Dear participants

It is our pleasure and honor to welcome you to the Midlands Society of Physiological Sciences (MSPS) Meeting 2021. Enclosed in this Program Booklet, you will find the meeting agenda, list of sponsors, the schedule for oral presentations, and links to all abstracts and poster-video presentations.

The goal of MSPS is to foster advances in physiology-related research, training, and public awareness. We are pleased to fulfill this goal by organizing this webinar meeting where undergraduate, graduate, and postdoctoral trainees as well as faculties from different institutions at Omaha, Lincoln, and Kearney in Nebraska, and South Dakota have enthusiastically participated. Although we will sincerely miss the in-person interactions, we are confident that this meeting will provide ample opportunities for virtual interactions and intercampus collaborations among the students and researchers.

We take this opportunity to humbly appreciate the Keynote Speakers for accepting our invitation, judges of abstracts and video-posters to select the best sciences for awards, students and trainees for their active participation, and sponsors and the American Physiological Society for their support. We would also like to thank Mr. Cody Anderson for his tireless and timely efforts to update our MSPS website with all relevant information.

With regards

Sincerely,

Paras Kumar Mishra, Ph.D.
President

Organizing committee members:

Xuejun Wang, M.D., Ph.D., President-Elect
Dustin Slivka, Ph.D., Past-President
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AGENDA

2021 Annual Meeting of the Midlands Society of Physiological Sciences
Webinar Meeting October 23, 2021 (Saturday)

Morning Sessions

8:30 - 9:00 AM Welcome and Opening Remarks - Introduction to the Keynote Speaker

9:00 AM - 10:00 AM Keynote Speaker Dr. Joseph A. Hill, M.D., Ph.D.
HFpEF: Malady, Model, and Meta-inflammatory Mechanisms

10:00 - 10:05 AM Vendor Presentation (Fujifilm Visual Sonics)

10:10 - 10:25 AM Postdoctoral Fellow Oral Presentation by Dr. Rahul Kaklij
Developing a mouse model to test the impact of metabolic syndrome on Lupus-induced organ damage

10:30 - 10:45 AM Postdoctoral Fellow Oral Presentation by Dr. Madan Kumar Arumugam
Loss of phosphatidylethanolamine methyltransferase promotes liver fibrosis In Mice

10:45 - 10:50 AM Vendor Presentation (Charles River)

10:55 - 11:10 AM Graduate Student Oral Presentation by Tyler Kambis
MiR-133a overexpression prevents ferroptotic cell death in the diabetic heart

11:15 - 11:30 AM Graduate Student Oral Presentation by Liz Pekas
Impacts of microcirculatory dysfunction on skeletal muscle mitochondrial function and oxygen utilization in Peripheral Artery Disease

11:35 - 11:50 AM Graduate Student Oral Presentation by Linda Berg Luecke
Cell surface phenotyping of human heart reveals cardiomyocyte-specific targets and surfaceome dynamics of explanted cardiac fibroblasts

11:50 - 12:35 PM Lunch Break

Afternoon Session

12:35 - 12:40 PM Vendor Presentation (DSI)
12:45 – 1:00 PM  Graduate Student Oral Presentation by Mingqi Cai  
*Soluble guanylate cyclase activation increases proteasome activities and protects against proteotoxicity in cardiomyocytes*

1:05 – 1:20 PM  Graduate Student Oral Presentation by Samiksha Giri  
*CSN5-mediated nuclear exclusion of P27 in VSMC contributes to exacerbation of neointimal hyperplasia by CSN8 hypomorphism*

1:20 - 1:25 PM  Vendor Presentation (Ad Instruments)

1:30- 1:45 PM  Undergraduate Student Oral Presentation by Lucas Wang  
*Overexpression of skeletal muscle Nrf2 protects against aging-associated dysfunction in skeletal muscle and heart*

1:50-2:05 PM  Undergraduate Student Oral Presentation by Tiffany Knecht  
*Constrictor responses of cerebral resistance arterioles in male and female rats exposed to alcohol prenatally*

2:05- 2:10 PM  Vendor Presentation (Beckman Coulter)

2:15- 2:20 PM  High School Award recipient presentation by Reema Guda  
*COVID-19: In-depth analyses of coronavirus mutations and their implications*

2:25- 2:30 PM  High School Award recipient presentation by Anuj Singh  
*The role of oxidative stress in mediating the effects of gut dysbiosis on host health*

2:30- 2:35 PM  Break

2:35 PM - 3:35 PM  Local Speaker Dr. William Chen, M.D., Ph.D.  
*Next-Generation Cardiovascular Regenerative Medicine and Engineering*

3:40 - 4:20 PM  Award Announcement and Closing Remarks

4:20 PM  Adjourn

4:20- 5:00 PM  Business Meeting
ORAL PRESENTATIONS
DEVELOPING A MOUSE MODEL TO TEST THE IMPACT OF METABOLIC SYNDROME ON SYSTEMIC LUPUS ERYTHEMATOSUS-INDUCED ORGAN DAMAGE

Rahul M. Kakalij, Erika I. Boesen
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Background/hypothesis: Metabolic syndrome (MetS) is common in SLE patients and associated with increased cardio-renal risk. The present study was carried out to develop a model to investigate further whether MetS accelerates SLE-induced organ damage.

Methods and Results: Female C57BL/6 mice were untreated or treated epicutaneously with the TLR7 agonist imiquimod (IMQ), and fed a high-fat (HF) “Western” diet (fat 42% kcal, sucrose 34% kcal) or control diet (fat 12.6% kcal, sucrose 34% kcal) for 6 weeks. Data at 6 weeks were compared by two-way ANOVA, with n=8 mice per group. Supporting early-stage induction of autoimmunity, spleen weights were significantly increased in IMQ-treated mice (PTreatment<0.001), and renal cortex IgG deposition was confirmed by Western blot. Increased body weight, gonadal fat pad mass, and plasma leptin levels were observed between HF and control animals (PDiet<0.001) for both IMQ and untreated mice. However, the increase in these parameters with HF diet was significantly diminished in IMQ-treated mice (PDiet*Treatment<0.05). Both the HF diet (PDiet<0.001) and IMQ treatment (PTreatment<0.05) significantly increased fasting blood glucose. IMQ treatment affected fasting insulin concentrations in a diet-dependent manner (PDiet*Treatment<0.01), with hyperinsulinemia observed in IMQ-HF treated mice (177.93±8) compared to IMQ-treated control diet mice (49.21±7.87; P<0.05 by Bonferroni post-hoc test), with plasma insulin similar between untreated mice on HF (112.09±18.84) and control diets (97.55±19.84; P>0.05).

Conclusion: Together, these data indicate that the IMQ model of SLE is associated with metabolic alterations, impaired glycemic control, and hyperinsulinemia under HF intake conditions. In the future, this model may be helpful to understand the complex relationship between MetS and organ damage in SLE.
Oral Presentation  
Category: Postdoctoral Fellow  

LOSS OF PHOSPHATIDYLETHANOLAMINE METHYLTRANSFERASE PROMOTES LIVER FIBROSIS IN MICE

Madan Kumar Arumugam1,2, Srinivas Chava1,2, Sathish Kumar Perumal1,2, Matthew C. Paal1,2, Terrence M. Donohue, Jr.1-3, Natalia A. Osna1,2, Kusum K. Kharbanda1-3*

1Research Service, Veterans Affairs Nebraska-Western Iowa Health Care System, Omaha, NE, USA. 2Department of Internal Medicine and 3Department of Biochemistry & Molecular Biology, University of Nebraska Medical Center, Omaha, NE, USA.

Background: Phosphatidylethanolamine methyltransferase (PEMT)-mediated catalysis generates phosphatidylcholine (PC) that is preferentially used in the assembly of very-low-density lipoprotein and is necessary for its normal secretion from the liver. Common genetic loss-of-function polymorphisms in the human PEMT gene reportedly confer susceptibility to non-alcoholic steatohepatitis. Here, we investigated the age-related spectrum of liver damage in PEMT KO mice.

Methods: Male and female C57BL/6 PEMT KO mice and wild type (WT) controls were fed standard chow. Livers were harvested from these mice at different ages and processed for histopathological and other biochemical analyses.

Results: At 10 weeks of age, both male and female PEMT KO mice had comparable body and liver weights as well as hepatic S-adenosylmethionine (SAM) levels as their respective WT controls. However, while PEMT KOs of both genders had a 2-3-fold higher levels of hepatic S-adenosylhomocysteine (SAH) (p<0.005) and a significant reduction in methylation potential (SAM:SAH ratio), only female KO mice exhibited ~2-fold higher hepatic triglyceride levels (p<0.01) with microvesicular steatosis in Zone 3 compared with their respective WT controls. With advancing age, there was a progressive worsening of liver injury. At 10 months of age, PEMT KO mice of both genders showed ~3-4-fold lower hepatic SAM levels and similar fold reduction in SAM:SAH ratio (p<0.001) compared with 10-week-old KO mice. The reduction in methylation potential was accompanied by a significant rise in hepatic triglycerides (p<0.05). Histopathological analysis revealed massive macro- and micro-vesicular steatosis, parenchymal inflammation, and granulomatous inclusions. Picro-Sirius Red staining showed evidence of pericellular fibrosis which was confirmed by immune-staining of collagen-1.

Conclusion: These results indicate that the loss of PEMT causes the spontaneous development of fatty liver in younger female mice and progressive liver fibrosis with advancing age in both genders. We suggest that loss-of-function polymorphisms in PEMT likely have similar effects in humans as they age.
GRADUATE STUDENTS

Oral Presentation
Category: Graduate Student

MIR-133A OVEREXPRESSION PREVENTS FERROPTOTIC CELL DEATH IN THE DIABETIC HEART

Tyler N. Kambis, Sumit Kar, Paras K. Mishra
Department of Cellular and Integrative Physiology, University of Nebraska Medical Center, Omaha, NE

Background/hypothesis: Myocardial lipid accumulation occurs prior to the development of cardiac dysfunction in patients with diabetes mellitus (DM). miR-133a prevents DM-induced myocardial lipid deposition and is decreased in the DM heart, however its cardioprotective mechanisms remain unknown. Ferroptotic cell death which results from an increase in lipid peroxidation combined with a failure of lipid-antioxidant mechanisms in the presence of iron, is upregulated in the DM heart. Thus, we hypothesize that overexpression of miR-133a protects against ferroptotic cell death via inhibition of lipid accumulation, leading to decreased lipid peroxidation in the DM heart. We developed a novel Akita/miR-133aTg (Ak/Tg) mouse model by crossbreeding DM Akita (Ak) with cardiac specific miR-133a Tg (Tg) to elucidate how miR-133a regulates ferroptotic cell death in the DM heart.

Methods and Results: Deep sequencing analysis revealed that miR-133a activated fatty acid oxidation in the DM heart (p-value=4.59E-11). Protein levels of transcription factor Peroxisome proliferator-activated receptor gamma (PPARG) (WT: 0.195±0.012, Ak: 0.23±0.012, Ak/Tg: 0.173±0.009, Tg: 0.16±0.012) and its coactivator Peroxisome proliferator-activated receptor-gamma coactivator (PGC-1α) (WT: 0.162±0.006, Ak: 0.224±0.012, Ak/Tg: 0.149±0.011, Tg: 0.145±0.010). Furthermore, citrate synthase activity was increased in the presence of miR-133a (WT: 0.793±0.043, Ak: 0.735±0.026, Ak/Tg: 0.994±0.055, Tg: 0.929±0.018), implying an increase of lipid clearance. Protein levels of glutathione peroxidase (GPX4), an antioxidant that inhibits the production of peroxidized lipids, were increased by the presence of miR-133a (WT: 0.038±0.003, Ak: 0.024±0.002, Ak/Tg: 0.040±0.004, Tg: 0.030±0.006). miR-133a also decreased levels of DM-induced peroxidized lipids (WT: 203.9±12.69, Ak: 231.9±7.252, Ak/Tg: 209.8±8.885, Tg: 251.9±14.26).

Conclusions: These data support that miR-133a decreases myocardial lipid deposition via increased lipid metabolism while simultaneously increasing levels of lipid antioxidant GPX4, leading to lower levels of peroxidized lipids resulting in the mitigation of ferroptotic cell death in the DM heart.
Peripheral artery disease (PAD) is an atherosclerotic disease that impairs circulatory function in the lower extremities. Skeletal muscle mitochondrial dysfunction and blunted muscle oxygen utility capacity have been reported in the ischemic limbs; however, the underlying mechanisms are not well-understood. For the first time, we investigated the impacts of chronic ischemia on skeletal muscle arteriole endothelial function and its contribution to skeletal muscle mitochondrial function and microvascular oxygen delivery and utilization capacity in PAD. Skeletal muscle and arteriole samples from patients with PAD (n=18, 68.4±10.2 years) and age-matched controls (CON, n=11, 64.6±9.3 years) were harvested. Endothelial-dependent and endothelial-independent vasodilatory function were assessed by flow, acetylcholine (ACh), and sodium nitroprusside (SNP), and skeletal muscle mitochondrial function was assessed by high-resolution respirometry. Skeletal muscle microvascular oxygen delivery and utilization capacity was assessed by near-infrared spectroscopy (NIRS) in-vivo. Endothelial-dependent vasodilation was attenuated in PAD in response to ACh (10⁻³ M, CON: 71.1±7%, PAD: 45.5±6%, p<0.01) and flow (CON: 46.6±6.8%, PAD: 29.2±4.5%, p<0.01), whereas endothelial-independent vasodilation was not different between groups (10⁻³ M, CON: 101.5±4%, PAD: 91.6±5%, p=0.12). Complex I + II state 3 respiration was lower in PAD (CON: 26.1±2.1, PAD: 7.8±1.4 pmol∙s⁻¹∙mg⁻¹, p<0.01), and skeletal muscle microvascular oxygen delivery and utilization capacity was blunted in PAD (CON: 67.2±10.9, PAD: 26.6±7.0%∙min⁻¹, p<0.01). Furthermore, flow-mediated dilation and ACh-mediated vasodilation were positively associated with complex I+II state 3 respiration (r=0.6 and r=0.5, respectively, p<0.05) and microvascular oxygen delivery and utilization capacity (r=0.5 and r=0.6, respectively, p<0.05). These findings suggest that conduit artery atherosclerotic blockage-mediated chronic ischemia attenuates skeletal muscle microcirculatory function (arteriolar endothelial function), which may be a key contributor to leg skeletal muscle mitochondrial dysfunction and attenuated leg skeletal muscle oxygen delivery capacity in patients with PAD.
In the heart, cell surface glycoproteins in cardiomyocytes and cardiac fibroblasts are essential for sustaining normal organ function and play critical roles in cardiac development, disease, and drug uptake. However, the lack of a detailed cell type- or chamber-resolved view of the cell surface proteome of the adult human heart currently limits discovery of new targets for precision drug delivery and the development of practical approaches for studying how different cell types contribute to the development of cardiac disease. CellSurfer, a new analytical platform, was applied to cardiac cells isolated from human hearts. Briefly, cell surface N-glycoproteins on ~1 million cells were labeled, digested, selectively enriched using streptavidin magnetic beads, cleaned using SP2, and analyzed by MS. Sample preparation was automated using liquid handling robotics. MS data were analyzed using Proteome Discoverer, Spectronaut, MSstats, and R. Results were curated and annotated using Veneer. Integrating CellSurfer with an optimized strategy for isolating intact primary cardiomyocytes and fibroblasts from human donor heart tissue resulted in the generation of the first chamber-, cell-type-, and patient-specific map of the cell surface N-glycoproteome in the adult heart. Overall, >1200 cell surface N-glycoproteins were detected, including >100 cell surface proteins not previously described in these cell types. Novel monoclonal antibodies generated for one cardiomyocyte protein uniquely localize to cardiomyocytes within human heart tissue sections and stem cell derivatives, suggesting its value for cell-type specific targeting and immunophenotyping. Comparisons of explanted cardiac fibroblasts within the first three passages reveals previously undescribed remodeling of the surfaceome, justifying caution when using cultured cells. These data represent the first major step towards a comprehensive, donor, cell-type, subtype, and chamber-resolved reference map of cell surface phenotypes in the adult human heart and reveal new targets for immunophenotyping, drug delivery, and benchmarking explanted cells and stem cell derivatives.
SOLUBLE GUANYLATE CYCLASE ACTIVATION INCREASES PROTEASOME ACTIVITIES AND PROTECTS AGAINST PROTEOTOXICITY IN CARDIOMYOCYTES

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Background: The ubiquitin-proteasome system (UPS) mediates the selective degradation of misfolded proteins and most cellular proteins that are normal but no longer needed, thereby playing important roles in diverse cellular processes. Proteasome malfunction and proteotoxicity are implicated in the genesis of a large subset of cardiovascular diseases. We previously discovered that activation of the cGMP-dependent protein kinase (PKG) primes the proteasome. Here we sought to test whether activation of the soluble guanylate cyclase (sGC) could activate the proteasome and improve protein quality control in cardiomyocytes.

Methods and Results: In cultured neonatal rat ventricular myocytes (NRVMs), treatment with an sGC activator (cinaciguat) led to increases in the levels of Ser239-phosphorylated vasodilator-stimulated phosphoprotein (VASP) in a dose-dependent fashion and increased the activities of 26S proteasomes as assessed with in-gel proteasome activity assays. Cinaciguat treatment decreased the protein levels of Thr41/Ser45 phosphorylated β-catenin, a representative substrate of the 26S proteasome. These results reveal that sGC activation increases PKG activity and stimulates proteasome activities in cardiomyocytes. Adenoviral vectors expressing degron CL1-fused green fluorescent proteins (GFPu, a proven UPS substrate) or red fluorescent proteins (RFP, a stable protein control) were used as a reporter system for UPS performance. Cinaciguat treatment enhanced the degradation of GFPu in NRVMs. In NRVMs with adenovirus-mediated overexpression of a human-disease-linked misfolded protein HA-tagged CryABR120G, the LDH leakage and PKCδ cleavage were significantly reduced by cinaciguat treatment. Consistently, the cinaciguat treatment improved cell viability in NRVMs infected with Ad-β-Gal or Ad-HA-CryABR120 as revealed by the MTT assay. The indices of cell injury and cell death were markedly attenuated by cinaciguat treatment.

Conclusions: Activation of sGC with cinaciguat increases PKG activity, activates the 26S proteasome, facilitates UPS functioning, and thereby protects against proteotoxicity in cardiomyocytes.
Background: Neointimal hyperplasia is a common pathological response to vascular injury, due to the proliferation and migration of vascular smooth muscle cells (VSMCs). CSN8, the smallest subunit of the COP9 signalosome (CSN) plays a role in maintaining the G1 phase of a cell cycle. Despite the strong evidence linking the CSN to cell cycle regulation, direct study of CSN in VSMC proliferation is lacking. The present study tests the hypothesis that increased CSN5-mediated nuclear exclusion of p27 contributes to the promotion of VSMC proliferation in injured vessels by CSN8 hypomorphism.

Methods and Results: Markedly smaller nuclear to cytoplasmic ratios for p27 and CSN5 proteins and significantly increased cytoplasmic CSN5 mini-complex or free CSN5 in the left common carotid artery (LCCA) were detected in CSN8 hypomorphic (CSN8-hypo) mice compared with non-hypomorphic littermate controls (CTL). We subjected adult CSN8-hypo and CTL mice to LCCA ligation and collected the LCCA segment proximal to the ligation for analyses. LCCA ligation increased in CSN8 proteins in wild type mice, suggesting an important role of the CSN in vascular pathology. Compared to CTL, the CSN8-hypo mice displayed more severe neointimal thickening at both 1- and 4-week after ligation, greater increases in PCNA and significantly greater prevalence of Ki67-positive VSMCs at 1 week after ligation. In cultures, in response to platelet derived growth factor (PDGF-BB), CSN8-hypo VSMCs displayed an increased proliferation as assessed by MTT assay, an accelerated G0/G1 progression, and significant increases in PCNA that could be attenuated by nuclear export inhibitor leptomycin B (LMB) but not by CSN denuedylase inhibitor CSN5i-3. CSN5i-3 significantly suppressed the PDGF-BB induced PCNA increases in CTL VSMCs.

Conclusion: CSN8 hypomorphism promotes neointimal hyperplasia in injured arteries and increases VSMC proliferation, likely through the nuclear exclusion of p27 protein that is mediated by CSN5.
OVEREXPRESSION OF SKELETAL MUSCLE NRF2 PROTECTS AGAINST AGING-ASSOCIATED DYSFUNCTION IN SKELETAL MUSCLE AND HEART

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Introduction: Nrf2 is a master transcription factor governing hundreds of genes involved in antioxidation, anti-inflammation, detoxification, and metabolism. Skeletal muscle (SkM) deletion of Keap1 upregulates over 100 cytoprotective proteins and enhances exercise capacity in adult mice. We hypothesize that Keap1 KO protects against aging-associated myopathy in SkM and in the myocardium.

Material & Methods: Experiments were carried out in 43 male SkM-Keap1^{floox/floox} mice assigned to 4 groups: young WT (11), young Keap1 KO (12), aging WT (11), and aging Keap1 KO (9). At 5 months in young mice and 25 months in aged mice a treadmill running test was done and echocardiography to evaluate myocardial function.

Results: We found that, aging WT displayed significantly shorter running distance than young WT (280.2 ± 35.1 vs 531.6 ± 38.4 m, p < 0.001) that which was improved in aging KO mice (508.2 ± 66.8 m, p < 0.01 vs aging WT). The echocardiography indicated that aging WT has exhibited lower ejection fraction (EF), longer isovolumic relaxation time (IVRT), and higher myocardial performance index (MPI) than young WT (EF: 60.3 ± 3.1 vs 68.1 ± 1.3 %, p < 0.05; IVRT: 22.6 ± 1.5 vs 16.9 ± 0.5 ms, p < 0.01; MPI: 0.71 ± 0.03 vs 0.55 ± 0.02, p < 0.001). These aging-associated cardiac dysfunctions were partially alleviated in aging KO (EF: 65.6 ± 0.65 %, p = 0.073; IVRT: 16.4 ± 1.5 ms, p < 0.01; MPI: 0.57 ± 0.03, p < 0.001 vs aging WT).

Conclusion: These data suggest that chronic activation of SkM Nrf2 not only attenuates aging associated SkM dysfunction but also improves cardiac aging parameters. The later effects, we speculate, are mediated by transference of Nrf2-upregulated proteins from the Keap1-deficient SkM to the myocardium through SkM-derived EVs.
CONSTRICCTOR RESPONSES OF CEREBRAL RESISTANCE ARTERIOLES IN MALE AND FEMALE RATS EXPOSED TO ALCOHOL PRENATALLY

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The purpose of this study is to determine whether responses of cerebral arterioles to vasoconstrictors are altered in rats exposed to alcohol in utero. While studies have shown that dilation of cerebral arterioles to NOS-dependent agonists is impaired in rats exposed to prenatal alcohol, no studies that we are aware of have examined the influence of prenatal alcohol on constrictor response of cerebral arterioles, thus proposing the goal of the present study. Our central hypothesis is that in utero alcohol exposure impairs the activity of the vascular smooth muscle, which will alter the ability of the arterioles to constrict in response to the vasoconstrictors. We fed Sprague-Dawley dams a liquid diet with or without alcohol (3% ethanol) for the duration of their pregnancy (21-23 days). We then examined the reactivity of cerebral arterioles to arginine vasopressin (0.1 and 1 nM), thromboxane (U-46619; 10 and 100 nM), and angiotensin II (1 and 10 µM) in four groups of rats: control male, control female, prenatal alcohol male, and prenatal alcohol female, at two different age ranges: 4-6 weeks of age and 14-16 weeks of age. We found that constrictor responses of cerebral arterioles to AVP and the thromboxane analog (U-46619) were similar in male and female rats regardless of exposure to prenatal alcohol at both age groups. In addition, while responses to angiotensin II were not altered by exposure to alcohol in male and female rats at 4-6 weeks of age, there were differences observed in responses to the high concentration in 14–16-week-old male and female rats exposed to prenatal alcohol. Based on the findings, we suggest that impaired dilation of cerebral arterioles, coupled with preservation of vasoconstriction, may increase the susceptibility of the brain to cerebral ischemic damage.
HIGH SCHOOL STUDENTS

Oral Presentation
Category: High School Student (Senior Division)

COVID-19: In-depth analysis of coronavirus mutations and their implications for future outbreaks

Reema Guda
Millard North High School

Coronavirus, SARS-CoV-2 that is responsible for the COVID-19 pandemic has around 30,000 letters of genetic code in its genome that constantly mutate to give rise to new strains of the virus. Mutations in a new strain could make it more infectious, hence studying their effects on the infectivity of the virus is very important for disease surveillance and vaccine development to combat the pandemic. SARS-CoV-2 produces a protein called spike (S), among others, where a specific part of this viral protein called the receptor-binding domain (RBD) needs to bind to a human protein called ACE2 to gain entry and infect human cells. Hence, my hypothesis is that mutations in the RBD region are more critical for the spread of the virus compared to mutations in other viral proteins. In this study, I carried out an in-depth analysis of observed coronavirus mutations using publicly available data obtained from around 130,000 COVID-19 patients. I used statistical tools to normalize and analyze these datasets to test my hypothesis. Overall, I conclude that the RBD region of the Spike protein accumulates both the highest and the most effective mutations than any other viral protein, and my hypothesis is proven correct with high statistical significance. In addition, virus strains with mutations in the RBD region are proven most infectious suggesting that new mutations in this region could be predictive of future outbreaks. My future research would focus on studying why some mutations are more dangerous than the others and predicting their effects on disease transmission rates.
Oral Presentation
Category: High School Student (Junior Division)

THE ROLE OF OXIDATIVE STRESS IN MEDIATING THE EFFECTS OF GUT DYSBIOSIS ON HOST HEALTH

Anuj D. Singh
Millard North High School

Background and Rationale: Last year, my project was focused on investigating if C. elegans can be a suitable model to test the effects of gut dysbiosis upon human health. Using stool from normal mice and mice subjected to inflammatory bowel disease, I demonstrated that gut dysbiosis dysregulates C. elegans growth. This year, my objective was to determine the effects of gut dysbiosis associated with colon cancer upon C. elegans health, and the underlying mechanism. An extensive literature search suggested a key role of oxidative stress in IBD, colorectal cancer, and C. elegans health.

Hypothesis: Oxidative stress plays a central role in mediating the effects of gut dysbiosis upon host health.

Methods: I used the stool from normal mice or mice susceptible to colon cancer. Effects on C. elegans growth (number, size, and mobility), were determined. Effects of inhibiting oxidative stress were determined. Probiotic treatment served as control. Data were analyzed and plotted using Microsoft Excel. Significance was calculated by the student's t-test.

Results: The C. elegans grown on dysbiotic gut microbiota were significantly smaller (size and number) as compared to those grown on microbiota from normal mice. Inhibiting oxidative stress had significant effects upon C. elegans growth, body texture and mobility as these worms were transparent and more agile. However, the effects were more pronounced in C. elegans cultured on colon cancer associated microbiota.

Conclusion: We concluded that oxidative stress plays a central role in mediating gut dysbiosis. My future studies will be focused on detailed analysis of the molecular mechanisms underlying the deleterious effects of oxidative stress.
POSTER PRESENTATIONS

(Poster titles are hyperlinked)
METABOLIC AND MICROENVIRONMENTAL FEATURES OF GRANULOSA CELL TUMORS IN PIK3CA* MICE

Seok-Yeong Yu, Yi Luan, Pauline Xu, So-Youn Kim.
Olson Center for Women’s Health, Department of Obstetrics and Gynecology, College of Medicine, University of Nebraska Medical Center, Omaha, NE

Background: Granulosa cell tumor (GCT) is related to the abnormal proliferation of GC in preovulatory follicles and accounts for approximately 5% of total ovarian cancer cases in women. GCT microenvironment is characterized by increased lipid accumulation, fibrosis and low macrophage infiltration. However, little is appreciated regarding the role of such microenvironmental feature on GCT development due perhaps to few mouse models available that resemble human GCT. PIK3CA* female mice (Gdf9-iCre+;Pik3ca*/w, Cre+) spontaneously develops GCT in postnatal day 65 (PD65).

Methods: We investigated the temporal gene expression related to fibrosis and metabolism in GCT, employing transcriptome analyses with ovaries at PD8, PD50 and PD65 from Gdf9-iCre-;Pik3ca*/w (Cre-) and Cre+ mice.

Results: We found that Cre+ mice had significantly upregulated gene expression related to anti-inflammatory macrophages (Arg1 and Chil3), fibrosis (Timp1, Fn1 and Col1a1) and cell proliferation (Cdk4 and 5) from PD8 to PD65 compared to Cre-. Also, the expression of glycolysis-related genes (Hk2, Pfkl and Idha) was remarkably higher in Cre+ mice than Cre- at PD65. Consistently, Cre+ mice had lower fasting blood glucose levels than Cre-. Regarding lipid metabolism, lipid oxidation-related genes were significantly downregulated in Cre+ mice, but the expression of genes related to lipid uptake and synthesis (Cd36 and Dgat2) was considerably upregulated at PD65. Consistently, the protein expression of PPARγ and DGAT2 was increased in Cre+ at PD65, and the nuclear localization of PPARγ immunofluorescence corroborates that lipid metabolism of GCTs in this model favors lipid synthesis over oxidation.

Conclusions: Our data indicate that PIK3CA* GCT mouse model exhibits the upregulation of genes for fibrosis, cell proliferation, glycolysis and lipid accumulation as evidenced in GCT patients. Such abnormalities, thus, would be considered for potential therapeutic targets for GCTs, supported by our PIK3CA* GCT mouse model which shows the progressive development of GCT.
Withdrawal of cardiac vagal activity is a common complication in patients with type 2 diabetes mellitus (T2DM) and is associated with arrhythmia-related sudden cardiac death and high mortality in T2DM patients. Although our recent study has demonstrated that reduction of N-type calcium (Cav2.2) currents and cell excitability of cardiac vagal postganglionic (CVP) neurons exacerbates myocardial infarction-evoked ventricular arrhythmias and mortality in T2DM rats, the mechanisms responsible for T2DM-reduced Cav2.2 channel activity in CVP neurons remain unclear. Since reactive oxygen species (ROS) has been reported to modulate calcium channel activity in peripheral postganglionic neurons, here we tested if hydrogen peroxide (H2O2, a type of ROS) overproduction inactivates Cav2.2 channels and further contributes to ventricular vagal dysfunction and ventricular arrhythmogenesis in T2DM. Rat T2DM was induced by a high-fat diet plus streptozotocin injection. Adenoviral catalase gene (Ad.CAT, 2 µl, 1 x 10^10 pfu/ml) was microinjected into CVP neurons to scavenge local H2O2 production. Reduced catalase expression accompanied by overproduction of H2O2 was detected in the CVP neurons in T2DM rats. In vivo transfection of Ad.CAT not only increased protein expression of catalase but also attenuated H2O2 production in CVP neurons in T2DM rats. Data from reverse-phase protein array also demonstrated that overexpression of catalase by transfection of Ad.CAT into CVP neurons markedly increased T2DM-reduced protein expression of Cav2.2 in CVP neurons. Local microinjection of Ad.CAT into CVP neurons also significantly restored T2DM-blunted ventricular vagal activity, as demonstrated by an improvement in the response of left ventricular systolic pressure to left vagal efferent nerve stimulation. Additionally, in the power spectral analysis of heart rate variability (HRV), transfection of Ad.CAT into CVP neurons increased the T2DM-attenuated high frequency power (an index of vagal activity), leading to further restoration of the low frequency to high frequency ratio. Moreover, the inducibility of ventricular arrhythmia measured in anesthetized condition was significantly reduced in T2DM rats transfected with Ad.CAT, compared with T2DM rats (0.75±0.48 in T2DM rats vs. 3.0±1.38 in T2DM+Ad.CAT group). Transfection of Ad. vector into CVP neurons had no effects on protein expression of catalase and Cav2.2, ventricular vagal activity, HRV, as well as susceptibility to ventricular arrhythmias in T2DM. Based on above data, we conclude that H2O2 overproduction inactivates Cav2.2 channels and further contributes to ventricular vagal dysfunction and ventricular arrhythmogenesis in T2DM, suggesting that H2O2 signaling pathway might be an effective therapeutic target to suppress ventricular arrhythmias in patients with T2DM.
ALTERED GLYCOSYLATION OF PLASMA HIGH DENSITY LIPOPROTEINS IN RHEUMATOID ARTHRITIS: A FRIEND OR FOE?

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Background: Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease and an independent risk factor for cardiovascular diseases (CVD). RA patients exhibit dyslipidemia and accelerated atherosclerosis and are two times more susceptible to CVDs than individuals without RA. Traditional risk factors such as lipoprotein levels and systemic inflammation fail to fully explain the excess risk of CVD among RA patients. Altered glycosylation of plasma proteins is a hallmark of RA. Aberrant glycosylation of IgG and acute phase proteins, for example, have been shown to correlate with disease progression in RA. Among plasma lipoproteins, High Density Lipoprotein (HDL) is an athero-protective molecule. HDL confers athero-protection particularly through reverse cholesterol transport activity, antioxidant, and anti-inflammatory properties. Recent studies have shown that apolipoproteins on HDL are glycosylated and changes in glycosylation affects the immunomodulatory and cholesterol efflux function of HDL. Nevertheless, little is known about the structure, site-specific glycosylation, and function of glycans on HDL in RA patients. Herein, we hypothesize that altered glycosylation of HDL could be cause for accelerated atherosclerosis in RA patients.

Results and Conclusion: Using ultrasensitive liquid chromatography and mass spectrometry-based approaches we determined the structure and relative levels of N-glycans on HDL isolated from RA and non-RA patients. Our results show alterations in the N-glycan profile of HDL isolated from RA patients compared to healthy controls. In addition to glycosylation changes, apolipoproteins of HDL show differences in modifications such as oxidation. Our results correlate with the severity of the disease and provide an index to assess the quality of HDL beyond its absolute levels in serum/plasma. This level of detail is essential for defining the precise mechanisms of how these modifications impact HDL function and to explain the accelerated atherosclerosis in RA.
ACUTE METABOLOMIC, PROTEOMIC AND TRANSCRIPTOMIC CHANGES DURING LUTEOLYSIS: INTEGRATION OF OMICS ANALYSIS AS TOOL TO IDENTIFY NEW PLAYERS IN LUTEAL MAINTENANCE

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Prostaglandin F2α (PGF) triggers regression of the corpus luteum, an endocrine gland crucial for establishment and maintenance of pregnancy due to progesterone production. The present study was designed to identify acute metabolomic, proteomic and transcriptomic changes occurring in the corpus luteum during the early stages of luteolysis. Omics analysis were performed using luteal tissue collected from midluteal phase cows (n=6/treatment) 4h after injection with saline (control group) or PGF (PGF group). Metabolomic, proteomic and transcriptomic analysis were performed using LCMS/MS, MS and microarray. Serum progesterone concentrations were analyzed using ELISA. Data were analyzed using Welch’s two-sample t-test or t-student test. The Benjamini-Hochberg (BH) adjusted p values were provided to adjust for multiple-testing caused false discovery rate (FDR). Progesterone concentration were 56% lower (P<0.001) in PGF group confirming initiation of luteolysis. Principal component analysis showed a distinct metabolite composition between groups. Among 50 significantly changed metabolites, 49 were upregulated and 1 was downregulated in PGF group. MetaboAnalyst revealed arginine biosynthesis; aminoacyl-tRNA-biosynthesis; arginine and proline metabolism; valine, leucine and isoleucine biosynthesis; phenylalanine, tyrosine and tryptophan biosynthesis; nicotinate and nicotinamide metabolisms, as significantly changed pathways in PGF group. 230 and 54 proteins were down- and up-regulated in PGF group. Ingenuity Pathway Analysis (IPA) demonstrated Clathrin-mediated endocytosis; oxidative phosphorylation and LXR/RXR Activation among top five significantly changed canonical pathways. Metabolism of cholesterol, concentration of cholesterol, transport of lipids and translation were recognized as inhibited biological functions in the PGF group. Integration of metabolomic-proteomic data indicated glutathione metabolism; arginine and proline metabolism; arginine biosynthesis; TCA cycle and aminoacyl-tRNA biosynthesis as significantly changed pathways. Gene Set Enrichment Analysis (GSEA) established base excision repair; DNA replication; lysine degradation and phosphatidylinositol signaling system among downregulated pathways in PGF group. Cytokine-cytokine receptor interaction; cytosolic DNA sensing pathway and NOD like receptor signaling pathway were found as upregulated pathways in the PGF group. Integration of metabolomic-transcriptomic data indicated inositol phosphate metabolism; lysine degradation; phosphatidylinositol signaling; arginine biosynthesis among significant pathways. Summarizing, luteolysis is associated with acute changes within metabolic pathways that reduce lipogenesis, translation, production of mitochondrial energy and free radicals, and stimulate activation of immune cells, and synthesis of vasoactive factors and collagen.
Immunotherapy has emerged at the forefront of cancer treatment. Checkpoint inhibitor pembrolizumab (KEYTRUDA), a chimeric antibody which targets programmed cell death protein 1 (PD-1), has been approved by the FDA for use in pediatric patients with relapsed or refractory classical Hodgkin’s lymphoma. However, there is currently no published data regarding the effects of pembrolizumab on the ovary of female pediatric patients. In this study, prepubertal immunocompetent and immunodeficient female mice were injected with pembrolizumab or anti-mouse PD-1 antibody. The number of primordial follicles significantly decreased post-injection of both pembrolizumab and anti-mouse PD-1 antibody in immunocompetent mice. However, no changes in follicle numbers were observed in immunodeficient nude mice. Superovulation test and vaginal opening experiments suggest that there is no difference in the number of COCs and the timing of puberty onset between the control and anti-mouse PD-1 antibody treatment groups, indicating that there is no effect on short-term fertility. Elevation of pro-inflammatory cytokine TNF-α following COX-2 upregulation was observed in the area that primordial follicles generally occupy inside the ovary. CD3+ T-cell infiltration was detected within some ovarian follicles and between stromal cells of the ovaries in mice following treatment with anti-mouse PD-1 antibody. Thus, PD-1 immune checkpoint blockade affects the ovarian reserve through a mechanism dependent on inflammation following CD3+ T-cell infiltration.
**HEPATOCYTE-SPECIFIC TP-R DELETION ATTENUATES XENOBIOTIC-METABOLIZING ENZYMES IN LIVER: IMPLICATIONS IN PROTECTION AGAINST ETHANOL-INDUCED LIVER INJURY**

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Alcohol-associated liver disease (AALD) is a major health concern with limited treatment options. Thromboxane prostanoid receptor (TP-R), a G-protein-coupled receptor, is expressed in liver. An alcohol diet increases TP-R mRNA levels in hepatocytes. However, the role of hepatocyte TP-R in AALD still remains unknown. Here, hepatocyte-specific TP-R knockout mice (KO) and their wild type (WT) littermate control mice were fed with the Lieber-DeCarli control or 5% (vol/vol) ethanol (ET) diet for 4 wk. Mice were divided into four groups: 1) WT:Cont, 2) WT:ET, 3) TP-R-KO:Cont, and 4) TP-R-KO:ET. RNA sequencing of liver samples showed that markers of xenobiotic metabolism (Cyp2b10, Cyp26b1, Cyp2c55), inflammatory response (Nfkb1, Tlr9, Ccl9, Il6ra, Cxcl2), and fibrosis (Col3a1, Col4a2, smad2, smad4) were lower in TP-R-KO:ET mice compared with WT:ET group. Further real-time PCR analyses showed that the mRNAs encoding Cyp2b10 and Cyp2c55, both members of xenobiotic metabolism system, were 132.6-fold (P<0.05) and 5.2-fold (P<0.001), higher, respectively, in WT:ET-fed mice than in WT:Cont mice. In contrast, livers of TP-R deficient ET-fed mice had lower mRNA levels of Cyp2b10 (P<0.001) and Cyp2c55 (P<0.05). Of note, the mRNA levels of pro-inflammatory markers including Ccl2 (P<0.05), Il6 (P<0.001), Il1b (P<0.01), Cxcl10 (P<0.01), and Lcn2 (P<0.01) were increased significantly only in WT:ET mice but not in TP-R-KO:ET-fed mice compared with controls. Interestingly, in hepatic nuclear extracts, the protein level of PPAR-γ2, which has anti-inflammatory and anti-fibrotic effects, was significantly higher in TP-R-KO:ET-fed mice (P<0.05) than in WT:ET mice. These findings lead us to suggest that hepatocyte TP-R plays an important role in regulating the expression of enzymes involved in hepatic xenobiotic metabolism, thereby contributing to development of AALD.
NOVEL MECHANISM OF NEURAL CONTROL OF BREATHING IN ACUTE LUNG INJURY

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Background: Acute lung injury (ALI) induces inflammation and impairs gas exchange to induce hypoxemia that reflexively increases respiration within a few hours to a few days after ALI. The neural mechanisms underlying the respiratory dysfunction after ALI are not fully understood. The purpose of this study involved investigating the role of carotid body chemoreceptor afferents and pulmonary spinal afferents in mediating abnormal ventilation in ALI.

Methods and Results: ALI was induced in male SD rats (200-250g) using a single intra-tracheal injection of bleomycin (bleo) (day 0) and measured RR, TV (Tidal Volume), and MV (Minute Ventilation) in response to 10% hypoxia and 5% hypercapnia weekly from W1-W4 using whole-body plethysmography (WBP). MV at 21%O₂ increased from baseline to W1 and W2 in bleo-rats (p≤0.01). Changes in MV were due to a significant increase in RR while TV showed no changes. Chemoreflex was assessed by measuring the absolute difference between 21% O₂ and 10% hypoxia, and, 5% hypercapnia. Our data indicated a blunted chemoreflex at W1 and W2 during hypoxia and hypercapnia which could be due to maximal pre-activation of chemoreceptors, called the ‘ceiling effect’. Chemoreflex was inhibited in bleo-rats through 90% hyperoxia which showed no changes in RR, suggesting absence of tonic activity. To assess the role of pulmonary spinal afferents in mediating an increased RR in ALI, we induced bleo-ALI on day 0, and ablated the TRPV1-positive lung spinal afferents by bilaterally injecting 5μl of a TRPV-1 specific neurotoxin, resiniferatoxin, RTX (50 μg/ml) into the stellate ganglion (day 3), and used WBP to measure resting respiratory variables (day 7). RTX ablation of pulmonary spinal afferents caused a significant reduction in RR in bleo-rats at W1 (p≤0.05).

Conclusions: These data suggest that chemoreflex activation in response to hypoxia and hypercapnia is blunted whereas, pulmonary spinal afferent activation may play a major role in the increased RR in ALI.
Poster Presentation
Category: Graduate student

ROLE OF P63 IN CYCLOPHOSPHAMIDE-INDUCED OOCYTE DEATH IN PRIMORDIAL FOLLICLE

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Background: Radiotherapy and cytotoxic chemotherapy cause detrimental side effects while advanced cancer therapies have increased 5-year survival rates of cancer survivals. Due to the high sensitivity of the ovary, cancer treatment causes ovarian damage and follicle depletion which leads to premature ovarian insufficiency (POI) in young female cancer survivals. POI, presented as endocrine dysfunction and infertility, becomes a critical status in prepubertal girls and premenopausal women. Cyclophosphamide (CPA) forms DNA crosslinks and leads to apoptosis in rapidly proliferating tumor cells. However, the underlying mechanism of the CPA-caused death in dormant oocytes from the ovary remains controversial. One group proposed that CPA induces apoptotic pathway in oocytes of primordial follicles through DNAPK->H2AX->CHK2->p53/TAp63a->PUMA and ABL kinase inhibitor, GNF2, prevents oocyte death against CPA. The other group suggested that CPA activates dormant oocytes of primordial follicles via the PI3K pathway, leading to burnout of follicle pool. Meanwhile, another group presented that p63 is not involved in the apoptotic pathway of oocyte death. In this study, we used oocyte-specific p63 and Abl conditional knockout mouse models to clarify the elemental mechanism regarding the CPA-induced POI in the ovary.

Experimental Design: We generated oocyte-specific Abl1 and p63 knockout mouse models using GDF9-iCre to investigate the role of ABL and p63 in the oocyte death with CPA treatment. Postnatal day 7 (PD7) female mice were i.p. injected with 100 mg/kg CPA and ovaries were harvested day 3 post-treatment for further analysis. We also tested GNF2 in PD7 mice in vivo to examine its feasibility of protecting oocytes of primordial follicles against CPA.

Results: We found that CPA reduced the number of primordial follicles without accelerating the number of growing follicles. The quantification of surviving follicles validated that 90% of the primordial follicles from oocyte-specific Abl1 knockout mice were lost following CPA treatment in vivo and in vitro, with most of the growing follicles maintained inside of the ovary. Accordingly, GNF2 did not prevent loss of primordial follicles after CPA treatment. However, ovarian morphology and follicle quantification revealed significant follicles were rescued in oocyte-specific p63 knockout mouse with CPA treatment. Consistently, high expression of CHK2 was detected in the oocytes of the ovary cultured with the 4-hydroperoxy cyclophosphamide (4HC, CPA effective metabolite) in vitro.

Conclusions: The number of primordial follicles rather than growing follicles dramatically declines post CPA treatment, suggesting that dormant follicles are more sensitive to CPA compared to growing follicles. ABL tyrosine kinase is dispensable for CPA-induced oocyte death in the mouse ovary. Most of all, this study suggests that TAp63 is the master regulator to induce follicle depletion caused by CPA. CHK2 inhibitors can be further tested as fertoprotective reagents to preserve follicles against CPA as the CHK2-p63 pathway regulates primordial follicle death without activating dormant follicles.
Poster Presentation
Category: Graduate student

CISATRACURIUM ATTENUATES LPS-INDUCED MODULATION OF MMP3 AND JUNCTIONAL PROTEIN EXPRESSION IN HUMAN MICROVASCULAR ENDOTHELIAL CELLS

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Acute respiratory distress syndrome (ARDS) is a life-threatening form of acute lung injury (ALI) associated with hypoxemic lung damage and inflammation. Matrix metalloproteinase protein-3 (MMP3 or Stromelysin-1) is known to promote vascular injury in ALI/ARDS. Cisatracurium, a nicotinic neuromuscular blocker, is used in ARDS patients to decrease mechanical ventilator dyssynchrony, increase oxygenation, and improve mortality. However, the magnitude and the underlying mechanisms of these potential benefits of cisatracurium remains unclear. We investigated the effect of cisatracurium on lipopolysaccharide induced MMP3 expression in human microvascular endothelial cells. In our results, cisatracurium treatment significantly decreased LPS-induced MMP3 expression and increased expression of cell junction proteins such as vascular endothelial cadherin (VE-Cadherin) and Claudin-5.
ENHANCEMENT OF THE PROBDNF- P75NTR PATHWAY IN DENERVATED SKELETAL MUSCLE

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Background/Hypothesis: Myokines are molecules released from muscle tissue. One such myokine is brain derived neurotropic factor (BDNF) and its precursor, proBDNF, which appear to play important and novel roles in inflammation. While BDNF is well characterized in the brain, its role as a myokine is far less understood, and even less is known about its precursor proBDNF. Understanding the role of proBDNF is critical in understanding skeletal muscle’s endocrine function. Skeletal muscle denervation, such as that seen with spinal injuries, peripheral neuropathies, and aging, induces muscle inflammation. Thus, we hypothesized that proBDNF is involved in denervation-induced skeletal muscle inflammation.

Methods and Results: In the unilateral sciatic denervation mouse model, proBDNF and its receptor p75NTR were increased with concurrent decreases in mature BDNF and receptor TrkB. Along with the upregulation of p75NTR protein levels, were corresponding increases in downstream phosphorylation of JNK and NFκB, both of which have been shown to play roles in inflammation. Blocking p75NTR decreased activation of these inflammatory pathways and reduced many of the inflammatory markers in the denervated muscle, indicating that the p75NTR pathway is critical for inflammation. Further, in skeletal muscle specific BDNF knockout mice, inflammatory markers were reduced after denervation as compared to wild type mice.

Conclusions: These results indicate that the activation of the proBDNF-p75NTR pathway may contribute to the adverse changes in denervated skeletal muscle. Therefore, targeting the proBDNF-p75NTR pathway could potentially mitigate denervation-induced inflammation and myopathy.
CATEGORIZING FILAMENTATION PHENOTYPES ACROSS DIVERGENT C. ALBICANS STRAINS

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Background/Hypothesis: The human pathogen Candida albicans causes systemic infections with a high mortality rate. Current research has demonstrated antifungal drug sensitivity and pathogenic differences between divergent clinical strains, but research has generally focused on a single strain background. This study categorized clinical isolates from the University of Nebraska Medical Center (UNMC) both genetically by Multilocus Sequence Typing (MLST) and phenotypically by filamentation. Filamentation is strongly correlated with pathogenesis in C. albicans, and we hypothesized that the clinical strains, isolated from systemic infections, will likely filament in most in vitro conditions. However, our work has shown that the various in vitro models used to predict in vivo filamentation have varying genetic requirements, so it is possible that strains may experience filamentation defects in one or more conditions. We may be able to use patterns of filamentation to identify in vitro conditions that best predict in vivo filamentation.

Methods/Results: For the MLST analysis, seven housekeeping genes were amplified using standard primers, and PCR products were analyzed by Sanger sequencing. Novel allelic profiles were found using the C. albicans MLST database tool. Filamentation was analyzed in four liquid and four solid inducing conditions, with solid and liquid non-inducing control condition. Several strains exhibited filamentation defects in at least one media condition.

Conclusions: Genetically, the clinical strains have known allele sequences but the allelic profiles are novel. Further eBURST analysis with known reference strains will need to be performed to determine clade designation. Although the clinical strains were isolated from systemic infections several exhibited filamentation defects in one or more conditions in vitro. For further phenotypic characterization these strains will be utilized in in vivo filamentation studies.
**Poster Presentation**
Category: Graduate student

**BIOLOGICAL ACTIVITY OF NOVEL CURCUMIN ANALOG (CIS-TRANS CURCUMIN) AT ADENOSINE RECEPTOR SUBTYPES A₁ AND A₃**

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**Background/hypothesis of the study:** A wide variety of plants produce compounds that contain the 4-hydroxy-3-methoxybenzyl or “vanillyl” group. These vanilloid compounds include such well-known food molecules as vanillin, capsaicin, gingerol, and curcumin. Vanilloid compounds have been widely studied for their medicinal properties, and one property that most of them have in common is the ability to induce analgesia. Studies have indicated multiple potential cellular mechanisms for this analgesic activity, including TRPV1 receptors, opioid receptors, and adenosine receptors (ARs). Our lab has become interested in the role that ARs might play. In this study, we measured the interaction of a curcumin analog (cis-trans curcumin or CTCUR) with the two AR subtypes that are linked the inhibitory Gα subunit (subtypes A₁ and A₃).

**Methods and Results:** We performed competitive binding assays on transfected Chinese hamster ovary cells to quantify the binding affinity of CTCUR for each receptor. We also performed confocal microscopy as an additional method for assessing binding affinity. Finally, we assayed cAMP production as a measure of receptor activity. Competition assay results indicate that CTCUR binds to subtype A₃ with a Kᵢ of 4.00 X 10⁻⁷ M, and confocal microscopy results indicate that CTCUR binds to subtype A₁ in the sub-micromolar range as well. Data from cAMP assays, alongside previous work in our lab that examined CTCUR’s action at AR subtypes A₂A and A₂B, suggest that CTCUR acts as a weak agonist of ARs. Competition assay results also indicate that unmodified (i.e. trans-trans) curcumin binds to subtype A₃, with a Kᵢ of 2.57 X 10⁻⁶ M.

**Conclusions:** These results are of interest because they demonstrate that the vanillyl moiety can indeed bind to ARs. This fact, in turn, suggests that ARs may mediate some of the medicinal effects of vanilloid compounds, including analgesia.
SER14-RPN6/PSMD11 PHOSPHORYLATION IS REQUIRED FOR ACTIVATION OF THE 26S PROTEASOME BY CAMP AND PROTECTS AGAINST CARDIAC PROTEOTOXIC STRESS

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Background. In cultured cells, cAMP-dependent protein kinase (PKA) was shown to activate the 26S proteasome through directly phosphorylating Serine 14 of Rpn6/PSMD11, a non-ATPase subunit of the 19S proteasome. Hence, we sought to test the hypothesis that Ser14-Rpn6 phosphorylation is required for PKA-mediated activation of the proteasome in vivo and protects against cardiac proteotoxicity.

Methods and Results. We generated via CRSPR/Cas9 gene editing two mouse models, in which the codon for Ser14 in the Rpn6 gene was mutated to encode either Alanine (Rpn6S14A) for blocking Ser14-Rpn6 phosphorylation or Aspartic acid (Rpn6S14D) for mimicking this phosphorylation. Treatments that activate the cAMP-PKA pathway increased Ser14-phosphorylated Rpn6 (p-Ser14-Rpn6) and enhanced proteasome activity in wild-type, but not Rpn6S14A, mouse embryonic fibroblasts (MEFs) and mouse hearts, indicating that p-Ser14-Rpn6 is required for the activation of 26S proteasome by PKA. To test the role of p-Ser14-Rpn6 in cardiac response to increased proteotoxic stress (IPTS) in mice, we cross-bred the Rpn6S14A mice with CryABR120G transgenic mice (a model of cardiac IPTS) and monitored cardiac phenotypes. Surprisingly, Rpn6S14A knock-in neither mitigated the progression of cardiac hypertrophy and malfunction induced by CryABR120G nor shortened lifespan of CryABR120G mice, suggesting that p-Ser14-Rpn6 is dispensable for cardiac response to IPTS, which is consistent with that myocardial p-Ser14-Rpn6 is not increased in CryABR120G mice. To further determine the effect of mimicry of Ser14-Rpn6 phosphorylation on myocardial proteasome functioning and cardiac proteotoxicity, we cross-bred the Rpn6S14D mice with CryABR120G transgenic mice. Preliminary data showed that Rpn6S14D knock-in is trending to increase function of the ubiquitin-proteasome system as well as decrease the accumulation of misfolded protein.

Conclusions. Ser14-Rpn6 phosphorylation is required for the activation of the 26S proteasome by PKA in mice. Genetic mimicry of Ser14-Rpn6 phosphorylation has potential to protect against cardiac proteotoxicity.
EFFECTS OF 3-WEEKS OF AEROBIC EXERCISE IN THE HEAT ON FITNESS AND MOLECULAR OUTCOMES IN FEMALES

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Background: The effects of exercise training in the heat has been well documented in men. However, the effects of active heat acclimation in females have been underrepresented. We have previously reported blunted aerobic power (VO₂peak) and mitochondrial-related gene expression in men after aerobic exercise training in the heat. It is unclear if these observations persist within females. Therefore, the purpose was to determine the impact of 3 weeks of aerobic exercise training in the heat vs. room temperature on thermoregulation, gene expression, and aerobic capacity in females.

Methods: Twenty-two untrained college aged females (24±1 yoa and 66±9 kg) were assigned to 3 weeks of aerobic exercise training in either 20°C (n=12) or 33°C (n=10) environmental temperatures.

Results: Aerobic capacity (Pre 39±6 to Post 41±6 mL·kg⁻¹·min⁻¹, p=0.021) and peak power (Pre 191±33 to Post 206±27 W, p<0.001) increased after three weeks of training but were not different between temperature conditions (aerobic capacity, p=0.440; power p=0.955). HR decreased with training from Day 1 to Day 20 (152±16 to 140±13 b·min⁻¹, p<0.001), but was not different among the temperature groups (p=0.341). Sweat rate increased with training from Day 1 to Day 20 (661±1 to 787±1 mL·h⁻¹, p<0.005) and was higher in 33°C than 20°C (p<0.001). Independent of temperature, PGC-1α mRNA increased with an acute exercise bout before (5.29±0.70 fold change, p<0.001) and after training (2.69±0.22 fold change, p=0.004). Similarly, gene expression of VEGF increased acutely (3.44±0.47 fold change, p<0.001), after training (1.81±0.23 fold change, p<0.001), and was also blunted(p<0.001), relative to the pre-trained acute exercise response.

Conclusions: Females can increase aerobic fitness and maintain an exercise induced PGC-1α mRNA response in the heat equally to that of room temperature conditions. These findings contrast with the blunted PGC-1α mRNA response and VO₂peak alterations previously observed in men.
**INFLUENCE OF LOCAL MUSCLE COOLING ON MITOCHONDRIAL RELATED GENE EXPRESSION AT REST**

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**Background:** Our lab has demonstrated physiological and molecular differences between exercising in ambient cold and an application of local cold. Local cold application has been shown to decrease the temperature of the skin and muscle, causing vasoconstriction, and decreasing blood flow. Currently, the effects of local cold application independent of exercise on mitochondrial related gene expression is unknown. Therefore, the purpose of this study is to determine the impact of localized cooling to the skeletal muscle during rest.

**Methods:** Thermal wraps were applied to the *vastus lateralis* (VL) of each limb of 12 participants. One limb received a cold application (randomized) in the form of circulated 10 °C fluid (COLD), while the other did not (CON). Wraps were removed at the 4-hour time point and measurements of skin temperature, blood flow, and intramuscular temperature were collected. Muscle biopsies were taken from the VL of each leg for later molecular analyses. Statistical significance (p<0.05) was analyzed via paired t-tests.

**Results:** Skin temperature, measured via laser thermometer, was lower in COLD (34.84±0.94 °C, to 13.15±0.99 °C, p<0.01). Similarly, thermal camera skin temperature measurements were (35.75±0.94 °C, to 12.81±0.93 °C, p<0.01). Intramuscular temperature, via hypodermic thermocouple, also was lower in COLD (35.57±0.76 °C, to 20.54±1.31 °C, p<0.01) after cold application. Blood flow, measured via ultrasound equipped with a transducer, did not change (0.64±0.12 cm/sec, to 0.62±0.17cm/sec, p=0.450). Arterial diameter was lower in COLD after 4h (0.62±0.05 mm, to 0.60±0.05 mm, p=0.018). Molecular outcomes (gene expression) will be measured soon via RT-qPCR and analyzed using the 2-ΔΔct method.

**Conclusion:** Mitochondrial related gene expression data will help interpret previous local cold and exercise findings.
Background: Physiological responses to hypoxic stimuli are assumed to be dependent upon the inspired partial pressures of oxygen. Decreasing the fraction of inspired oxygen has been used to simulate atmospheric partial pressure of oxygen decreases at terrestrial altitude. Therefore, the purpose of this investigation was to determine the independent effects of altitude and hypoxia.

Methods: Eighteen subjects completed 3 trials (sea level, hypoxia, altitude). Hypoxic stimuli lasted 90 minutes via ascent to 4,200 m (altitude) or simulated via decreased fractional inspired oxygen (hypoxia). Body fluid, cardiovascular variables, and skeletal muscle and brain oxygenation were measured at rest and exercise (3-minute step test).

Results: Muscle $O_2$Hb decreased and muscle HHb increased across environments during exercise ($p<0.001$). Resting brain $O_2$Hb at altitude and hypoxia was lower than sea level ($p<0.012$). Exercising brain $O_2$Hb was lower at altitude than sea level ($p=0.007$), trending similarly when compared to hypoxia ($p=0.066$). Brain HHb increased during exercise across environments ($p<0.001$) with no difference between altitude and hypoxia ($p>0.05$). $SPO_2$ at altitude (rest: 79±1.4%, exercise: 80±0.9%) and hypoxia (rest: 79±1.4%, exercise: 82±1.0%) were not different ($p=0.208$). HR at altitude (rest: 72±3, exercise: 141±3 beats·min$^{-1}$) and hypoxia (rest: 70±2, exercise: 141±4 beats·min$^{-1}$) were not different ($p>0.397$). Stroke volume and cardiac output during exercise at altitude (109.6±4.1 mL, 15.5 ±0.7 L, respectively) were higher than hypoxia (97.8±3.3 mL, 13.8±0.6 L, respectively) ($p<0.002$). Total body water decreased at altitude (43.0±1.8 L) compared to hypoxia (43.7±1.8 L) and sea level (43.8±1.8 L) ($p<0.016$) with plasma volume also decreasing at altitude (-16.6±2.0%) compared to hypoxia (-3.8±2.2%, $p<0.001$).

Conclusions: There appears to be a tissue specific response to environment, wherein the brain but not muscle becomes hypoxic. Caution is warranted when using alterations in fractional oxygen concentrations as a surrogate for altitude.
IMPACT OF LOCAL HEAT APPLICATION ON MITOCHONDRIAL RELATED GENE EXPRESSION AT REST

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BACKGROUND: Mitochondrial health is characterized by the constant cycle of growth (biogenesis) and breakdown (mitophagy) within the mitochondria to allow for optimum function. Previous research has observed a difference in the response of PGC-1α mRNA while exercising in ambient heat compared to local heat. There was a blunting of PGC-1α in ambient heat during exercise whereas there was a non-blunting response of PGC-1α when local heat was applied during exercise. However, the effects of local heat application alone, without exercise (i.e., at rest), on skeletal muscle mitochondrial gene expression are unknown.

PURPOSE: To determine the impact of local heat at rest on skeletal muscle gene expression related to mitochondrial biogenesis and mitophagy.

METHODS: Twelve healthy, active subjects sat in a semi-reclined resting position with a heated thermal wrap (HOT) and a neutral temperature wrap (CON) for 4-hours. Skin temperature, blood flow, intramuscular temperature, and a Bergström biopsy were obtained from each leg following the 4-hour intervention.

RESULTS: Skin temperature was higher during HOT (37.4±0.1°C) than CON (34.9±0.2°C) (p<0.001). Intramuscular temperature was also higher in HOT (36.3±0.4°C) than CON (35.2±0.8°C) (p<0.001). There was no difference in local blood flow between HOT (0.67±0.1 m/sec) and CON (0.63±0.1 m/sec) (p=0.125). NFE2L2 mRNA increased with local heat application (1.49±0.9 fold change, p=0.045). However, there were no differences in the other mitochondrial biogenesis genes or any mitophagy related genes in response to temperature (p>0.05).

CONCLUSION: These data suggests that local heat application has an impact on NFE2L2 mRNA in human skeletal muscle whereas other genes related to mitochondrial homeostasis are unaffected.
Background: Prolonged sitting (PS) and sedentary lifestyle (SL) in the US has significantly increased in recent decades, leading many adults to spend a majority of their workday sedentary. Additionally, elevated carbon dioxide (hypercapnia), commonly observed in crowded environments such as workplaces and/or classrooms, have been known to impair cognitive and respiratory function in humans. However, it is currently unknown how exposure to hypercapnia combined with PS affects the cardiovascular and autonomic function in middle-aged adults.

Purpose: Therefore, the purpose of this study was to examine the effects of prolonged sitting with hypercapnia on cardiovascular and autonomic function in middle-aged adults. Furthermore, we sought to examine the impacts of muscular contraction, specifically, group III/IV muscle afferent activation via passive and active leg movement in adults during prolonged sitting with mild hypercapnia.

Methods: Four healthy sedentary middle-aged adults (48 ± 7 years old) were recruited for this study. Utilizing a randomized control study design participants completed two (2) visits: control (CON) and active (ACT). Each visit included 2.5 hours of PS in a mild-hypercapnic environment to determine how activation of group III (mechanoreceptors) and group IV (metaboreceptors) afferents affect vascular function.

Results: Responses to ACT between 0-min and 250-min showed a 12% increase in blood flow (BF) with a 27.74% increase in shear rate (SR), while CON showed a 0.23% decrease in BF with a 8.9% increase in SR. Flow mediated dilation (FMD) of the popliteal artery increased following ACT (2.45 ± 0.82%) and decreased in CON (-1.45 ± 1.21%) while brachial artery FMD found increases in both conditions (ACT: 4.075 ± 0.92%, CON: 0.76 ± 0.54%). Conclusion: These findings provide a novel insight towards the vascular effects of PS within mild hypercapnia in sedentary middle-aged adults and the mechanisms behind preserving vascular function via muscular contraction during PS with mild hypercapnia.
Background: Pulse wave velocity (PWV) is a gold standard measure to assess arterial stiffness, which is a marker of cardiovascular dysfunction, that is measured by the time delay between wave reflections in the arterial network and the distance between sampling points. Although PWV assessment is well-characterized in the conduit arteries, PWV in the skeletal muscle microcirculation has not been well-studied. Therefore, the purpose of this study was to differentiate the conduit artery and microcirculatory responses to an arterial occlusion by measuring PWV from conduit artery pressure waves and velocity waves derived from near-infrared spectroscopy (NIRS).

Methods: In 13 healthy-young subjects (23±3.8 years), piezoelectric force sensors were placed on the carotid artery and the radial artery, and a NIRS sensor was situated on the medial forearm. After 10 minutes of rest in a supine position, a 5-minute baseline was collected, followed by 5 minutes of arterial occlusion (250 mmHg), and 5 minutes of recovery. Pulse-transit-time (PTT) was derived from the difference in time between the respective wave-feet, and the PTT was normalized by the distance between the sensors. Results: PWV in the microcirculation (PWVm) exhibited a pattern distinct from PWV in the conduit artery network (PWVc). PWVc showed no change within the first 10 second of post-occlusion reactive hyperemia (PORH) followed by a rapid and significant decrease from baseline (-0.78±0.13 m/s, p<0.01), whereas PWVm exhibited a large reduction in the first 10 seconds of PORH, which trended towards significance (-0.51±0.19 m/s, p=0.08), followed by a rapid increase back to baseline.

Conclusions: While previous literature suggests that the NIRS reoxygenation recovery rate taken in the first 30 seconds following PORH may elucidate microcirculatory function, our initial findings indicate that only the first 10 seconds of PORH may be relevant because of conduit artery involvement after 10 seconds.
LOSS OF CARDIAC COP9 SIGNALOSOME SUBUNITS: DIFFERENCE IN PHENOTYPIC EXPRESSION, BUT SHARED UPREGULATION OF PKCδ

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Background: The COP9 signalosome holocomplex (CSN) is formed by 8 unique protein subunits (COPS1~COPS8) and regulates Cullin-RING ligases via Cullin deneddylation. Cardiomyocyte-restricted knockout (cko) of Cops8 causes massive cardiomyocyte necroptosis, dilated cardiomyopathy, and shortened lifespan in mice. CSN’s deneddylase resides in Cops5 and Cops5-cko has yet to be explored. Myocardial protein kinase Cδ (PKCδ) is upregulated in Csn8-cko mice but its role in cardiomyocyte necroptosis remains unknown.

Methods and Results: cko of Cops8, Cops5, or Cops8+Cops5 double cko (double-cko) initiated in adult mice were achieved using a tamoxifen-inducible Cre-LoxP system. Echocardiography performed 21 days after tamoxifen withdrawal showed no significant difference between Myh6-MerCreMer transgenic (MCM) and Cops5-floxed/Cops8-floxed control groups. Compared with either control group, all cko groups displayed dilated cardiomyopathy, but the severity in the Cops5-cko and double-cko groups were comparably greater than that in the Cops8-cko group. Kaplan-Meier survival analyses revealed shortened lifespans for all cko groups, compared to the MCM group. The post-tamoxifen lifespans of Cops5-cko and double-cko mice were comparable (42 days) but significantly shorter than that of Cops8-cko (81 days). We observed a greater number of cardiomyocytes positive for Evans blue dye uptake and greater increases of myocardial CD45 proteins in Cops5-cko and double-cko mice than in Cops8-cko mice. Concurrent with this pathology, both Csn8-cko and Csn5-cko mice exhibit a significant upregulation of PKCδ. Coupling perinatal Cops8-cko with heterozygous germ-line knockout of PKCδ decreased key protein markers for the necroptotic (RIP1-RIP3-MLKL) pathway, attenuated cardiac malfunction (as shown by stroke volume and cardiac output), and delayed premature death induced by Cops8-cko.

Conclusions: (1) Cullin-deneddylation activity is required for the CSN to suppress cardiomyocyte necroptosis; (2) upregulation of PKCδ contributes to activation of the necroptotic pathway by Cops8 deficiency in cardiomyocytes.
Poster Presentation
Category: Graduate student

HIGH GLUCOSE CONDITIONS AFFECT POLYAMINE BIOSYNTHETIC PATHWAY BY INCREASING CELL PROLIFERATION IN TRIPLE NEGATIVE BREAST CANCER

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Breast cancer is the second leading cause of mortality in women worldwide. Studies have shown that the co-existence of diabetes and breast cancer, termed diabetic breast cancer, can be lethal and lead to higher mortality (15-40%) than breast cancer alone. In triple negative breast cancer (TNBC) patients, chance of recurrence is about 3 months, and the chance of mortality can be as high as 75%. TNBC is highly refractive, heterogeneous, and resistant to most chemotherapies. Polyamines, which are ubiquitous in most cells and play a role in cell growth, have been shown to be elevated in cancer, though their role in diabetic breast cancer has not been explored. Here we hypothesized that high glucose conditions increase TNBC cell proliferation through the polyamine biosynthesis pathway. For this purpose, we used the MDA-MB-231 TNBC cell line and treated it with low glucose (5mM) and high glucose (25mM) concentrations. Cell proliferation was monitored with varying glucose concentrations and with polyamine inhibition. Efficacy of polyamine inhibition to cause cytotoxicity was monitored by re-supplementation with the polyamines. Further, polyamine levels were monitored in these cells and polyamine metabolic enzyme expression was monitored at the genetic and protein level using RT-PCR and Western Blots. Results indicated that under high glucose conditions, polyamine enzyme expression increased, and this was correlated with elevated polyamine levels and an increase in cell proliferation. Supplementation of polyamines indicated that the current polyamine inhibitor is only cytostatic, hence there is a need for a more targeted approach to regulate polyamine levels and mitigate cancer growth in diabetes. Future studies will identify potential targets in the polyamine biosynthesis pathway for efficient cancer therapeutics.
IMPROVING TRANSFECTION EFFICACY IN CARDIOMYOCYTES

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Background: Transfection is a method to introduce foreign genetic material into a cell in vitro. It is a major approach to study loss and gain-of-function of a gene in cardiomyocytes. However, transfecting cardiomyocytes is a difficulty technique and there is a lack of technical knowledge on how to improve transfection efficacy in cardiomyocytes.

Aim: To improve transfection efficacy in cardiomyocytes without increasing transfection-induced cell death.

Methods and Results: We transfected two established cardiomyocytes cell lines, HL1 and H9C2, by three widely used transfection methods: lipofectamine-mediated, polyjet-mediated, and via electroporation. We have standardized the dose of each of three transfection agents for the best transfection efficacy. In addition, we have evaluated transfection-induced cell death in cardiomyocytes through lactate dehydrogenase (LDH) assay. By combining different doses of transfecting agents and cell death caused by them, we achieved the best combination where transfection efficiency is highest and transfection-induced cell death is lowest. Moreover, the comparative analyses of the three transfection methods revealed that polyjet has highest transfection efficacy with least cell death. Furthermore, in our ongoing studies, we are determining the best cost-effectiveness approach to obtain the best quality data of transfection in cardiomyocytes by comparing three instrumentations: InfinityM200 plate reader, Incucyte, and Arrayscan.

Conclusion: This is the first extensive study on improving transfection efficacy in cardiomyocytes. Our results will facilitate the researchers in the area of cardiovascular diseases by improving their technical skill on genetic manipulation of cardiomyocytes.
DEVELOPMENT OF A NOVEL FLOW CYTOMETRY-BASED HUMAN NK CELL-MEDIATED ADCC COMBINATION IMMUNOTHERAPY ASSAY

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Background/hypothesis of the study: Our team is currently developing a flow cytometry-based protocol for a human natural killer (NK) cell-mediated antibody-dependent cellular cytotoxicity (ADCC) combination immunotherapy against B-cell lymphoma. ADCC is one method by which NK cells can kill target cells, where antibodies (e.g., α-CD20 in B cell lymphoma) are used to facilitate recognition and killing of target cells by NK cells. We hypothesize a combination immunotherapeutic approach can be developed with high efficacy in improving human NK cells' capacity to mediate ADCC.

Methods and Results: Developing our flow cytometry-based NK cell-mediated ADCC assay has involved: determining the optimal target cell discrimination strategy; testing various incubation conditions such as time and volume; incorporating relevant B-cell lymphoma target cell lines; assessing multiple effector to target ratios; establishing an NK cell pipeline from human procurement to assay incorporation; identifying promising therapeutics for combination assays; and building an NK cell immunophenotyping panel to complement NK cell killing efficacy data.

Conclusions: Research for this project is ongoing. Assay development progress to date will be presented and discussed.
Adenosine receptors (ARs) are G-protein coupled receptors (GPCRs) which have been shown to have therapeutic potential for conditions such as chronic pain, cancer etc. ARs are made up of four subtypes, A1, A2A, A2B, and A3. A3ARs have previously been indicated to lower inflammation, prevent or treat cancer, and produce antihyperalgesic effects in many preclinical pain models, specifically for neuropathy. We have previously published in animal models that ferulic acid dimer, a cinnamic acid derivative, has non-opioid antinociceptive properties through primarily binding to A3ARs. However, research is lacking on the in vitro effects of the binding ability of trans-cinnamic acid dimers to A3ARs. We hypothesized that a specific modification of trans-cinnamic acid would increase binding to A3ARs as compared to their monomeric counterparts. For this study, we performed fluorescent competitive binding assays using cells transfected with ARs: Chinese Hamster Ovary (CHO) cells for A3 and Human Embryonic Kidney (HEK) cells for A2A and A2B receptors. The compounds analyzed included monomers and dimers of 3-methoxy cinnamic acid. The binding assays performed suggested that 3-methoxy cinnamic acid dimer was the most effective in binding to A3ARs, while the monomer did not bind as well. There was very little to no binding observed at A2A and A2B receptors. Further experiments will be performed to investigate receptor activation by monitoring cyclic AMP production with these compounds, and further investigate the biological action in an animal pain model.
**Poster Presentation**  
Category: Undergraduate

**EFFECT OF AGE AND FOOD DEPRIVATION ON ANXIETY-LIKE BEHAVIOR IN MICE**

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**Background/hypothesis of the study:** Stress and anxiety motivate and influence behavior, but the mechanisms by which they do so are not completely understood. Hormones are a key biological factor that may mediate anxiety-like behavior. Androgens, such as testosterone, act through the androgen receptor (AR) to ameliorate anxiety-like behaviors in rodents, as wild-type (wt) males exhibit lower anxiety-like behavior relative to wt female mice and male mice with dysfunctional ARs (tfm male mice). Gonadal hormone levels are affected by both an animal’s age as well as its nutritional state. Therefore, the objective of this study is to determine if food deprivation induces anxiety-like behavior in juvenile and adult wt male, wt female, and tfm male mice. Furthermore, we would like to determine if such anxiety-like behavior is due to changes in gonadal hormone levels.

**Methods and Results:** Mice were isolated for one week before anxiety testing to prevent social interaction influences. The age groups we used were adult (about 11-13 weeks old) and adolescent (about 4-5 weeks old). For each age group we had three treatments: 24hr food deprivation, 6hr food deprivation, and a 0hr control. Blood samples were taken a few days before testing. For the anxiety test, we used an elevated plus maze. We measured the time spent in and number of entrances to the open arms, closed arms, or the middle. Following the anxiety test, animals were perfused and brain tissues collected for c-fos analysis.

**Conclusions:** Due to the slowing of research due to a lab flood and covid shut down, our data set is not large enough to draw conclusions, yet. The current data show the adult female mouse spending less time in the open arms than male mice exposed to the same 24hr food deprivation treatment. Data are still being collected for this ongoing project.
According to the World Health Organization, worldwide obesity has tripled since 1975 and is still on the rise, putting people at a higher risk of some cardiovascular diseases and kidney disease. Growth differentiation factor 15 (GDF15) is a stress-responsive cytokine that has been studied recently for its role in multiple biological processes and diseases. The member of the transforming growth factor beta (TGF-β) family has been investigated as a therapeutic agent and biomarker for obesity and associated cardiovascular diseases, stroke, diabetes, osteoarthritis, cancer, and kidney disease. Studies have shown the anti-inflammation, anti-obesity, and anti-diabetic effects of GDF15, but few have focused on the role of GDF15 in the kidneys. This study examined the in vivo protective effects of GDF15 in the kidneys of both diet-induced obese mice and GDF15 transgenic mice, as well as investigated whether GDF15 prevents norepinephrine (NE)-induced kidney fibrosis and pro-inflammatory cytokine production in cultured kidney cells. The in vitro portion of the study included two kidney cell lines, HK-2 and LLC. Western blot analysis of these cell lines after various treatments of either NE, GDF15, or both revealed some markers of inflammation (NF-κB) and fibrosis (α-SMA, TGF-β) were significantly greater in cell cultures treated with NE. Higher GDF15 concentrations attenuated these responses. Kidney tissue samples were analyzed by Western blot, HE staining and Sirius Red staining. Analysis of wild-type (WT), high-fat diet (HFD), transgenic GDF15 (NAG), and high-fat transgenic (NAG+HFD) tissues illustrated the renoprotective effects of GDF15. NAG+HFD levels of some kidney injury markers (α-SMA, TIM-1, TNF-α) were not significantly different from the NAG tissue, while WT and HFD levels were significantly different. These results show GDF15 is a possible therapeutic target for kidney injury due to its protective effects on the body. Further investigation into GDF15 and its role in the kidneys, as well as other biological processes, is necessary to understand the overall effect of elevated GDF15 levels on the body.
Regulation of blood pressure is essential in bodily health. Hypertension and hypotension can be associated with varying diseases that could potentiate further disease. Understanding the mechanisms that regulate blood pressure is important within medicine. In the study, we focused on the cardiac sympathetic afferent reflex (CSAR). This work examined sex differences in CSAR in overall function and the involvement of the paraventricular nucleus (PVN) of the hypothalamus. We tested the hypothesis that there was a significant difference between male and female rats regarding bradykinin (BK)-induced CSAR response. To approach this study, we used a random selection of genetically similar rats. The rats were instrumented to measure mean blood pressure (MAP) and heart rate (HR) and to allow pericardial injection of BK that is known to activate the CSAR. Pericardial injection of BK was observed to increase MAP and HR by 24 +/- 4 mmHg and 20 +/- 6 bpm in male rats. In females, we observed an increase in MAP and HR of 18 +/- 2 mmHg and 12 +/- 4 bpm. Thus, MAP and HR responses appeared attenuated in female rats. However, these differences did not reach statistical significance. We assessed PVN involvement by immunostaining for cFos, an immediate-early response gene product. We observed cFos staining in the PVN indicative of neural activation in this area in both male and female rats. The degree of immunostaining was an average of 5.2 stained cells counted on each image collected, with a minimum of 3 and a maximum of 7 cells for each image. The total area of staining averaged 4.486 square units per image, with a minimum area of 2.517 and a maximum area of 6.982. The average cell area staining was 0.8616 square units per cell, with a minimum of 0.567 and a maximum of 0.997 per cell. This work has uncovered new aspects regarding the CSAR. Learning how this mechanism relates to blood pressure control can help to understand the blood pressure regulation in humans, the differences between males and females. Ultimately, this may lead to more effective medications relating to blood pressure management customized to gender.
Transient receptor potential (TRP) channels are a family of membrane proteins evolved to interpret environmental stimuli through ion channels. TRPM8 is responsible for the sensation of innocuous cool temperatures, and it might be responsible for noxious cold temperatures as well. When applied topically, menthol acts upon a cold transduction enzyme to decrease the thermal sensitivity of TRPM8. This decreased thermal sensitivity allows activation of TRPM8 at warmer temperatures, simulating a “cool” sensation in warm environments. The “cool” menthol feeling is used widely in everyday commercial products, but most popular when used as an analgesic in medicated pain-relieving cream. 12 male subjects will participate in three visits to the Exercise Physiology Laboratory. The first visit will consist of a maximal aerobic fitness test, body composition, and skin irritation test. These data will be used to describe the population and to calculate the intensity of the subsequent trials (50%Wpeak). The experimental exercise trials will occur on two subsequent, randomized visits (EXP, CON) where the participant will complete a 60 min exercise session within a hot environment (33°C, 40%RH) with either an over-the-counter menthol cream (EXP) or a non-menthol cream (CON) applied topically to the torso. Thermoregulatory variables (core body temperature, skin temperature, and heart rate) will be collected continuously and averaged upon completion. Thermal sensation will be recorded throughout the study by the ASHRAE thermal sensation and comfort scales. Current research suggest that menthol is an ergogenic aid due to its effect on thermal sensation; however, by decreasing the thermal sensitivity of TRPM8 receptors, there is a physiological response to the perceived external “cooling.” The most salient response is an increase in heat conservation, which means menthol should decrease exercise capacity due to fatigue associated with core body temperature. This incongruence requires further investigation into the effect of menthol on exercise performance.
IMPACT OF LOCAL HEAT APPLICATION ON MARKERS OF MYOGENESIS AND PROTEOLYSIS

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Background: Previous exercise research indicates that ambient and local heat may induce independent effects on muscle gene expression during exercise. However, it is currently unclear if local heat stimulus at rest causes changes in markers of myogenesis and proteolysis. Purpose: To determine the impact of local heat application at rest in markers of myogenesis and proteolysis in human skeletal muscle.

Methods: Twelve subjects had thermal wraps placed on the experimental limb, and a neutral temperature wrap on a control limb for a total of 4 hours. After the 4-hour intervention a Bergström biopsy was performed to collect muscle samples. Muscle samples were homogenized, and protein concentration was found using a Protein Assay Kit. Proteins were then separated via electrophoresis. Membranes were blotted for total and phosphorylated proteins for multiplex detection and phosphorylation state. Real time quantitative PCR was ran on a myriad of myogenic and proteolytic related genes to find changes in post heat gene expression.

Results: There was no effect of temperature on protein; (mTOR, p=0.449; P70, p=0.526; 4E-BP1, p=0.548). Furthermore, mTOR\textsuperscript{ser2448} phosphorylation was not different (p=0.981), p70\textsuperscript{Thr389} phosphorylation was not different (p=0.583), and 4E-BP1\textsuperscript{Thr37/46} phosphorylation was not different (p=0.238). There was no effect of temperature on proteolytic genes (FBOX32, p=0.573; FOXO3a, p=0.452; TRIM63, p=0.284; RPL-3L, p=0.577), nor was there an effect of temperature on myogenic genes (MYF5, p=0.445; MYF6, p=0.895; MEF2a, p=0.810; RPS3, p=0.321; MSTN p=0.321; MYO-D, p=0.118; MYO-G, p=0.766). Conclusion: Localized heat application at rest has no influence on markers of myogenesis and proteolysis.
CHARACTERIZATION OF VIRUS INFECTION OF PSEUDOMONAS FLUORESCENS USING RNA SEQUENCING

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Background/Hypothesis: The *Pseudomonas* genus is a large and diverse group of bacteria that occupy nearly every environmental niche. Some examples of the genus, such as *Pseudomonas aeruginosa*, are commonly found in hospitals and cause serious human diseases. Other examples, such as *Pseudomonas fluorescens*, cause mild to no symptoms in humans. Despite their relatively widespread nature, all members of the *Pseudomonas* genus are susceptible to viruses known as bacteriophages. Surprisingly, despite extensive research, both the bacteria and their bacteriophages still have many proteins and genes that are termed “hypothetical”, and as a result, many of their functions are also unknown. Using novel Podoviruses that have been isolated in our laboratory and the *Pseudomonas fluorescens* host, we seek to clarify the mechanisms and functions of these “hypothetical” genes and gene products by studying gene expression changes during viral infection. These gene expression changes, determined using RNA sequencing, will elucidate the phage genes previously unrecognized during infection of *P. fluorescens* over time and as they induce a stress response.

Methods/Results: These genes are initially found through next generation sequencing and functionally annotated for characterization of phylogenetic homology. Validation of this work is performed using co-occurrence network analysis to identify frameshift mutations or gene hubs of various *Pseudomonas* bacteriophages that correlate with virus pathogenicity. By mapping the course of gene expression of *P. fluorescens* and an infecting bacteriophage, we learn more about the genes and gene products of both the host and the virus. Initial analyses have already identified proteins involved in this infection mechanism.

Conclusion: Overall, using such bioinformatics temporal gene expression analyses, we validate a genetic surveillance workflow. This methodology elucidates virulence factors of common nosocomial *Pseudomonas* bacteriophages, and how they dysregulate the growth of *Pseudomonas* bacteria. This will provide greater insight into bacteria in the Pseudomonas genus, including those that cause human disease.
**Poster Presentation**  
Category: Undergraduate

**METHOD FOR VSV-G AND SPIKE VIRAL PSEUDOTYPING**

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**Introduction**  
We are using a virus pseudotyping system to screen drugs that interfere with SARS-CoV-2 entry. To date, we have been focused on establishing the system in our laboratory.

**Methods**  
We use a triple transfection approach in HEK-293T cells for pseudotyped virion production. Plasmid 1: The backbone virus for virion production is a Moloney Murine Leukemia Virus (MoMLV). Plasmid 2: This is a psi-element containing plasmid that encodes an EGFP reporter gene. Plasmid 3: This is a plasmid encoding the SARS-CoV-2 spike protein (variants will be introduced). Control Plasmid 3 encodes the envelope for Vesicular Stomatitis Virus G (VSVG). Viral particles are transduced onto VERO E6 cells which express the receptor for spike-mediated entry – ACE2. Transduction efficiency (proxy for viral entry) is quantitated via flow cytometry. Transfection was also monitored via fluorescence microscopy.

**Results and Discussion**  
Initial results yielded a transduction efficiency of 3.4% with VSVG and lower with spike. This is not as high as we believe is possible based on reports by others and the result reveals that our pseudotyping method can be enhanced. Next steps include performing transductions in the presence of transduction enhancing agents (e.g polybrene). Other next steps include modulating plasmid rations during transfection to enhance virion production with maximal viral envelope incorporation. Once this system is functioning as expected, we will work with our collaborators at UNMC to screen drugs with potential for interfering with SARS-CoV-2 entry into target cells.
PROMISING ANTI-SCHISTOSOMA ANALOG SA01 SUGGESTS PRO-INFLAMMATORY IMMUNE SYSTEM RESPONSE

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Schistosomiasis, a commonly neglected tropical disease, is a waterborne parasitic worm infection able to infect through direct skin penetration. This disease affects approximately 270 million people worldwide and ranks only second to malaria as a leading infectious disease. Although some possible alternatives are emerging, currently, the most effective drug treatment is praziquantel (PZQ). However, PZQ is only effective against the adult stage of the worm, allowing juvenile worms to progress in the infection. Additionally, Schistosoma worms are developing resistance to this drug as reduced efficacy has been noted. Thus, the need for drug discovery and testing is increased. SA01, a worm clearing derivative of aryl hydantoin Ro 13-3978 is being investigated to treat Schistosomiasis. Previous data points to the compound acting on the host’s immune system as opposed to directly on the worms. Single cell transcriptomics was conducted, and a notable change was significant increase in neutrophil population. Furthermore, there were significant changes in the erythroidal lineage indicative of splenic erythropoiesis, which is consistent with an inflammation type response. To explore these immunological phenotypes, transcriptomics is conducted to analyze expression patterns for genes associated with neutrophil granulopoiesis as well as splenic erythropoiesis to elucidate SA01’s mechanism of action.
AGING AND THE DEVELOPMENT OF ALCOHOL-ASSOCIATED LIVER DISEASE

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Background/Hypothesis: Alcohol-associated fatty liver disease (AFLD) is a prominent health issue globally. Gender, lifestyle, genetics, and viral infection exposure all contribute to the development and advancement of AFLD. Though aging is known to influence hepatic structure and function, a comprehensive understanding of the role of aging in AFLD development is notably lacking. This study aimed to determine how age affects the variety of alcohol-induced liver injury.

Methods and Results: Younger (8-week-old) and middle-aged (6-month-old) rats were pair-fed Lieber-DeCarli control or ethanol diet for 6 weeks. After the feeding period, animals were sacrificed with collection of serum and tissues (liver, gut and adipose) for biochemical analysis. The average food intake was similar in both younger and older rats, though only younger rats showed significant increase in body weight during the 6-week feeding. In ethanol-fed animals, younger rats experienced a greater increase in liver/body weight ratio and greater decrease in epididymal white adipose tissue mass compared to older rats. Serum cholesterol, bile acids, and non-esterified free fatty acids were heightened to a similar extent in both ethanol-fed groups. However, the elevation in serum alanine aminotransferase and endotoxin levels was moderately higher in older ethanol-fed rats compared to younger ethanol-fed rats. Similarly, while quantitative analysis revealed similar ethanol-associated increases in liver triglycerides for both groups, histopathological evaluation revealed increased ballooned hepatocytes and inflammatory markers in older ethanol-fed rats, indicating heightened progressive liver injury. Furthermore, both older and younger ethanol-fed rats experienced decreased expression of ileal tight junction proteins Claudin-1 and Claudin-7, though the change was most profound in the older rats. This increased intestinal permeability in ethanol-fed animals is indicative of increased liver damage, supporting previously mentioned histopathological findings.

Conclusion: Age, possibly in relation to increased intestinal permeability, plays a significant role in the development of advanced liver disease with alcohol consumption.
Introduction: The autoimmune disease systemic lupus erythematosus predominantly affects women and can lead to lupus nephritis (LN) as a common and severe complication in approximately 50% of patients. The F1 progeny (NZBWF1) of the New Zealand Black (NZB) X New Zealand White (NZW) mice is a well-established mouse model to study LN. Our previous data indicated that the onset of albuminuria in female 34-week NZBWF1 mice was associated with renal iron accumulation and iron may contribute to renal injury. This study tests the hypothesis that NZBWF1 mice are sensitized to the injurious and pro-inflammatory effects of iron compared to healthy NZW control mice.

Methods and Results: Female 8-week (pre-autoimmune) and 34-week NZBWF1 (autoimmune) mice together with age-matched healthy control NZW mice underwent 24h urine collection to confirm non-albuminuric status, then were injected i.v. with Iron-Sucrose (2mg elemental iron) or vehicle (saline) and sacrificed 24h later. Plasma blood urea nitrogen (BUN) was measured to determine renal health, and plasma monocyte chemoattractant protein-1 (MCP-1) was measured by ELISA as an inflammatory marker. Groups were compared within strains by 2-way ANOVA (n=7-12/group). BUN was significantly increased with age in both strains (P_Age<0.05), but there was no significant effect of iron-sucrose (P_Treatment>0.05). Iron-sucrose treatment significantly increased plasma MCP-1 concentrations in both strains (P_Treatment < 0.01). There was no significant effect of age on this effect for NZW mice (P_Treatment*Age = 0.2), however in NZBWF1 mice the iron-sucrose induced increase in MCP-1 was significantly greater in 34-week compared to 8-week mice (P_Treatment*Age < 0.05; P<0.05 by post-hoc test).

Conclusion: Our data suggest that 34-week-old NZBWF1 mice (which have lupus) show increased pro-inflammatory responsiveness to iron-mediated stress. Further studies to determine whether the enhanced pro-inflammatory effects of iron contribute to pathology in lupus nephritis are ongoing.
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