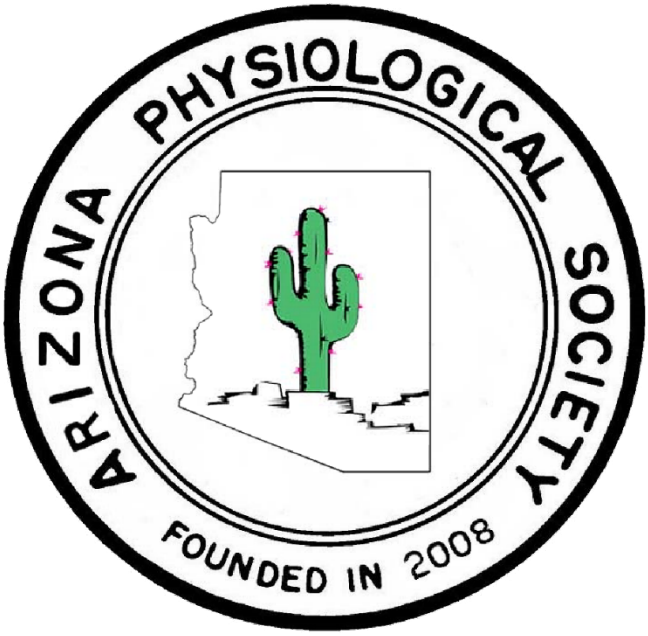
The Arizona Physiological Society



11th Annual Meeting

October 5-6, 2018

Arizona State University

Ventana Ballroom, Memorial Union, Tempe Campus

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We are extremely grateful for the institutional support we have received this year and in the past. Thank you so much for believing in our society's mission and providing the help we need to keep it alive.

Arizona State University – Phoenix

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Directions to the ASU Tempe Campus
(to the Apache Rd parking structure and The Graduate Hotel)

[Directions via Google]

From Phoenix Sky Harbor Airport

Take Loop 202 East to Scottsdale/Rural Rd. (Exit 7).
Turn right onto Rural Rd.
Drive south to Apache Blvd.

From West Valley

Take I-10 East toward Tucson.
Exit onto Loop 202 toward Tempe.
Exit Scottsdale Rd. and turn right.
Turn right onto Apache Blvd.

From Northeast Valley

Take Scottsdale Rd. South toward Tempe.
Turn right onto Apache Blvd.

From Southeast Valley

Take the 60 West into Tempe.
Exit at Mill Ave. and turn right.
Turn right on Apache Blvd.

From Tucson

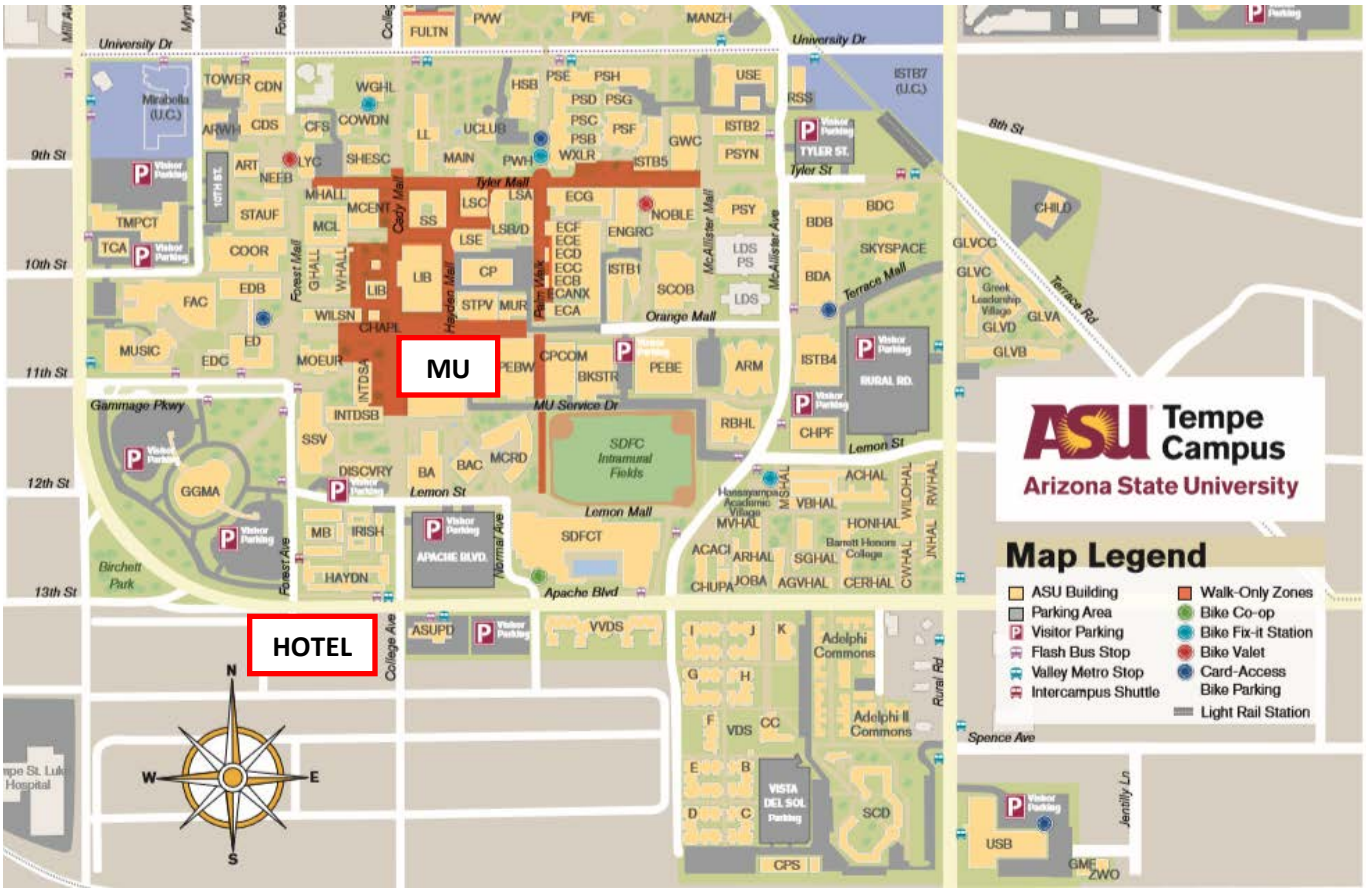
Take I-10 West to Tempe
Exit onto US 60 East
Exit Mill Ave. and head North
Turn right on Apache Blvd.

From Flagstaff

Take I-17 south to Phoenix
Exit I-10 East
Exit 202 Loop East
Exit Scottsdale Rd./Rural Rd and turn right
Turn right onto Apache Blvd

ASU Tempe Campus Map

P Visitor parking



Hotel = The Graduate

PROGRAM SCHEDULE

Note: *All sessions will take place in the Ventana Ballroom located on the second floor of the ASU Tempe campus Memorial Union.

FRIDAY, October 5

10:00 – 10:30 am	**Registration/Set up Posters**
10:30 – 10:45 am	**Welcome**
10:45 – 11:45 am	**Session 1: Physiological Responses to Stress**
	Chairs: Taben Hale (UA-Phoenix) and Jordan Glass (ASU)
10:45-11:00am	Trevor Fox, Graduate Student, ASU - School of Life Sciences *Aedes aegypti eggs likely require protected microclimates to survive desert southwest winters*
11:00-11:15am	Haley Owen, Graduate Student, MWU *Determining the prevalence of Rickettsia rickettsii in geographically distinct populations of Rhipicephalus sanguineus in Arizona*
11:15-11:30am	George Brusch, Graduate Student, ASU - School of Life Sciences *A mechanistic approach to understanding the relationship between dehydration and enhanced immune function*
11:30-11:45am	Stephanie Olzinski, Graduate Student, ASU - College of Health Solutions *Sun radiation in moderate environmental conditions does not affect fluid balance in female collegiate soccer players*
12:00 – 1:00 pm	**Lunch with the Vendors and Raffle Entries**
1:00 – 1:45 pm	**One-Minute Poster Session and Raffle**
1:45 – 3:00 pm	**Session 2: Neuro and Cerebrovascular Physiology**
	Chairs: Ann Revill (MWU) and Claire DeLucia (UA-Tucson)
1:45-2:00pm	Tyler Quigley, Graduate Student, ASU - School of Life Sciences *Focusing on the honeybee blood-brain barrier*
2:00-2:15pm	Wesley Tierney, Graduate Student, California State University Northridge *The long-term effects of human neural progenitor cells on a rat model of ataxia*
2:15-2:30pm	Yu-Jing Li, Postdoctoral Fellow, University of Arizona – Phoenix *Novel selective S1PR1 ligand attenuates hypoxia plus glucose deprivation-induced inflammatory mediator levels in human brain vascular smooth muscle cells*
2:30-2:45pm	Benjamin Rivera, Graduate Student, University of Arizona – Tucson *Impact of developmental nicotine exposure on cholinergic airway signaling*
2:45-3:00pm	Julia Lorence, Undergraduate Student, ASU - West Campus *Impact of sex differences and tumor location on survival outcomes in glioblastoma patients*
3:00 - 3:30 pm	**Break**
3:30 - 4:30 pm	**Keynote Speaker: Dr. Michael Joyner, Mayo Clinic, Rochester, MN** *Physiology: An antidote for excessive reductionism*
4:30 - 5:00 pm	**Break**

5:00 - 5:45 pm **Session 3: Comparative Physiology**
Chairs: Tinna Traustadottir (NAU) and Jill Azzolini (ASU)

5:00-5:15pm Anthony Basile, Graduate Student, ASU - School of Life Sciences
Mourning doves, Zenaida macroura, are resistant to metabolic and vascular effects of a mammalian diabetogenic refined carbohydrate diet

5:15-5:30pm Jon Vimmerstedt, Graduate Student, Midwestern University
Which precise mechanisms set thermal limits in animals? Testing the OCLTT hypothesis in Japanese quail embryos

5:30-5:45pm Christopher Olson, Assistant Professor, MWU
Black jacobins reveal a unique hummingbird solution to communicating in a noisy tropical forest

6:00 - 7:00 pm **Dinner**

7:00 - 9:00 pm **Poster Session, HS Teacher round table**

SATURDAY, October 6

8:00 - 8:30 am **Continental Breakfast**

8:30 - 9:40 am **Session 4: Undergraduate Research Symposium**
Chairs: Scott Boitano (UA-Tucson) and Meli'sa Crawford (ASU – SOLS)

8:30-8:40am Elizabeth Hanson, ASU – Psychology and Elliot Smith, ASU – Biophysics and Psychology
How hippocampal CA3 dendritic complexity is quantified using the intermittent restraint stress paradigm

8:40-8:50am Andrew Alamban, University of Arizona – Tucson
Truncated isoform of Cx37 is not sufficient to suppress proliferation of rat insulinoma cells

8:50-9:00am Christi Williams, NAU
The role of nitric oxide and CaMK gene expression on the heart

9:00-9:10am Sanna Rahman, University of Arizona – Phoenix
Hypoxia plus glucose deprivation increases NF-κB activation and downstream pro-inflammatory enzyme levels in human brain VSM cells

9:10-9:20am John Son, ASU/Mayo Clinic – Center for Metabolic and Vascular Biology
Expression of insulin-like growth factor-1 (IGF-1) mRNA isoforms in muscle of humans with obesity

9:20-9:30am Kaylin Sweeney, ASU - West Campus
Autophagy markers in human skeletal muscle following acute aerobic and resistance exercise

9:30-9:40am Sarah Livingston, ASU
Pomegranate-derived nutraceuticals activate the vitamin D signaling pathway

9:45 - 10:45 am **Arizona Distinguished Lecture, Dr. Janis Burt, University of Arizona, Tucson**
An integrative approach to defining the role of connexins in vascular development and remodeling (*from proteins to systems)*

11:00 - 12:15pm **Session 5: Cardiovascular, Respiratory, and Exercise Physiology**
Chairs: Michael Zawada (AT Still U) and Alexandra Garvin (UA-Phoenix)

11:00-11:15am Tia Alexander, Graduate Student, Midwestern University
Evaluation of the effects of combination of mild aerobic exercise and angiotensin-II type-I receptor blocker, losartan, on aortic function and structure in a mouse model of Marfan syndrome

11:15-11:30am	Candy Rivas, Graduate Student, University of Arizona – Tucson *Development of a protease activated receptor-2 (PAR2) antagonist for the treatment of asthma*
11:30-11:45am	Katon Kras, PhD, Designated Campus Colleague, University of Arizona – Phoenix *Assessment of skeletal muscle mitochondrial metabolic flux and control using polarographic and luciferase-based techniques*
11:45-12:00pm	Corey Mazo, Graduate Student, ASU – College of Health Solutions *mTOR signaling in human skeletal muscle following acute aerobic and resistance exercise*
12:00-12:15pm	Ethan Ostrom, Graduate Student, Northern Arizona University *Aerobic exercise in older adults maintains Nrf2 signaling compared to inactive controls*
12:15 - 2:00 pm	**LUNCH, BUSINESS MEETING AND AWARD CEREMONY**

2018 Arizona Distinguished Lecture

Dr. Janis M. Burt
University of Arizona, Department of Physiology

Research in Dr. Burt's laboratory over the last 30+ years has centered on the mechanisms underlying connexin-and gap junction-mediated coordinated tissue function and controlled growth, with particular emphasis on vascular function in injury and disease settings.

Originally, the contribution of gap junctions to controlled growth was assumed to reflect their abilities to mediate intercellular exchange of growth-related molecules including metabolites, nucleotides and other signaling molecules; however, in recent years it has become clear that connexins contribute to controlled growth through mechanisms independent of formation of functional gap junction channels.

2018 Keynote Lecture

Dr. Michael Joyner

Mayo Clinic, Rochester, MN

The laboratory of Michael J. Joyner, M.D., is interested in how humans respond to various forms of physical and mental stress during activities such as exercise, hypoxia, standing up and blood loss.

Dr. Joyner and his team study how the nervous system regulates blood pressure, heart rate and metabolism in response to these forms of stress. They are also interested in how blood flow to muscle and skin responds to these stressors. These responses are studied in young healthy subjects, healthy older subjects and people with conditions such as heart failure.

Finally, Dr. Joyner is personally interested in the role of integrative approaches in science as a powerful tool to integrate and critique data from reductionist approaches.

ORAL PRESENTATION ABSTRACTS

S1. Aedes aegypti eggs likely require protected microclimates to survive desert southwest winters

***Trevor Fox**, A Gil, JF Harrison

School of Life Sciences, Arizona State University, Tempe

The World Health Organization declared a state of emergency in 2016 regarding the rapid, global spread of the Zika, Dengue, and Chikungunya viruses, which are spread by the mosquito Aedes aegypti (A.a.). Populations of A. aegypti are increasing exponentially in Phoenix and Tucson, posing an imminent threat to human health. Populations of A. a. in Maricopa County are extremely low during in the winter months, suggesting that survival of eggs through winter is a key bottleneck. We tested whether A.a. eggs can survive outdoors from February to May, 2017, in Tempe, AZ. Egg survival dropped rapidly and was less than 3% by early May ($\chi 26, 219 = 110, p < 0.0001$). As eggs must survive until the monsoon which begins in mid-June, it is likely that outdoor overwinter survival of A.a. eggs was locally trivial. In contrast, over 80% of eggs survived this four-month period if they were maintained identically except at 80% relative humidity, suggesting that desiccation is the primary mode of death. Additionally, we tracked the abundance of A.a. larvae in Tempe throughout 2017-18 in 16 buckets, refilled approximately every two weeks. These confirmed the inability A.a. to reproduce and develop through the coldest winter months (Jan-March), and additionally, showed that A.a. avoid sunny locations in summer months. These data suggest that A.a. are unlikely to survive Arizona winters outside, and that educational and management efforts focused on preventing indoor reproduction or survival in protected microclimates such as irrigation or storm drains could be very successful in reducing A.a. populations, Zika-caused birth deformities, and dengue and Chikungunya-caused deaths. *Funding: This research was partially supported by NSF 1558052.*

S2. Determining the prevalence of Rickettsia rickettsii in geographically distinct populations of Rhipicephalus sanguineus in Arizona

***Haley E Owen**, SR Lisowski, JW Allen, TT Yao, CP Schaefer, JA Hernandez, MC Quinlan, JK Lee, RE Kreisler, NG Goetz, JM VandenBrooks

Midwestern University, Glendale, AZ

Rocky Mountain Spotted Fever (RMSF) is a rapidly progressing disease with a mortality rate of up to 20% in humans. Tick-borne vectors transmit the pathogenic bacteria Rickettsia rickettsii to both canines and humans. Human populations in close proximity to canines with high prevalence of ticks and high infection rates of R. rickettsii are at an elevated risk for contracting RMSF. Traditionally, the major tick vectors for R. rickettsii in the United States were identified as Dermacentor variabilis and Dermacentor andersoni. However, during a recent outbreak of RMSF in Arizona, a previously unknown vector, Rhipicephalus sanguineus (the brown dog tick), was identified. Yet, while the brown dog tick is present in every state in the United States, currently it is only known to act as a vector in Arizona and the surrounding regions. Therefore, we investigated why R. sanguineus can act as a vector in Arizona, but not elsewhere. Hypotheses that may explain this phenomenon include: 1) variation in R. sanguineus, 2) variation in R. rickettsii, 3) changing climate, habitat, and dog-keeping practices, or 4) more likely a combination of the aforementioned factors. We have collected brown dog ticks from 13 locations across Arizona, New Mexico, and Mexico. Previously, we have shown that there are two distinct lineages of R. sanguineus that coexist in Arizona (tropical and temperate) by phylogenetic analysis of three mitochondrial genes. Additionally, there are significant differences in morphology and geographic distributions between these two lineages. Here, we have measured the variation in rickettsial presence across the tick lineages and geographic locations. We isolated rickettsial DNA from the tick samples, amplified the OmpA gene with polymerase chain reaction, and verified R. rickettsii presence by gene sequencing. Based on our analysis of rickettsial prevalence, 34% of all sampled R. sanguineus ticks within Arizona carry R. rickettsii. There was also a significant difference in rickettsial prevalence between temperate (22%) and tropical (40%) populations of R. sanguineus ($p=0.14$). We hypothesize that this variation combined with the variation in geographic distribution of these tick lineages across Arizona may be contributing to the difference in incidence of RMSF cases in the state. This data is currently being used to model the feasibility of a canine vaccine to help prevent the future spread of RMSF both within and outside of Arizona. *Funding: MWU One Health Award 2017 to JVB; MWU Student One Health Award 2017 to JA; MWU Student One Health Award 2018 to SL; MWU MBS Funding to HO and TY*

S3. A mechanistic approach to understanding the relationship between dehydration and enhanced immune function

***George Brusch IV**[a], T Webster[a], M Wilson Sayres[a], J Blattman[a], A Baldwin[b], DF DeNardo[a]

[a]School of Life Sciences, Arizona State University, Tempe, AZ; [b]Mesa Community College, Mesa

The performance of the immune system varies with the physiological state of the organism, with immune function often suppressed during periods of negative resource balance due to scarcity or heavy investment (e.g., reproductive activity). In contrast, recent studies have shown that immune function is enhanced when water, a fundamental resource, is limited naturally or experimentally. The purpose of this study was to understand the mechanisms responsible for the peculiar finding that dehydration, while deleterious to most major physiological systems, somehow improves innate immunity in multiple species of reptile. First, we used a combination of temperature and proteinase treatments on plasma samples to explore the potential involvement of small innate peptides. Next, we looked at RNA expression in liver samples between hydrated and experimentally dehydrated animals to identify immune-related proteins that are upregulated with dehydration. Finally, we will perform western blot analyses on stored plasma aliquots to verify upregulated proteins identified by RNA-seq analysis are elevated in the blood. We found that various components of the complement and non-complement pathways are upregulated in dehydrated animals. These results provide a molecular and cellular basis for immune modulation and provide a mechanistic understanding of biochemical and proteonomic changes involved in resource-based upregulation of innate immunity. Understanding how animals cope with resource restrictions will enable us to predict how they might be impacted by future climate change, where, in many regions, rainfall events are predicted to be less reliable, resulting in more frequent drought. *Funding: School of Life Sciences RTI grant and NSF GRFP to GABIV*

S4. Sun radiation in moderate environmental conditions does not affect fluid balance in female collegiate soccer players

***Stephanie Olzinski**, FC Wardenaar, J Beaumont

College of Health Solutions, Arizona State University, Phoenix

The purpose of this study was to determine the effects of sun radiation (SR) on fluid balance (FB) and hydration status (HS), as radiation with ambient temperature may influence athletes' sweat loss and performance in different environments. Initial FB and HS were assessed in female athletes (n=10) of a single soccer team at the NCAA DI level in moderate, dry conditions (55-68°F, 18-48% humidity) who were monitored during 3 training days of equal intensity of exercise: 2 outdoor practices in direct sunlight (cold and moderate temperature) and 1 day indoors without SR (moderate temperature). Humidity, temperature, and wet bulb globe temperature (a measurement partly based on SR) were recorded using two Kestrel 5400 heat stress meters in the direct sun and in the shade. Each athlete's semi-nude dry body weight was recorded (896 Flat Scale, Seca) before and after exercise. Urine samples were obtained before, after, and the morning after exercise, and urine specific gravity (USG) was tested (PEN-S.G. Atago) to assess HS. Athletes wore heart rate and activity monitors to estimate energy expenditure (EE) (BioModule, Zephyr) and were provided with water and/or a 0-calorie sports drink. Weights of consumed food and drink (PT 1400, Sartorius AG) constituted their total intake. Fluid and sweat rates were calculated as intakes of liquid and outputs minus urine losses/hr based on urine samples collected. Two-way repeated measures ANOVA analyzed differences on a group level; Mann-Whitney test and one-way ANOVA estimated within-subject coefficient of variation (CVw) of differences between hydrated and dehydrated groups. No significance was found in FB (1.01±0.32 L/hr) or EE (444±97.1 kcal/hr) across all days ($p > .05$). For individual result analysis, 4 out of 10 athletes had consistent USG >1.025 ($p < .001$) suggesting potential dehydration. This group was steadily dehydrated on all days indicated by a small CVw of 5.7%; the higher CVw (6.9%) in the n=6 group (USG <1.025) indicates that on average the athletes are not dehydrated but must be aware of incidental dehydration. The 4 dehydrated athletes all selected water as their preferred beverage during exercise, of which is known that consumption of water on its own does not stimulate drinking behavior as would an electrolyte drink. The conclusion is that in moderate temperatures, athletes self-regulate their drinking habits and achieve FB during exercise both with and without SR; however, athletes with average USG >1.025 are more likely to stay dehydrated even in moderate temperatures. The findings suggest that for this group additional education and counseling would be beneficial to ensure proper HS throughout all types of environments. *Funding: Athletics Research Grant from the Graduate and Professional Student Association (GPSA) of Arizona State University*

S5. Focusing on the honeybee blood-brain barrier

***Tyler Quigley[a],** G Amdam[a,b]

[a]School of Life Sciences, Arizona State University, Tempe; [b]Norwegian School of Life Science, Aas, Norway

A honeybee's behavior is influenced by her physiological state and by stressors in her environment. However, we do not know the mechanism by which endocrine signals and foreign chemicals enter the honeybee brain. Elucidating this pathway requires both a structural and functional exploration of the honeybee blood-brain barrier (BBB). We have elucidated the structure of the honeybee BBB using electron microscopy and electron tomography. We've located and described two glial subtypes that compose the barrier, and have found them to be similar in composition to that of other insects. In addition, we examined how the fungicide Pristine, the parasite Varroa, and aging influence the permeability of the honeybee BBB. We used a dye permeability assay to determine that while the fungicide does not seem to have an effect on the honeybee BBB, high levels of Varroa and natural senescence are associated with an increase in honeybee BBB permeability. From this work, we have concluded that differences in the honeybee blood-brain barrier may help explain certain behavioral and cognitive changes in honeybees exposed to various types of stress. *Funding: Research Council of Norway, Awards #213976 and #262137*

S6. The Long-Term Effects of Human Neural Progenitor Cells on a Rat Model of Ataxia

***Wesley Tierney**[a], B Ortega[a], A Lemus[a], T Uhlendorf[a], J Ochoa[b], W Van Trigt[b], A Kopyov[b], O Kopyov[b], R Cohen[a]

[a]California State University, Northridge; [b]Celavie Biosciences, Oxnard, CA

The Spastic Han Wistar (sHW) rat serves as our model for Ataxia showing similar symptoms of hind-limb rigidity, motor deterioration, decreased weight and a shortened lifespan. In addition, the sHW rat shows progressive loss of Purkinje cells starting at 30 days. Our lab has previously reported that direct cerebellum injection of human Neural Progenitor Cells (NPCs) were effective in alleviating the symptoms of treated sHW mutants. After 20 days post-injection mutant rats injected with NPCs showed improvements in motor activity, weight gain and lifespan. This new study examined whether human NPCs (under chronic infusion of the immunosuppressant cyclosporine for 60 days post-transplantation) would not only survive but begin to differentiate into cerebellar neurons. For this experiment, rats were placed into four treatment groups: A normal sibling control group that received no treatment, a mutant sHW rat control group that received no treatment, a mutant control group that received a injection of dead NPCs, and an experimental mutant group that received live NPCs. Direct bilateral cerebellar injections (AP -11.0 mm; ML ±2.0 mm; DV 5.5 mm) of 500,000 of either live or dead cells were performed at 40 days of age on rats already implanted with Alzet pumps that chronically perfused cyclosporine for 25 days. The pumps were replaced twice allowing for longer term NPC survival inside sHW mutants. Starting at day 40 (prior to transplantation), we tested the mutant motor activity via open field testing and rotarod twice per week to examine the effectiveness of transplanted NPCs. The results showed that all mutant rats regardless of treatment started to decline in open field testing around day 40. However, around day 50 (ten days post-transplantation), the live NPC-treated mutants began to exhibit increased motor activity while dead NPC and untreated mutants continued to display decreased motor abilities. Starting around Day 60 (20 days post-transplantation), live NPC-treated mutants moved at the same rate as normal rats, and this trend continued until the end of the experiment (100 days; 60 days post-transplantation). Rotarod data also showed similar motor decreases with the dead NPC and untreated mutants while the live NPC group were statistically similar to normal siblings throughout the experiment. At 100 days of age, live NPC rats were perfused with 4% paraformaldehyde, their brains removed and sliced for histological staining. Through immunostaining, we observed migration of these NPCs towards the damaged Purkinje cell layer in mutant cerebellums. Selective immunostaining will be utilized to determine the differentiation fate of these cells. *Funding: California State University Northridge*

S7. Novel Selective S1PR1 Ligand Attenuates Hypoxia plus Glucose Deprivation-Induced Inflammatory Mediator Levels in Human Brain Vascular Smooth Muscle Cells

***Yu-Jing Li**[a], S Rahman[b], FD Shi[c], RJ Gonzales[a]

[a]Department of Basic Medical Sciences, College of Medicine, University of Arizona, Phoenix; [b]School of Life Sciences, Arizona State University, Tempe; [c] Barrow Neurological Institute, Phoenix

Sphingosine-1 phosphate receptor 1 (S1PR1) ligands are proven effective in phase III clinical trial studies to reduce the peripheral and central immune response of multiple sclerosis patients. Similarly, current clinical trial studies are now underway investigating the beneficial impact of S1PR1 immune modulation during acute ischemic stroke. In a murine experimental stroke model, we previously observed that selective S1PR1 modulation reduced lesion volume.

However, the role of S1PR1 on function and health at the level of the cerebrovasculature during ischemic injury has not been investigated. Therefore, the purpose of this study was to examine the impact of ozanimod, a selective S1PR1 ligand, on S1PR1 receptor expression and cerebrovascular pro-inflammatory mediator levels following ischemic-like conditions in vitro. Specifically, we examined whether ozanimod would attenuate hypoxia plus glucose deprivation (HGD)- induced COX-2 and iNOS levels in primary male human brain vascular smooth muscle cells (HBVSMC). We further examined the impact of HGD on HBVSMC density and morphology. Cells were studied at passage 7 and exposed to normoxia (room air) or HGD (1% O2) for 3, 6, 9 h. Protein levels were assessed via western blot and light microscopy was used to visualize cell density and morphology. Similar to our previous findings in murine whole brain lysate, S1PR1 was basally expressed in HBVSMCs and levels were significantly increased following HGD. HGD exposure (6 and 9 h) decreased HBVSMC density, however, ozanimod had no impact on this response. HGD exposure also resulted in changes in cell morphology (shape and size) and ozanimod attenuated these in vitro ischemic-like modifications. Ozanimod also reduced the amount of vacuoles in cultured cells following HGD suggesting cell health preservation. HGD increased protein levels of the proinflammatory mediators, iNOS and COX-2. Increases in HGD-induced COX-2 levels were reduced with ozanimod treatment. In conclusion, these data suggest that ischemic-like conditions contribute to vascular inflammation and selective S1PR1 modulators may serve as important mediators of ischemic damage by altering the structure, function, and health of the cerebrovasculature, specifically at the level of the vascular smooth muscle. *Funding: University of Arizona Sarver Heart Center and University of Arizona Valley Research Project Grant VRP37 P2 (RG, FS).*

S8. Impact of Developmental Nicotine Exposure on Cholinergic Airway Signaling

***Benjamin D Rivera**, NG Borrero, S Cross, RF Fregosi, S Boitano
Asthma and Airway Disease Research Center, University of Arizona, Tucson

In 2014, 8.4% of US women disclosed smoking during pregnancy. Conservatively, this translates to approximately 360,000 smoke-exposed infants born in 2015 in the U.S. Nicotine exposure of the fetus causes a wide variety of aberrant cholinergic signaling through activation of Nicotinic Acetylcholine Receptors (nAChRs) in the developing lung and nervous system. Altered expression of nAChRs can persist and result in lasting changes in lung function. The impact on airway nAChRs, which can influence ion transport to control the airway surface liquid layer and mucociliary clearance, remains poorly defined. Here we begin to discern the impact of an acute nicotine challenge on airway epithelial signaling by evaluating the tracheae of developmental nicotine exposed (DNE) and control rat neonates. Tracheae were excised from neonate rats between post-natal days 7 - 11 and were mounted in Ussing Chambers to measure transtracheal resistance (TTR) and short circuit current (Isc) in response to nicotine. The resting TTR in DNE animals was significantly higher than that in sham treated control animals. Additionally, it was found that trachea from DNE-treated animals displayed larger peak Isc in response to nicotine when compared to control tracheae. Isc experiments of whole neonate trachea in the presence of epithelial Na+ channel (ENaC) and/ or Ca-induced Cl Channel (CaCC) inhibitors (amiloride and DIDS, respectively) suggest that the peak Isc is mediated by CaCC secretion in response to the Ca2+ influx triggered by activation of nAChRs. Preliminary experiments to evaluate changes in nAChR subunit expression between DNE and control animals using mRNA extraction and qPCR suggest altered subunit expression in the DNE-treated animals. In summary, we report the first Ussing Chamber recordings from rat neonate trachea and demonstrate significant changes in transtracheal resistance and Isc following DNE. These data highlight the potential physiological changes of early life exposure of nicotine, independent of cigarette smoke. *Funding: NIEHS/SBRPP ES094940; ES025494; HD071302*

S9. Impact of sex differences and tumor location on survival outcomes in glioblastoma patients

***Julia Lorence**[a], SC Massey[b], SK Johnston[c], S Ranjbar[b], P Whitmire[b], C Rickertsen[b], G De Leon[b], H White[b], K Singleton[b], L Hu[c], JR Mitchell[b], JB Rubin[d,e], KR Swanson[b,f,g],
[a]College of Liberal Arts and Sciences, Arizona State University, Tempe; [b]Mathematical NeuroOncology Lab, Mayo Clinic, Phoenix, AZ; [c]Department of Radiology, University of Washington, Seattle, WA; [d]Department of Pediatrics, Washington School of Medicine, St. Louis, MO; [e]Department of Neuroscience, Washington School of Medicine, St. Louis, MO; [f]Department of Neurosurgery, Mayo Clinic, Phoenix, AZ; [g]School of Mathematical and Statistical Sciences, Arizona State University, Tempe, AZ

Glioblastoma (GBM) is the most common malignant primary brain tumor in adults with a median survival of 14—16 months. Patient sex plays an important role in GBM as there is a difference in incidence rates and outcome between males and females, which may be attributable to differences in genetic makeup and physiology. The purpose of the study was to investigate the impact of tumor location and sex differences on survival outcomes based

on tumor location, laterality, age, handedness, and extent of resection. Patients (129 males and 87 females) who received standard-of-care were included. Pre- and Post MR imaging was available to determine tumor location and laterality. Analyses were performed using Cox proportional hazard modeling and Kaplan-Meier analysis (log-rank test) to determine which variables impacted patient survival. Overall survival was significantly longer in females in comparison to males (197 days, $p = 0.0391$). Investigating specific tumor locations, females with a tumor in the left frontal lobe ($n = 12$) showed a survival advantage compared to females with a right frontal ($n = 15$) GBM (2853 days, $p = 0.0160$). Significant differences in median OS were also associated with age. Female patients below the age of fifty showed significantly longer survival (2602 days, $n = 84$, $p<0.001$). Interestingly, 70% of IDH1 mutant tumors ($n = 10$) and 76% of MGMT methylated tumors ($n = 26$) were found in the frontal lobe and were found in the right hemisphere. Together, our results demonstrate that age, sex, and specific brain locations are associated with differences in genetics and survival in GBM. *Funding: Bidstrup Undergraduate Fellowship (JL)*

S10. Mourning Doves, Zenaida macroura, are resistant to metabolic and vascular effects of a mammalian diabetogenic refined carbohydrate diet

***Anthony Basile**[a], W Clark[a], X Shi[b], H Gu[b], P Deviche[a], KL Sweazea[a,b]

[a]School of Life Sciences, Arizona State University, Tempe; [b]College of Health Solutions, Arizona State University, Phoenix

Birds are an enigma: their plasma glucose is 1.5-2 times that of similar sized mammals, yet they do not normally exhibit symptoms of diabetes. We hypothesized that feeding adult Mourning Doves a refined carbohydrate diet (white bread: WB) for four weeks would result in diabetes-like pathologies including hyperglycemia, endothelial dysfunction, and altered metabolic profiles when compared to birds receiving a nutritionally balanced diet (bird seeds: SD). Following the four-week long diets, we euthanized birds with an overdose of sodium pentobarbital and collected cardiac blood, liver, kidney, and pectoralis muscles for metabolomics analyses and biochemical assays. We also dissected cranial tibial arteries to measure acetylcholine-mediated vasodilation. Contrary to the hypothesis, doves fed WB developed few changes in metabolite concentrations compared to control doves (number of altered metabolites: 9 of 123 in plasma, 7 of 92 in liver, 6 of 91 in pectoralis muscle; $p<0.05$). Moreover, pathway analyses revealed only three significantly altered pathways (liver: glutathione metabolism and histidine metabolism; pectoralis muscle: glyoxylate and dicarboxylate metabolism; $p<0.05$). Plasma glucose, uric acid, insulin, liver triglyceride, glycogen, and pectoralis muscle glycogen concentrations did not differ between groups, but liver glycogen was increased (2.12-fold) in the WB group ($p=0.015$). Diet type had no significant effect on vasodilation. However, compared to results from a prior study on wild-caught doves, captivity improved vasodilation (WB vs wild birds: $p=0.002$; SD vs wild birds: $p=0.022$). In conclusion, a four-week long diet consisting of white bread increased liver glycogen but did not instigate symptoms of diabetes.
Funding: School of Life Sciences and Office of Knowledge Enterprise Development (KLS & PD); American Physiological Society IOSP Summer Research Fellowship (WC) and the Center for Evolution and Medicine Graduate Fellow Award (AJB)

S11. Which precise mechanisms set thermal limits in animals? Testing the OCLTT hypothesis in Japanese quail embryos

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Amazingly, the primary mechanism establishing an animal's upper thermal limit is unknown. The classic protein denaturation hypothesis remains unlikely as most proteins denature at temperatures much higher than the critical thermal limits for organisms. Here, we focus on testing an alternate hypothesis – the oxygen and capacity-limited thermal tolerance (OCLTT) hypothesis. The OCLTT hypothesis predicts that increasing temperatures cause a mismatch between oxygen supply and demand, forcing the animal to transition to anaerobic metabolism. Eventually, anaerobic metabolism fails to meet energetic demand and the organism dies. Most support for the OCLTT hypothesis has come from aquatic animals with relatively little support in terrestrial animals. Yet, certain life stages, such as the embryonic stage where animals have underdeveloped respiratory systems and exist in an aquatic medium, may be more susceptible to oxygen limitation. We tested this hypothesis in Japanese quail (Coturnix coturnix) embryos by attempting to artificially increase basal metabolic rate with treatment of 3,3',5-triiodo-L-thyronine (T3). Halfway through development, T3 dissolved in DMSO was injected into the yolk of half of the eggs, while the other half received vehicle only. The next day, all embryos were exposed to 48.0°C for one hour to

measure survivorship. If the OCLTT hypothesis were correct, the embryos injected with T3 should show reduced survivorship at high temperatures due to increased oxygen demand. In fact, survivorship in the T3-injected group was one quarter of that in the control group suggesting that oxygen availability may be limiting thermal tolerance. However, the relationship between mass-specific metabolic rate and survivorship was weak indicating a possible secondary effect of T3 independent of metabolic rate. Therefore, further studies are necessary to elucidate the direct mechanisms behind this effect. *Funding: Midwestern University*

S12. Black jacobins reveal a unique hummingbird solution to communicating in a noisy tropical forest

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Birds have a phenomenal capacity for vocal communication, yet all species examined to date show a very limited range in their vocal and auditory frequencies (0.4-8 kHz). Using specialized equipment to acquire vocal recordings at a wide range of frequencies in the sonic and ultrasound ranges, we recorded the vocalizations from a hummingbird species that occurs in the Brazilian Atlantic Forest. The predominant vocalization (~98% of observations) of black jacobins (Florisuga fusca) consists of a triplet of syllables with high fundamental frequency (mean F0 ~11.8 kHz), rapid frequency oscillations (300 Hz) and strong ultrasonic harmonics (up to 80 kHz). Importantly these vocalizations had no detectable elements below ~10 kHz, thus were not harmonics or extensions of lower frequency syllables. Rather their frequencies range above the known hearing range of any bird species recorded to date, including hearing specialists like owls. Furthermore, these jacobin vocalizations were produced in multiple behavioral contexts, including close one-on-one antagonistic interactions among individual birds. These vocalizations where also produced in isolation and across seasons with detectable differences in some acoustic parameters. Overall, these findings suggest that black jacobins either have an atypically high frequency hearing range, or alternatively their primary vocalization type has a yet unknown function unrelated to vocal communication. The contexts of these high-frequency vocal signals in a complex tropical forest, as well as the physiological adaptations to vocal production and hearing that allow these vocal signals to be matched are future directions that will be addressed. Thus, black jacobin vocalizations challenge current notions about avian vocal communication and add to the numerous distinctive features that hummingbirds have evolved, including vocal learning, backwards flight, hovering, torpor, and ultraviolet vision.

Funding: OHSU Tartar Trust Fellowship (Olson); Eastlick Distinguished Professorship (Portfors); NSF grant #IOS-1456302 (Mello)

S13. How Hippocampal CA3 Dendritic Complexity is Quantified using the Intermittent Restraint Stress Paradigm

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Our research team is interested in how chronic restraint stress affects the functionality and morphology of neurons within the hippocampus. As a consequence of chronic stress, pyramidal neurons, especially within the CA3 region, show reduced apical dendritic complexity. Stress-induced morphological changes to the apical dendrites correspond to functional deficits in spatial memory abilities in rats (Conrad, 2010). By reducing CA3 apical dendritic complexity, chronic stress disrupts the hypothalamic-pituitary-adrenal axis, causing poor negative feedback and the overproduction of stress hormones, which is believed to be the mechanism behind the spatial memory deficits (Conrad 2006). The present investigated whether an intermittent restraint paradigm would be as effective, if not more so, in producing CA3 apical dendritic retraction and spatial memory deficits compared to a daily restraint paradigm. The rationale for the intermittent restraint paradigm is discussed in more detail in another abstract/poster from our team. The goal of this poster is to discuss the importance of the process in quantifying hippocampal CA3 dendritic complexity. Male, young adult Sprague-Dawley rats were placed in four groups (n=12/group): controls, daily restraint (6h/d/21 = DR6), or intermittent restraint, consisting of 5 days of restraint for 2 or 6 hours a day, with two days off, and then repeated for 21 days (IR2 or IR6). After behavioral testing over seven days, brains were collected on the eighth day and processed for Golgi stain (Ortiz et al., 2018). The brains were cut on a cryostat (120 µm) and prepared. The next steps, from identifying neurons to quantification, can take anywhere from six months to two years. We are in the midst of quantification from 48 rats with about 10 neurons each, equivalent to 480 neurons. Several steps are challenging to make this process slow. For example, each neuron must be fully impregnated or dyed so that the full dendritic tree could be discerned, somewhat isolated in order to avoid confusion with nearby

neurons, in the designated region of the dorsal CA3 hippocampus, and categorized properly as either a short shaft (SS) or long shaft (LS) neuron, given that SS neurons are inherently more complex than LS neurons. Multiple rounds of checking are necessary to ensure that all these requirements are met. Once neurons are drawn effectively they are quantified through Scholl analysis and number of branching points both apical and basal. Both of these provide a holistic measure of dendritic complexity, as it pertains to hippocampal plasticity. *Funding: College of Liberal Arts and Sciences and Barrett, The Honors College*

S14. Truncated isoform of Cx37 is not sufficient to suppress proliferation of rat insulinoma cells

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Connexin (Cx) proteins are known to regulate direct cellular communication via gap junction channels, as well as support communication of cells with their environment via hemichannels. Upon the discovery of a short "fragment" of Cx43, which assists in trafficking full-length Cx43 to the membrane, the possibility arose that such fragments of other connexins might serve a similar role. This 20 kDa fragment of Cx43 is a product of internal translation at a methionine site downstream from the typical 5' translation start site. Interestingly, Cx37 has an analogous internal methionine that, if active, would produce a 13 kDa isoform spanning residues 213-333. Cx37 has growth suppressive properties in the vascular endothelium as well as in our model cell line, rat insulinoma (Rin) cells. In this cell line, growth suppression by Cx37 requires both the 213-333 region of Cx37 as well as the regions that form the channel pore. Consequently, we hypothesized that the 13 kDa fragment by itself, would not exert a growth suppressive effect, but might participate in protein-protein interactions with full-length Cx37 to alter its effects on cell growth. Cx-deficient Rin cells with the tet-on promoter (iRin), were stably transfected with full-length mouse Cx37 (iRin37) or the 13 kDa piece (iRin13k), for doxycycline-inducible expression of these proteins. In the continuous presence of doxycycline, iRin, iRin37, and iRin13k cells were plated at an initial amount of 3 x 104 cells and their proliferation monitored every 3rd day over a 21-day period. Results show that both induced and non-induced iRin13k, as well as non-induced iRin37 cells, proliferated much more ((3.78 ± 0.376) x 10^6; n=4) than the induced iRin37 line ((1.08 ± 0.341) x 10^6; n=4) by day 21. This result suggests that the 13 kDa piece of Cx37 does not mediate growth suppression like full-length Cx37 and, further, supports previous data indicating that a functional channel is necessary for Cx37 to be growth suppressive. Moving forward, similar proliferation assays using an iRin cell line that co-expresses full-length Cx37 and the 13 kDa isoform will be used to determine whether the 13kDa fragment alters the growth suppressive effect of full-length Cx37 and if it does, determine the underlying mechanism of this effect. *Funding: NIH grant HL 122443; University of Arizona College of Medicine (COM); University of Arizona Undergraduate Biology Research Program (UBRP)*

S15. The role of nitric oxide and CaMK gene expression on the heart

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Research on the diabetic heart has suggested that there is a difference in the way the CaMKII protein is activated and functions compared to a non-diabetic heart. CaMKII is known to help mediate ion channels and Ca2+ handling in normal functioning hearts however, in human and mouse models that have diabetes CaMKII is up regulated in the heart leading to heart dysfunction. Further research has shown that nitric oxide has protective effects on the heart by helping calcium and cellular signaling. Additionally, nitric oxide helps activate CaMKII. Nitric oxide and CaMKII work together to regulate the heart's homeostasis and excitation-contraction coupling (ECC) effects by targeting important ion channels such as, Ca2+/Camodulin channel. While the effects of the CaMKII protein and exposure to nitric oxide on the Ca2+/Camodulin channel have been studied significantly, the genetic component of the CaMKII gene with exposure to nitric oxide has not been studied. The importance of this research is to understand the physiological effects of nitric oxide and CaMKII gene expression to better understand the normal heart function. Furthermore, this study utilised Echocardiography and the Langendorff heart technique to better understand the contractility of the heart models. The heart models that were used were wild type mice that had CaMKII gene expression and transgenic mouse models without CaMKII gene expression. Lastly, the results showed that the knock out mouse models had a reduced heart rate and an increased cardiac output compared to wild type mouse models. The contractility of the heart was preserved in both models suggesting that CaMKII is altered in the diabetic heart and CaMKII inhibitors could be a potential therapeutic target for diabetes. *Funding: NIH, Minority Health and Health Disparities International Research Training, T37MD008636*

S16. Hypoxia plus Deprivation Increases NF-κB Activation and Downstream Pro-Inflammatory Enzyme Levels in Human Brain VSM Cells

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Vascular inflammation is a key component for cerebrovascular disease and ischemic injury is suggested to be a significant contributor. Our previous studies have demonstrated that hypoxia plus glucose deprivation (HGD), an in vitro model of ischemia, increases the proinflammatory mediator, cyclooxygenase-2 levels (COX-2), in vascular tissues. Nuclear factor kappa B (NF-κB) activation is an upstream transcription factor of COX-2 and had been suggested to be involved in "sterile" inflammation in experimental stroke models. Mechanisms underlying the development and progression of inflammation in the cerebrovasculature following ischemic injury in human tissue has not been addressed. Thus, purpose of this study was to examine the impact of HGD on NF-κB expression and activation in HBVSM. In addition, we assessed pro-inflammatory mediator levels of downstream NF-kB transcription products, COX-2 and iNOS, and level of its upstream receptor, TLR4. Primary HBVSMC at passage 7 were treated with normoxia (room air) or HGD (1% O2). Following exposure to HGD (3 h), cells were isolated, homogenized, and total protein content determined. Lysates, either whole cell or nuclear and cytosolic fractions, were prepped for western blot and analysis. Anti-α-Smooth muscle actin was used to verify HBVSMC origin and NF-κBp65, phosphorylated NF-κBp65, COX-2, and TLR4 protein levels were all measured post HGD. NF-κBp65 total protein was expressed in HBVSMC and a trend for an increase in levels following HGD was observed. Indirect activation of pNF-kB65 was assessed via nuclear fractionation studies and was increased following HGD. COX-2 and iNOS protein levels were also increased following HGD. Additional findings suggested that HBVSMC expressed TLR4 however total protein levels of TLR4 were not altered by HGD. In conclusion, these studies indicate that HGD alters proinflammatory enzyme levels, potentially by altering NF-κBp65 activation in human vascular smooth muscle. *Funding: University of Arizona Sarver Heart Center and University of Arizona Valley Research Project Grant VRP P1 (RG)*

S17. Expression of insulin-like growth factor-1 (IGF-1) mRNA isoforms in muscle of humans with obesity

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Isoforms of insulin-like growth factor-1 (IGF-1) gene encodes different IGF-1 isoforms by alternative splicing, and which are known to play distinct roles in muscle growth and repair. These isoforms in humans are known as IGF-1Ea, IGF-1Eb and IGF-1Ec (the latter is also known as mechano-growth factor). We sought to determine whether IGF-1 mRNA isoform expression is impaired in skeletal muscle of humans with obesity, and given that humans with obesity display reduced protein synthesis in muscle. We studied 10 subjects (3 females/7 males) with obesity (body mass index: 34 ± 1 kg/m2) and 14 subjects (6 females/8 males) that were lean (body mass index: 24 ± 1 kg/m2) and served as controls. The groups represented typical populations of individuals that differed ($P < 0.05$) in body fat (obese: 32 ± 2; lean: 22 ± 2) and insulin sensitivity (Matsuda insulin sensitivity index, obese: 5 ± 1; lean 11 ± 2). Total RNA was extracted from 20-30 mg of tissue from muscle biopsies performed after an overnight fast. Isolated RNA was used to perform cDNA synthesis. Real-time PCR was performed using the following predesigned TaqMan® gene expression assays (Thermo Fisher Scientific Inc): assay ID: Hs01547657_m1 (detects IGF-1Ea), assay ID: Hs00153126_m1 (detects IGF-1Eb+IGF-1Ec) and assay ID: Hs03986524_m1 (detects IGF-1Ec), as well as assay ID: Hs01060665_g1 (detects ACTB, which was used to adjust the IGF-1 mRNA expression data. Responses for mRNA expression were calculated using the comparative CT method (2-ΔΔCT). Combined IGF-1Eb+IGF-1Ec mRNA expression was lower in the subjects with obesity compared to the lean controls (0.67 ± 0.09 vs 1.00 ± 0.13; $P < 0.05$) but that of IGF-1Ea (0.99 ± 0.16 vs 1.00 ± 0.33) or IGF-1Ec (0.83 ± 0.14 vs 1.00 ± 0.21) were not different between groups ($P > 0.05$). These data show altered muscle IGF-1 mRNA isoform expression in humans with obesity. Decrease in the expression of specific isoform of IGF-1 mRNA in muscle of humans with obesity may explain the lower protein synthesis we have previously observed in these individuals. Furthermore, these findings may have direct implications for muscle growth and repair in humans with obesity.
Funding: ASU Barrett, The Honors College, Thesis funding

S18. Autophagy markers in human skeletal muscle following acute aerobic and resistance exercise

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The specific adaptive response of skeletal muscle to exercise is dependent on both anabolic and catabolic processes. Autophagy is a catabolic process by which damaged and dysfunctional cellular components, such as proteins and organelles, are removed and recycled. Consequently, autophagy represents a key cellular process related to muscle function, and previous research has shown autophagy to be sensitive to exercise. However, the extent to which different forms of exercise impact autophagy in skeletal muscle has not been thoroughly investigated. The purpose of this study was to determine how markers of autophagy in skeletal muscle are impacted by different forms of acute exercise. In a counterbalanced, crossover design, six healthy, recreationally active young men (27±3 yr) completed acute aerobic (AE, 40 min of cycling, ~70% maximal HR) and resistance exercise [RE, 8 sets, 10 reps, ~65% 1-repetition maximum (1RM)], separated by ~1 wk. Western blot was used to investigate autophagy markers in muscle biopsies (vastus lateralis) obtained before and at 1 and 4h post-exercise. LC3BI was not changed from baseline in either group, however, there was a trend ($P=0.08$) for LC3BI to be lower at 1h in AE vs. RE (0.71±0.16 vs. 1.1±0.36 fold). In contrast, LC3BII was reduced ($P<0.05$) in both groups at 1h (AE: 0.38±0.11; RE: 0.35±0.10 fold) and 4h (AE: 0.65±0.15; RE: 0.53±0.12), however, no differences were observed between groups ($P>0.05$). Further, both p62 and cytosolic FOXO were reduced in both groups at 4h ($P<0.05$). These data support that autophagy is likely increased in skeletal following acute exercise and that this response is similar following acute aerobic and resistance exercise. Further work is warranted to identify the role of autophagy for mediating exercise mode specific adaptation of skeletal muscle. *Funding: Supported by GPSA and ASU*

S19. Pomegranate-derived nutraceuticals activate the vitamin D signaling pathway

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The active vitamin D hormone, 1,25-dihydroxyvitamin D (1,25D), mediates its biological effects by binding to the nuclear vitamin D receptor (VDR) and promoting heterodimerization with retinoid X receptors (RXRs). The VDR-RXR heterodimer regulates gene transcription in vitamin D target tissues including the colon, kidney, and brain thus effecting epithelial cell proliferation, differentiation, and chemoprevention. The vitamin D signaling pathway has been postulated to interact with various nutraceuticals such as resveratrol and curcumin. More recently, health benefits attributed to pomegranate have been associated with its high content of polyphenols (e.g., ellagitannins) which are metabolized by the gut microbiota to produce urolithins. Urolithin is thought to be the putative bioactive compound underlying the health benefits derived from pomegranate. Urolithin A (UA) is the most abundant of the urolithins and is found in the greatest concentration in human serum. In this study, we investigated the ability of UA to modulate 1,25D signaling via transcription-based assays and qPCR. We hypothesized that 1,25D in combination with UA will stimulate VDR activity more than 1,25D alone, and will increase transcription of 1,25D target genes. The increased activation of anti-oxidation genes by 1,25D and UA could help combat reactive oxygen species and attenuate cellular "aging". Our results indicate that there is a significant increase in VDR transcriptional activity when HEK-293 kidney cells are treated with UA in conjunction with 1,25D, and the stimulation by UA is apparent at several 1,25D concentrations. A similar effect was also observed using three structurally-distinct 1,25D response elements resulting in subtle differences in UA-mediated enhancement of VDR activity. Additionally, the expression of exogenous RXR did not augment the effect of UA, nor did UA promote an enhancement in VDR-RXR heterodimerization in a mammalian 2-hybrid (M2H) assay, suggesting that UA does not facilitate increased VDR activity by stimulating VDR-RXR heterodimerization. The potentiation of VDR activity via UA retains receptor-selectivity since UA did not promote further activation of estradiol-bound ER, or RXR-RXR homodimerization as assayed via the M2H platform. Finally, HEK-293 cells treated with 1,25D and increasing concentrations of UA demonstrated a dose-dependent, positive trend in transcriptional activation employing both luciferase and qPCR assays. Taken together, our novel results position urolithin A as a putative VDR modulator, suggesting that the influences of 1,25D and UA converging on VDR may potentially mediate anti-aging and promote longevity. *Funding: This work was supported by National Institutes of Health (NIH) grants DK033351 to MRH and CA140285 to PWJ. Arizona State University New College Undergraduate Inquiry and Research Experiences (NCUIRE) Program researcher grants were awarded to SL and MSS.*

S20. Evaluation of the effects of combination of mild aerobic exercise and angiotensin-II type-I receptor blocker, losartan, on aortic function and structure in a mouse model of Marfan syndrome

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Marfan syndrome (MFS) is a connective tissue disorder that can cause morbidity and mortality in patients through aortic aneurysms, dissections, and ruptures. Previously, we have shown that mild aerobic exercise improves aortic function and structure in a mouse model of MFS. It is also known that angiotensin II type I receptor (AT1R) signaling pathway contributes to the progression of MFS aneurysms. Considering previous findings that losartan, an AT1R blocker, could also slow down progression of MFS aneurysms in both the mouse model and human patients, we aimed to investigate potential beneficial effects of combination of mild exercise and losartan during the progression of aortic aneurysm in the mouse model of MFS. Our evaluation of aortic function and structure in MFS mice subjected to exercise only, losartan only, or combinational therapy provides additional information on the most effective therapeutic approach to delay progression of aneurysm in MFS. Drug therapy consisted of 0.6g/L or 0.3g/L of losartan in drinking water. The mild aerobic exercise regimen consisted of 8m/min, 30min/day, 5days/week (55% VO2 max). Mice were divided into experimental groups: control, MFS, MFS + exercise, MFS + 0.6g/L losartan, MFS + exercise + 0.6g/L losartan, and MFS + exercise + 0.3g/L losartan. The biophysical properties of the aorta were determined by high-resolution high-frequency ultrasound imaging (Vevo2100, FUJIFILM VisualSonics). Small chamber myography was used to determine the aortic function by measuring phenylephrine-induced aortic contraction in aortic segments. Aortic diameters at the aortic annulus and sinus of Valsalva were higher in MFS mice when compared to control at the 3, 6, and 9 months of age. All treatment groups had no effect on aortic root growth in MFS mice at 3 months; however, a decrease in aortic diameter was observed at 6 months. These differences were more pronounced at 9 months. MFS mice exhibited higher PWV when compared to control mice, indicating increased stiffness of aortic wall in MFS mice. All treatment groups had lower PWV when compared to the MFS group at 6 and 9 months. Maximum contraction in response to phenylephrine (Emax) was lower in MFS mice compared to controls, which was reversed in MFS mice subject to combinational therapy. No differences were seen between the combination of 0.6g/L of losartan with exercise and 0.3g/L of losartan with exercise. Our data suggests combination of mild exercise with lower dose of losartan can be beneficial in delaying the progression of aortic aneurysm in the mouse model, and can be explored as a potential therapeutic regimen in MFS patients, especially in young adolescence with lower tolerance for high dose of losartan. *Funding: The Marfan Foundation, Midwestern University Graduate Fund*

S21. Development of a protease activated receptor-2 (PAR2) antagonist for the treatment of asthma

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Asthma is a complex heterogeneous disease of the airways affecting 24.6 million people in the United States. Current trends indicate this number will increase both in incidence and severity. Despite a growing population of people diagnosed with asthma and a subset of patients experiencing refractory disease, there exists a paucity of efficacious therapies for difficult-to-treat asthma. We seek to address this healthcare need by employing novel approaches to asthma drug development through targeting of Protease-Activated Receptor-2 (PAR2) in allergic asthma initiation and development at the level of the airway epithelium. To this endeavor, we have developed a novel PAR2 antagonist, C391. The purpose of our study was to determine the efficacy of C391 in reducing hallmarks of asthma: inflammation and airway hyperresponsiveness. Using the asthma-inducing fungus Alternaria alternata as our model, we have demonstrated that C391 can inhibit PAR2-dependent Ca2+ and mitogen activated kinase (MAPK) signaling in human bronchial epithelial cells (16HBE14o- cells) as well as cytokine secretion by polarized 16HBE14o- cells grown at air-liquid interface. Preliminary data suggests that C391 may inhibit PAR2-mediated migration of inflammatory cells as a mechanism for the reduction of inflammatory cells recruited to the airways. In an in vivo model using Balb/C mice C391 provided protection against A. alternata-induced airway hyperresponsiveness and inflammatory cell recruitment. Together, these data indicate PAR2 is a viable pharmacological target and PAR2 antagonists could provide a novel therapy for the treatment of allergic asthma. *Funding: NIH NS098826; NIH AI140257; APS Porter Fellowship*

S22. Assessment of skeletal muscle mitochondrial metabolic flux and control using polarographic and luciferase-based techniques

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The purpose of this study was to assess both maximum oxidative capacity and control sensitivity of isolated mitochondria (MITO) using two techniques, polarography (POL) and luciferase (LUC). MITO were isolated from C57BL6J mouse quadriceps skeletal muscle (n=6). MITO oxidative flux is typically assessed by measuring O2 consumption rate (Jo) using POL. In contrast, LUC measures ATP production rate (Jp) and offers enormously greater assay sensitivity. For example, per assay in this study, POL and LUC used 25.3 ± 2.0 μg and 127 ± 9 ng MITO protein, respectively. Thus, LUC was roughly 200-fold more sensitive. In POL assays, MITO maximum ATP production rate can be calculated by multiplying two measured values, maximum Jo stimulated by saturating ADP times the ADP/O ratio. MITO oxidizing glutamate + succinate (G+S, 10 mM + 10 mM) in POL had maximum Jp of 2535 ± 374 nmol ATP/min/mg at 37 degrees Celsius. Due to temperature sensitivity, LUC assays must be carried out < 28 degrees Celsius, but with Q10 adjustment to 37 degrees Celsius, LUC gave a corresponding max Jp of 1467 ± 188. Thus, LUC assessment of maximum Jp was 56 ± 3% of the POL rate. These maximum Jp data agree with retrospective analyses of human muscle MITO data collected by Kras, Katsanos, and coworkers; with n=5 MITO preparations, LUC rates were 59 ± 7% of POL rates. The kinetics of [ADP] control of oxidative flux were also determined with both techniques. In POL a creatine kinase energy clamp was used to establish 5 levels of [ADP] (21, 31, 47, 70, and 109 μM) while Jo was continuously measured. Eadie-Hofstee analysis gave KmADP for Jo of 13.1 ± 1.3 μM. In LUC ADP was simply added at 8 levels (0, 1.5, 3, 6, 12.5, 25, 50 and 125 μM) and Jp was measured. The LUC determined KmADP for Jp was 12.7 ± 0.5 μM, similar to the POL value. However, the ionic form of ADP that binds to the adenine nucleotide translocase (ANT) is ADP3-, which depends on the total ADP (ΣADP), pH, and the free [Mg2+] of the assay buffer. Under the conditions of the two assays, the corresponding KmADP3- values were 4.75 ± 0.67 μM and 1.92 ± 0.08 μM for POL and LUC, respectively. The 2.5-fold higher KmADP3- in POL likely reflects the creatine kinase clamp, which contains 5 mM ΣATP, a competitor with ADP for binding at ANT. Thus, an apparent KiATP equal to 3.2 mM can be calculated by comparing the POL and LUC assessments of ADP kinetics. We conclude that LUC provides a high sensitivity assay to assess both maximum oxidative flux and the kinetics of ADP control. Moreover, when combined with POL, the comparison of the two KmADP3- determinations offers insight into adenylate competition for binding at ANT.

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S23. mTOR signaling in human skeletal muscle following acute aerobic and resistance exercise

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Aerobic (AE) and resistance exercise (RE) training are known to elicit unique adaptions in skeletal muscle. However, the precise molecular mechanisms mediating these unique adaptations remain to be completely resolved. The purpose of this study was to investigate the response of the mammalian/mechanistic target of rapamycin (mTOR) signaling pathway, a known regulator of muscle protein synthesis and fiber size, during the immediate hours following acute AE and RE. In a counterbalanced, crossover design, six healthy, recreationally active young men (27±3 yr) completed acute AE (40 min of cycling, ~70% maximal HR) and RE [8 sets, 10 reps, ~65% 1-repetition maximum (1RM)], separated by ~1 wk. Muscle biopsies (vastus lateralis) were obtained before and at 1 and 4h postexercise and western blot analyses were used to examine changes in the phosphorylation of mTOR signaling proteins. Skeletal muscle mTOR phosphorylation (ser2448) was increased in both groups at 4h, however, this response was greater in RE vs. AE (6.2±1.7 vs. 3.7±0.68 fold, $P<0.05$). S6K1 phosphorylation (thr389) was also increased in both groups at 4h (9.91±4.0 vs. 10.2±3.6 fold, $P<0.05$). In addition, S6K1 phosphorylation was also greater in RE vs. AE at 1h (4.45±0.42 vs. 1.93±0.78 fold, $P<0.05$) and there was a trend ($P=0.08$) for increased S6K1 phosphorylation in RE at 1h. 4E-BP1 phosphorylation (thr37/46) was unaffected by exercise, however, postexercise 4E-BP1 phosphorylation was greater in RE vs. AE ($P<0.05$, main effect of group). No time or group differences were observed for eEF2 phosphorylation (thr56). These data highlight subtle differences in the response of the mTOR signaling pathway to divergent acute exercise. In particular, our findings indicate that acute RE may target mTOR signaling more than acute AE, at least during the immediate hours postexercise. Further research is warranted to determine whether these acute responses relate to exercise-mode specific skeletal muscle adaptations.

Funding: GPSA and ASU

S24. Aerobic Exercise in Older Adults Maintains Nrf2 Signaling Compared to Inactive Controls

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We have previously shown that older men have impaired Nrf2 signaling response to acute exercise as compared to young controls. The present RCT investigated whether exercise-induced Nrf2 signaling would improve with a more regular exercise stimulus. Inactive men and women (62-77y) were randomized to an 8-week exercise intervention (EX, n=9) or a non-exercise control group (CON, n=8). EX performed supervised aerobic exercise 3d/wk for 45-min/d. The effectiveness of the exercise intervention was measured by changes in maximal aerobic capacity (VO2max). Nrf2 signaling was measured in response to acute exercise (30-min cycling at 70% VO2max) in isolated PBMCs. Nrf2 protein quantification was measured at 7 time points (Pre, +10, +30, +1, +4, +8, +24h) and gene expression of HO-1, NQO1, and GCLC were measured at 5 time points (Pre, +1, +4, +8, and +24h) before and after the 8-week intervention. There were no significant differences between sexes for any variable or baseline differences between EX and CON. The exercise intervention improved VO2max by 19% ($p<0.05$) while CON did not change. The acute exercise trial significantly increased Nrf2 nuclear expression in both groups ($p<0.05$). The response did not change in EX pre-post intervention, however CON had an attenuated response at post-testing ($p<0.05$). Interestingly, basal levels of nuclear Nrf2 were 3-fold greater post-intervention compared to pre in both groups ($p<0.05$). The intervention had a significant effect on NQO1 gene expression ($p<0.05$) which mirrored the Nrf2 response: maintained in EX and attenuated in CON. There were no significant effects for HO-1 and GCLC. These preliminary results indicate that an 8-week exercise intervention is not sufficient to increase exercise-induced Nrf2 signaling, despite improving aerobic fitness. Nevertheless, the magnitude of the response after exercise training was more robust than observed in the inactive controls. Additional testing and analyses, including young individuals, are underway. *Funding: NIH R15AG055077*

POSTER PRESENTATION ABSTRACTS

F1. Varied effects of dietary carotenoid supplementation on oxidized lipoproteins in tissues of two waterfowl species

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The antioxidant potential of dietary carotenoids is widely debated for birds and current literature is limited to specific tissues and carotenoid dosages. We investigated the effects of dietary carotenoid supplementation on plasma, liver, adipose, heart and breast muscle oxidative damage in two phylogenetically-matched species of ducks. We hypothesized that dietary carotenoids would exhibit dose, sex and species-dependent effects on oxidative damage and that there would be tissue priority in oxidative damage. After a 6-week depletion period, captive adult Northern pintail (Anas acuta) and mallard (Anas platyrhynchos) ducks of both sexes were fed a diet containing <3, 50 or 100 μg/g xanthophylls for 17 weeks. Oxidized lipoproteins were quantified in all samples using a commercially available kit. For all doses liver and heart exhibited the highest oxidative damage, whereas adipose had the lowest. Further, 100 μg/g carotenoids had pro-oxidant effects in plasma, while 50 μg/g carotenoids were pro-oxidant in adipose from female mallards. Male Northern pintails supplemented with <3 μg/g carotenoids exhibited significantly greater oxidative damage in breast, while females had elevated oxidative damage in heart. No significant effects were observed in liver. High doses of dietary carotenoids did not have additional benefits in reducing oxidative damage. The current study does not support a strong antioxidant role for dietary carotenoids in the tissues of these species of ducks. In addition, this study demonstrates differential effects on tissue oxidative stress depending on sex, species, and concentration of dietary carotenoids suggesting carotenoids play a complex, tissue-specific role in the balance of oxidative stress in birds. *Funding: University of Poitiers, France (MG), NSF/IOS-0746364 and 0925633 (KJM)*

F2. Effects of Fascial Stretch Therapy (FST) on Pain Index and Activities of Daily Living (ADL) in Patients with Chronic Non-specific Low Back Pain (LBP)

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BACKGROUND: Numerous fascia-focused therapies are used to treat pain, most relying on direct manipulation and/or tool-mediated techniques. FST, on the other hand, uses distally applied techniques to yield both local and global desired tissue outcomes and subjective pain improvement, including those related to LBP. We hypothesize that subjects receiving FST will have reduced nonspecific LBP and enhanced activities of daily living (ADL) scores.
METHODS: Eleven subjects who met study criteria (7F, 4M; Age 22-32 y/o) underwent 1 (N=11), 2 (N=7), or 3 (N=5) successive FST treatments (Tx in table below) which consisted of 30 min of 3-strap stabilization-mediated body stretch (8 per side). Subjects had pain and ADL scores (Bathing: BAT; Car egress/ingress: CEI; Toilet use: TOI; Forward bending: FOB; Dressing: DRE) measured pre- and 1- and 3-day post-FST. We used a linear mixed effects model to ascertain the relative % change in scores over time using the pretreatment time point as the reference group. All p-values were 2-sided and $p<0.05$ was considered statistically significant.
RESULTS: Statistically significant improvements in pain and ADL scores (*) were found at the time points shown in the table: SCORE 1 Tx; 1 day post 1 Tx; 3 day post 2 Tx; 1 day post 2 Tx; 3 day post 3 Tx; 1 day post 3 Tx; 3 day post PAIN 1 Tx; 1 day post* 1 Tx; 3 day post* 2 Tx; 3 day post* 3 Tx; 1 day post* BAT 2 Tx; 3 day post* CEI 1 Tx; 1 day post* 1 Tx; 3 day post* 2 Tx; 1 day post* 2 Tx; 3 day post* 3 Tx; 1 day post* 3 Tx; 3 day post* TOI FOB1 Tx; 1 day post * 1 Tx; 3 day post*2 Tx; 1 day post * 2 Tx; 3 day post* 3 Tx; 1 day post* 3 Tx; 3 day post* DRE 1 Tx; 1 day post* 2 Tx; 1 day post* 3 Tx; 1 day post* 3 Tx; 3 day post* Score improvements noted in the table ranged between 31% and 57% compared to pretreatment time
point.
CONCLUSION: This pilot study shows that both single as well as multiple, successive 30-minute FST treatments improve pain and ADL scores, with the highest improvements seen in pain and FOB. Future studies will determine optimal treatment frequency and measure additional variables aimed at mechanistic understanding of treatment effects. [All subjects were consented as part of a UA-approved IRB].

F3. Coccidioidomycosis Detection Using Targeted Plasma and Urine Metabolic Profiling

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Coccidioidomycosis, also known as Valley Fever (VF), is a potentially lethal fungal infection that results in an estimated 200 deaths/year in the United States, disproportionately affecting Arizona residents. Between 1998 and 2016, Arizona accounted for 51-79% of all reported cases of VF in the United States. In highly endemic areas such as the Phoenix and Tucson metropolitan areas of Arizona, VF is estimated to account for 15-30% of all community-acquired pneumonia (CAP). Current diagnostic methods such as chest imaging, serology, skin tests, and fungal cultures are either too costly, time-consuming, invasive, or indeterminant. Despite the important role of metabolism in the molecular pathogenesis of VF, robust metabolic markers to enable the screening, surveillance, therapeutic monitoring and timely treatment of VF are still lacking. In this study, we present the first-ever targeted liquid chromatography-tandem mass spectrometry (LC/MS-MS) metabolic profiling approach for identifying metabolic marker candidates to enable highly sensitive and specific VF detection. In this targeted approach, 207 plasma metabolites and 231 urinary metabolites from many metabolic pathways of potential biological relevance were reliably detected and monitored in 147 samples (59 plasma and 88 urine) taken from two groups of subjects (47 VF patients and 100 non-VF controls). The results of our univariate significance testing and multivariate model estimation informed the construction of a 3-metaboltie panel of plasma biomarkers and a 9-metabolite panel of urinary biomarkers. Receiver operating characteristic (ROC) curves generated based on enhanced orthogonal partial least squares-discriminant analysis (OPLS-DA) models showed near-perfect classification performance of the plasma metabolite model [AUC=0.995 (95% CI: 0.983-1.00), sensitivity=0.944, specificity=0.976] and excellent overall accuracy of the urinary metabolite model [AUC=0.929 (95% CI: 0.873-0.985)] with high sensitivity (0.897) and specificity (0.881). Bioinformatics analyses were also performed on plasma and urine metabolite data in order to elucidate affected pathways. Enrichment, pathway, and network analyses ubiquitously revealed significant disturbances in glycine and serine metabolism in VF patients and should be studied further to identify potential therapeutic targets for VF treatment. Future studies should also validate the performance of our biomarker panels in discriminating VF patients from subjects with similar fungal infections that commonly present with overlapping clinical features such as histoplasmosis and blastomycosis, as well as bacterial and viral CAP. *Funding: College of Health Solutions, Arizona State University*

F4. Stressing out the honeybee blood-brain barrier

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Aging and parasitic load are both stressors associated with extreme behavioral changes in honeybees. Although these behaviors may be partially explained by degradation of neuronal function, dysfunction of the glial blood-brain barrier may also be involved. First, we demonstrate how Varroa mite load during development influences the integrity of the blood-brain barrier. Next, we show how blood-brain barrier integrity changes due to aging and age reversal. This work has broad implications for social insect research; knowledge about the honeybee blood-brain barrier can inform those interested in everything from pollinator conservation to decoding miniature brains.

F5. 10-Week high fat diet promotes endotoxemia and alters microbial taxonomy in male adolescent rats

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Diet-induced changes in gut microbiota are associated with increased risk of obesity and diabetes. Prior research shows that a proprietary soil-derived compound decreases weight gain and blood glucose in diabetic mice. The aim of this study was to examine the effects of a similar organometallic complex (OMC) on gut inflammation in rats fed a high fat diet (HFD). Six-week-old male Sprague-Dawley rats (n=36) were divided into two dietary groups: chow (18.9% protein, 57.33% carbohydrates, 5% fat) or HFD (20% protein, 20% carbohydrates, 60% fat) for 10 weeks. Animals were further divided (n=6/group) and administered 0, 0.6 or 3.0 mg/mL OMC in their drinking water. Microbial taxonomy of cecal contents were evaluated by LEfSe analysis and showed a significant abundance of Rikenellaceae, Alistipes, Erysipelotrichaceae and Oceanospirillales in samples from chow rats (LDA <2.0) after 10 weeks. In contrast, HFD cecum contents were more enriched in bacteria classified as "other" and "mitochondria" (LDA <2.0). Additionally, HFD rats developed endotoxemia, which was prevented by OMC. Western blot analyses

showed no changes in protein expression of the inflammatory cytokines NF-kB, IL-1B, or IL-6 (Two-Way ANOVA, p=0.362, p=0.702, and p=0.746, respectively) in rats fed HFD. Likewise, protein expression of NF-kB, IL-1B, and IL-6 were not altered (Two-Way ANOVA, p=0.402, p=0.062, or p=0.953 respectively) by OMC-treatment. Thus, while 10-week HFD induces gut dysbiosis and endotoxemia, inflammatory biomarkers were not significantly altered by diet or OMC. Further studies are needed to determine whether OMC alters intestinal permeability. *Funding: Isagenix International, LLC.*

F6. Approaches for testing hypotheses for the hypometric scaling of aerobic metabolic rate in animals

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Hypometric scaling of aerobic metabolism (larger organisms have lower mass-specific metabolic rates (MR/g)), is nearly universal for interspecific comparisons among animals, yet we lack an agreed upon explanation for this pattern. If physiological constraints on the function of larger animals occur and limit MR/g, these should be observable as direct constraints on animals of extant species, and/or as evolved responses to compensate for the proposed constraint. There is evidence for direct constraints and compensatory responses to oxygen-supply constraint in skin-breathers but not vertebrates with gas exchange organs. Larger birds and mammals retain food in the gut for longer, consistent with a direct constraint on nutrient uptake across the gut wall, but there is little evidence for larger animals evolving compensatory responses to gut transport constraints. Larger placental mammals (but not marsupials or birds) show evidence of greater challenges with heat dissipation, but there is little evidence for compensatory adaptations to enhance heat loss in larger endotherms, suggesting MR more generally balances heat loss for thermoregulation in endotherms. Size-dependent patterns in many molecular, physiological and morphological properties are consistent with size-dependent natural selection, such as stronger selection on neurolocomotory performance and growth rate in smaller animals, and stronger selection for safety and longevity in larger. Hypometric scaling of MR very likely arises by different mechanisms in different taxa and conditions, consistent with the diversity of scaling slopes for MR.
Funding: This research was partially supported by NSF IOS 1256745 and 1558127.

F7. Augmented thermogenic response in mice overexpressing lipid droplet-interacting protein (LIP) in brown adipose tissue

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Adipose tissue is the primary energy storage organ in humans and rodents. Cellular energy is stored as triglyceride within phospholipid-coated droplets (LDs) and released as free fatty acid (FA) according to metabolic demand. Brown adipose tissue (BAT) increases its energy output as heat during acute responses to cold exposure. Brown adipocytes contain multilocular LDs, numerous mitochondria and demonstrate a high rate of fatty acid oxidation and glucose uptake. Recently we identified a novel LD-Interacting Protein, LIP, which is highly expressed in BAT. The functional role of LIP in FA metabolism and thermogenic responses has not yet been evaluated. Using an adipose-specific promoter, we generated a transgenic mouse line that selectively overexpresses LIP in BAT (LIP-BAT). LIP-BAT mice exhibited no difference in food consumption, body weight, or insulin sensitivity as demonstrated by insulin and glucose tolerance tests. By 20 weeks of age, transgenic animals displayed a significant increase in fat mass (9.3±0.4% vs 7.6±0.3% WT p=0.002) and a slight reduction in lean mass percentage (75.6±0.4% vs 76.9±0.4% WT p=0.023). Because transgenic animals overexpress LIP in BAT, we used a Comprehensive Lab Animal Monitoring System to compare heat production at room temperature (RT) and 4oC over a 48h period. While no difference was observed between LIP-BAT and WT animals at RT, heat production was increased in LIP-BAT mice at 4oC (0.70±0.004kcal/hr LIP-BAT vs 0.62±0.005kcal/h WT p<0.0001). The largest difference occurring within the first 24h of cold exposure (0.68±0.005kcal/h LIP-BAT vs 0.59±0.007kcal/h WT p=0.001). Over a 24h period at RT, respiratory exchange ratios (RERs) were similar between LIP-BAT and WT animals. Over the initial 24h period at 4oC, LIP-BAT animals showed higher RER than WT mice (0.89±0.006kcal/h vs 0.85±0.003kcal/h WT p<0.0001). Expectedly, cold exposure induced a reduction of RER in WT mice (0.91±0.006kcal/hr RT vs 0.85±0.003kcal/hr 4oC) p<0.0001), indicative of a switch of energy substrate from carbohydrates to FAs. Interestingly, overexpression of LIP in BAT prevented this switch in transgenic animals (RER: 0.89±0.006kcal/h at RT vs 0.89±0.003kcal/h at 4 oC). Consistent with energy expenditure (heat) findings, LIP-BAT mice had higher body temperatures (35.4±0.2oC vs 33.0±0.7oC WT, p=0.014) after 3h of fasting cold exposure. Upon 2h cold exposure, there was a reduction in BAT mass in WT (0.26±0.028% RT vs 0.20±0.006% 4oC, p=0.035) but not

transgenic animals (0.25±0.0.009% RT vs 0.27±0.010% 4oC, p=0.718). LIP overexpression in BAT enhanced thermogenic responses likely through enhanced utilization of carbohydrates as a fuel source. *Funding: Mayo Clinic Center for Biomedical Discovery Platform Award and NIH Initiative for Maximizing Student Development (IMSD) at Arizona State University*

F8. Loss of SETD2 induces a metabolic switch in renal cell carcinoma cell lines toward enhanced oxidative phosphorylation

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SETD2, a histone H3 lysine trimethyltransferase, is frequently inactivated and associated with recurrence of clear cell renal cell carcinoma (ccRCC). However, the impact of SETD2 loss on metabolic alterations in ccRCC is still unclear. In this study, SETD2 null isogenic 38E/38F clones derived from 786-O cells were generated by zinc finger nucleases, and subsequent metabolic, genomic, and cellular phenotypic changes were analyzed by targeted metabolomics, RNA-sequencing, and biological methods, respectively. Our results showed that, compared to parental 786-O cells, 38E/38F cells had elevated levels of MTT/Alamar blue levels, ATP, glycolytic/mitochondrial respiratory capacity, citrate synthase (CS) activity, and TCA metabolites such as aspartate, malate, succinate, fumarate, and α-ketoglutarate. The 38E/38F cells also utilized alternative sources beyond pyruvate to generate acetyl-CoA for the TCA cycle. Moreover, 38E/38F cells showed disturbed gene networks mainly related to mitochondrial metabolism and the oxidation of fatty acids and glucose, which was associated with increased PGC1α, mitochondrial mass, and cellular size/complexity. Our results indicate that SETD2 deficiency induces a metabolic switch toward enhanced oxidative phosphorylation in ccRCC, which can be related to PGC1α-mediated metabolic networks. Therefore, this current study lays the foundation for the further development of a global metabolic analysis of cancer cells in individual patients, which ultimately will have significant potential for the discovery of novel therapeutics and precision medicine in SETD2 inactivated ccRCC. *Funding: This work was supported in part by the China Scholarship Council, Arizona State University (ASU), and the Gloria A. and Thomas J. Dutson Jr. Kidney Research Endowment. THH is supported by National Cancer Institute (R01CA224917) and the Department of Defense (W81XWH-17-1-0546).*

F9. Novel Organometallic Complex Prevents High Fat Diet-Induced Liver Injury In Male Sprague-Dawley Rats

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Nonalcoholic fatty liver disease is the most common form of chronic liver disease in the United States. Diets high in saturated fats are known to promote obesity and hepatic steatosis. The consumption of a high-fat diet (HFD) can increase the risk factors associated with insulin resistance, which can lead to the onset of diabetes and obesity. A prior study of a soil-derived organometallic complex (OMC) showed that supplementation reduces glucose and body mass in diabetic mice. The goal of this study was to test the efficacy of a similar OMC compound on the prevention of hepatic steatosis induced from a HFD. Six-week-old male Sprague-Dawley rats (n=42) were divided into the following diet groups: standard rodent chow or 60% kcal from fat high fat diet (mainly lard) for 10-weeks. Rats were further divided into OMC treatment groups with OMC added to their drinking water: 0 mg/ml, 0.6 mg/ml or 3.0mg/ml OMC. At 10 weeks, study animals were euthanized with sodium pentobarbital (200 mg/kg, i.p.) and cardiac plasma, as well as liver samples, were collected and stored at -80° C until further analyses. Plasma ALT and AST, as well as liver triglyceride and free glycerol concentrations, were measured using commercially available kits. To assess liver damage, aspartate transaminase (AST) and alanine transaminase (ALT) are biomarkers of inflammation and cell injury to the liver, which were examined. Rats fed HFD had elevated plasma ALT activity, which was prevented by treatment with the high dose of OMC ($p<0.05$). No changes in plasma AST activity were detected. Examination of liver triglyceride and free glycerol concentrations showed increased fat accumulation in the liver of rats consuming HFD (Two-Way ANOVA, $p<0.001$). OMC did not prevent this increase. These findings suggest that, although OMC does not prevent the accumulation of lipids in the liver of rats fed HFD, it does mitigate liver injury resulting from excess dietary intake of saturated fats. *Funding: Isagenix International, LLC*

F10. Can Daytime Measures of Respiratory Sinus Arrhythmia and Breathing Stability Serve as Biomarkers for OSA?

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Obstructive sleep apnea (OSA) is by definition a disorder of nighttime breathing however, there is good evidence that OSA results in daytime impairments in cognition and cardiovascular function. Traditionally, the diagnosis of OSA has required the individual undertake an in-home or in-laboratory overnight sleep study. However, both these tests are expensive and, due to health insurance quotas, are often limited in their availability. Here, we outline simple methods for assessing daytime respiratory sinus arrhythmia (RSA) and respiratory stability that show promise as potential diagnostic tools to identify adults at risk for OSA. We recorded heart rate (finger oxy-photoplethysmography) and respiration (inductance plethysmography) in 8 adults (n= 4M; 4F) with mild, moderate and severe obstructive sleep apnea (age: 32-78 years; BMI: 32 +/-6) and 9 (n= 5M; 4F) healthy adults (age 32-70 years; BMI: 26 +/-2). Subjects completed five minutes of eupneic breathing, five minutes of paced breathing (7.5 breaths/minute) and after equilibration, five minutes of hyperoxic, hypercapnia (5% FICO2). Using de-identified data, we obtained estimates of RSA amplitude, RSA stability, and respiratory stability for each subject in each condition and performed a regression analysis to determine the relationship between each of these variables and sleep apnea severity. Our results indicate that daytime measures of respiratory stability and RSA reliably differentiate healthy adults from adults diagnosed with OSA ($p<0.006$). These analyses require minimal instrumentation and very brief sampling periods i.e., ~5 minutes. Summary schematics arising from the analyses may prove useful as early indicators for OSA risk. *Funding: This work was supported by start-up monies (Department of Physiology, University of Arizona) awarded to EFB and by The Finley and Florence Brown Predoctoral Fellowship (Sarver Heart Center, University of Arizona) awarded to JRV.*

F11. A truncated isoform of Cx37 may regulate trafficking and channel function of full-length Cx37

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Connexin (Cx) 37 is a transmembrane protein expressed by the endothelial cells of the arterial vasculature where it supports phenotypic switching between growth arrested (differentiated), proliferative and death states. The possible mechanisms underlying this phenotypic switching function include phosphorylation-dependent regulation of 1) intercellular and transmembrane exchange of signaling molecules and metabolites via gap junction channels and hemichannels, respectively, or 2) intracellular signaling cascades via regulated protein-protein interactions mediated by the carboxyl terminus. The Cx37 mRNA has a potential internal ribosomal entry site (IRES) available at methionine 213 homologous to that occurring in Cx43, which supports translation of a truncated version of Cx43 essential for protein trafficking. In Cx37, this site could support translation of a truncated 13 kDa (13k) piece that contains the entire carboxyl terminus with all its putative phosphorylation sites, and a portion of the 4th transmembrane domain, which would tend to anchor the protein at the membrane. To determine whether a death-inducing mutation, S321D, in the full length protein would induce death as the 13k form and to determine whether this mutant form altered the trafficking of the full length form, we transfected naïve and Cx37-expressing rat insulinoma (Rin) cells with Cx37-13k-S321D. We hypothesized that expression of the 13k-S321D protein would induce cell death by itself and regulate the trafficking, channel functions, and growth phenotype of cells expressing the full-length Cx37 protein. Proliferation assays and immunocytochemistry indicated that 13k-S321D alone did not induce cell death nor alter the proliferative properties of Rin cells. In addition, 13k-S321D co-localizes with endoplasmic reticulum and Golgi apparatus markers. Patch-clamp electrophysiology studies of hemichannel behavior in cells co-expressing full-length Cx37 and i13k-S321D revealed a preference of the hemichannel for a low (110 pS) conductance state, with occasional transitions to high conductance states (600 pS), as compared to the intermediate conductance (300 pS) state preferred in Rin cells expressing only the full length Cx37wt. The data suggest that i13k-S321D alone does not form functional channels and does not alter the growth phenotype of Rin cells, but when co-expressed with Cx37wt may influence trafficking and function of the hemichannels formed by full-length Cx37. *Funding: NIH grant HL 122443*
University of Arizona Senior Vice President for Research (SVPR); University of Arizona Undergraduate Biology Research Program (UBRP)

F12. Influence of storage conditions and preservatives on metabolite fingerprints in urine

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Urine is rich in human metabolites and is widely used in metabolomics research. Maintaining the stability of the metabolites in the urine is of great importance for the authenticity and reliability of research results. Herein, the influence of several factors, including storage time (24 h and 48 h), storage temperature (4℃, 22℃ and 40℃) and use of preservatives (boric acid and thymol), on metabolites fingerprints in urine was systematically investigated based on HILIC/UPLC-MS/MS data. Statistical analysis results showed that metabolites in urine could be well preserved at 4℃ for 48 h but deterioration could easily occur to urine under high temperature (40℃, stimulating the environment in Arizona) storage condition. At room temperature (22℃), significant metabolites changes were observed between 24 h-storaged sample and 48 h-storaged sample, which was due to the gut microbes activities existing in urine. Besides, preservative thymol was proved to be effective in maintaining metabolites stability in urine under 22℃/48h condition and high temperature (40℃) condition, probably because of the inhibitory effect of thymol on gut microbes in urine. A guidance on the usage of preservatives was also proposed with the purpose of preventing the metabolites changes in urine. In conclusion, the results of this study could provide valuable information for urine sample storage, which could be helpful in preventing the wrong conclusions and misleading results caused by metabolites changes in urine in the field of research and laboratory medicine. *Funding: College of Health Solutions, ASU*

F13. Detrimental effects of caveolae disruption with methyl-β-cyclodextrin on aortic function & structure in a mouse model of Marfan syndrome

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Marfan syndrome (MFS) is a systemic connective tissue disorder caused by mutations in the fibrillin-1 gene. Previous studies have shown that transforming growth factor β (TGF-β) and angiotensin II type 1 receptor (ATII/AT1R) signalling play important roles during the progression of MFS aneurysm. Interestingly, both pathways are shown to be regulated by caveolin-1 (Cav-1), a structural protein within caveolae, which is highly expressed in vascular smooth muscle and endothelial cells. Considering the complexity of caveolae and CAV-1 regulatory functions, we aimed to investigate the effects of caveolae (CAV-1) on the progression of aortic aneurysm. Four-week old MFS (Fbn1C1039G/+) and control C57BL/6 mice received (500mg/kg) intra-peritoneal injection of cholesterol depleting agent methyl-β-cyclodextrin (MβCD). Cardiac and aortic structure/function were measured at 3 & 7 months of age using Vevo 2100 ultrasound imaging system (FUJIFILM VisualSonics). Measurements for aortic annulus, sinus of Valsalva, showed an increase in MFS at 7 months. Measurements for pulse wave velocity (PWV), which is a reliable proxy for aortic wall stiffness, showed an increase in 3- and 7-month old MFS mice. All these effects were exacerbated in MFS mice treated with MβCD. Furthermore, the dimensions of the left ventricle were also evaluated systole and diastole. Cardiac function was evaluated measuring the cardiac output, stroke volume, ejection fraction, which showed no difference in MFS and control mice in the presence or absence of MβCD treatment. Early ventricular filling velocity and E/A ratio were decreased in MFS mice at 3 and 7 months. No significant difference was found in the atrial filling velocity in both MFS and control groups. The measurements for blood pressure showed higher systolic values in 7-month old treated MFS mice as compared to 3-month old groups. MFS (Fbn1C1039G/+) and control C57BL/6 mice were also injected intraperitoneally with a cell-permeable Cav-1 scaffolding domain (CSD) peptide or vehicle. Aortic wall elasticity was markedly decreased in MFS groups, but significantly improved in CSD-treated MFS mice. In addition, elastin fiber fragmentation within the aortic wall was evident in aortic cross sections isolated from MFS mice. Interestingly, CSD injection improved elastin organization in MFS mice aorta. Our data shows that disruption of caveolae structure in MFS mice has negative impact on aortic wall structure and function, while indicating that increased Cav-1 activity in MFS has protective effects. This study provides insights into the role that caveolae may play during the progression of aortic aneurysm, and warrant further investigation into the potential value of CAV-1 as a therapeutic target in MFS associated aortic aneurysm. *Funding: Marfan Foundation*

F14. Impact of Chronic Angiotensin Treatment on the Responses of Isolated Cardiac Fibroblasts to Acute Pro-Fibrotic Stimuli

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Pathological cardiac remodeling involving fibrosis can lead to heart failure in people with hypertension. We recently demonstrated that angiotensin II (AngII)-induced increases in left ventricular (LV) collagen content and macrophage infiltration are attenuated by concomitant bradykinin-B1 receptor (B1R) antagonism in Sprague-Dawley (SD) rats. Given that AngII upregulates B1R, the goal of the present study was to determine the impact of B1R stimulation on cardiac fibroblast (CF) physiology following chronic (in vivo) or acute (in vitro) AngII treatment. Adult male SD rats were infused with AngII (200 ng/kg/min) or saline for 4 weeks with osmotic minipumps. Isolated CFs from the LV of saline and AngII-treated rats were passaged to P1 then treated in vitro with AngII (1uM), a selective B1R agonist (Lys- [Des-Arg9], 100 nM), AngII+B1R agonist or vehicle for 48 hr. Transforming growth factor beta 1 (TGF-β1) and monocyte chemoattractant protein 1 (MCP-1) were assessed in culture media. Matricellular protein periostin and the pro-oxidant NADPH oxidase 2 (Nox2) levels were determined in cell lysate by western blot. CFs isolated from AngII-treated rats secreted more MCP-1 in vitro ($p<0.05$), compared to control CFs. Although MCP-1 secretion was not further modified by acute AngII treatment, B1R activation tended to reduce MCP-1. Chronic AngII did not alter TGF-β1 release from CFs. However, in vitro incubation with AngII significantly increased TGF-β1 secretion in all rats. B1R stimulation had no impact on TGF-β1 levels. Periostin tended to increase in CFs isolated from AngII infused rats, while Nox2 was not different. Acutely, AngII tended to increase Nox2 in vitro to a greater degree in CFs that were isolated from control rats. Selective B1R stimulation in vitro resulted in a significant 1.77-fold upregulation of Nox2 only in CFs isolated from control rats ($p<0.05$). Taken together, these data demonstrate that increased MCP-1 production and periostin induced by chronic AngII persist at least to P1 in isolated CFs. Moreover, chronic AngII resulted in an attenuation of increased Nox2 by acute AngII stimulation. Notably, we demonstrate for the first time that selective B1R activation markedly increases Nox2 in CFs. Although B1R antagonism has been shown to offset fibrotic and inflammatory effects of AngII in vivo, acute effects of AngII stimulation are not enhanced in the presence of B1R stimulation. Future studies will determine the extent to which in vivo or in vitro B1R antagonism prevents AngII-induced CF activation. Investigation of oxidative stress and Nox2 regulation by B1R activation may provide greater insight into the impact of bradykinin on CF physiology. *Funding: University of Arizona College of Medicine – Phoenix Springboard grant*

F15. Skeletal Muscle Transcriptome Response to Acute Interval and Continuous Exercise in Older Adults

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Exercise represents a powerful strategy to preserve skeletal muscle function with advancing age. However, less understood are the molecular mechanisms that may be targeted by different exercise strategies and intensities. The purpose of this study was to identify the unique transcriptional response of skeletal muscle in older adults after acute high intensity interval (HIIE) and moderate intensity continuous (MOD) cycling exercise. In a counter-balanced, cross over design, eight older adults (5M, 3F; 67±2yr; BMI: 26.0±1.8kg·m-2) completed a bout of HIIE (ten, 1-min intervals, 85-95% heart rate max, 1-min rest between intervals) and MOD cycling (30-min, 60-65% VO2peak), separated by ~1 week. Muscle biopsies (vastus lateralis) were obtained before exercise and at 4h after each exercise bout. Whole transcriptome next-generation sequencing was performed on cDNA synthesized from skeletal muscle RNA. Sequencing data were analyzed using HTSeq and differential gene expression from preexercise was identified using DESeq2. Relative to preexercise, HIIE increased expression of 247 genes and decreased expression of 42 genes, whereas MOD increased expression of 158 genes and decreased expression of 38 genes. While 55 genes were responsive to both HIIE and MOD, we identified 234 genes that were only responsive to acute HIIE and 149 genes that were only responsive to acute MOD. Several of the top differentially expressed genes uniquely responsive to HIIE included genes involved in muscle growth. These data highlight mutual and unique transcriptome responses of aging skeletal muscle to acute HIIE and MOD. These preliminary findings indicate that acute HIIE performed by older adults appears to elicit greater transcriptional activity compared to MOD, at least in the immediate hours post exercise, and that this greater transcriptional activity may include processes related to muscle growth. *Funding: Supported by a JumpStart grant, College of Health Solutions, ASU.*

F16. The effect of genistein and exercise on metabolic pathways in mice fed a high fat/high sucrose diet

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A westernized diet, which is composed of high fat and high sugar content, not only increases the risk of obesity but also increases the risk of individuals becoming prediabetic and potentially type 2 diabetic. Impairments of insulin signaling not only affect blood glucose levels but also cause numerous degenerative processes, including apoptosis, increased lipolysis, and depletion of glycogen stores. One of these impairments can be induced by corticosterone which is thought to exacerbate metabolic syndrome, furthering hyperinsulinemia and hyperglycemia. C57BL/6J mice aged5-6-weekswere randomly assigned mice to one of the following groups (n=8-10/group): lean control, high fat diet high sucrose (HFD), HFD+Gen, HFD+Ex, and HFD+Gen+Ex. The HFD consisted of 60% saturated fat, 20% carbohydrate, 20% protein and included sucrose and fructose in the drinking water. Exercise consisted of daily moderate treadmill running for a total duration of 150 minutes/week for 12 weeks. C57BL/6J mice fed HFD will exhibit phenotypes seen in T2DM pathology which has a direct impact on metabolic pathways such as gluconeogenesis. We have determined that serum glucose and insulin levels in males are rescued with HFD+Gen+Ex, compared to HFD. We are currently determining total protein expression levels of enzymatic proteins, such as 11βHSD1 (responsible for the conversion of corticosterone to its active form) and 11βHSD2 (responsible for the conversion of active cortisol to inactive cortisone), We predict that serum corticosterone levels will be elevated in mice fed a HFD diet. Assessments of metabolic pathways may lead to novel treatments or more importantly, address the protective role that genistein, and exercise may provide. *Funding: Layla Al-Nakkash, Tom Broderick and Jeffrey Plochocki were supported by Midwestern-Arizona Alzheimer's Consortium. Chaheyla St Aubin was supported by the Department of Biomedical Sciences, College of Health Sciences*

F17. Differences in lipid metabolism in high fat- high sucrose fed mice treated with genistein and exercise

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Intake of diets high in saturated fat and high overall fat with sucrose have been associated with chronic diseases including type 2 diabetes and Alzheimer's disease (AD). This study will vary diet and exercise and assess the changes in adipose metabolism by measuring serum markers, liver hepatosteatosis, and lipid metabolism in skeletal muscle . Male and female 5-6 week old C57BL/6J mice were randomly into one of the following groups for a study duration of 12 weeks (n=8-10/group): lean control (LN), high fat diet and sucrose (HFD), HFD with genistein (GEN), HFD with exercise (HFDX), HFD with genistein and exercise (GENX). Exercise consists of 150minutes/week of treadmill running. We predict that exercise and genistein will help to improve deficits in lipid metabolism associated with diabetes and obesity.

In males, body weight was 12% and 18% ($P<0.05$) lower in HFDX or GENX mice respectively compared to those fed HFD. Body weight was significantly 42% ($P<0.05$) lower in GENX males compared to those fed HFD. In females, body weight was unchanged by HFDX and decreased only 8% with GEN supplementation compared to those fed HFD. Body weight was significantly 16% ($P<0.05$) lower in the GENX females compared to those fed HFD. Weight loss was not attributed to reduced food intake. Glucose tolerance tests (GTT) in subgroups of mice (n=3-5/group) were performed. Rescue of serum glucose was noted in GENX males (not females), which reflected concomitant changes in serum insulin. We are currently determining total protein expression of adipose triglyceride lipase and carnitine palmitoyl transferase-b in skeletal muscle along with assessments of liver steatosis and serum T3. We aim to provide mechanistic evidence for sex-dependent effects and ultimately to demonstrate an association between genistein- and exercise-mediated improvements in diabetic obesity. *Funding: Layla Al-Nakkash and Tom Broderick were supported by Midwestern-Arizona Alzheimer's Consortium. Amy Fisher was supported by the Department of Biomedical Sciences, College of Health Sciences.*

F18. Relationships among skeletal muscle satellite cells, capillarization, and VO2peak in older adults

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A reduction in satellite cells has been reported to contribute to muscle loss with aging. Exercise presents a powerful strategy to stimulate satellite cells, however, to what extent various forms of exercise stimulate skeletal muscle satellite cells in older adults is less understood. The purpose of this study was to 1) examine relationships among satellite cell density, capillary density, and VO2peak in older adults, and 2) to identify changes in satellite cell density following two different intensities of aerobic exercise. In a counter-balanced, cross-over design, six older

adults (4M, 2F; 67±2yr; BMI: 26.6±2.0 kg·m-2) completed an acute bout of high intensity interval (HIIE; ten, 1-min intervals, 85-95% heart rate max, 1-min rest between intervals) and moderate intensity continuous cycling (MOD; 30-min, 60-65% VO2peak), separated by ~1 week. Muscle biopsies (vastus lateralis) were obtained before exercise and at 24h after each exercise bout. Immunofluorescence was utilized to identify fiber type, satellite cells, and capillaries. A significant relationship between baseline capillary density and baseline satellite cell density ($P=0.018$; $R2=0.7885$) was observed. Further analysis revealed significant correlations between baseline satellite cell density and VO2peak ($P<0.001$; $R2=0.9898$), capillary density and VO2peak ($P=0.019$; $R2=0.7854$), satellite cells/myosin heavy chain (MHC) I fiber and VO2peak ($P=0.026$; $R2=0.7508$), and satellite cells/MHC II fiber and VO2peak ($P=0.002$; $R2=0.93$). Total satellite cells/fiber and fiber type-specific SC/fiber were unchanged in response to acute MOD or HIIE ($P>0.05$) and no differences were observed between exercise trials ($P>0.05$). These data reveal a positive relationship between skeletal muscle capillary density and satellite cell density in older adults. Further, while no changes in satellite cell density were observed 24h following acute MOD or HIIE, our preliminary findings suggest an association between skeletal muscle satellite cell density and VO2peak in older adults. Thus, future research is needed to examine whether these exercise strategies differentially impact changes in the proliferation or differentiation of satellite cells in older adults, and to what extent capillary density may be related to chronic adaptations in satellite cell density and VO2peak. *Funding: Supported by a JumpStart grant, College of Health Solutions, ASU.*

F19. Understanding the Role of Caveolin-1 Protein (Cav1) in Marfan Syndrome-Associated Aortic Aneurysm

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Marfan Syndrome (MFS) is an autosomal dominant connective tissue disorder resulting from mutations in the Fibrillin-1 gene. MFS reveals several clinical manifestations, with the most life-threatening being aortic aneurysm which reportedly results from crosstalk between the Angiotensin-II (AngII) pathway and over activation of transforming growth factor-beta (TGF-β) signaling. Interestingly, inhibition of the AngII type 1 receptor delays aneurysmal growth, and it has been shown that Losartan, an angiotensin receptor blocker, is beneficial in treating MFS, partially due to its inhibitory effects on TGF-β expression. Studies have shown that caveolin-1 (Cav1), a coat protein of Caveolae regulates these pathways, and may pose a method to regulate this crosstalk in MFS-associated aortic aneurysm. Cav1 knockout animal models illustrate increased elastin synthesis and nitric oxide (NO) production. Previous studies in the MFS mouse model have reported reduced NO production in the aortic wall. Considering the important role that Cav1 plays in regulating the above pathways, elastin fiber structure and organization, and NO production, we aim to investigate the potential role of Cav1 protein during the progression of MFS-associated aortic aneurysm by generating MFS mice lacking Cav1 expression (MFS/Cav1 KO). This study will provide new insights on the role of Cav1 in pathogenesis of aortic root aneurysm. In vivo analysis of biophysical properties in 3 months old MFS/Cav1 KO mice was performed using Vevo 2100 high-resolution ultra sound imaging system (FUJIFILM VisualSonics). Diameter measurements included aortic annulus, sinus of Valsalva, and sinotubular junction, where MFS and MFS/CAV1KO had significantly greater diameters than control but were not significantly different from each other. Pulse wave velocity, an indicator of aortic wall stiffness, was significantly increased in MFS compared to control and significantly increased in MFS/Cav1KO compared to control and MFS. Cardiac parameters were measured and included cardiac output, stroke volume, heart rate, ejection fraction, fractional shortening, and left ventricle dimensions and filling velocities, where no significant differences were seen. Interestingly, left ventricular mass was significantly greater in MFS/CAV1KO compared to MFS and control mice. At this time, this study reveals information on the biophysical properties of genetic manipulation of Cav1 in MFS. Based on our preliminary observation, Cav 1 seems to have a protective role during the progression of aortic aneurysm in MFS mice with some beneficial effects on aortic wall elasticity and left ventricular function. *Funding: This study was funded by a Faculty Grant from The Marfan Foundation and Midwestern University Graduate Fund.*

F20. The role of deep breaths in vocal production

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Peripheral vocal motor dynamics describes how complex interactions of laryngeal, respiratory and vocal tract movements generate and shape vocal sounds in mammals. Glottal airflow provides the energy, and laryngeal movements set important boundary conditions. The integration of laryngeal and breathing movements can be

investigated through simultaneous recordings of subglottal pressure and electromyographic (EMG) activity of laryngeal muscles. Laboratory rats allow the comprehensive recording of vocal movements in awake and spontaneously behaving animals. We studied the role of augmented breaths (aka 'sighs' or 'deep breaths') during vocal production. Sighs were characterized by a two-phase deep inhalation followed by a longer than average exhalation. From 10 Sprague-Dawley rats we recorded 1757 augmented breaths during sleep, resting and during social interactions. The individual sigh rates ranged between 14.9 and 61.2 sighs per hour. During 13% of the sighs, animals produced vocalization during the extended exhalation immediately following the deep inhalation. We found single calls and multiple calls during the extended exhalation. The data suggest much less stereotypy in the vocal motor patterns of a nonhuman mammals than previously assumed.

F21. Impact of Prenatal Dexamethasone on Plasma Cytokines in Neonatal and Adult Rats

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Dexamethasone (DEX) is a synthetic glucocorticoid administered to mothers at risk for pre-term labor. Current research suggests DEX administered in utero confers a pro-inflammatory state that contributes to the development of chronic disease, like obesity, in adult offspring. However, there is limited information regarding the longitudinal impact of in utero DEX exposure on the immune system. In the present study, we determined the plasma levels of 20 cytokines in male and female neonatal and adult rats that had been exposed to DEX in utero. Pregnant dams were administered DEX (0.4mg/kg or 0.1mg/kg per day, s.c.) or vehicle on gestation days 18-21. A subset of rats was sacrificed at PND0 and remaining rats were weaned at day 21 and sacrificed at 2.5 – 3 months of age for measurement of plasma cytokine levels. DEX reduced plasma Il-1β in males but not females at PND0, but increased MCP-1 and IP-10 in both males and females. However, differences in these neonatal inflammatory markers did not persist into adulthood. Specifically, DEX reduced the pro-inflammatory markers MCP-1, Il-2, Il-17α, Il-18, and TNFα in adult males, but not females. Further studies will determine the long-term consequence of age- and sex-related changes of DEX on immune function. *Funding: ABRC ADHS14-082990*

F22. Can Intermittent Restraint Stress Be Used to Alter Hippocampal Plasticity, as Measured by Dendritic Complexity?

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Much research has demonstrated that chronic stress leads to poor spatial learning and memory, a function requiring the hippocampus (Kim et al 2015; Conrad, 2010). Moreover, when spatial memory deficits are observed, a parallel change in hippocampal morphological restructuring occurs (Kim et al 2015). In our lab, we assess the apical dendritic complexity of hippocampal CA3 neurons because these neurons become simplified at the same time that spatial memory deficits are observed (Conrad et al., 2017). While rodents can be stressed through different means (Campos et al 2013), our lab uses daily restraint (6 hours/day for 21 days) because it has been shown to more consistently cause dendritic retraction and spatial memory deficits compared to other models (McLaughlin et al., 2007). Interestingly, a study by Zhang and Colleagues (2014) reported that restraint for three hours could be more robust on stress responses and anxiety profile when the chronic stressor is administered for five days, followed by two days off for the weekend, and then resuming for two more days. They suggest that a "workweek" schedule may produce a higher stress response and anxiety due to intermittent nature of the stressor. However, it is unclear whether an intermittent stressor for a longer duration (3 weeks) would be more robust than a daily stressor for a comparable time. Moreover, Zhang and colleagues (2014) assessed anxiety profile and so it is unknown as to whether an intermittent stressor would impact spatial ability. Finally, our past work found that daily restraint for 2 hrs/d/21days or 6hrs/d/10 days failed to impair spatial memory and alter apical CA3 dendritic complexity (McLaughlin et al., 2007). Consequently, the goal of the present study was to determine whether chronic restraint, given intermittently with five days of restraint, followed by two days off (for three weeks) would produce spatial memory deficits and CA3 dendritic atrophy. In the present study, Sprague-Dawley male adult rats were used and assigned to four groups (n=12/group): control, daily restraint (6h/d/21d = DR6), intermittent restraint for 2h/d (IR2), and intermittent restraint for 6h/d (IR6). Spatial memory was assessed using various tasks over seven days and then brains were collected on the eight day after restraint ended. If the intermittent restraint is more robust than daily restraint, then we expect to find greater CA3 apical dendritic retraction than compared to daily restraint. The data are

still in the process of being quantified and so we will present the current status with predicted and alternative outcomes and the potential implications. *Funding: College of Liberal Arts and Sciences*

F23. Cardiometabolic changes During the Hormonal Transition of a Male-To-Female Athlete: a Case Study

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The cardiovascular consequences of female sex hormone exposure on human male biology is currently unknown. This level of investigation is critical given the potential adverse outcomes reported in rodent models. The present case study aimed to comprehensively assess the cardiovascular phenotype before and during estrogen treatment for gender reassignment. This is the case of a biologically male, Caucasian distance runner (28 yr) undergoing male-to-female gender reassignment. The subject had completed the social male-to-female transition prior to testing and two baseline assessments were made prior to the initiation of hormone treatment. Testing following the initiation of estrogen treatment took place at 4-8 week intervals depending on the subject's availability. Testing included resting echocardiography for assessment of biventricular function, dual energy x-ray absorptiometry (DXA; for body composition), and central vascular blood pressures and stiffness assessments. Treadmill-based VO2 peak and running economy, as well as non-invasive cardiac output and a-VO2 at rest and at peak exercise were also quantified at each visit. Throughout the first 12 months of treatment, stroke volume decreased (135.6 to 80.1 ml/beat) with an initial reduction in peak heart rate (ranged 188 to 180 bpm). Consequently, peak cardiac output declined (28.37 L/min to 15.55 L/min) while a-VO2 difference increased (11.6 to 19.9 ml O2/100 ml blood). This resulted in only a relatively minor decrease in absolute VO2 (3.3 to 3.1 L/min). Ejection fraction (calculated using modified Simpson's method via echocardiogram) decreased (61% to 57%) along with left ventricular diastology (mitral valve E/e 5.98 to 4.1). Right Ventricular Fractional Area change did not change (53% to 53%), while measures of right heart diastology increased (tricuspid valve E/e 3.93 to 4.6). Both right ventricular (RV) and left ventricular (LV) strain initially improved with the addition of estrogen, before worsening over the course of hormone treatment (RV strain ranged from -36 to -31.5%; LV strain ranged from -23.5 to -19%). In summation, therapeutic estrogen administration and testosterone blockade may adversely affect cardiopulmonary fitness mainly via reduction in myocardial performance at peak exercise, while also being associated with a worsening of LV and RV strain at rest. More research is to examine the long-term consequences of gender reassignment therapy on cardiovascular function. *Funding: Subsidized Student Research*

F24. Hot and Dry: Effects of heat waves and water limitation on metabolic and evaporative water loss rates

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Environmental temperature is important because it influences a range of animal processes, including behavior, energy use, locomotion, and reproduction. In addition to warming (increasing mean temperature), environments are expected to continue to exhibit an increased frequency of extreme temperature events, such as heat waves. Some animals can respond to heat waves by reducing their metabolic rates (after controlling for test temperature) to conserve energy. Heat waves often coincide with reduced water availability (e.g., drought), and water is critical to homeostasis. Therefore, a combined heat wave and drought may reduce rates of both energy and water use. To investigate, we employed a 2 x 2 factorial manipulation of temperature (field-parameterized heat wave vs. control diel regimes) and water availability (ad libitum vs. absent) in fasted variable field crickets (*Gryllus lineaticeps*). After 4 days of treatment, we used flow-through respirometry to estimate metabolic rate (VCO2) and evaporative water loss rates at 28°C. We will discuss whether temperature regime and water availability exhibit additive, synergistic, or antagonistic effects on rates of metabolism and water loss. Together, our results will provide new insight into the effects of shifts in co-varying environmental factors (e.g., combined heat wave and drought) on animals' water and energy budgets. *Funding: Pacific Fund*

F25. Mechanisms of metabolic scaling in California Seed Harvester Ants (*Pogonomyrmex californicus*)

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In social insect colonies, metabolic rates have been found to scale hypometrically with their mass. This relationship at the colony level is called social scaling. We hypothesized the social scaling relation of metabolism was mainly

determined by the maturation of sociality, which was estimated by the complexity in the colony, e.g. division of labor index. It has been known that a social insect colony newly founded would take time to grow into matured with the feature of division of labors. Therefore, we tracked changes of metabolic rate, mass and division of labor ontogenetically on 20 seeds harvester ant (Pogonomyrmex californicus) colonies founded in 2017 for 8 months. We found it was feasible to predict metabolic budget of colonies based on the task performances of ant workers and the proportion of broods. Also, during the ontogeny, metabolic cost of ant workers was driven by two factors: 1. The proportion of brood vs matured ant workers. Specifically, colonies with a higher relative proportion of broods tend to recruit ants to work harder; 2. The task allocation of ant workers. It means the proportion of tasks with relatively low/high metabolic cost could dampen down/rise up the energy budget of whole colonies. Those results suggest that hypometric scaling in the social insect colonies are mainly caused by the declination of relative broods' proportion and percentage of tasks with high metabolic cost during ontogeny. *Funding: NSF 1558127*

F26. Effect of Angiotensin II and Bradykinin Receptor Antagonism on Reactive Oxygen Species Enzyme Expression

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We have demonstrated that bradykinin B1 receptor antagonist, R-954, treatment attenuates macrophage infiltration and collagen deposition in the left ventricle of angiotensin-II infused Sprague Dawley rats. It has also be shown that R-954 reduces inflammation in a model of renal ischemia reperfusion injury. Cardiac and renal fibrosis and inflammation have been shown to be mediated, in part, by increased oxidative stress. The present study evaluates the extent to which R-954 altered expression levels of pro- and anti-oxidant proteins in the left ventricle and kidney of angiotensin II-infused rats. Adult male Sprague Dawley Rats received a four-week treatment of either saline or Ang-II (200 ng/kg per min, s.c.), R-954 (R-954, 400 µg/kg per day, s.c.), or combined R-954+Ang-II. Kidneys and hearts were harvested, and protein expression of oxidant producing (NOX2) and scavenging (SOD-1, SOD-2, catalase) enzymes was measured by Western Blot. In the heart, treatment with AngII tended to decrease the levels of the scavenging protein SOD-1 and this effect was offset by concomitant R-954 treatment. Treatment had no notable effect on the levels of NOX2, SOD2, or catalase in the heart. In the kidneys SOD2 levels were decreased in AngII treated rats and this was not affected by R-954. NOX2, SOD1, and catalase levels were unchanged by any treatment. Taken together, these results suggest that AngII reduces the capacity for scavenging of reactive oxygen species in both the heart and the kidney, thereby resulting in increased oxidative stress. These changes appear to be mediated, at least in the heart, through the bradykinin B1 receptor. *Funding: APS Undergraduate Summer Research Fellowship*

F27. Assessment of novel VDR antagonists that mediate suppression of vitamin D signaling

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Antagonists are chemical compounds that bind to receptors without eliciting activation of signaling, therefore the biochemical response to the receptor ligand can be blocked or significantly decreased. Receptor antagonists display varying levels of binding affinity but do not produce the agonist-mediated response upon binding. The vitamin D receptor (VDR) binds with high affinity to its endocrine agonist, 1,25-dihydroxyvitamin D3 (1,25D) to regulate the expression of a suite of genes in target tissues including kidney, intestine, and bone to control biological processes such as calcium and phosphate bone mineral homeostasis. In conditions of VDR hyperactivity, excess vitamin D production, or significantly increased intake via diet or supplementation, there are risks associated with potential resultant hypercalcemia including kidney stones, GI dysfunction, behavioral changes such as depression, and cardiac problems. In the present study, we synthesized novel VDR antagonists to create synthetic ligands that have high affinity for VDR, and consequently inhibit the activation of 1,25D-VDR regulated genes that lead to hypercalcemia. It is well established that agonist-bound VDR forms a functional DNA-binding heterodimer with the retinoid X receptor (RXR) that associates with vitamin D responsive elements (VDREs) to either induce or repress transcription. Therefore, we exploited this agonist-dependent VDR-RXR heterodimerization pathway as an initial screening assay to evaluate the efficacy of our putative VDR antagonists. The initial test employed the mammalian two-hybrid assay (M2H), which can assess if the novel antagonists compete with 1,25D and bind to VDR to prevent the formation of a heterodimer with RXR, a prerequisite step in VDR signaling. A second assay utilized VDRE-linked luciferase reporter plasmids to measure antagonist activity in the more natural environment of the VDRE DNA platform. This assay was followed by a third screen to determine the specificity of antagonist binding to VDR

versus other closely related receptors in the nuclear receptor superfamily. Results from these assays revealed that two novel analogs bind as bona fide antagonists to the VDR, albeit with different affinity. Our findings also suggest that additional chemical modification of antagonists may result in analogs with enhanced ability to suppress 1,25D-VDR activation. Thus, this study further broadens our understanding of VDR-ligand binding interactions, and may facilitate the development of novel therapeutic compounds with the potential for clinical applications in treating VDR-directed hypercalcemia. *Funding: School of Mathematical and Natural Sciences at Arizona State University*

F28. Characterization of the Aortic Function & Structure in the Human Apolipoprotein E Mouse Model

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Apolipoprotein E (ApoE) is a multifunctional protein with three isoforms (epsilon 2, 3, and 4) that correspond with diverse functional and pathological manifestations. Most notably, the epsilon 4 allele has been associated with an increased risk of cardiovascular disease and is the most prominent risk factor for the development of Alzheimer disease (AD). AD is a progressive neurodegenerative disease and the leading cause of dementia. Although research in AD has been ongoing, the pathogenesis of the disease remains elusive. The discovery of vascular abnormalities in AD patients and the association of ApoE epsilon 4 with cardiovascular disease has led to the hypothesis that the condition may initially arise from a vascular abnormality. However, little is known about the pathological consequences associated with the apolipoprotein isoforms in major peripheral arteries, such as the aorta. In this study, we aimed to characterize the functional (vasoconstriction and vasodilation) and structural (aortic wall elasticity and integrity) characteristics of the thoracic aorta in wild type C57BL/6, targeted replacement ApoE epsilon 3, and targeted replacement ApoE epsilon 4 mice, at 12 and 18 months of age, using small chamber myography. Our data showed a significant drop in phenylephrine (PE)-induced contraction in aortic segments isolated from 12-months old ApoE epsilon 4 mice as compared to control C57BL/6 mice, with no differences observed between the groups at 18 months of age. A significant increase in EC50 for PE was also observed in 12-month old APOE epsilon 4 mice. There was no evident difference in acetylcholine-induced relaxation between experimental groups at 12 and 18 months of age. In addition, the stretch-induced wall stress and reversibility of elasticity were significantly reduced in aortic segments isolated from ApoE epsilon 4 mice indicating an increase in aortic wall stiffness and reduced wall integrity in ApoE epsilon 4 mice. This study provides the first preliminary evidence of peripheral vascular dysfunction in the well-established mouse model of AD (ApoE epsilon 4). *Funding: Midwestern University Graduate Funds*

F29. Opening for common goals: the transient (calcium) connection of TRP and Cx channels

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We are currently exploring a possible functional link between Connexin (Cx) and Transient Receptor Potential (TRP) channels. The Cxs are membrane proteins best known for making gap junction channels (GJCh, cell-to-cell conduits permeable to small cytoplasmic molecules) that support tissue coordination. With moderate increases of cytoplasmic Ca2+ ([Ca2+]i), undocked half-GJCh or hemichannels (HCh) may open in the cell membrane and support diffusive exchange between intra- and extracellular spaces. Cx isotypes are present in essentially all vertebrate cells, but they are particularly important for nutrition, waste disposal and cell signaling in avascular tissues such as the cornea and eye lens. Cx43 and Cx50 are found in the latter. Another numerous and ubiquitous protein family forms TRP channels, which mediate a variety of sensations (e.g. pain, pressure, temperature, flavors), can be stimulated by natural chemicals (e.g., capsaicin, menthol, camphor, pH) and are relatively non-selective cation (Na+, Ca2+, Mg2+) permeable. TRP Vanilloid 4 (TRPV4) channels have been implicated in osmotic control. Mouse lens epithelium exposed to hyposmotic solutions displays [Ca2+]i increase, ATP release and propidium iodide (PI) uptake. These responses are also induced or enhanced by TRPV4 agonists and prevented by TRPV4 antagonists. Because TRPV4 channels are cation selective, ATP release must occur through other structures, for instance, Cx43 HChs, which can mediate both ATP release and PI uptake. Using single electrode whole cell patch-clamp in primary cultures of lens epithelium, we found that depolarized cells displayed membrane current (Im) transitions of variable amplitude (range: 30 to 300 pS) compatible with Cx43 HCh activity. The frequency and recurrence of these presumptive HCh events were variable and not consistently modulated by GSK1016790A, a TRPV4 agonist. However, this agonist induced an overall increase in cell membrane permeability, as revealed by larger macroscopic Im. Intriguingly, such (TRPV4-associated) currents were greatly enhanced with

hyperpolarization and inactivated with depolarization. During inactivation, Im transitions indicating channel closures can be readily observed. These data suggest that, in physiological conditions, TRPV4-dependent [Ca2+]i fluctuations regulates the gating of Cx HChs. This illustrates how the opening of different channels types intersects for coordinated cell function. *Funding: NIH, HLBI Grants HL058732 and HL131712; NIH, Grant number EY006915*

F30. Examination of an Organometallic Complex on Insulin Resistance in Adolescent Male Rats Following a 10-week High Fat Diet

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With the rising prevalence of obesity and diabetes, novel treatments to help mitigate or prevent symptoms of these conditions are warranted. Prior studies have shown that fossilized plant materials found in soil lowers blood sugar in a mouse model of diabetes. The goal of this study is to determine whether a similar organometallic complex (OMC) could prevent insulin resistance in the skeletal muscle brought on by chronic high fat intake by examining the protein expression of key enzymes in the insulin signaling pathway and examining glucoregulatory measures. Six-week-old periadolescent male Sprague-Dawley rats (n=12) were randomly chosen to be fed either a high fat diet (HFD) (20% protein, 20% carbohydrates [6.8% sucrose], 60% fat) or a standard chow diet (18.9% protein, 57.33% carbohydrates, 5% fat) for 10 weeks. Rats from each diet group were then randomly assigned to one of three doses of OMC (0, 0.6, 3.0 mg/mL), which was added to their drinking water and fasting blood glucose was measured at baseline and again at 10 weeks. After 10 weeks, rats were euthanized, and soleus muscle samples were isolated, snap-frozen, and stored at -80°C until analyses. Fasting plasma glucose was measured using a commercially available glucose oxidase kit. Following 6 and 10 weeks, HFD rats developed significant hyperglycemia ($p<0.001$ and $p=0.025$) compared to chow controls which was prevented by high dose OMC ($p=0.021$). After 10 weeks, there were significant differences in fasting serum insulin between diets ($p=0.009$) where levels were higher in HFD rats. These results suggest that OMC could prevent insulin resistance by reducing hyperglycemia. Further studies are needed to characterize the effects of diet and OMC on the insulin signaling pathway in skeletal muscle, the main site of postprandial glucose disposal. *Funding: Isagenix International LLC*