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APSSG21.5

Long-Term Effects of Estrogen Loss on Cardiac Function Following Chronic Sympathetic Stimulation

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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 (LVESD), left ventricular end-diastolic 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 compared to the 12M-OVX+ISO 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.

APSSG21.6

Endothelial Function in Transgender Women on Gender-Affirming Estrogen Therapy: A Protocol

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Transgender women (individuals assigned male sex at birth who identify as women) have increased cardiovascular risk as compared to their cisgender (gender identity aligns with sex at birth) counterparts. Oral, as compared to transdermal, estrogen use has been associated with impaired endothelial function and increased cardiovascular risk in cisgender women, though whether this applies to the transgender women population using gender-affirming estrogen therapy is unknown. The aim of this study is to determine the associations between oral and transdermal

estrogen use and endothelial function in healthy transgender women. We hypothesize that oral estrogen use will be associated with impaired endothelial function as compared to transdermal estrogen use. Healthy transgender women (≥18 years old) who have taken gender-affirming estrogen therapy for ≥3 months and can provide informed consent will be recruited using study posters and word-of-mouth through our partnerships with local transgender community organizations. Flow-mediated vasodilation, a non-invasive measure of endothelial function and a validated assessment of cardiovascular risk, will be assessed by high-resolution vascular ultrasound examination of the brachial artery. After resting in a supine position for a 5-minute period, participants' right brachial artery diameter will be measured at baseline and in response to hyperemia using a blood pressure cuff inflated to supra-systolic pressure (200mHg or 50mmHg above systolic blood pressure) to cause distal limb ischemia. Flow-mediated dilation will be measured between 45 seconds and 2 minutes post-cuff release, and calculated as a function of baseline arterial diameter. Assuming a 6% difference in flow-mediated dilation between oral and transdermal estrogen use, 80% power with 2-sided alpha 0.05, 28 healthy transgender women (14 oral estrogen users, 14 transdermal estrogen users for minimum 3 months) will be studied. Institutional research ethics approval has been obtained and study recruitment is currently ongoing. Analysis of Covariance will be employed to evaluate the association between flow-mediated dilation and route of estrogen administration, while adjusting for additional covariates including age, body mass index, and smoking/vaping status. Follow-up sensitivity analyses will be performed excluding current smokers/vapers. This research will determine the difference in cardiovascular risk between oral and transdermal gender affirming estrogen therapy use. The results of this study have the potential to better inform transgender women and their health care providers regarding gender-affirming therapies and cardiovascular health. Funding: This study is unfunded.

APSSG21.7

The systemic inflammatory response induced by LPS administration is more pronounced in women than in men

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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 endotoxin 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.

APSSG21.8

Estrogen Regulates Kisspeptins Signaling in Human Airway Smooth Muscle

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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 NFkB 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.

APSSG21.9

Cardiovascular risk of gender affirming hormone therapy in transgender men

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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.

APSSG21.10

Sex, Mitochondria and Metabolism

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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 metabolic phenotypes have been amply described 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 mitochondria 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.

APSSG21.11

Sex-specific gene expression signature in obese human and rat cardiac hypertrophy

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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 Nppa 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.

APSSG21.12

Obesity Mediates Cardiovascular Sex Differences in Polycystic Ovary Syndrome for Genetically Predisposed Individuals

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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

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

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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 60g 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

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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±11.7cm/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

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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-plantar 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

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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

euthanasia (15 weeks of age), kidneys and blood were collected. Renal cortical and medullary RAS protein expression was assessed by Western blotting using total protein content for normalization. Angiotensinogen (Agt) ELISA and angiotensin-converting enzyme 2 (ACE2) activity were performed on plasma. Student's t test was used for statistical analyses. HAF rats had increased body weight and kidney weight normalized by tibial length (0.5410 ± 0.022 vs 0.3827 ± 0.013 g/cm, p<0.0001). Agt expression was increased in the cortex of HAF rats (1.102 ± 0.020 vs 1.000 ± 0.033, p=0.02), but not in the medulla. Agt was also increased in the plasma of HAF rats (375.8 ± 13.2 vs 329.4 ± 9.3 ng/mL, p<0.01). ACE2 expression was increased in both the cortex (1.735 ± 0.118 vs 1.000 ± 0.066, p<0.0001) and medulla of HAF rats. However, plasma ACE2 activity was decreased (65.0 ± 2.3 vs 72.6 ± 2.4 pmol/min/mL, p<0.05) in HAF rats. The angiotensin II receptor type II (AT2R) was upregulated in the cortex (1.763 ± 0.163 vs 1.000 ± 0.05004, p<0.0001) and medulla of HAF rats. HAF rats exhibited an upregulation of kidney cortical Agt as well as plasma Agt. Since the substrate for the rate-limiting step of the RAS is Agt, intervening at this step by administering a renin inhibitor may be suited for targeted therapy. Increasing renal ACE2 and AT2R may attempt to compensate for the androgen-mediated elevation in BP, although ACE2 activity is reduced in HAF rats. This finding suggests that using an ACE2 agonist may ameliorate increased BP in PCOS. In conclusion, chronic hyperandrogenemia in a female rat model results in pathological and compensatory deviations in RAS component expression that may be targeted using novel pharmacological approaches. Supported by NIH grants: NIGMS P20GM121334 (LLYC & DGR), NIDDK R21DK113500 (DGR), NIGMS P20GM104357, NHLBI P01HL51971

APSSG21.17

Reduced Uterine Perfusion Pressure prevents Seizure-induced reductions in neurotransmitter transporters in pregnant mice

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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<0.5; 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±0.4 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, T32HL105324, William Townsend Porter Pre-doctoral Fellowship from the American Physiological Society

APSSG21.18

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

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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

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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 cubilin 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

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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

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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 they 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

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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

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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 ass 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

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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 uM 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 estrodial was added to the purfasate 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

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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.

APSSG21.28

Cardiovascular Disease and Menopause

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Despite the recent declines in cardiovascular disease (CVD) burden, CVD remains the leading cause of death in women. Notably, women develop coronary heart disease (CHD) several years after men, with visible increases in CHD risk seen after menopause. This observation led to hypothesize that the menopause transition (MT) contributes to this rise in CHD risk. Over the past 20 years, longitudinal studies of women traverse menopause have contributed significantly to our understanding of the relationship between the MT and CVD risk. By following women over the MT, researchers are able to unravel chronologic aging from ovarian aging effects in relation with CVD risk. Longitudinal studies on menopause have documented dramatic changes in sex hormones, and adverse changes in body fat deposition, lipids/lipoproteins, and vascular remodeling over the MT. These changes can, collectively, increase women's risk of developing CVD later in life. Interestingly, patterns of sex hormones and vasomotor symptoms over the MT have been linked to greater risk of subclinical atherosclerosis after menopause. Most recently, new potential CVD risk markers/issues relevant to menopause have been pointed out. One is heart fat which has been found to be greater in postmenopausal women and related to lower estradiol levels. A second evolving issue is higher levels of high-density lipoprotein cholesterol (HDL-C), which has not been consistently cardioprotective in postmenopausal women. Findings so far underline the significance of the MT as a time of accelerating CVD risk, which emphasizes the importance of monitoring women's health during midlife, a critical window for applying early intervention strategies to reduce CVD risk as women age.

APSSG21.29

G Protein-Coupled Estrogen Receptor Involvement in Sex Differences in Pathophysiology in a Preclinical Model of Hyponatremia

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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 hypothalamo-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 hematocrit 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

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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

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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 response to ganglionic blockade (2-way ANOVA, $P<0.0413$) but preserved HR responses to propranolol and atropine respectively. Allo-Aca treatment significantly decreased (2-way ANOVA, $P<0.0011$) 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:

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The hemodynamic response to sympathetic activation differs in women with natural menstrual cycles and women using oral contraceptives

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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

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Sex differences in the immune system: placenta and beyond

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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). EVTs remodel the SUAs by replacing the endothelial and smooth muscle cells from these vessels so that they are no longer vasoactive and able to contract. This remodelling results in arteries with large lumens and low-resistance, ensuring consistent blood flow to the placental bed and fetus. Pregnancies 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 substantial 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

pregnancy and, in case of pregnancy complications, personalised interventions should be employed that target specific cellular and molecular mechanisms.
Primary Author is a(n): Investigator

APSSG21.38

Sex Differences in Vascular Stiffening with Age

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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.

APSSG21.39

Selection of Extraction Methodology and Baseline Gender Differences in Rodent Gut Microbiomes: Important Considerations for Conducting Pre-Clinical Microbiome Research

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Background: Rodent models are used to test hypotheses about gut microbiota (GM) in health and illness. The ability to compare results across GM studies has been challenging, however, because pre-clinical researchers employ different methods that alter the GM including telemetry implantation. Furthermore, sex as a biological variable is an important component of reproducible research, yet baseline sex GM differences in pre-clinical rodent models are understudied. Aim: Evaluate if sex, surgical transmitter implantation, prophylactic subcutaneous antibiotic administration or DNA extraction methods alter GM community structure. Methods: The GM was assessed by high throughput 16S rRNA gene amplicon sequencing. To evaluate sex-related GM differences, we collected fecal samples from 5 male and 5 female Wistar Kyoto rats (no surgery/no antibiotic) for 5 days. To examine the effect of a surgical procedure, we implanted a permanent telemetry device into 5 male Wistar Kyoto rats. Following standard prophylaxis procedures, these rats received enrofloxacin intraoperatively (5mg/kg SQ). To determine whether surgery and antibiotic prophylaxis affected the GM, we compared pre-surgery results with post-surgery results (days 1, 3, and 7 postop) and also compared to an antibiotic only group (n = 2; no surgery). Alpha diversity was quantified by Shannon index and beta diversity using Bray-Curtis dissimilarity matrices. Differential abundance of bacteria was calculated using analysis of composition of microbiomes (ANCOM). Results: In male and female rats, DNA extraction method influenced alpha diversity and bead beating (Qiagen) was associated with increased alpha diversity versus Promega (P=0.01). In males versus females, when daily replicates were averaged (to control for female estrus), alpha diversity was not different by sex (P=0.12). Longitudinally, there was a sex (F=8.6, P=0.004) and time (F=4.6, P=0.03) effect on alpha diversity, but no interaction between sex*time (F=0.4, P=0.55). Nevertheless, male and female rats had significantly different microbial community structure (beta diversity; PERMNOVA F=8.3, P=0.001). The relative abundance (RA) of several Bacteriodetes bacteria were significantly higher in females and males had higher RA of Firmicutes bacteria (ANCOM). For the antibiotic and surgery comparisons, the antibiotic only group had no change in alpha diversity over time. In the surgery + antibiotic group, there was a postop reduction in alpha diversity in the samples in the Qiagen extracted samples (group*time F=4.8, P=0.038), but this was not reproduced in Promega samples (group*time F=0.001, P=0.97). Conclusions: The study is important for defining appropriate experimental controls in rodent

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

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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, only 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

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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-g; 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.
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APSSG21.42

Maternal B Cell Depletion Decreases Blood Pressure and Improves Fetal Weights in Offspring of a Rat Model of Preeclampsia

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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 depletion, 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 123±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 (5.11±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, 1.6±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

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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, and meta-regression and subgroup analyses will be used to explore potential source 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

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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), 1.029-3.616]; 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

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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 pre-contraction 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

increased. Female vessels but not male vessels exhibited increased myogenic reactivity. Mouse aortas and mesenteric arteries showed no difference in vessel elasticity after prenatal BPS exposure. Conclusions: Although BPA has been linked to negative health outcomes, little is known regarding the effects of its substitutes and their role in developmental programming of cardiovascular disease. Here, we demonstrate that prenatal BPS exposure leads to alterations of vascular function in a sex-specific manner via differential regulation of vascular development. Funding sources: The Canadian Institute of Health Research, The Cumming School of Medicine, The Libin Cardiovascular Institute, and the University of Calgary Faculty of Graduate Studies References: 1. Alonso-Magdalena P, Quesada I, Nadal Á. Prenatal Exposure to BPA and Offspring Outcomes: The Diabesogenic Behavior of BPA. 2015 Jun 15;13(2). 2. Liao C, Liu F, Alomirah H, Loi VD, Mohd MA, Moon HB, et al. Bisphenol S in urine from the United States and seven Asian countries: Occurrence and human exposures. Environ Sci Technol. 2012 Jun 19;46(12):6860–6. 3. Gingrich J, Pu Y, Ehrhardt R, Karthikraj R, Kannan K, Veiga-Lopez A. Toxicokinetics of bisphenol A, bisphenol S, and bisphenol F in a pregnancy sheep model. Chemosphere. 2019 Apr 1;220:185–94. 4. Duckles SP, Miller VM. Hormonal modulation of endothelial NO production. Pflugers Arch. 2010 May;459(6):841.

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Microbiome in Obese Pregnancy and Preeclampsia

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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 placentation 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

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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 MetaboAnalyst 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-5'-thioadenosine, were different in male vs 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, acetyllysine 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)

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Guanylate cyclase-C and anxiety-like behavior: gender and estrus cycle differences

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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 oestrous 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.

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Regulation of brown adipose tissue activity by brain uroguanylin is dependent on gender and phase of estrous cycle

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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 T-1020). 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).

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Sex-specific bone matrix signatures divergently influence endothelial cell survival.

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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. Following 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.

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Cluster of Differentiation 14 (CD14) Attenuates Salt Sensitive Hypertension and Renal Injury in Females but not Males

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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

marrow transplant studies then confirmed that the blood pressure and renal damage effects are due to knockout of CD14 in hematopoietic cells. Finally, ovariectomy completely abrogated the effect of CD14 knockout. These studies provide a novel treatment target and evidence of a new dichotomy in immune activation between sexes within the context of hypertensive disease where CD14 inhibits immune cell activation and prevents salt-sensitive hypertension and renal injury in females.

APSSG21.56

Role of the Renal Androgen Receptor in Ammonia Metabolism

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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.

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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 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 (iSAT) will be collected and flash frozen. Western blot analysis of protein isolated from the liver or iSAT will be used to 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

iSAT. Expected Results: We expect that female offspring of HetDB pregnancies will retain insulin-mediated inhibition of lipolytic and lipogenic processes, while male offspring will show a phenotype characteristic of insulin resistance. Significance: Obesity is now considered endemic, and as a greater number of pregnancies are confounded by maternal obesity, it is critical to understand the effects on the offspring.

APSSG21.58

Acclimation to a High Salt Diet is Sex Dependent

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Premenopausal females are less likely to develop hypertension and salt-related complications than are males, yet the impact of sex and sex hormones on mechanisms regulating Na+ homeostasis is poorly defined. We determined whether female rats have a more efficient capacity to acclimate to increased dietary salt intake challenge. Male and female rats maintained on a normal salt (NS) diet were challenged with a 5-day high salt (HS) diet. We assessed serum, urinary, skin, and muscle electrolytes, total body water, and kidney Na+ transporters during the NS and HS diet phases. During the HS challenge, natriuresis increased more rapidly in females, whereas serum Na+ and body water concentration increased only in males. To determine if females are primed to handle changes in dietary salt, we tested whether the renal endothelin-1 (ET-1) pro-natriuretic system is more active in females, compared to males. During the NS diet, female rats had a higher urinary ET-1 excretion rate than males. Ingenuity Pathway Analysis of RNA sequencing data identified the enrichment of ET signaling pathway transcripts in the inner medulla of kidneys from NS-fed female rats compared with male counterparts. Notably, in human subjects who consumed a Na+-controlled diet, women had a higher urinary ET-1 excretion rate than men consistent with our rats. Estrogen signaling via G protein-coupled estrogen receptor 1 (GPER1) can elicit cardiovascular and renal protective actions. Renal GPER1 expression is significantly higher in female rats than in male rats. We recently found that activation of GPER1 in the renal medulla of female rats, but not males, evokes ET-1-dependent natriuresis. Using pharmacological and genetic approaches, we revealed that ET receptors in the renal medulla of female rats work co-operatively to mediate the diuretic and natriuretic response to GPER1 activation. In addition, GPER1 deletion decreased urinary ET-1 excretion in female mice but not males. Of note, wild-type female mice had significantly greater urinary ET-1 excretion than wild-type males, whereas knock-out mice had no sex differences in ET-1 excretion. Importantly, urinary excretion of ET-1 reflects intrarenal ET-1 production/release. Overall, these results suggest that female sex confers a greater ability to maintain Na+ homeostasis during acclimation to dietary Na+ challenges. Our data uncover an important role for renal medullary GPER1 in promoting Na+ excretion via an ET-1–dependent pathway in females, but not in males. 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

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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 the gut-brain-axis, 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 defecation 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

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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 achetylcholine 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 tethered 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 sFlt1-induced preeclampsia

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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

signaling to mitigate the increased risk of cardiovascular disease in women exposed to PE.

APSSG21.62

Effects of the COVID-19 pandemic on type 2 diabetes mellitus incidence and sex disparities.

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Background: Sex differences in the interaction between Type 2 diabetes mellitus (T2DM) and cardiovascular diseases exist, however early diagnosis and frequent screenings for T2DM are essential for both sexes because T2DM increases the risk of heart disease and stroke regardless of sex. Several new studies have reported that obese male patients are associated with severe outcomes with Corona Virus Disease (COVID-19). Yet, the potential impact of the pandemic on T2DM diagnosis and screening is unknown, and it is not clear if sex has affected the characteristics of T2DM patients during the pandemic. Thus, the aim of this retrospective study was to explore the impact of COVID-19 on T2DM incidence and sex-based differences in characteristics during the COVID pandemic. We tested the hypothesis that the number of newly diagnosed T2DM patients would decrease, and sex disparities in T2DM patients would be seen during the pandemic. Methods: Using electronic medical records (EHRs) obtained from the USA-based TriNetX database, prevalence and incidence of “T2DM (ICD-10-CM: E11)” pre- and during the COVID pandemic were determined. Aggregate lab data from EHRs were extracted and statistical analysis were performed using lab values and patient demographics such as sex, race, and comorbidities. Results: Noticeable declining trends in T2DM incidence were identified, with April 2020 exhibiting the largest decrease. Compared to April 2018, both sexes displayed the largest decrease in April 2020. Monthly T2D patients’ lab data revealed worsening T2DM parameters such as hemoglobin A1c in the April 2020 group. Demographic data during 2020 revealed that male T2DM patients had a significantly higher prevalence of comorbidities including hypertension (M vs F: 66% vs 64%), CDK (M vs F: 17% vs 14%), heart failure (M vs F: 14% vs 11%), but female T2D patients had a significantly higher asthma comorbidity (M vs F: 6% vs 12%). Conclusion: As a result of the COVID-19 pandemic, there has been a marked decrease in T2DM diagnosis, as well as its prevalence and the April 2020 data appeared to show worsening T2DM severity. These data may suggest the possibility of a future increase in the diagnosis of T2DM related heart disease. Furthermore, our analysis indicated that sex disparities persisted in T2DM patients during 2020, and these pandemic-related sex and racial disparities in T2DM patients may magnify the existing sex differences in heart attack and stroke patients in the post-COVID era

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Sex Differences in HPA Axis Dynamics in the Rat: Interaction of Opioid Abstinence and Sleep Restriction

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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 ambulation 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,

thereby affecting vulnerability to relapse. Funding: NIH R01 HL150523 National Institutes of Health HEAL (Helping to End Addiction Long-Term) Initiative

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Sodium-Glucose Cotransporter-2 Inhibition Decreases Mitochondrial Dysfunction in White Adipose Tissue in Hyperandrogenemic Female Rats

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Introduction: Polycystic Ovary Syndrome (PCOS) is the most common endocrinopathy in reproductive-age women. It is characterized by androgen excess and ovulatory dysfunction; 80% of this population is obese. Obesity may be caused by mitochondrial dysfunction (MD) leading to oxidative stress by production of reactive oxygen species (ROS). Sodium-glucose cotransporter-2 Inhibitors (SGLT2i) decrease fat mass in women with PCOS and improves MD in white adipose tissue (WAT) in diabetic male mice. We have previously reported that hyperandrogenemic female (HAF) rats have increased body weight (BW), fat mass, and insulin resistance (IR), and that SGLT2i decreases their fat mass without decreasing food intake or IR. We want to test the hypothesis that SGLT2i improves obesity in HAF rats by ameliorating MD in WAT. Methods: 40 four-week old female Sprague Dawley rats were randomized to either placebo (PBO) or dihydrotestosterone (DHT) exposure (7.5 mg/90days). After 10 weeks of DHT, rats received drinking water alone or with the SGLT2i empagliflozin (10mg/kg/day) for another 3 weeks. Body composition was analyzed by EchoMRI before and after SGLT2i. Upon euthanasia, subcutaneous WAT was collected. qRT-PCR data was normalized by the geometric mean of 3 housekeeping genes and log2 transformed. Protein expression was normalized by actin-beta. Results: When corrected by BW, HAF rats increased their fat mass compared to PBO while SGLT2i in HAF prevented this (-25.9 ± 3.9 vs 4.8 ± 6.0 %, $P<0.0001$). Compared to PBO, HAF rats had decreased cytosolic superoxide dismutase (SOD1) gene and mitochondrial SOD (SOD2) gene & protein expression. In HAF rats, SGLT2i increased SOD1 gene (-0.13 ± 0.10 vs -0.85 ± 0.15, $P<0.05$) expression, and increased SOD2 gene (-0.18 ± 0.11 vs -0.88 ± 0.14, $P<0.05$) & protein (1.08 ± 0.09 vs 0.78 ± 0.07, $P=0.095$) expression. Nuclear respiratory factor 1 (NRF1) gene expression was downregulated in HAF rats compared to PBO (-0.88 ± 0.10 vs -0.08 ± 0.19, $P<0.05$), with NRF1 being a prominent regulator of mitochondrial biogenesis. SGLT2i in HAF upregulated NRF1 (0.27 ± 0.14 vs -0.88 ± 0.10, $P<0.001$). In PBO, SGLT2i had no significant impact on the above parameters. In summary, androgen excess in female rats led to increased fat mass compared to controls. This was associated with decreased cytosolic and mitochondrial SOD expression in WAT, suggesting increased susceptibility to oxidative damage. Additionally, androgen excess also decreased NRF1 expression, pointing to decreased mitochondrial biomass. SGLT2i decreased fat mass in HAF rats, which was accompanied by increased expression of SODs and NRF1. Our data suggest that SGLT2i 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

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

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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.

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Angiotensin II-induced hypertension and kidney injury: lack of significant sexual dimorphism in PT-Agtr1a-/- mice

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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 infused 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.
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Exploring sex differences in renal sodium transporters with Four Core Genotype (FCG) model

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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. FXX and FXY (ovaries); whereas, traits influenced by SCC are similar between MXX and FXX 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 are 60% higher in FXX vs MXY 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 SPAKp 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*

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Adoptive transfer of T regulatory cells prevents Ang II-induced postmenopausal hypertension

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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

hypertension. The goal of the present study was to determine if adoptive transfer of donor T regulatory cells into postmenopausal females could prevent the development of Ang II-induced hypertension. Postmenopausal females (VCD mouse model of menopause) received T regulatory cells (Meno/Treg), T effector cells (Meno/Teff), or vehicle control (Meno/PBS) via tail vein injection at two-week intervals, followed by Ang II infusion (800ng/kg/min) for 14 days. Blood pressure was monitored via tail cuff. Adoptive transfer of Teff or Treg cells did not alter baseline blood pressures prior to Ang II infusion. Following Ang II infusion for 14 days, there was a significant increase in mean arterial pressure in both menopausal groups that received PBS and Teff cells (14-day delta MAP: Meno/PBS 32.3 + 4.9 mmHg *, Meno/Teff 30.3 + 4.7 mmHg*, *p<0.05 vs baseline). In contrast the menopausal group that received T reg cells had no significant increase in mean arterial pressure following 14 days of Ang II infusion (14-day delta MAP: Meno/Treg 2.9 + 4.4 mmHg). Flow cytometric analysis determined no significant differences in the frequency of splenic T helper (CD4+) or T reg cells (FoxP3+) following Ang II infusion in any group of postmenopausal females, irrespective of the T regulatory or T effector transfer. Protection against Ang II induced hypertension in the postmenopausal females that received the adoptive transfer of Tregs (Meno/Treg) was associated with a significant increase in T regulatory cells in the kidneys, as measured via flow cytometry (Renal FoxP3: Meno/PBS 11.0%, Meno/Teff 12.1%, Meno/Treg 16.9%*; * p<0.05 vs Meno/Teff and Meno/PBS). The data suggest that postmenopausal expansion of T reg populations could protect postmenopausal females from susceptibility to hypertension and that T regulatory cell infiltration into the kidneys may contribute to this protection.

APSSG21.71

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

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Background: Activation of endothelial small conductance calcium-activated K+ channels (KCa2.3) and intermediate conductance calcium-activated K+ channels (KCa3.1) leads to vascular relaxation. Our previous studies have shown that endothelial KCa2.3 down-regulation in corpus cavernosum diminishes erectile function. Aim: We hypothesized that in type-2 diabetic mice KCa2.3 channel function is impaired in erectile tissue. Methods: C57BL/6 (WT) and hetero(db/+)- or homo(db/db)- zygote a diabetic type 2 mice model were sacrificed in accordance to APS Guiding Principles for the Care and Use of Animals in Research and Training. Erectile function was measured, and corpus cavernosum strips were mounted for functional studies, and processed for qPCR and immunoblotting. Results: In anesthetized diabetic db/db mice, erectile function was markedly decreased compared to non-diabetic heterozygous db/+ mice, and the impairment was even more pronounced compared to normal WT mice. qPCR revealed KCa2.3 and KCa1.1 channel expressions were upregulated in corpus cavernosum from db/db mice. Immunoblotting showed down-regulation of KCa2.3 and the KCa1.1β subunit in the corpus cavernosum from db/+ mice and for KCa1.1β subunit in the db/db mice. Acetylcholine relaxations were impaired only in Phenylephrine pre-contracted but not in Noradrenaline ones, while relaxations induced by the nitric oxide donor, SNP were unaltered in corpus cavernosum from db/db compared to db/+ mice. NS309 (0.5 μM), an activator of KCa2 and KCa3.1 channels, leftward shifted concentration-response curves for acetylcholine in corpus cavernosum from db/+ mice, but this was not the case in corpus cavernosum from db/db mice. Apamin, a blocker of KCa2 channels, inhibited acetylcholine relaxation in corpus cavernosum from db/db and db/+ animals, being less effective in db/db compared to db/+ animals. Iberotoxin a blocker for KCa1.1 channels, inhibited acetylcholine 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.

APSSG21.72

Peripheral hypercapnic chemosensitivity during exercise in males and females.

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Hypercapnic chemosensitivity is the ventilatory response to increased partial pressure of CO2 and is the result of central and peripheral chemosensors 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 chemosensitivity 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 (161±31.5 L/min) vs female subjects (102±19 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

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

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[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

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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% O2) 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”, “KRAS SIGNALING UP”, “TNFA SIGNALING VIA NFKB”, “ANDROGEN RESPONSE”, “UV RESPONSE DN”, “EPITHELIAL MESENCHYMAL TRANSITION”. In normoxia “SPERMATOGENESIS” was enriched in male 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.
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Maternal plasma proteome profiling of biomarkers for stratification of early-onset and late-onset preeclampsia

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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.

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Membrane androgen receptor-induced neurodegeneration

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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.

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Interaction of Sex, Chronic Pain and Obesity on Cortisol in Adolescent Humans

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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 by 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

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Effect of Gonadal Hormones on Autoimmunity, Pathology and Behaviour in the 3×Tg-AD Mouse Model of Alzheimer's Disease

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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 Aβ42 levels in females, whereas male sex hormones increase expression of Aβ42 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.

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The Effect of Inorganic Nitrate on Arterial Stiffness and Blood Pressure Across the Menstrual Cycle in Healthy Subjects

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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, AIx 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; $p = 0.3$), AIx (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.

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Sex Differences in Dental-Associated Cardiovascular Disease

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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 males 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.

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Long-term cardiovascular impact of maternal depression with peripartum onset: gender-based differences in the adult offspring

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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(1) and 1MA/PM2/18).

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Sex differences in alcohol consumption and alcohol-associated liver disease

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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.

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New studies in mechanisms of polycystic ovary syndrome

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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 syndrome1. 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 pregnancy2, 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 offspring3, suggesting that androgen-receptor pathways are molecular gateways to PCOS transmission3. 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 transmission1. Recent studies indicates that not only PCOS daughters but also their sons also have an increased risk of developing disorders associated with PCOS4-6. 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 health7,8. 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:S1471-4914(21)00129-5. doi: 10.1016/j.molmed.2021.05.005. Online ahead of print.PMID: 34127396

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Odysseus was right-Mentoring is Important!

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The term ‘mentor” comes from the Odyssey by Homer, and is the name of the person that Odysseus (or Ulysses) asked to help his wife, Penelope, educate and take care of his son, Telemachus, over the years when he was away fighting the Trojan war. You will need mentors for every stage of your career from your earliest days as a student to when you reach the top of your field as an administrator, educator, industry CEO, etc. In this short talk we will discuss how to choose mentors, the responsibilities that the mentor has to the mentee, and the responsibilities the mentee has to the mentor.

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Men at higher risk of mortality during New York COVID Surge: A case for physiological mediators

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Background/purpose- Previous studies have shown that men have a higher mortality rate and more severe COVID-19 infection than women. The mechanism for this disparity is not clear. We hypothesize that innate sex differences in hormonal and immune system milieu drive the higher mortality in men. · Methods- A cohort study was conducted to compare male mortality during the COVID-19 surge in New York as compared to a pre-COVID period. A population of 364,992 adult patients receiving care at Bronx Montefiore Health System (BMHS) between 1/1/2018 and 1/1/2020 was defined. Those individuals hospitalized during the pre-COVID period (1/1/2020-2/15/2020) and during the COVID surge (3/1/20-4/15/20) were examined for outcomes. Individual addresses were linked to the American Community Survey to define census tract level socio-demographic data. Descriptive and bivariate statistical methods were used to evaluate differences by sex in clinical and socio-demographic variables of the BMHS population defined. Unadjusted and adjusted (for those variables significant in bivariate testing) logistic regression modeling were used to measure association of male sex with hospital mortality through 8/15/2020 for both pre-COVID and COVID time periods. · Results- Men were older (mean of 50.0 years versus 49.9 years; $p<0.001$), had more comorbidities (20.1% had a Charlson comorbidity score >3 versus 19.2% for women; $p<0.001$), more had diabetes (17.8% versus 16.5%; $p<0.001$), hypertension (47.2% versus 43.6%; $p<0.001$), more prevalence of smoking (36.4% versus 24.0%; $p<0.001$), lower BMI (median of 28.0 versus 29.2; $p<0.001$) and lower prevalence of

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

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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.

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Establishment of novel placenta-on-a-chip model

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Background: Preeclampsia is a cardiovascular disorder diagnosed post 20 weeks of gestation. It is the leading cause of morbidity and mortality in pregnancy. Inappropriate placentation 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 vasculogenesis potential (CD31; $p<0.005$). Conclusions: Our novel 3D microfluidics model of human placental tissue can recapitulate aberrant placentation 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.

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Quantifying dynamic cerebral autoregulation during repeated squat-stand maneuvers with 20% resistance above body weight

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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 weighted 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

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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

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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 (1g, 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 density bone 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 micro-gravity 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: 80NSSC21K0311 and 80NSSC19K9518.

APSSG21.92

Squat-stand maneuvers alter cardiovascular and autonomic recovery in females

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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 second 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

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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-cl1 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

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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 diepoxide 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

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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-wk 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.
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APSSG21.97
Circulating cell-free mitochondrial DNA in preeclampsia

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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

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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, were 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 (HH:1.010 ± .007, LH:1.012 ± .009; p =.542) or 24-hr osmolality (HH:388 ± 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 (HH:1.005 ± .001, LH:1.005 ± .001; p =.656) or 3-hr osmolality (HH:193 ± 40, LH:203 ± 60mOsm/kg; p =0.485). NBW change did not differ between trials (HH:0.29 ± 0.36, LW:0.44 ± 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.

APSSG21.99

Advanced Age and Female Sex Protect Cerebral Arteries during Acute Oxidative Stress

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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 (H_2O_2) 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 H20) and exposed to H_2O_2 (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, H_2O_2 killed ~10% in each group. Consistent with elevated $[Ca^{2+}]i$ initiating cell death, peak $[Ca^{2+}]i$ responses (Fura-2 fluorescence) to H_2O_2 were greater in PCAs of young (ΔF340/F380=0.51±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 vanilloid 4 (TRPV4) channels (HC-067047, 1 μM) attenuated the rise of $[Ca^{2+}]i$ and reduced SMC death in response to H_2O_2 most effectively in young males (3±1%) but had negligible effect in PCAs from old males or females. Activating TRPV4 channels (GSK-1016790A, 50 nM) during H_2O_2 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 ($\Delta\Psi m$) can lead to cell death. To evaluate $\Delta\Psi m$ during H_2O_2 exposure, PCAs were equilibrated with tetramethylrhodamine methyl ester (TMRM; 10 nM). Over 30 min, H_2O_2 induced greater depolarization of $\Delta\Psi m$ (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 Ca^{2+} attenuated $\Delta\Psi m$ 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 H_2O_2, $\Delta\Psi m$ 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 H_2O_2 in young males is associated with greater TRPV4-dependent Ca^{2+} entry and $\Delta\Psi m$ depolarization. Funding source: AHA 10TPA34850102, R01HL136292.

APSSG21.100

Sex bias in murine bone studies

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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 (37.8%, 785 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.

APSSG21.101

Maternal High Fat Diet-Induced Obesity Confers Sex Differences in Offspring Body Composition and Glucose Clearance

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Background: Maternal obesity and high-fat diet (HFD) during pregnancy increase offspring's cardiovascular disease risk. How the early life environment underlies sex differences in metabolic disease risk is not yet fully understood. Methods: To examine the effects of maternal high fat diet (HFD)-induced obesity on offspring body composition and glucose clearance, female C57BL6/J mice were fed a control (17% fat, TD95092) or HFD (45% fat, TD8811) ad libitum for 12 wks prior to and during pregnancy and during lactation (n = 19-30). Female mice were bred with lean male mice at 17 wks of age. At weaning, male and female offspring from control and HFD-dams were randomized to control (C) or HFD (H), provided ad libitum, generating 4 groups of offspring: CC, CH, HC, and HH, where the first letter corresponds to maternal diet and the second to offspring postweaning diet. Body composition was assessed using Echo-MRI and glucose clearance measured by intraperitoneal glucose tolerance test (IP-GTT). Results: After 16 wks on postweaning HF diets, both male (40.4g ± 1.3) and female (28.0g ± 1.1) offspring from HFD-fed dams exhibited hyperresponsive weight gain compared to CH offspring (35.4g ± 1.5 & 25.0g ± 0.7). Female HH offspring preferentially gained fat mass (Δ23.4% ± 1.1) over lean mass (Δ2.9% ± 1.3) when compared to CH female offspring (Δ12.4 ± 1.4/13.2% ± 1.7), while in male offspring, postweaning HFD was associated with increase in fat mass independent of maternal diet. In females, neither maternal nor postweaning HFD had any effect on glucose clearance at weaning, after 6 wks on diets, or after 16 wks on diets as measured by IP-GTT. In males, there were no differences in glucose clearance at weaning or after 6 wks on diets. After 16 wks on diets, male HH offspring had significantly higher blood glucose levels after ip glucose injection at all times points (15, 30, 60 and 180 min after glucose injection), while HC males had the lowest blood glucose levels. Conclusions: These data suggest that in our mouse model of maternal HFD programming, both male and female mice exhibit hyperresponsive weight gain to the double hits of maternal and postweaning HFD, while there are sex differences in body composition and glucose clearance. Funding: USDA ARS Project 6026-51000-012-06S

APSSG21.102

Linking maternal obesity and fecal microbiome in hypertensive offspring of preeclamptic-like mice

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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 Firmicutes, 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

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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 meditator 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). Agonistic 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 1.3±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

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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 (QIK, MLK, PKCH, MST, STE7, PEK, FRAY) and 5 in males (AKT, SGK, P38, MARK, CDK). While 15 were affected in both sexes (DMPK, PKA, PKG, RSK, PLK, DYRK, NMO, CAMK1, JNK, PAKA, RAD53, ERK, PAKB, PKD, PIM, AMPK). This data provides new insights into the dysregulation of adipose tissue expansion in females, by identifying

possible target phosphokinases important in the adipocyte hypertrophy as a direct consequence of MSEW. Normalized lipidomic data show that female MSEW mice have significantly increased triacyclglycerols (TAG, $p<0.05$), while phosphoethanolamines and phosphatidylglycerols are downregulated ($p<0.05$). This data confirms the consistent increase in adiposity observed in MSEW female mice. In contrast, male MSEW mice display higher phosphatidylglycerol and phosphoethanolamines concentrations ($p<0.05$) and downregulated TAG compared to controls ($p<0.05$). Interestingly, phospholipids have been shown to activate Transient Receptor Potential Vanilloid 1 (TRPV1) an important component of the Adipose Afferent Reflex (AAR). This data supports previous studies in MSEW male mice that points to the AAR as the mechanism for exacerbated MSEW neurogenic hypertension. Taken together, this data indicates that MSEW fed a HF show a sex-specific signature of metabolic pathways affecting the pgWAT homeostasis. This data supports the hypothesis that MSEW induces exacerbated adiposity only in female mice fed a HF. Identifying functional metabolic signatures in this model is critical to elucidate the underlying molecular mechanisms behind the sex-specific obesity risk associated with early life stress.

APSSG21.106

Does task complexity impact the neurovascular coupling response similarly between males and females?

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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 (1: 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 (ρ) 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 ($\rho=0.58$) and PCA total activation ($\rho=0.60$) metrics in males, whereas these had weak correlations for females ($\rho=0.43$ and $\rho=0.38$, 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

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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 Il-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 AhRf/f- 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.

APSSG21.108

The importance of T and B cells in the production of autoantibodies to the angiotensin II type I receptor and the pathophysiology associated with preeclampsia

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Preeclampsia (PE) is new onset hypertension in pregnancy which is associated with activated CD4+T cells, natural killer (NK) cells, and B cells secreting autoantibodies to angiotensin II type 1 receptor (AT1-AA). We have previously shown CD4+T cells isolated from women with PE cause hypertension, increased TNF-α, endothelin-1 and sFlt-1 when injected into pregnant nude-athymic rats, compared to CD4+T cells from normal pregnant (NP) women. However, their role to cause B cells to secrete the AT1-AA or activated NK cells as mechanisms of hypertension was not known. Our goal was to determine the role of PE CD4+T cells to stimulate B cell secretion of AT1-AA and/or NK cells in pregnant nude-athymic rats. CD4+T cells or B cells were magnetically isolated from NP and PE placentas, cultured and injected into nude-athymic rats on gestation day (GD) 12. In order to examine the role of B cells secreting AT1-AA, a subset of the rats receiving PE CD4+T cells were treated with either rituximab on GD14 or anti-CD40 ligand on GD12. On GD19, mean arterial pressure (MAP) and tissues were collected. In a separate group of Nude athymic pregnant rats, PE placental B cells were transferred on GD12 and MAP was determined at GD19. A student's t-test and ANOVA were used for statistical analysis. MAP and AT1-AA increased to 114+1 mmHg (n=9) and 19.8 ± 0.9 bpm (n=6) in NPnude+PE CD4+T cells compared to 98+2 mmHg (n=7, $p<0.05$), and 1.3 ± 0.9 bpm; (n=6, $p<0.05$) in NPnude+NP CD4+ T cells. Pup and placenta weights were 1.5+0.1g and 0.5+0.02g in NPnude+NP CD4+ T cells which decreased to 1.2+0.08g and 0.37+0.02g with PE CD4+ T cells. Circulating cytolytic NK cells increased to 1.6+0.3% gate (n=6) with PE CD4+T cells compared to NP CD4+T cells, 0.6+0.3% gate (n=4,$p<0.05$). Rituximab (103+2 mmHg n=3, $p<0.05$) and anti-CD40L (102+1 mmHg, n=3, $p<0.05$) lowered MAP compared to NPnude+PE CD4+ T cells. Moreover, MAP increased in Nude athymic pregnant rats that received placental PE B cells (114 ±5 mmHg)(n=3) compared to control Nude athymic pregnant rats (102±1 mmHg)(n=3) or NPnude+PE CD4+T cells. These results show that placental CD4+ T cells and B cells play an important role to cause hypertension and contribute to other causal factors in the pathophysiology of PE. This study is supported by NIH grants RO1HD067541-06, P20GM121334.

APSSG21.109

Sex differences in offspring of maternal methamphetamine use

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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

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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-islet 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 GMD 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

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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 mmm Hg (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 ($p<0.005$) 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±4.3 % 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

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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

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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-to-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 undergone 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.

APSSG21.116

Sex Differences in Response to Sepsis

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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.

APSSG21.117

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

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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 (ER?) and beta (ER&[beta]). 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 ER&[beta]. 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 ER&[beta]-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 quantitated 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-androstanediol (3-&[beta]-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 ER&[beta] 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-&[beta]-diol activity at ER&[beta], and deleterious effects on vWAT mitochondria.

APSSG21.119

Sex-dependent impact of postnatal BPS on vascular and cardiometabolic health

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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/J6mice were exposed to BPS (250nM) or vehicle control 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.

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APSSG21.120

Sex differences in atherosclerosis

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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 seperately remain uncovered. In this presentation, the consequence of pooling data on gene regulatory networks of atherosclerosis between sexes is shown as well as seperate 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.

APSSG21.121

How to convince your grandmothers and government agencies that research is important. Speak up to make a difference. Advocacy for science.

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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.

APSSG21.122

Low energy expenditure during adolescence/adulthood predicts diet-induced weight gain more than energy expenditure during breeding/rearing

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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 is 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 house at 20°C or 30°C. F1 offspring from litters 2 – 4 were individually house 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 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 30C 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.

APSSG21.124

Digital Health Sciences towards Clinical Excellence and Better Health

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The application of data science, informatics, and artificial intelligence (AI) to biomedicine and healthcare has received a great attention recently with the demonstration of the huge poptential. Given the disruptive nature of those technologies and the pace of scientific and technological advances exceeds the capacity of any one individual, innovations in healthcare AI requires a team effort in collectively designing and developing optimal solutions. However, the success implementation of the techniques highly depends on trust, hope, and science which requires us to take people-centric, value-based, and evidence-driven strategies. In this talk, I will discuss opportunities and challenges faced when advancing AI in the healthcare domain which requires us to approach those challenges systematically and collaboratively.

APSSG21.125

Sex differences in lipids and lipoprotein metabolism

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Prior to menopause, women are protected from the development of atherosclerotic cardiovascular disease (ASCVD) compared to age-matched men and have lower risk of myocardial infarction (MI). The age of onset of ASCVD and MI is also delayed for women. Some of this protection seen by women is due to the effects of sex hormones estrogens and androgens on lipid and lipoprotein metabolism. Other aspects are sex hormone independent and are mediated by the sex chromosomes. In this talk, we will discuss the contributions of estrogens and androgens to several steps of lipid and lipoprotein metabolism that are important to the development of ASCVD with obesity including the regulation of plasma free fatty acid levels, the hepatic steps of fatty acid uptake, triglyceride synthesis, very low density lipoprotein (VLDL) secretion and de-novo lipogenesis. We will also discuss the role of hepatic estrogen signaling in mediating the atheroprotective functions of high density lipoprotein (HDL) particles including HDL particle composition and cholesterol uptake from HDL by the liver for secretion as bile, termed reverse cholesterol transport (RCT).

APSSG21.126

Sexual dimorphism in extracellular matrix receptor, integrin expression in kidney

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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 qRTPCR. 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. qRTPCR 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. qRTPCR 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.

APSSG21.128

The Role of Cajal Bodies and miRNA Regulation in the Expression of sFlt1 in Preelampsia

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Preeclampsia (PE) affects approximately 1 in 25 of pregnancies in the United States annually. PE is defined by the presence of new hypertension and new-onset of significant proteinuria or other maternal organ/utero-placental dysfunction occurring at or after 20 weeks of pregnancy. The mechanisms responsible for PE are unclear, but are thought to be due to inadequate remodeling of the maternal spiral arteries leading to ischemic placental tissue. At the cellular level, low oxygen signaling induces an adaptive response that ultimately results in aberrant cell signaling and expression of factors that are associated with PE pathology, such as elevated secretion of soluble Fms-like tyrosine kinase 1 (sFlt1) which acts as a potent vasoconstrictor. However, there is a lack of knowledge on the exact cellular processes that occur in response to hypoxia that lead to the pathology of PE. One cellular process affected by hypoxia is nuclear organization. Our lab has published, for the first time, a hypoxia-dependent increase of Cajal bodies (CBs) in fibroblast cell nuclei. CBs are highly dynamic subnuclear domains that contribute to the biogenesis and maturation of ribonucleoproteins (RNPs) which take part in fundamental cellular activities such as translation, pre-mRNA splicing, and telomere maintenance. They also spatially associate with gene loci such as the Chromosome 19 MicroRNA Cluster (C19MC). Knockdown of the CB marker protein coilin, reducing CB formation in return, decreases the biogenesis of C19MC encoded miRNAs. These miRNAs collectively play important roles in normal placental function such as trophoblast migration and invasion and have been shown to be aberrantly expressed in hypoxia. Additionally, we have novel data showing CBs are induced in placental trophoblasts from the well-characterized clinically relevant model of PE, the reduced uterine perfusion pressure model of placental ischemia (RUPP) in the rat. The RUPP model mimics many characteristics of women with PE, including an altered miRNA profile and upregulation of sFlt1 levels. Interestingly, previous work shows that CB numbers are increased in placenta of women with PE, as well. The upregulation of CBs and altered miRNA expression in response to hypoxia suggests a cellular pathway that contributes to the PE pathology and gives rise to the central hypothesis that placental ischemia induced CBs leads to an increase in the production of C19MC miRNAs and ultimately, an increase in vasoconstrictors such as sFlt1 leading to maternal hypertension. To investigate the relationship between induced CBs and sFlt1, we screened several C19MC miRNAs and have found miR-517-3p to be positively regulated by hypoxia and coilin and is an indirect positive regulator of sFlt1. Knockdown of miR-517-3p decreases sFlt1 mRNA, but not full-length fms related receptor tyrosine kinase 1 (Flt1). Therefore, hypoxia upregulation of miR-517-3p promotes Flt1 alternative splicing through TNFAIP3

interacting protein 1 (TNIP1) mediated increase of NF- kB 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

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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 house 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

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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 when 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).

APSSG21.131

Polycystic Ovary Syndrome and COVID-19

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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 sheddase 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 NIGMS P20GM-121334 to LLYC and DGR, and NIH NIDDK R21DK-113500 to DGR.)

APSSG21.132

MicroRNA-21 Modulates Brown Adipose Tissue Adipogenesis and Thermogenesis in a Mouse Model of Polycystic Ovary Syndrome.

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Background and aim: Hyperandrogenemia and ovarian dysfunction characterize polycystic ovary syndrome (PCOS), the most common endocrine disorder in premenopausal women. PCOS is associated with increased adiposity and brown adipose tissue (BAT) dysregulation. Altered adiposity via increased circulating adipokine leptin is associated with impaired thermogenic responses in non-PCOS models. However, the molecular mechanisms that mediate BAT adiposity in PCOS and its relation to altered thermogenesis are poorly understood. MicroRNAs play critical functions in brown adipocyte differentiation and maintenance. MicroRNA-21 (miR-21) is upregulated by androgens and by increased adiposity in the adipose tissue in non-PCOS models. We aim to study the role of microRNA-21 (miR-21) in androgen-mediated adiposity and browning derangements in the BAT in PCOS. Methods: Three week-old miR-21 knockout (miR21KO) or wild type (WT) female mice were treated with dihydrotestosterone (DHT, 8 mg/silastic tube) or vehicle for 90 days (n=12/grp). Body composition was measured by EchoMRI. BAT (interscapular) weight was measured by gravimetry. Energy expenditure (EE), oxygen consumption (VO2), and carbon dioxide production (VCO2) were measured by indirect calorimetry. Serum leptin levels were detected by ELISA. Markers of adipogenesis (adiponectin, PPAR-γ, C/EBP-α), fatty acid synthesis (fatty acid synthase [FAS], acetyl-coA carboxylase [ACC]), and browning (UCP1, Cox7a1, Cpt1, Elov3, Dio2 and Cidea) were quantified by RT-qPCR and/or western blot in BAT. Results: DHT increased body weight (25.07 ± 0.52 vs 21.79 ± 0.47 g, $p<0.05$), fat mass (4.60 ± 0.46 vs 1.98 ± 0.12 g, $p<0.05$), BAT mass (73.40 ± 10.51 vs 46.18 ± 2.43 mg, $p<0.05$), and serum leptin (2.6-fold), and did not significantly change EE, VO2, VCO2 or adipogenesis markers expression in WT mice. Adaptive downregulation of fatty acid synthesis markers was observed in DHT-treated WT mice. All browning markers were not altered by DHT except for iodothyronine deiodinase 2 (Dio2) which was significantly downregulated by 40% compared with the vehicle-treated WT mice. DHT-treated miR21KO mice showed attenuated DHT-mediated increase in body weight s (23.84 ± 0.99 vs 25.07 ± 0.52 g, $p<0.05$) compared with WT mice. MiR-21 ablation did not modify DHT-mediated increases in fat mass, BAT mass or circulating leptin. However, DHT-treated miR21KO mice showed a trend to reduced EE, VO2 and VCO2 values compared with DHT-treated WT. Interestingly, the adaptive reduction in fatty acid synthesis by DHT was lost in miR21KO mice which showed a significant increase in FAS protein level. Additionally, the adipogenesis marker PPAR-γ

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)

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Polarity and Diversity in Gender Expression: A novel measurement for sex and gender-based analysis conducted in clinical research with cisgender female participants

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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 gender 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 multivariate 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 multivariate modelling. Results: As part of a larger longitudinal study on women's experiences with prolapse and their outcomes after surgery, we analyzed 198 individuals, 89.4% (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.

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High Plasma Soluble Prorenin Receptor Is Associated With Elevated Systolic Blood Pressure In Aged And Super-aged Males But Not In Female Mice

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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±2) 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

(R=0.821, P<0.001) in males but not in females. BUN and urinary creatinine levels were reduced in aged (BUN 18±2, creatinine 12±1.6mg/dL) and super-aged (BUN 10±1.8 mg/dL, P=0.001; creatinine 10±1.4 mg/dL, P<0.001) male mice but not in females. Urine volume, sodium concentration, and eGFR (P=0.06) did not differ among groups. In aged mice, only super-aged males experienced decline in testosterone levels (P=0.001) but levels of 17β-estradiol and progesterone did not. We conclude that age impacts plasma sPRR, SBP, and renal function in a sex dimorphic manner, which may help to explain sex-dependent differences in the predisposition to CV and renal diseases during elderly. In perspective, the use of ageing models will accelerate mechanistic insight and advances in precision medicine in older subjects. Support from NIH: DK104375 and FAPESP 17/17027-0 to MCP; AG047296 to RM; and HL133619 to SHL

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The Inflammatory Response to Downregulation of Dopamine D2 receptor in the Renal Proximal Tubule is Sex-dependent.

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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 fl/fl, PSGLT2::Cre+ mice (D2R PT-/-) that lack D2R only in the renal proximal tubule and Drd2 fl/fl, 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 Fn-1 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.

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Sex Differences in Irritable Bowel Syndrome

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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.

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Sex Differences in Developmental Origins of Adult Cardiovascular Disease

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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 male and 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 interventions) 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, Kirschenman R, Cooke C-LM, Phillips TJ, Case CP, Davidge ST. Pharmacological Research, 165:105461, Jan 26, 2021 Acknowledgement This research is supported by This study was funded by a Canadian Institutes of Health Research Foundation grant and by the generosity of the Stollery Children's Hospital Foundation and the Alberta Women's Health Foundation through the Women and Children's Health Research Institute

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Women, Opioids and Addiction in the Time of COVID-19

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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.

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Intestinal Epithelial Axin1 Drives Sex Differences in the Gut Microbiome and Obesity

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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

metabolic disorders. Gut dysbiosis plays a critical role in the development of metabolic conditions. It is known that healthy women have an upregulation intestinal Wnt/β-catenin signaling, indicating increased turnover and regeneration of the gut mucosa. Diet induced obesity has been shown to activate Wnt/β-catenin and cause shifts in the microbiota resulting in systemic inflammation. However, it is unknown whether gut-derived signaling explains the gender difference in metabolic diseases. Axin1 is a scaffold protein in the Wnt/β-catenin signaling pathway. Our previous studies have shown the importance of the Axin1/Wnt/β-catenin signaling pathway in intestinal 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 Axin1?IEC 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 Axin1?IEC mice have blooms in Odoribacter and Clostridales genera while Axin1?IECmales have enriched abundances in Akkermansia. After high fat diet challenge, Axin1?IEC female mice gained more weight compared to their Axin1LoxP mates. Inversely, Axin1?IEC males gained less weight compared to Axin1LoxP males. Despite this, high fat diet fed Axin1?IEC 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 to 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.

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

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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 (IR-CON). 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 SBP in AE-PCOS was higher at BSL versus IR-CON (148±11 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 Hb/mmHg, P=0.03), and T attenuated the improved BRS (T=0.488± 0.178 bursts/100 Hb/mm Hg, 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=0.023±0.013 vs. ANT=0.027±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

regulation, but not entirely, suggesting factors other than testosterone contribute to poor BP control in AE-PCOS. Changes in resting BP and MSNA were less notable in IR-CON. Hormone suppression had little impact on CVBRS in AE-PCOS or IR-CON, but CVBR gain was lower than BSL in both groups after androgen administration. Our findings suggest that testosterone impacts both the sympathetic and parasympathetic nervous system contributions to BP control in AE-PCOS. However, only the parasympathetic system appears responsive to androgens in IR-CON women, which might be related to glucose dysregulation.
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Acute kidney injury prior to pregnancy decreases late-gestation urine volume and increases plasma aldosterone levels

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

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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 empagliflozin (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 vWAT, 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

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

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Background: PCOS is characterized by hyperandrogenemia and elevated blood pressure (BP). Due to exposure to prenatal hyperandrogenemia, male and female offspring of hyperandrogenemic 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 F1HAF, HAF offspring maintained a normal baseline BP compared to age and sex-matched F1Contr. Yet, both male F1HAF and male offspring of control dams (F1Contr.) have higher baseline BP compared to age-matched female F1HAF and F1Contr. The present study tested the hypothesis that aging female F1HAF have an upregulation of the vasodilator arm of RAS (AT2R and ACE2) that protects 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 F1HAF compared to both female F1contr. and female F1HAF (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 F1HAF compared to male F1contr., female F1HAF, 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 F1HAF compared to both female F1contr. and female F1HAF (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 F1HAF 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 F1HAF and F1contr. could be a compensatory mechanism against further elevated BP. Upregulation of ACE2 in aging male F1HAF 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 F1HAF in order to better understand the sex differences in their response to Ang II. Funding Sources: R01HL135089, P01HL051971, P20GM121334, P20GM104357

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

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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/grp). 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.)

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Cardiovascular disease in transgender and gender diverse adults taking feminizing gender-affirming hormone therapy

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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 orchiectomy, 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-BIRCWH (K12 HD057022; PIs: 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 5135 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

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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 peri-bronchial 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

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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 nM BQ-123). Laser-Doppler flowmetry (LDF) and local heaters were used to measure skin blood flow and induce local thermal 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.

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Sex differences in the cardiorespiratory response to isometric exercise or passive movement of the lower limb

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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±2 vs. 90±3 mmHg, Main effect of time p<0.001). During PLM in both 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±4 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 PLM compared to baseline and remained elevated at the end of PLM (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 PLM 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.

APSSG21.153

Transwomen competing in women's sports: What we know, and what we don't

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The purpose of this presentation is to summarize the differences between males and females regarding athletic performance, review the current knowledge regarding the effects of gender affirming hormone therapy in transwomen on factors that influence athletic performance, and provide an update on legislation regarding the participation of transwomen in women's sports. It is well documented that males outperform comparably aged and trained females in most measures of physical fitness and athletic events. Generally speaking, males have 20-40% more body mass, 45% more lean body mass, run 10-15% faster, and have 30-60% greater muscle strength than females. These male athletic advantages originate from sex based physiological differences. In 2015 the International Olympic Committee adopted a new transgender participation policy with much less stringent requirements for transwomen to compete in women's sports. As a result, the inclusion of transgender athletes in the Tokyo Olympic Games has brought to the forefront the issues of safety, fairness, and inclusion of transwomen in women's sports. To date research indicates that gender affirming hormone therapy in transwomen eliminates the male advantages in hemoglobin concentrations. Gender affirming hormone therapy in transwomen causes 0-4% reductions in body mass, 4-5% reductions in muscle mass, and concomitant increases in fat mass. Furthermore, there have been only 5 papers published evaluating the effects of gender affirming hormone therapy in transwomen on handgrip strength (showing 0-9% reductions in strength), 1 paper published evaluating knee extensor and flexor strength (showing no reductions in strength), and 1 paper published evaluating push-ups (27% reduction), sit-ups (16% reduction) and 1.5 mile running performance (8% reduction in speed). Otherwise the effects of gender affirming hormone therapy in transwomen on athletic performance and factors influencing athletic performance (e.g. VO2max, lactate threshold, isotonic 1 repetition maximum) remain unknown. However, on the basis of safety and fairness, in the past year legislation has been introduced in 37 states to limit participation in girl's and women's sports to cisgender women, with the legislation being signed into law in seven states. This information is pertinent to the health of transwomen as their choice to engage in gender affirming hormone therapy may influence their eligibility to participate in sports.

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Biological sex influences glomerular podocyte endowment in rats

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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 pathology 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

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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), a 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- Wolpern et al. BMC Sports Science, Medicine, and Rehabilitation, 2015; 7:16; 2- Weatherwax, R. M (2019). Medicine & Science in Sports & Exercise, 51(4), 681-691; 3- Dalleck, L., et al. (2016). Journal of Fitness Research, 5(3),15-27.

APSSG21.156

Care of the transgender adolescent

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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

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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

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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

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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

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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 under hemodialysis treatment (men=M, n=31, 63±2 years old vs. women=W, 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.

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

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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.

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Sympathetic transduction during hypoxia and hypercapnia in healthy young women and men

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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. Hypoxaemic 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 (TPRi). 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.
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Sex-based Differences in Immunological and Physiological Responses to Systemic Inflammation in Rodents

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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 naïve 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, lung, 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 naïve rats did not vary with sex, but 12 hours after inoculation, frequency (fR) was greater in males than females (?79.57±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 (?0.19±0.12 vs ?0.28±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 (?503.08±268.68 vs ?138.28±95.54 pg/mg, p<0.0001) and the correlation of NLCI with liver IL-6 levels was greater in males than females (?r=0.5790 vs ?r=0.2099). This was supported by the fact that compared to inoculated males, inoculated females exhibited a lower NLCI (?0.19±0.12 vs ?0.28±0.10, p=0.009), lower IL-17 level in lung tissue (?1.52±1.32 vs ?5.38±3.07, p<0.0001 pg/mg), and lower correlation between these indices (?NLCI-IL-17 correlation: r=0.5917 vs ?NLCI-IL-17correlation: r=0.6175; p<0.001) at 12 hours post-inoculation. In summary, these data indicate that there are differences in ventilatory pattern predictability, central and peripheral cytokine expression, and their correlation between sexes in response to E. coli infection. Taken together, these findings indicate that female rats may be able to produce a more robust protective response to systemic infection and additional studies to determine sex-based differences in immune and ventilatory responses to systemic inflammation are warranted.

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The diagnostic potential of oxidative stress biomarkers for preeclampsia: systematic review and meta-analysis

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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.

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Impact of Maternal Alcohol Intake on Development and Long-term Health of Offspring

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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.

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ACE2 contributes the normal regulation of arterial pressure and immunity in females of reproductive age

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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.

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Orchiectomy exacerbates lung mechanical consequences of intermittent hypoxia in C57BL/6J mice

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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. Orchiectomy 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 orchiectomy. 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 orchiectomy 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 orchiectomy 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.

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

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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 ± 0.17s ; 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.

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

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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 simulataneously 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

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Sex Differences in Transplantation

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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 alloimmunity 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 phenomenum 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.

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Obesity, Pregnancy, and Hypertension

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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

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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 mm Hg, $p<0.05$). 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: 0.31±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 (YF-P: 0.27±0.02 mg/24h vs. YF-MMF: 0.1±0.04 mg/24h and YF-P: 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.

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Sex-dependent role of adipose tissue HDAC9 in diet-induced obesity and metabolic dysfunction.

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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 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 4C 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.

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Chest Pain in a Young Transgender Woman: A Case Report

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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 myopericarditis. 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.

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Estrogen Receptor α modulates the action of the lupus susceptibility locus Sle1b

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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, Sle1b, controls immune cell hyperactivation, loss of tolerance, and autoantibody development. The impact of Sle1b 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 Sle1b is not known. Sle1b enhances lupus by dampening BCR signaling and thereby allowing autoreactive B cells to evade tolerance induction mechanisms. Sle1b consists of linked polymorphisms in the SLAM gene cluster resulting in differential splicing of the Ly108 (Slamf6) gene. Slamf6 interacts with phosphatases to negatively regulate B cell receptor (BCR) signaling, and this activity is amplified in mice carrying Sle1b. 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 Sle1b 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.Sle1b.ERα+/+ and B6.Sle1b.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.Sle1b.ERα+/+ mice. These data suggests that ERα, like Sle1b, 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.Sle1b.ERα+/+ females proliferated more robustly upon BCR activation than B cells from either B6.Sle1b.ERα-/- females or B6.Sle1b.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

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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, ERβ functionality is enhanced, and has a suppressive effect on [Ca2+]i, 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.

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Estradiol protects females from the deleterious effects of Interleukin-17A on neurovascular coupling

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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

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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

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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.

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Modulating Effects of Castration on Vas Deferens Smooth Muscle Electrical Activities: Insights from A Quantitative Study

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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 conductnaces 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 simulated in the whole cell model by applying an external stimulus current (10-30 pA), as a brief square pulse of 10 ms duration. The results showed both L-type Ca2+ and Na+ channel are indispensable for generating the spike, although the L-type Ca2+ channel is the major contributor to the total inward current. The results also revealed that both BK and A-type K+ channel channels are essential in maintaining the RMP and repolarization. Because of castration, A-type K+ current is reduced, and as a result, it elevated the RMP from ? 50 mV to ? 47 mV (more positive). The model was able to evoke an AP with a reduced current stimulus. Conclusions: To date, a biophysically detailed computational model does not exist for VDSM cells. Our model, constrained heavily by physiological data, provides a powerful tool to investigate the ionic mechanisms underlying the genesis of VDSM electrical activity. In the guinea-pig, following castration, VDSM was accompanied by cell membrane depolarization, which caused to evoke more spontaneous contractions. Impact Statement: The testosterone-mediated regulation of A-type K+ channels provide important information for the male sexual disorder induced by testosterone therapy for men with castration.

Reference: Ohya S, Ito K, Hatano N, Ohno A, Muraki K, Imaizumi Y. Castration Induces Down-Regulation of A-Type K+ Channel in Rat Vas Deferens Smooth Muscle. International journal of molecular sciences. 2019 Jan;20(17):4073.

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Sex differences in vulnerability to addiction

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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 ERa, 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 dorsalatral 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 dorsalateral 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.

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Adverse Cardiometabolic Effects of Severe Food Restriction in Males and Females

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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

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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 inulin/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.
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Differential Activation of Renal Cell Death Pathways in Diabetic Male and Female Vascular Endothelial Cell ET-1 Knockout Mice

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Contrary 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 K01HL145324 and UAB Diabetes Research Center Pilot Project grant to CDM, and UAB KURE R25 DK115353 to VGM and JSP.

APSSG21.189

Development of a New Mouse Model to Study Menopause-Associated Asthma that Aligns with the Human Condition.

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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 Diepoxide (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

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Colonic mitochondrial function is altered in hyperandrogenemic female rats

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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 adipositiy 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 simulataneously 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=0.0011). 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 hyperandrogenemia. 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.

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Gonadal Steroid Hormones Do Not Alter Stress-Induced Cardiovascular Responses in Adult Male and Female Rats Exposed to Dexamethasone In Utero

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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

estradiol. 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.
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APSSG21.193
Sex Differences in Risk for Intestinal Inflammation and Disease: The Role of Exercise

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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

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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 16-18-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: 5T32GM092715-14 and R01-HL-137694-01

APSSG21.195

Sex Differences in Exercise Capacity

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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

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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

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Biological sex affects the risk of developing type 2 diabetes (T2D): ~40% more men develop T2D than pre-menopausal 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.

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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: 1.02±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, HsPRR: 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: 11.1±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

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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=4-5 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 AIH (-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 AIH and remained below baseline at 5 minutes post-AIH (-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

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Every cell is sexed, every person is gendered, and every organism is stressed. Whereas sex refers is 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 presented. 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

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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 results 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 (1P30 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.

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Sex Differences in Basal Protein Expression of eNOS and NRF2/HO-1/NQO1 in HAECs and HUVEC

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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 ($P \leq 0.05$) and are expressed as arbitrary densitometry units as mean ± standard deviation. Results: In HAECs, phospho-eNOSSer1177 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 -derived HAECs. The opposite was observed in HUVECs as male had higher phospho-eNOSSer1177 (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.

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Juvenile female rats have decreased Myelin Basic Protein expression following in utero T cell suppression

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Background: Offspring of hypertensive pregnancies are at an increased risk of cognitive dysfunction, adverse cardiovascular events, and deficiencies in neurological development compared to offspring of normal pregnancies. In preliminary data, we have seen offspring from dams with hypertensive pregnancies who have and have not been subjected to a single immunotherapy event, express increase hyperactivity. Interestingly, offspring from women with hypertensive pregnancies have an increased risk of developing attention-deficit/hyperactivity disorder (ADHD) and/or behavioral opposition disorders. In the current study we aimed to determine if 1) offspring born to dams with hypertension treated with or without Orencia had changes in myelin basic protein (MBP) and neuronal nuclei (NeuN) at early adolescence; and 2) if there were any gender differences between the offspring. We hypothesize that offspring from hypertensive pregnancies will have a differential expression of MBP and NeuN in the prefrontal cortex compared to normal pregnant (NP) offspring. Methods: On gestational day (GD) 12, miniosomtic pumps are placed in the abdominal cavity infusing antiangiogenic factors soluble fms like tyrosine kinase-1 (sFlt-1) and soluble endoglin to induce hypertension during pregnancy. sFlt-1 dosage was 7 µg/kg for PreE model, and sFlt-1 and soluble endoglin was dosed at 7 µg/kg and 4.7 µg/kg respectively for the HELLP model. On GD13, a subset of HTN rats and NP rats underwent infusion with Orencia (prevents T cell activation; 2mg; kg). Shortly following birth, all pups were weighed, tagged and cross-fostered with naïve lactating dams. Rat pups underwent a series of behavioral tests and were euthanized between post-natal day 60-65. At that time organs were saved and the prefrontal cortex was used to measure MBP and NeuN protein expression via western blot (n=3/group/gender). Data was analyzed via a 2-way ANOVA with $p<0.05$ serving as significant. Results: At birth offspring from the Orencia treated HELLP dams weighed significantly less than NP offspring ($p<0.05$). Both NP and HELLP female juvenile rats born to dams treated with Orencia had less MBP in the PFC compared to male rats. However, there were no gender differences seen among untreated NP and HELLP offspring ($p>0.05$). Female rats born to NP dams had less NeuN compared to males, an imbalance that remained unchanged with Orencia administration. There were no significant differences in HELLP rats born to untreated dams ($p>0.05$), however Orencia administration did decrease NeuN expression in female offspring relative to male offspring from HELLP+O dams. Discussion: This preliminary protein expression study suggests that offspring born to hypertensive dams do not have changes

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.