with and modulate the activity of other molecules that also participate in the BCR signaling cascade, such as MAP kinases. We hypothesize that ERα synergizes with Sleiβ by interacting with and modulating the activity of proteins in the BCR signaling cascade. To begin to test this hypothesis, we used in vitro BCR activation assays followed by flow cytometry and western blotting to assess activation of the BCR signaling cascade in B cells from male and female B6.Sleiβ.ERα+/+ and B6.Sleiβ.ERα−/− mice. Through these studies, we found that ERα dampens BCR signal strength, as measured by BCR activation-induced calcium flux, in B cells of both male and female B6.Sleiβ.ERα+/+ mice. These data suggests that ERα, like Sleiβ, may promote autoimmunity by attenuating BCR signal strength. We also examined the impact of ERα on BCR-induced activation of kinases within the BCR cascade. These studies revealed that ERα promotes greater and more sustained phosphorylation of p38 MAPK after BCR engagement and that this effect is more dramatic in females. As p38 MAPK phosphorylation promotes proliferation in B cells, we investigated the impact of ERα on BCR-induced proliferation in vitro. B cells from B6.Sleiβ.ERα+/+ females proliferated more robustly upon BCR activation than B cells from either B6.Sleiβ.ERα−/− females or B6.Sleiβ.ERα+/+ males. Altogether, our results indicate that ERα not only dampens BCR signal strength, likely thereby allowing autoreactive B cells to survive, but also promotes more robust proliferation after BCR engagement. This enhanced proliferation may be mediated via ERα-dependent augmentation of p38 MAPK activation. Future studies will include further examination of the impact of ERα on the activation of kinases and phosphatases in the BCR cascade as well as exploration of physical interactions between ERα and p38 MAPK and other signaling components in B cells.

APSSG21.180
Sex, Aging and Lung Disease
Y S Prakash1
1Anesthesiology and Perioperative Medicine, Mayo Clinic

Asthma is more common in pre-pubescent males, but increases in women and aging males, highlighting roles for sex steroid effects in airways, beyond any intrinsic differences in the structure and function of the lung or respiratory system. Separately, aging is associated with an increase in asthma (asthma of the elderly), particularly in women, that is immunologically different from asthma in younger individuals, involves airway fibrosis, and is more severe and relatively steroid resistant. Thus, understanding of the relationships between sex, aging and airway disease is clinically significant. A limitation to understanding how sex steroids influence asthmatic airways is their complex, cell- and context-dependent effects, only further complicated by age-related changes and life-long exposures. Effects of sex steroids on bronchial epithelium and airway smooth muscle (ASM) are relevant, given their roles in modulating airway tone and structure. The focus should probably be on estrogens given increase in asthma among young women that is reduced post-menopausally, but returns with the use of estrogen replacement, while progesterone does not modulate estrogen effects. Whether and how estrogens are protective or deleterious in asthmatic airway a topic of intense research. Emerging data show that 1) Human epithelium and ASM express the receptors ERα and ERβ, while aging ASM shows reduced ERα; 2) Estrogens non-genomically reduce ASM calcium responses to agonist and increase cAMP, overall aiding bronchodilation effects retained with aging; 3) Asthmatic or cytokine-exposed ASM express more ERβ than ERα, suggesting a shift in ER profile; 4) With inflammation, ERα functionality is enhanced, and has a suppressive effect on [Ca2+], cell proliferation and fibrosis: effects that are retained in aging; 5) ERα and ERβ signaling diverge in inflamed or asthmatic airways. In mouse models of allergic asthma, ASM ERβ is increased (less so in epithelium), while conversely absence of ERβ results in greater airway thickening, reactivity, and ASM expression of Ca2+ regulatory and fibrosis proteins. Conversely, ERβ-specific
agonists blunt airway reactivity and remodeling, and ASM expression of fibrosis proteins. Overall, these emerging data highlight the need for further research into differential effects of estrogen receptors particularly with aging, and how they affect the airway. Here, ERβ may have an “anti-inflammatory” role, while ERα is pro-inflammatory. Accordingly, in the aging airway of women, adverse effects of hormone replacement on asthma may involve a differential effect on ERα that needs to be better understood.

APSSG21.181
Estradiol protects females from the deleterious effects of Interleukin-17A on neurovascular coupling
Jessica Youwakim, Diane Vallerand, Helene Girouard
Pharmacology and Physiology, Université de Montréal

Introduction: Sex and menopausal status exerts great influence on the pathogenesis of cerebrovascular diseases. These conditions have also been associated with an imbalance between pro- and anti-inflammatory status in favor of a greater production of pro-inflammatory cytokines. We recently observed that interleukin-17A (IL-17A) decreases cerebral blood flow responses to whisker stimulations in male mice and therefore compromise the dynamic link between neuronal activity and local cerebral blood flow; a phenomenon called neurovascular coupling (NVC). However, females are protected from cerebrovascular alterations induced by IL-17A. Our hypothesis thus stipulates that estradiol protects females from NVC impairment caused by IL-17A. Method: In this study, C57BL/6J male and female mice were perfused with IL-17A through an osmotic pump. Female mice were divided into three subgroups: non-OVX female, OVX female and OVX female receiving estradiol. Cerebral blood flow changes in response to whiskers stimulations was assessed by laser Doppler flowmetry. Results: IL-17A administration decreases the vascular responses to whiskers stimulations in male compared to non-OVX mice (p<0.05). In OVX mice, this protection is lost (p<0.05) but restored by an estradiol treatment (p<0.05). Conclusion: These results suggest that females are protected from the deleterious effects of IL-17A on NVC due to their higher estradiol levels. Thus, treatment with estradiol could prevent cerebrovascular dysfunction induced by pro-inflammatory conditions involving IL-17A in menopausal women.

APSSG21.182
Women & Stroke: The Importance of Age and Sex
Louise McCullough
Neurology, McGovern Medical School

Despite several advancements in stroke care, disparities continue to exist with regard to sex differences in cerebrovascular disease. These sex differences are due to a combination of several factors, many of which are unique to the female sex. Some of these unique factors, such as pregnancy and menopause, are related to hormonal changes seen throughout the female life cycle. Hormonal fluctuations, which impact the protective effects of the female sex hormones, can be induced by the use of hormonal contraception. Other risk factors, although present in both sexes, have a higher prevalence in elderly females, such as atrial fibrillation leading to cardioembolic strokes. Similarly, differences in premorbid modified Rankin Scale have an impact on the differences in stroke outcome between the two sexes. Clinical research aimed toward highlighting potential causes of these disparities has shown important differences in the calibers of blood vessels in the cerebral circulation between the two sexes, whereas basic science research has shown differences in circulating endothelial progenitor cell pools between males and females, with higher levels being more protective. With the increasing awareness of these sex differences, future research is being geared toward gender-specific modes of therapy, focusing on the molecular level, as well as the individual patient. [Dr. McCullough is supported by the NIH (National Institute of Neurological Disorders and Stroke) and the American Heart Association (AHA)].

APSSG21.183
Estrogen regulates voluntary running behavior in rats
Victoria Mathis, Lauren Points, Brock Pope, Lori Winter, Sarah Clayton, LiLian Yuan
Physiology and Pharmacology, Des Moines University

Despite the myriad social and health benefits of exercise, humans display heterogeneous levels of participation. Significant progress has been made in identifying molecular events, systems, and mechanisms that support exercise’s beneficial effects, but it is not clear yet what regulates exercise behavior itself or serves to maintain prolonged chronic exercise behavior. The human heterogeneity in voluntary exercise can be recapitulated in a rodent model of wheel running, a behavior with high rewarding properties. While rats given continuous access to running wheels all began with low running activity, a 3-week training program dramatically increased running activity and uncovered a wide range of individual differences in running behavior. In addition, we have also identified intriguing sex differences in this model. Compared to age-matched males, female rats exhibited significantly higher levels of average daily running. When assessing individual female rats’ running behavior, we also observed a repetitive peak-valley pattern of running activity, with peaks coinciding with the proestrus stage (highest estrogen level) in the rat estrous cycle. Bilateral ovariectomy (OVX) not only lowered their overall running activity, but also completely eliminated cyclical variations. Furthermore, low dose estrogen replacement via osmotic mini-pumps in an OVX background restored running activity to pre-OVX levels, and acute estradiol injections were able to replicate running peaks. Collectively, our results suggest estrogen regulates running activity which offers a unique opportunity to examine the mechanisms responsible for driving exercise behaviors. Further studies identifying molecular mechanisms that mediate estrogen’s effects are currently underway.
Objective: The vas deferens smooth muscle (VDSM), which is dependent on testosterone, generates spontaneous contraction. Although the factors modulating the spontaneous contraction are not completely understood, different experimental studies have supported that the VDSM cell electrophysiological phenomena is eminently correlated to it. According to a recent study, the castration has down regulated the A-type K+ channel activities in VDSM cell [Ohya et al., 2019]. In the present time, computational modeling plays a powerful role in understanding various complex biological/physiological systems. To explore the quantitative contribution of castration into the VDSM membrane electrical activities, a biophysically detailed single VDSM cell model is presented. Materials And Methods: First, we constructed computational models for seven ion channels found in guinea-pig VDSM cells based on published experimental data: One voltage gated Na+ ion channel, two voltage gated Ca2+ ion channels, a hyperpolarization-activated ion channel, two voltage-gated K+ ion channels, one Ca2+-activated K+ ion channels and a nonspecific background leak ion channel. All ion channel models were validated by comparing the simulated currents and current-voltage relationship with those reported in experimental work. Then, all ion channels were integrated to simulate the VDSM electrical activities towards neurotransmitter/current stimulus. We investigated the contribution of the castration by mimicking the testosterone as down regulation of A-type K+ channel on VDSM cell excitability. Results: The ion channel conductances are set to maintain the resting membrane potential (RMP) at ? 50 mV as the physiological range of RMP in VDSM cell varies from ? 45 mV to ? 70 mV. The action potential (AP) and membrane depolarization are mediated regulation of A-type K+ channels. Results: The ion channel activities in VDSM cell [Ohya et al., 2019]. In the present time, computational modeling plays a powerful role in understanding various complex biological/physiological systems. To explore the quantitative contribution of castration into the VDSM membrane electrical activities, a biophysically detailed single VDSM cell model is presented. Materials And Methods: First, we constructed computational models for seven ion channels found in guinea-pig VDSM cells based on published experimental data: One voltage gated Na+ ion channel, two voltage gated Ca2+ ion channels, a hyperpolarization-activated ion channel, two voltage-gated K+ ion channels, one Ca2+-activated K+ ion channels and a nonspecific background leak ion channel. All ion channel models were validated by comparing the simulated currents and current-voltage relationship with those reported in experimental work. Then, all ion channels were integrated to simulate the VDSM electrical activities towards neurotransmitter/current stimulus. We investigated the contribution of the castration by mimicking the testosterone as down regulation of A-type K+ channel on VDSM cell excitability. Results: The ion channel conductances are set to maintain the resting membrane potential (RMP) at ? 50 mV as the physiological range of RMP in VDSM cell varies from ? 45 mV to ? 70 mV. The action potential (AP) and membrane depolarization are mediated regulation of A-type K+ channels.

Sex differences in addiction are seen for all classes of abused drugs in humans and animal models. Females exhibit a greater response to psychomotor stimulants such as amphetamine and cocaine than males, at least in part due to the gonadal hormone estradiol. Females also tend to be more susceptible to addiction-like behaviors and this is also modulated by estradiol. Sex differences in the way that the gonadal hormone, estradiol, interacts with the ascending telencephalic dopamine system are thought to result in these sex differences in motivated behaviors, including drug seeking. In rodents, repeated psychostimulant exposure enhances incentive sensitization to a greater extent in females than males. Estradiol increases females’ motivation to attain psychostimulants and enhances the value of drug related cues, which ultimately increases their susceptibility towards spontaneous relapse. This, along with females’ dampened ability to alter decisions regarding risky behaviors, enhances their vulnerability for escalation of drug use. In males, recent evidence suggests that estradiol may be protective against susceptibility towards drug-preference. The distribution of ERα, ERβ, and GPER1 throughout the brain may be key to understanding how estradiol can differentially regulate drug-taking between the sexes. Recent work has shown that conditioned place preference (CPP), induced by exposure to cocaine, is modulated by treatment with the GPER-1 agonist, G1, locally in dorsolateral striatum; G1 reduced preference for cocaine in males, but not in females. Preference for saccharine was also reduced in males, but not females. On the other hand, activation of GPER-1 in the dorsolateral striatum potentiated female rats’ motivation to self-administer cocaine. There was no effect of prior treatment with the GPER-1 agonist, G1, on extinction of cocaine-taking in females, however, G1 treatment resulted in greater drug-induced reinstatement (10 mg/kg cocaine, i.p.). There were no effects of intra-dorsolateral striatum GPER1 activation on motivation for cocaine or cocaine-induced reinstatement of responding in males. These results support the conclusion that activation of GPER1 in the dorsolateral striatum enhances established cocaine seeking behaviors for female rats, while in male rats activation of GPER1 attenuates establishment of a preference for cocaine. Sex differences in the actions of estradiol in both sexes are key to understanding how future research might enhance understanding of the mechanisms of sex differences in addiction-related
behaviors, which are dependent on estradiol receptor subtype and the region of the brain they are acting in.

APSSG21.186
Adverse Cardiometabolic Effects of Severe Food Restriction in Males and Females
Kathryn Sandberg¹, Aline de Souza¹, Jônathas Almeida¹, Natalia Shults², Hong Ji¹, Carolyn Ecelbarger¹
¹Medicine, Georgetown University, ²Pharmacology & Physiology, Georgetown University

Little is known regarding the long-term effects of severe food restriction (sFR) after body weight has recovered as a result of refeeding. The few studies in people suggest prior exposure to sFR is a risk factor for cardiometabolic disease later in life, though the mechanisms are poorly understood. Female sFR rats developed insulin resistance during the three month refeeding period (sFR-Refed). Hypertension response sensitization also persisted three months after refeeding through activation of angiotensin type 1 receptors. Within the first week of the sFR diet, female rats stopped cycling through their four day estrus cycle. After two weeks, uterine wet weights were less than half of the control (CT) group. Ischemia/reperfusion-induced cardiac arrhythmias were two-fold higher and cardiomyocyte pathology was more severe compared to CT rats. In contrast, male rats were less susceptible to the long term adverse cardiac effects of sFR. While male sFR rats had twice as many ischemia/reperfusion-induced cardiac arrhythmias immediately after the sFR period ended, they recovered during the refeeding period and no differences in arrhythmia frequency were detected between the CT and sFR-refed rats. Most promising is our recent discovery that treatment of female sFR-refed rats with the angiotensin converting enzyme inhibitor captopril during the middle of the refeeding period and well after body weight recovered, attenuated ischemia/reperfusion-induced cardiac arrhythmias. We conclude that the long term adverse cardiometabolic effects observed in female rats after refeeding stem from the acute impact of sFR on insulin, angiotensin II and estrogen signaling cascades, all of which become chronically dysregulated. The maladaptive allostatic state of these major endocrine systems causes long-lasting, insulin resistance, hypertension response sensitization and cardiomyocyte damage months after refeeding. Our findings have implications for women exposed voluntarily or involuntarily to an extended period of sFR. In this regard, the COVID-19 pandemic is creating a healthcare crisis within a crisis by nearly doubling globally the number of low-income women who have experienced sFR.

APSSG21.187
Sex-Dependent Differences in Hypertension and Urinary Angiotensinogen Excretion in 2-Kidney 1-Clip (2K1C) Goldblatt Hypertensive Rats are Mitigated in Ovariectomized Rats
Emily Pemberton¹, Weijian Shao¹, Akemi Katsurada¹, Annie Bell¹, L. Gabriel Navar¹
¹Department of Physiology and Hypertension and Renal Center of Excellence, Tulane University

Previous studies have showed sex dependent differences in hypertension in rats with unilateral renal artery stenosis (2K1C), with females developing a lesser hypertension than male rats, which exhibit a robust activation of the intrarenal RAS. The present study was focused on the responses in female 2K1C rats and on the role of estrogen in mitigating the development of hypertension, by comparing responses in intact female 2K1C rats and in 2K1C ovariectomized (OVX) female rats in comparison to those in male 2K1C rats. A 0.2mm silver clip was placed on the left renal artery of male rats, female rats and OVX rats with one group of female rats left intact. Blood pressures (BP) of all groups were measured on days 1, 3, 7, 14, and 21 using tail cuff plethysmography. Following these measurements, rats were placed in metabolic cages for 24 hours to measure water intake and collect urine. Rats underwent clearance studies on day 22. Following pentobarbital anesthesia, the jugular vein was catheterized and an infusion with an insulin/PAH/albumin saline solution at a rate of 1.2Ml/hour. A femoral artery catheter was placed for direct measurement of arterial pressure. Both ureters were catheterized, and urine flow was collected in 30min periods over 2 hours for assessment of renal function and analysis of urinary constituents. Following clearance periods, both kidneys were collected for histology and immunohistochemistry to determine degree of kidney injury. At day 21, the degree of hypertension in intact 2K1C females was statistically lower than in OVX female and male 2K1C rats, while the BP in OVX rats was not different from that in male rats. Importantly, the urinary angiotensinogen (uAGT) excretion rates in female 2K1C, while greater than in control female rats was lower than in male 2K1C rats. The OVX 2K1C rats did not show statistical differences in uAGT excretion rate compared to 2K1C male rats. Nevertheless, urinary protein excretion rates in intact 2K1C females and OVX rats were lower than in male 2K1C rats. Non-clipped kidneys of intact 2K1C females and OVX 2K1C rats had significantly greater uAGT/uCre than the clipped kidneys. In conclusion, the increased uAGT in OVX rats indicate augmented activity of the intrarenal renin-angiotensin system levels in OVX 2K1C rats compared to intact 2K1C females. Our data suggest that removal of estrogen source partially reduces the protective role against hypertension in females. Preliminary tissue analysis suggests less degree of interstitial fibrosis in the 2K1C female rats compared to male 2K1C rats. Our findings help explain sex-differences in the renal responses to unilateral arterial stenosis in females compared to males. This study was supported by the ASPIRE Program, the Warren R. Bourgeois III, MD and Usha Ramadhyani Bourgeois, MD Student Research Endowed Fund and the Carol Lavin Bernick fund at Tulane University, New Orleans, LA.
Conversely to other renal diseases that progress faster in males than females, diabetic kidney disease advances at a similar rate in both sexes. The vasoactive peptide endothelin-1 (ET-1) is critical in diabetic kidney disease; however, the specific role of ET-1 derived from the endothelium in the development of this disease remains unclear. These studies were designed to examine the role of vascular ET-1 in diabetic kidney disease, and to determine if it plays a role in the loss of renal protection observed in diabetic females. Hyperglycemia was induced in male and female vascular endothelial cell ET-1 knockout (VEET KO) and floxed ET-1 control mice with streptozotocin (STZ, 50 mg/kg i.p., 5 consecutive days). 10 weeks later, urine and kidneys were collected and kidney damage and cortical expression of genes involved in cell death pathways were assessed. In response to diabetes, female VEET KO mice displayed greater interstitial fibrosis and cortical tubule dilation than male VEET KO mice, as well as increased cortical T cell numbers compared to female diabetic controls. Glomerulosclerosis, GFR and NGAL and nephrin excretion were not different between sexes or genotypes. Interestingly, the lack of vascular ET-1 led to decreased albumin excretion with diabetes, although no sex difference was found (VEET KO vs. floxed ET-1: 51.6 ± 4.3 vs. 104.1 ± 3.3 μg/day, p<0.05; n=5-7/group). We also found a sex effect in protein and KIM-1 excretions, with greater excretion of these parameters in females than males (females vs. males, protein: 3.8 ± 0.6 vs. 1.8 ± 0.1 mg/day; KIM-1: 3.5 ± 0.0 vs. 1.6 ± 0.5 pg/day; p<0.05; n=5-7/group). Absence of endothelium-derived ET-1 in diabetic males resulted in cortical downregulation of 1 necrosis gene (Hspbap1, p<0.005, n=2-3/group). In contrast, diabetic VEET KO females displayed upregulation of 13 genes (6 for autophagy, 4 for necrosis, 3 for apoptosis, p<0.005, n=3/group) vs. floxed-ET-1 females. When compared to diabetic male VEET KO mice, a total of 48 genes were upregulated in cortex from VEET KO females (16 genes each for autophagy, necrosis, and apoptosis; 2-4 fold increase, p<0.05, n=3/group). These results highlight the protective role that vascular ET-1 plays in females, but not males, against the development of diabetic nephropathy.

Funded by NIH KO1HL145324 and UAB Diabetes Research Center Pilot Project grant to CDM, and UAB KURE R25 DK115353 to VGM and JSP.

Objective: Women who develop asthma after menopause tend to have more severe symptoms and respond poorly to standard treatment. Details of the mechanisms underlying this specific type of asthma are limited, as researchers have been unable to appropriately model phenotypes observed in menopausal asthmatics using animal models. We aim to demonstrate the first successful model of menopause associate asthma that mimics the human condition. Methods: We used the 4-Vinylcyclohexene Dieneoxide (VCD) mouse model of menopause followed by subsequent house dust mite (HDM) sensitization and challenge (VCD/HDM) to mimic menopause-associated asthma phenotypes in wild type C57BL/6 mice. Across two independent experiments, VCD/HDM (menopausal asthmatic, n=20), VCD/Saline (menopausal non-asthmatic, n=19), Vehicle/HDM (non-menopausal asthmatic, n=15), and Vehicle/Saline (non-menopausal non-asthmatic, n=15) underwent invasive airway function tests, after which blood, bronchoalveolar lavage fluid (BALF), and lung and ovarian tissues were harvested for analysis. Results: Menopausal asthmatic mice had significantly increased airways hyperresponsiveness (AHR) during methacholine challenge as detected by increased resistance and elastance, both in the entire respiratory system, as well as the distal airways and tissues, compared to non-menopausal asthmatic mice. While Type-2 cytokines, IgE, mucin production and eosinophil recruitment were similar in both HDM challenged groups, menopausal asthmatic mice had significantly more neutrophil recruitment detected in BALF, alveolar inflammation in the lung tissue, and extracellular matrix (ECM) deposition, independent of asthma status. Conclusions: In line with human studies, menopausal asthmatic mice have enhanced AHR, neutrophilia, and alveolar inflammation compared to non-menopausal asthmatic mice. Thus, the VCD HDM mouse model provides a translational tool with similar phenotypes to the human condition that will allow us to better study mechanisms driving menopause-associated asthma, and is the first to do so. NIH Funding: AI135935, HL125602, HL142769, HL131834.
APSSG21.190
Colonic mitochondrial function is altered in hyperandrogenemic female rats
Kristin Edwards1, Ngoc Hoang1, Karen Brooks1, Jacob Pruett1, Steven Everman1, Jonathan Hosler1, Licy Yanes Cardozo1,2,3,4
1Cell and Molecular Biology, University of Mississippi Medical Center, 2Medicine (Division of Endocrinology, Diabetes and Metabolism), University of Mississippi Medical Center, 3Women’s Health Research Center, University of Mississippi Medical Center, 4Cardiovascular-Renal Research Center, University of Mississippi Medical Center

Introduction: Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder in women during their reproductive years. Approximately 80% of PCOS women suffer from hyperandrogenemia. PCOS women are reported to have a high prevalence for developing irritable bowel syndrome (IBS). When IBS is present with PCOS, patients have a higher BMI and higher levels of body fat compared to PCOS alone. However, the underlying pathophysiology for the development of IBS remains unknown. Hyperandrogenemia has been shown to cause alterations to mitochondrial function. Therefore, the goal of this project is to investigate the mechanisms linking PCOS with the development of IBS. Methods: The hyperandrogenemic female (HAF) rat model exhibits characteristics similar to PCOS women such as increased body weight, fat mass, and food intake. At 4 weeks of age, female rats received dihydrotestosterone (DHT, 7.5mg/90 days) pellets. Body weight, food intake, and adiposity were monitored throughout the study. At 15 weeks of age, the rats were sacrificed to collect colon tissue. A tissue homogenate containing colon mitochondria was prepared from washed colons. Mitochondrial respiration was measured using glutamate/malate, succinate, oleate, or octanoate as substrates. Mitochondrial reactive oxygen species (mtROS) was measured simultaneously with mitochondrial respiration using an Oroboros Fluorespirometer. Activities of individual mitochondrial electron transport complexes were also measured. Data was normalized to mitochondrial content using citrate synthase activity, which confirms that the observed changes are not due to changes in mitochondrial content. Results: Colon mitochondrial respiration showed a significant decrease in HAF rats compared to controls using glutamate/malate (0.2287 ± 0.03282 vs. 0.5219 ± 0.05491, p=0.0012), succinate (0.6475 ± 0.07953 vs. 1.223 ± 0.05826, p=0.0001), oleate (0.2626 ± 0.02274 vs.0.4252 ± 0.02547, p=0.0004), or octanoate (0.3699 ± 0.04513 vs.0.6809 ± 0.02182, p<0.0001). mtROS production was significantly increased compared to controls (0.4277 ± 0.04197 vs. 0.1954 ± 0.02265, p<0.0001). Complex IV of mitochondrial oxidative phosphorylation, a marker for oxidative phosphorylation capacity, also showed a decrease in activity by 60% in HAF rats compared to controls (1.568 ± 0.2475 vs. 3.932 ± 0.5372, p<0.0001). Conclusions: The decrease in colon mitochondrial function in the HAF rat model of PCOS suggests that mitochondrial dysfunction is involved in the development of IBS in PCOS women with hyperandrogenemica. The increase in mtROS production in the HAF rat suggests that oxidative damage may also be involved in the development of IBS. This study provides a better understanding of the role of mitochondria in the development of IBS with PCOS and an avenue for the development of strategies to re-establish normal mitochondrial function. This could provide options for preventive and therapeutic interventions for IBS where there are limited treatment options in PCOS women. Supported by NIH grants: 1P20GM121334 (JFR, LLYC, and JPH)

APSSG21.191
Gonadal Steroid Hormones Do Not Alter Stress-Induced Cardiovascular Responses in Adult Male and Female Rats Exposed to Dexamethasone In Utero
Lakshmi Madhavpeddi1, Taben Hale1, Robert Handa2
1Basic Medical Sciences, University of Arizona: College of Medicine - Phoenix, 2Biomedical Sciences, Colorado State University

It is well established that even transient prenatal insults can impact cardiovascular function in adulthood, and that men and women demonstrate a different risk for and progression of cardiovascular disease. We have hypothesized that adult cardiovascular disease may have its origins in utero as a result of exposure to elevated levels of glucocorticoids. In support of this, we have shown that when pregnant rat dams are treated with the glucocorticoid, dexamethasone (DEX), for the last 4 days of gestation, female-specific changes resulting in enhanced pressor and tachycardic responses to stress occur in adult offspring. We hypothesize that the sex-specific impact of prenatal stress on cardiovascular stress responses is due to the activational effect of gonadal steroid hormones. Pregnant dams were administered DEX (0.4mg/kg per day, s.c.) or vehicle on gestation days 18-21. This resulted in a significant reduction in birthweight in DEX-exposed males and females. At 8 weeks, rats underwent a gonadectomy (GDX) or sham surgery, or remained intact, and at 10 weeks rats were instrumented with radiotelemetric transmitters for direct recording of arterial pressure in conscious, freely moving male and female rats. At 11-12 weeks rats were placed in a restraint tube for 20 minutes, followed by a 3-hour recovery period, to assess whether GDX alters the sex-specific stress responses in DEX-exposed offspring. Restraint-stress testing was performed on diestrus in intact and sham females, and absence of cycling in GDX females was confirmed via cytological analysis. We demonstrate that intact females, but not males, that were exposed to DEX in utero exhibit an exaggerated pressor response to restraint, as compared to vehicle exposed females. We found that GDX did not alter stress-responsive MAP in males regardless of prenatal treatment, suggesting testosterone does not play a role in acute cardiovascular stress responses adult rats. In vehicle exposed females, when compared to intact, both sham and GDX surgery resulted in an exaggerated pressor response to restraint. However, in females that were prenatally exposed to DEX, there was no difference in the pressor response to restraint between intact, sham, and GDX rats. This suggests that the exaggerated pressor response observed in DEX females compared to males is not due to activational effects of
estrogen. It is possible that gonadal steroids act at an organizational level to mediate the sexual dimorphism observed in rats exposed to DEX in utero. Future studies to identify the mechanisms by which prenatal dexamethasone produce long-term changes in cardiovascular function will be important for better understanding the sex-specific consequences of prenatal programming over the lifespan. Funding: NIH U54 MH118919

APSSG21.193
Sex Differences in Risk for Intestinal Inflammation and Disease: The Role of Exercise
Sara C. Campbell1, Paul J. Wisniewski2, Stanley A. Lightfoot3, Dorothy Vatner4, Stephen Vatner4, Laurie B. Joseph5
1Kinesiology and Health, Rutgers, the State University of New Jersey, 2School of Medicine, University of South Carolina, 3Center for Cancer Prevention and Drug Development, University of Oklahoma Health Science Center, 4Cell Biology & Molecular Medicine, Rutgers-New Jersey Medical School, 5Ernest Mario School of Pharmacy, Rutgers, the State University of New Jersey

Colon cancer is third most diagnosed cancer and fourth cause of death worldwide with males having a greater incidence of colon cancer compared to females. Between 1990 and 2017 there was an increase from 37 million to more than 68 million people diagnosed with inflammatory bowel disorder, which includes colon cancer. Exercise is known to prevent inflammatory bowel disease and colon cancer. It is estimated that exercise may prevent approximately 15% of the colon cancers and may decrease the mortality. However, the mechanisms by which this may occur and how they may differ in males and females are not known. The purpose of this study was to determine sex differences on the effects of exercise on colon inflammation and proliferation index in male and female mice. First, to establish a baseline, we measured the pro-inflammatory marker cyclooxygenase-2 (COX-2) using immunohistochemistry in the colons of male and female mice on a control diet. Males showed higher baseline COX-2 expression compared to females (p<0.05). However, after ad libitum access to free wheel running exercise for 12-weeks, inflammation was reduced in the male mice to the level of female mice. Next, we sought to accelerate inflammation in colon by using a 45% high fat diet, which increased colon inflammation in both males and females. When exercised on the 45% high fat diet, only females showed a reduction (50%) in inflammation. Finally, we examined the proliferative phenotype via proliferation index using PCNA (proliferating cell nuclear antigen). This is a clinical measure to assess disease risk by dividing total nuclei counted per colonic crypt by immunoreactive nuclei. The higher the proliferation index the greater the risk for disease. Results showed that there was a 14.7% reduction in proliferation index in females compared to males suggesting females have less risk of disease compared to males. Further, exercise reduced proliferation index by 30.4% compared to results in sedentary behavior. Taken together these results suggest that exercise reduces the risk of developing colon cancer, more in females than males. In conclusion, males have a higher level of baseline colon inflammation compared to females, which is exacerbated by high fat diet. Exercise can reduce colon inflammation and the risk of colon cancer more in females on a high fat diet, whereas males have a higher risk of disease along with those who are sedentary. It will be important to understand the mechanisms by which exercise protects against colon cancer, in general, and specifically mediates the gender differences in colon cancer.

APSSG21.194
Female Mice Are Protected from Impaired Parenchymal Arteriole TRPV4 Activation and Cognitive Dysfunction During Hypertension
Laura Chambers1, Martina Yen1, William Jackson1, Anne Dorrance1
1Pharmacology and Toxicology, Michigan State University

Vascular dementia is the second most common subtype of dementia following Alzheimer’s disease and accounts for 10-20% of all dementia cases. However, its prevalence is likely highly underestimated, as 50% of Alzheimer’s disease patients are found to have cerebrovascular damage at autopsy. Hypertension, a leading risk factor for vascular dementia, damages cerebral arteries, leading to impaired cerebral blood flow autoregulation and cognitive decline. There is a complex sex difference in the relationship between hypertension and vascular dementia development. Women who develop hypertension pre-menopause have a 65% increased risk of dementia development later in life; this increased dementia risk does not exist in hypertensive men of similar age. This suggests female sex hormones may play a protective role against hypertension-associated cerebrovascular damage, and that the absence of these hormones has a detrimental effect on the cerebral vasculature. Endothelium-dependent dilation in cerebral parenchymal arterioles (PAs) is highly dependent on transient receptor potential vanilloid 4 (TRPV4) channel activation. We have previously shown that hypertensive male mice and rats have impaired TRPV4 function that is associated with cognitive impairment. We hypothesized that age-matched, cycling female mice are protected from impaired PA TRPV4-mediated dilation and impaired cognitive function during hypertension. To test this, AngII-filled osmotic minipumps were implanted in 16-week-old female C56BL/6 mice. Female mice received either an AngII dose that matches the dose previously used in male mice (800ng/kg/min) or a higher dose (1200ng/kg/min) that produces a similar elevation in blood pressure to male mice. Sham mice served as control. Blood pressure was measured by tail-cuff plethysmography. Pressure myography was used to assess TRPV4-mediated dilation in PAs. Barnes maze was used to assess spatial memory. Data are presented as means ± SEM unless otherwise stated (n=4-9 per group). Systolic blood pressure was elevated in 1200ng AngII-treated female mice vs sham (sham: 146 ± 8, 800ng AngII: 161 ± 13, 1200ng AngII: 179* ± 7mmHg; p=0.0482 by one-way ANOVA). PA dilation in response to the TRPV4 agonist GSK1016790A (10-9-10-5M) was not impaired in AngII-treated female mice (sham: 85 ±
5, 800ng AngII: 73 ± 7, 1200ng AngII: 77 ± 8% maximum dilation; p=0.4381 by one-way ANOVA). Female AngII-hypertensive mice were also protected against impaired spatial memory in Barnes maze (sham: 71 ± 5, 800ng AngII: 76 ± 4, 1200ng AngII: 67 ± 4% time spent investigating holes in target maze quadrant; p=0.3010 by one-way ANOVA). These data provide novel evidence that female sex hormones play an essential protective role in maintaining TRPV4 function in PAs and proper cognition during hypertension. Future studies will identify the role of these hormones in protection against cerebrovascular damage during hypertension. Determining the mechanisms behind the sex differences in hypertension-associated vascular dementia is crucial for the development of better targeted therapies for hypertensive women. Funding: ST32GM092715-14 and R01-HL-137694-01

**APSSG21.195**

**Sex Differences in Exercise Capacity**

Marko Oydanich1, Jie Zhang1, Sara C. Campbell2, Robert A. Dowden2, Dorothy Vatner2, Stephen Vatner2

1Cell Biology & Molecular Medicine, Rutgers-New Jersey Medical School, 2Kinesiology and Health, Rutgers, the State University of New Jersey

The study of sex differences has become an important requirement for all NIH grants. Since most experimental studies now are conducted in mouse models, it becomes important to address sex differences in mice. Accordingly, the goal of this investigation was to determine sex differences in exercise capacity, measured on a treadmill, in mice and the mechanisms mediating these differences. There are many reasons why the literature is controversial on this topic, e.g., studying mice of the same age is difficult because male mice weigh more than female mice of the same age, and different results are derived from different exercise techniques. We studied C57 female wild-type (WT) mice (5 months old), which exhibited greater, p<0.05, maximal exercise capacity for running distance (489 ± 15 m; n=6) than age-matched male WT mice (307 ± 17 m; n=8) as well as a 21% improvement in work to exhaustion. Female mice also exhibited a 12% increase in peak oxygen consumption (peak VO2), a 14% increase in peak carbon dioxide production (peak VCO2), and 13% in peak energy expenditure when compared to age-matched male mice. One of the most important mediators of sex differences in exercise capacity is the presence of sex hormones. Therefore, we also studied the effect of estrogen on exercise capacity. After ovariectomy (OVX), female mice no longer demonstrated enhanced exercise compared with males, including a decrease in power. Conversely, chronic administration of estrogen to male mice improved capacity in running distance and work to exhaustion by 35% after 4 weeks of treatment. Next, we investigated nitric oxide (NO), a downstream target of estrogen. Total NO synthase (NOS) activity was higher in female mice compared with male mice (0.11 ± 0.02 mU/mg vs. 0.052 ± 0.007 mU/mg; p<0.05), but was no longer different after OVX. Furthermore, males chronically treated with estrogen exhibited an 81% increase in NOS activity. NO blockade with L-NAME eliminated the enhanced exercise capacity observed in both females and males treated with estrogen. The microbiome may also mediate the enhanced exercise in female mice, since female mice after exercise have enhanced microbes from Muribaculaceae, which promote exercise tolerance and are reduced with OVX. Thus, as expected, estrogen is a key mechanism mediating the enhanced exercise capacity in female mice. However, this investigation also demonstrated other novel key mechanisms, increased nitric oxide activity and the microbiome.

**APSSG21.196**

**Early life stress: impact and consequences of sex differences on cardiovascular and immune disease outcomes**

Jennifer Pollock1

1Medicine, University of Alabama at Birmingham

Exposure to adversity in childhood or early life stress, such as abuse, neglect, or severe household dysfunction, has an impact on the development of negative chronic health conditions at younger ages and with more severe outcomes. In the United States, about 50% of the population experience at least one or more adverse events in childhood. The impact of sex differences in the types of early life stress are now being investigated as well as sex differences in the outcomes of disease severity. This presentation will focus on early life stress with the impact and consequences of sex differences on cardiovascular and immune disease outcomes in adolescents, young adults, as well as in animal models. Briefly, potential causal mediators induced by early life stress will be discussed in novel translational studies.

**APSSG21.198**

**Sex Differences in Islets Endoplasmic Reticulum Stress Response**

George Brownrigg1, Yi Han Xia1, Søs Skovsø1, Evgeniy Panzhinskiy1, James Johnson1, Søs Skovsø1, Elizabeth Rideout1

1Cellular and Physiological Sciences, University of British Columbia

Biological sex affects the risk of developing type 2 diabetes (T2D): ~40% more men develop T2D than premenopausal women. To gain insight into mechanisms underlying this male-biased risk, we analyzed islet scRNAseq to monitor diabetes-associated changes in human insulin-producing β-cells of pancreatic islets. While both sexes showed a significant upregulation of genes associated with unfolded protein response (UPR) function/regulation in T2D, we observed sex differences in the magnitude of gene expression changes in which UPR-associated genes were altered. In support of a sex difference in UPR regulation, unbiased pathway analysis of islet RNAseq data from 20-week-old male and female mice showed significant enrichment of UPR pathway genes. Specifically, our results suggest that females show higher expression of genes involved in protein synthesis and folding compared with males. Because dysfunction of protein folding machinery triggers endoplasmic reticulum...
(ER) stress, we hypothesized sex differences may exist in the cellular ER stress response. Indeed, when we treated mouse islets with ER stress-inducing thapsigargin (Tg), we found that Tg-treated islets from females, but not males, were able to restore protein synthesis following acute ER stress induction. Further, kinetic cell death assays showed significant Tg-induced cell death in males at 0.1μM and 1μM Tg concentrations, with no effect in females until a higher 10μM dose. These results indicate that female islets maintain increased protein synthesis and lower cell death in an ER stress context. Lastly, we performed RNAseq on Tg-treated islets at two time points (6hr/12hr) and as before we performed pathway enrichment analysis. As expected, each sex had significant upregulation of UPR pathway genes compared to unstressed controls. However, between time point pathway analysis showed that females had significant upregulation of UPR pathway genes at 12hrs while there was no change in males. This suggests that the time course dynamics of the UPR response to ER stress is differentially regulated between sexes. Given that ER stress has been implicated in the pathogenesis of T2D, these findings provide insight into potential mechanisms underlying the male prevalence in T2D.

**APSSG21.201 Renal-derived human sPRR increases blood pressure in female but not in male mice.**

Gertrude Arthur1, Audrey Poupeau2, Kellea Nichols2, Jacqueline Leachman3, Analia Loria3, Jeffrey Osborn3, Frederique Yiannikouris4

1Pharmacology and Nutritional Sciences, University of Kentucky, 2Pharmacology and Nutritional Science, University of Kentucky, 3Biology, University of Kentucky

Recent studies showed that soluble prorenin receptor (sPRR) plays an important role in blood pressure regulation and in water balance. In rodent models, infusion of sPRR contributes to AngII production by increasing renin activity, systolic blood pressure (SBP) and aquaporin2 (AQP2)-dependent antidiuretic action. However, there is a gap of knowledge concerning the functional role of locally produced sPRR from the kidney and the relative contribution of sex. Additionally, clinical research indicated that sPRR may influence SBP in humans. Therefore, we examined the role of kidney-derived human sPRR in SBP control and fluid homeostasis in male and female mice. Human sPRR-Myc-tag transgenic mice were bred with mice expressing Hoxb7/Cre to selectively express human sPRR in the collecting duct (RHsPRR). RHsPRR and control (CTL) male and female mice were fed a standard diet for 10 months (n=8-11/group). Body weight and urine volume were examined and SBP measured by radiotelemetry. Western blot analysis depicted the presence of human sPRR-Myc-tag (28 KDa) in the cortex and medulla of RHsPRR mice validating the humanized mouse model. Renal-derived human sPRR did not change body weight in male or female mice (Male: CTL: 34±1, RHsPRR: 33±1g; Female: CTL: 28±1, RHsPRR: 30±1g). Renal-derived human sPRR did not significantly increase circulating sPRR (Male: CTL: 3995±643, RHsPRR: 4342±500pg/ml; Female: CTL: 3479±194, RHsPRR: 3948±238pg/ml) suggesting that kidney is not a source of circulating sPRR. In male mice, renal-derived human sPRR did not change SBP (CTL: 124±2 and RHsPRR: 116±5 mmHg) but tend to decrease urine volume by around 50% (M: CTL: 10±0.2, RHsPRR: 0.57±0.2 ml/day). In line with those results, renal aquaporin 2 (AQP2) protein expression in the kidney was significantly increased in RHsPRR male mice (CTL: 9±3, RHsPRR: 44±14 AU, P<0.05) indicating a role of human sPRR in water balance in male mice. Interestingly, SBP was significantly higher in RHsPRR female mice compared to CTL (CTL: 119±2, RHsPRR: 127±3 mmHg, P<0.05). Additionally, neither urine volume (CTL: 0.4±0.1, RHsPRR: 0.5±0.1 ml/day) nor AQP2 was influenced by human sPRR in female (CTL: 11±0.5, RHsPRR: 6.9±1.5 AU). Overall, our data suggest that renal human sPRR contributes to increase blood pressure in female mice and participate to water reabsorption in male mice. Whether the local renin angiotensin system or the sympathetic nervous system are involved in human sPRR-mediated increase of SBP in female mice

**APSSG21.202 Chronic Intermittent Hypoxia Adversely Affects Renal Microcirculatory Regulation and Tissue PO2 In Ovariectomized Female Rats**

Rania Gerritts1, Benjamin G. Madigan1, Katherine A. Harbeck1, Kelsey S. Schwarz2, James A. Lange3, Abbie Voas1, Sarah C. Clayton1, Noah J. Marcus3

1College of Osteopathic Medicine, Des Moines University Medicine and Health Sciences, 2Kinesiology, Iowa State University, 3Physiology and Pharmacology, Des Moines University Medicine and Health Sciences

Introduction: Epidemiological evidence indicates that sleep apnea, which increases in prevalence in post-menopausal women, is a major risk factor for development of chronic kidney disease. The mechanisms underlying this association are poorly understood, but abnormal renal hemodynamics, neurohormonal activation, and hypoxemia are hypothesized to play prominent roles in this process. In this study, we sought to determine if chronic intermittent hypoxia (CIH, a model of sleep apnea) would adversely affect renal microcirculatory regulation and tissue PO2 in ovariectomized (OVX) female rats and identify potential mechanisms by which this might occur. Hypothesis: CIH will exacerbate reductions in renal microcirculatory blood flow (RBF) and PO2 in OVX female rats during exposure to hypoxia that will persist after return to normoxia. Methods: Adult female Sprague Dawley rats (n=4-6 per group) underwent ovariectomy and after 4 weeks were randomized to either CIH or sham treatments. At the conclusion of CIH or sham, renal microcirculatory regulation was assessed under light isoflurane anesthesia (1.5% in air) during exposure to a series of 10 acute episodes of intermittent hypoxia (AIH, FiO2 10%, FiCO2 3%). Renal perfusion (RP) was measured using laser speckle contrast imaging (Moor FLPi-2, Moor Instruments) and PO2 was measured using fiber optic probes (Oxford Optronix). At the conclusion of the physiological experiments renal cortical tissue was collected for assessment of eNOS mRNA (qRT-PCR) and protein
(western blot) expression. Results: RP was significantly decreased (p<0.05) relative to baseline in both groups after 10 hypoxic episodes of AlI (-2±0.9% OVX-AIR, -8±3% OVX-CIH, p<0.05 vs. baseline), but to a greater extent in OVX-CIH animals. Similarly, cortical PO2 decreased significantly in both groups relative to baseline (-9±3% OVX-AIR, -19±6% OVX-CIH, p<0.05 vs. baseline), and decreased to a greater extent in CIH-OVX vs. CIH-AIR. Normoxic cortical PO2 also decreased relative to baseline during AlI and remained below baseline at 5 minutes post-AlI (-18±11% OVX-CIH). Expression of eNOS mRNA and protein was decreased in OVX animals relative to intact females but was not different between OVX-AIR and OVX-CIH.

Conclusions: Exposure to CIH in OVX female rats alters renal hemodynamic regulation and tissue PO2 in a manner which may contribute to tissue damage and development of CKD.

APSSG21.204
Sex and gender advances in allostatic load research
Robert-Paul Juster1
1Psychiatry and Addiction, University of Montreal

Every cell is sexed, every person is gendered, and every organism is stressed. Whereas sex refers to a multi-dimensional construct that includes genes, anatomy, gonads, and hormones central to our field, gender refers to an array of socio-culturally constructed roles, responsibilities, and restrictions that influence stress and coping. Diverse sexual orientations and gender identities are also related to unique sets of exposures and experiences that correspond with health inequalities that the allostatic load model is well suited to study. In this presentation, a selective review of allostatic load studies that nuance sex, gender, and sexual orientation will be proposed. Sex and gender matter and methods for conducting sex- and gender-based analysis in physiological research will be proposed. To better address sex and gender, it is important to account for both biological factors like sex hormones and gender-based factors like gender-roles as well as sexual orientation along continuums. These considerations provide a powerful framework to help solve health and wellness problems that cannot be easily explained by focusing solely on binary sex.

APSSG21.205
Thermoneutral modulation of sex differences in acute kidney injury
Lisa Curtis1, Rohan Balkawade1, Hannah Eckenrode1, Chunlan Fan1
1Division of Nephrology, Department of Medicine, University of Alabama at Birmingham

Acute kidney injury (AKI), an abrupt decrease in kidney function, has demonstrated sex differences as illustrated in clinical and pre-clinical studies. In most cases, females show lesser susceptibility to AKI with the exception of AKI resulting from cardiac surgery. Mitochondrial energetics are noted consistently as a contributor to the outcomes of AKI. The kidney is second only to the heart for mitochondrial content and resting metabolic rate. Mitochondrial transplantation via intra-arterial injection in female Yorkshire pigs reduced measures of renal injury in a model of AKI. Female rodents housed at thermoneutrality, the ambient temperature at which mitochondrial energetics are at a nadir, was shown to lessen the resistance to acute liver injury in females. In this study, we tested the hypothesis that housing at thermoneutrality can alter sex differences in AKI. C57BL/6J mice (female=6, male=4 each group) were housed at 22°C or 30°C, before undergoing bilateral ischemia reperfusion injury (IRI) for 20 minutes, a model of AKI. Sham mice underwent the exact surgical procedure except the bilateral occlusion of the renal pedicle. After 24 hours, mice were euthanized, blood and kidney were harvested to observe renal indicators of injury. Serum creatinine (SCR) values, a measure of kidney function, were elevated in mice that underwent IRI, with higher elevations seen in males relative to females, but showed a dichotomy with different housing conditions. While females demonstrate slightly higher SCR after IRI when housed at 30C relative to those housed at 22C, while in males, SCR elevation was decreased in the injured mice housed at 30C relative to those housed at 22C. The SCR levels in injured females and males housed at 30C approximate each other. Protein markers of renal injury kidney injury molecule 1 (KIM1) and Neutrophil gelatinase-associated lipocalin (NGAL), were also investigated. KIM1 was significantly elevated in males relative to females at 22C housing temperature. However, housing at thermoneutrality resulted in decreased levels in both males and females. NGAL expression after IRI was elevated in all mice with males showing higher elevations than females. Housing at thermoneutrality resulted in decreased NGAL relative to that found in mice housed in standard temperature in both males and females. Taken together, these data suggest that sex differences are diminished at thermoneutral housing by a reduction in kidney injury resulting in lesser sex difference. Alteration of resistance to AKI by thermoneutrality, and the resulting changes to mitochondrial energetics, may provide insight into our understanding of the mechanisms that underpin sex-based susceptibility to AKI. This study was supported by an Administrative Supplement for Research on Sex/Gender Influences to the UAB-UCSD O’Brien Center for Acute Kidney Injury Research (IP30 DK079337). All studies were conducted in accordance with guidelines for experimental procedures as set forth in the Declaration of Helsinki and APS “Guiding Principles for the Care and Use of Animals in Research and Training” and were reviewed and permitted by the Institutional Animal Use and Care Committee of UAB.
Background: Nitric oxide (NO) from endothelial NO synthase (eNOS) is cardioprotective and data indicate reductions in endothelial NO-dependent vasodilation occurs in men approximately 10 years earlier than in women. Evidence also suggests men have higher levels of oxidative stress than women, which may underlie decreased production and bioavailability of NO in men relative to women. Thus, we aimed to investigate sex differences in protein expression of eNOS and markers of antioxidant capacity in human aortic endothelial cells (HAECs) and human umbilical vein endothelial cells (HUVECs) under basal conditions. Methods: HAECs and HUVECs (Cell Applications, San Diego, CA) derived from a healthy, young male and female were cultured and collected for assessment of protein expression of nuclear erythroid factor 2-related factor 2 (Nrf2), a major transcription factor involved in regulation of antioxidant genes, and its products heme oxygenase (HO)-1, a cytoprotective enzyme, and NADPH quinone dehydrogenase (NQO1), an antioxidant enzyme, using western blot. Proteins were normalized to β-actin. Data were analyzed by unpaired t-tests (P ≤ 0.05) and are expressed as arbitrary densitometry units as mean ± standard deviation. Results: In HAECs, phospho-eNOS Ser1177 protein expression (0.02 ± 0.005 vs 0.01 ± 0.001, P = 0.006) was greater in female compared to male while total eNOS (2.38 ± 0.05 vs 2.09 ± 0.23, P = 0.0005) protein expression was derived HAECs. The opposite was observed in HUVECs as male had higher phospho-eNOS Ser1177 (1.54 ± 0.23 vs 1.21 ± 0.15, P = 0.04) and lower total eNOS (0.11 ± 0.05 vs 0.04 ± 0.006, P = 0.008) expression compared to female. NRF2 protein expression was greater in HAECs from female in comparison to male (0.17 ± 0.02 ± 0.08 ± 0.02, P = 0.0002) while in HUVECs female had lower NRF2 expression than male (0.25 ± 0.05 vs 0.41 ± 0.12, P = 0.01). HO-1 protein expression was greater in male compared to females in HAECs (0.69 ± 0.07 vs 0.16 ± 0.02, P <0.0001) and HUVECs (1.29 ± 0.52 vs 0.21 ± 0.10, P = 0.001). No significant difference in NQO1 expression was observed between genders in HAECs (2.48 ± 0.36 vs 2.36 ± 0.37, P = 0.6). However, HUVECs from male had greater NQO1 compared to female (0.24 ± 0.03 vs 0.14 ± 0.02, P = 0.0007). Conclusion: These preliminary data suggest a divergent sex response where there is an inverse expression of total eNOS, phosphorylation of eNOS and NRF2 between HAECs and HUVECs, while HO-1 expression is similar in both cell lines, but not between sexes. Careful consideration should be taken when choosing endothelial cells as an in vitro model to study disease, as expression of eNOS and antioxidant responsive element-associated proteins appear region and sex-specific which may confound findings and decrease physiological relevance.
in myelination or neuron growth during the early stages of adolescence. However, female pups who were exposed to a single immunotherapy event in utero had decreased expression of MBP suggesting that T cell suppression in utero affects neuronal development of only female pups. Paired with behavioral data suggesting hyperactivity among rat pups from Orencia dams, these studies suggest a possible link between immune cell dysregulation during pregnancy and ADHD.