2025 7th Annual Meeting Midlands Society of Physiological Sciences

Platte River Room, Nebraska Union,
University of Nebraska-Lincoln
Lincoln, Nebraska
October 10-11, 2025

MSPS

UNIVERSITY OF Nebraska® Lincoln

Dear MSPS Community,

On behalf of the Midlands Society of Physiological Sciences (MSPS) Council, I am delighted to officially welcome you to the **7th Annual Meeting of MSPS**, to be held **October 10–11, 2025**, at the **Nebraska Union, City Campus, University of Nebraska–Lincoln**. We are thrilled to have many of our members from across **Nebraska and South Dakota** joining us for this exciting event.

As an official chapter of the **American Physiological Society (APS)**, MSPS is dedicated to fostering advancements in physiology-related research, education, and scientific communication. Our mission is to promote collaboration among members and institutions, provide high-quality scientific training, and enhance public awareness of the importance of physiology. The annual meeting represents a cornerstone of these efforts.

This year's meeting will highlight **multidisciplinary and translational approaches** that extend beyond traditional physiology. We are honored to host a **keynote lecture** alongside **three featured local faculty presentations** exploring topics such as fetal programming of cardiac health, molecular nutrition, environmental dust exposure and lung injury, and educational physiology.

We are pleased to announce that we received **62 abstract submissions** this year. From these, **8 have been selected for oral presentations**, with the remaining abstracts showcased as **poster presentations**. In addition, we are introducing an **innovative flash talk session**—a fast-paced format where presenters deliver a three-minute summary of their research with no Q&A. This session will feature **15 flash talks** by high school, undergraduate, graduate, and postdoctoral trainees from our member laboratories, fostering communication skills and professional networking among the next generation of physiologists.

We extend our heartfelt gratitude to everyone who has made this meeting possible—our **volunteers**, **abstract and poster judges**, **invited speakers**, and **institutional and industry partners**. We especially thank the **American Physiological Society**, **Office of Research & Innovation–UNL**, **College of Education & Human Sciences–UNL**, **Department of Nutrition & Health Sciences–UNL**, **College of Medicine–UNMC**, **Department of Pharmacology & Neurosciences–Creighton University**, **Center for Heart & Vascular Research–UNMC**, **Frontiers in Physiology**, and our industry sponsors **Avantor** and **Fisher Scientific** for their generous support.

Thank you to all participants for contributing to the success of the 7th Annual MSPS Meeting. I wish you a productive and inspiring experience filled with engaging presentations, meaningful networking, and potential collaborations.

Sincerely,

Sathish Kumar Natarajan

Sathish Kumar Natarajan, Ph.D.
President, Midlands Society of Physiological Sciences
On behalf of the 2025 MSPS Council:
William Chen (President-Elect), Hong Zheng (Past President), Surabhi Chandra (Secretary), Harold Schultz (Treasurer), Ivan Vechetti (Councilor), Cassandra Hays (Councilor), Jee-Yeon Hwang (Councilor), Amir Abazarikia (Postdoctoral Councilor), Flobater Gawargi (Postdoctoral Councilor), and Colman Freel (Graduate Student Councilor).

The 7th Annual Meeting for Midlands Society of Physiological Sciences (MSPS)
University of Nebraska-Lincoln (UNL)
PROGRAM AGENDA

DAY ONE: October 10, 2025

3:00 – 3:40 PM	Registration in **Platte River Room, Nebraska Union, UNL**
3:40 - 4:00 PM	Welcome address by Sathish Kumar Natarajan PhD President of MSPS, **Jeff Reese PhD,** Dean, College of Education & Human Sciences, UNL **Heather Rasmussen PhD**, Chair, Department of Nutrition & Health Sciences
4:00 – 5:00 PM	**Flash Talks by trainees in Platte River Room, City Union**, UNL *Moderators: Surabhi Chandra PhD, Colman Freel BS, Flobater Gawargi PhD, Amirhosssein Abarzarikia PhD* People's Choice Award: All Participants vote via QR Code; Judge for Best Flash Talk Award: Moderators are judges
5:00 - 5:30 PM	Coffee Break
5:30 – 8:00 PM	**Session 1 presentations:** *Chairs: Jee-Yeon Hwang PhD, Chandan Krishnamoorthy MTech*
5:30 – 6:00 PM	Faculty presentation: **Tomasz Bednarski PhD**, (University of Nebraska) *The effect of T4 and adiponectin receptor agonist supplementation on the left ventricular lipid metabolism. Chair: Jee-Yeon Hwang, PhD*
6:00 – 6:20 PM	*Trainee Talk:* **Md Salim Ahammed**, University of South Dakota. Cardiomyocyte Specific Knockout of p62/SQSTM1 Exacerbates CryABR120G-based Cardiac Proteinopathy in Mice
6:20 – 6:40 PM	*Trainee Talk:* **Anna Kosmach**, University of Nebraska Medical Center. Studying the thoracic aorta using pressure myography: technique and functional characterization
6:40 – 7:00 PM	*Trainee Talk:* **Uma Maheswari Deshetty PhD**, University of Nebraska Medical Center, Protective effect of a small molecule, C381 in attenuating HIV Tat-induced microglial activation and neuroinflammation.
7:00 – 8:30 PM	Dinner and Poster Set up in NU Ballroom located at the Second Level of City Union, UNL.

****

DAY TWO:

October 11, 2025

9:00 – 9:30 AM	Registration / Breakfast in **Platte River Room, Nebraska Union, UNL**
9:30 – 9:45 AM	Welcome / Opening Remarks for day 2: Sathish Kumar Natarajan, UNL
9:45 – 10:45 AM	***Keynote***: **Perrie O'Tierney-Ginn PhD** (Tufts University). *The Placenta-heart connection: Impact of the maternal metabolic milieu.* *Chair: Sathish Kumar Natarajan, PhD*

The 7th Annual Meeting for Midlands Society of Physiological Sciences (MSPS), University of Nebraska-Lincoln (UNL)
PROGRAM AGENDA (continued)

Session #2:

10:45 –11:15 AM Faculty presentation: **Tara Nordgren PhD**, University of Nebraska Medical Center. *Dust and Diet: Environmental factors impacting inflammation and lung health. Chair: William Chen PhD*

11:15 –11:30 AM Coffee Break and vendor table discussions

11:30 – 12:30 PM Session 2 Trainee presentations:
Chairs: Ivan Vechetti PhD, and Amirhosssein Abarzarikia PhD

11:30 –11:50 AM *Trainee Talk:* **Colman Freel** BS, University of Nebraska Medical Center. Duration and Severity of Maternal Hyperglycemia Drive Fetal Endothelial Injury and Newborn Blood Pressure Elevation

11:50 –12:10 PM *Trainee Talk:* **Amin Foroughi-Nezhad**, University of Nebraska Medical Center. Chronic In Utero Oxycodone Exposure Alters Placental EV Proteome and Fetal Cardiomyopathy-Linked Pathways

12:10 –12:30 AM *Trainee Talk:* **Bunmi Owolabi**, University of Nebraska Medical Center, Metabolic and Mitochondrial Dysfunction in Human Granulosa Cells from poor Responders.

12:30 –01:00 PM **Lunch Break and Poster Session in NU Ballroom,** located at the Second Level of Nebraska Union.

01:00 – 02:00 PM Poster Presentation (Odd Number Posters)

02:00 – 03:00 PM Poster Presentation (Even Number Posters)

Best Poster Award Judges: Attending faculty and Council members

Session #3 in Platte River Room, Ground Level, Nebraska Union, UNL:

03:00 – 03:30 PM Faculty Presentation. **Cassandra Hays PhD** (Creighton University)
In sync with students: Making physiology matter to modern medical trainees. Chair: Hong Zheng PhD

3:30 – 04:10 PM Session 3 Trainee presentations:
Chairs: So-Youn Kim PhD, Flobater Gawargi, PhD

03:30 – 03:50 PM *Trainee Talk:* **Ryan Antony**, University of Nebraska Medical Center. Leg dysfunction and synaptic degradation in a mouse model of peripheral artery disease

03:50 – 04:10 PM *Trainee Talk:* **Darshan Jadhav**, University of South Dakota. Single-Cell Transcriptomics Reveals Progressive Proximal Tubule Reprogramming in Diabetes

04:10 – 04:30 PM **MSPS Business Meeting** and Presentation of Awards

04:30 PM Thank you notes, and Closing Remarks

- Best poster presentation will be selected from poster presentations
- Best Flash talk and people's choice will be selected from flash talk presentations

Greetings!

This is a pre-annual meeting email to all registered participants for the 7th Annual Meeting for Midlands Society of Physiological Sciences. Registrants who are external to the University of Nebraska (NU) must be age 19 or older to attend the Symposium. All MSPS members and students are eligible to register to attend.

Please review this email for information about logistics, parking, and the annual meeting schedule.

Overview
7th Annual Meeting for Midlands Society of Physiological Sciences starts on Friday, October 10, 2025. Registration check-in and continental breakfast is 3:00-3:30 PM at the Nebraska Union, 1400 R Street, Lincoln, NE 68588. Look for us outside the Platte River Room located on the first floor, use KIOSK in the Nebraska Union to find Platte River Room, if you enter Nebraska Union from 1400 R street, Platte River Room is at the right side after front entrance. There is often a rush toward the end of the check-in, so please aim to check in early if possible and be on time for our student's flash talks. Please note that presentations will take place on the first floor of the Nebraska Union, with talks in the Platte River, poster session, Friday dinner and Saturday lunch will take place in NU Ballroom located on the second floor of Nebraska Union, If you take stairs right outside Platte River Room to second level, NU Ballroom close to Chimney Rock Room.

Our 7th annual meeting of MSPS officially begins with a welcome from organizers at 3:40 PM on the University of Nebraska-Lincoln campus at the Nebraska Union in the Auditorium. The Nebraska Union is at the corner of 14th and R streets. Flash talks will start at 4:00 PM and talks from invited guests will take place from 5:30 PM. Please see our MSPS website for updated information on program agenda. https://msps-online.org/msps-2023-2/

For a map of the area with the Nebraska Union and parking options, use the following link: https://www.google.com/maps/d/u/0/edit?mid=10sufLFJryrL3f8CYFwJPcYc3dHaNUDw&usp=sharing

Parking:

Attendees from External Institutions

- Consult the map listed above for the location of two public parking lots which are located closest to the Nebraska Union. These lots require on site payment of a fee which you will need to be prepared to pay in order to park.

Attendees from University of Nebraska Campuses (UNO, UNK, UNMC)

- If you are driving a state vehicle, you are allowed to park on campus in an area “A” parking lot without any special permit. State and government vehicles may not park in reserved parking areas.
- If you will be driving a personal vehicle. Please contact your campus' parking services office to receive a reciprocal permit that will allow you to park in an area “A” permit UNL parking lot. Vehicles may not park in reserved parking areas.
- Consult the map listed above for the closest area “A” permit parking lot.

UNO: For UNO permit holders email UNO Parking and Transit Services (unoparking@unomaha.edu).
UNMC: For UNMC permit holders email UNMC Parking Services (parking@unmc.edu).
UNK: For UNK permit holders email UNK PD Parking Division (parking@unk.edu).

Nebraska Union is in the heart of UNL city campus. Address: 1400 R St, Lincoln, NE 68588

UNL Permit Holder Reciprocal Parking

Parking permits from UNK, UNO, UNMC and Nebraska Innovation Campus may be used on the UNL Campus for academic and business related activities; all other uses are prohibited unless authorized by Parking and Transit Services. University of Nebraska faculty, staff and students who are primarily assigned to UNL must purchase a permit from UNL Parking and Transit Services and may not use permits from other campuses.

Regularly priced UNL parking permits (A, C, F and Garage) may be used on other University of Nebraska campuses. Be familiar with each campus's reciprocal parking regulations before parking. Contact Parking and Transit Services to determine eligibility.

With the implementation of the virtual permit system License Plate Recognition, UNL permit holders must request and display a permit validation sign for parking at UNMC and UNK campuses. The sign verifies License Plate Recognition System participation. It is available at no cost upon request. Permit holders can request and receive the sign, and display for all visits to these campuses for the effective period of the actual permit. Permit holders must contact Parking and Transit Services at unlpts@unl.edu from their UNL email accounts or come to the office at 625 Stadium Drive to receive a permit sign.

To park on the UNO and Nebraska Innovation campuses, UNL permit holders must notify Parking and Transit Services and request their permit be added to the reciprocal visitors list. Requests must be made from official UNL email accounts to unlpts@unl.edu

UNO and NIC campuses are notified and your license is added to their permit roster. Access remains for the effective period of the UNL permit.

https://parking.unl.edu/parking/other-permits/#reciprocal-parking

City campus parking map

https://parking.unl.edu/maps/parking-maps/#pdf-maps

https://maps.unl.edu/parking

Check in and Annual Meeting Materials:
At check in, you will receive the printed schedule, a listing of the poster titles and presenters, and guest Wi-Fi information. Complete list of program abstracts will be available on our MSPS website.

Food/Dietary:
During the Annual Meeting, Friday, dinner buffet, continental breakfast and lunch buffet, as well as snacks during the poster session will be provided. Coffee, tea, and water will be available during the beverage break and at the specified mealtimes. Please let me know as soon as possible If you have any dietary restrictions, we will have catering staff to provide you with a food option that fits your needs. You will need to check with the catering staff if you have questions regarding which foods will meet your needs.

Poster Presentations:
The poster session will take place 1:00-3:00 PM in the NU Ballroom (Nebraska Union 2nd floor). Poster presenters have been emailed guidelines for preparing, printing, and hanging their posters. If you submitted a poster but have not received this detail from meeting hands and please read the entire email including notes section, or if you have other questions regarding your poster, please contact snatarajan2@unl.edu

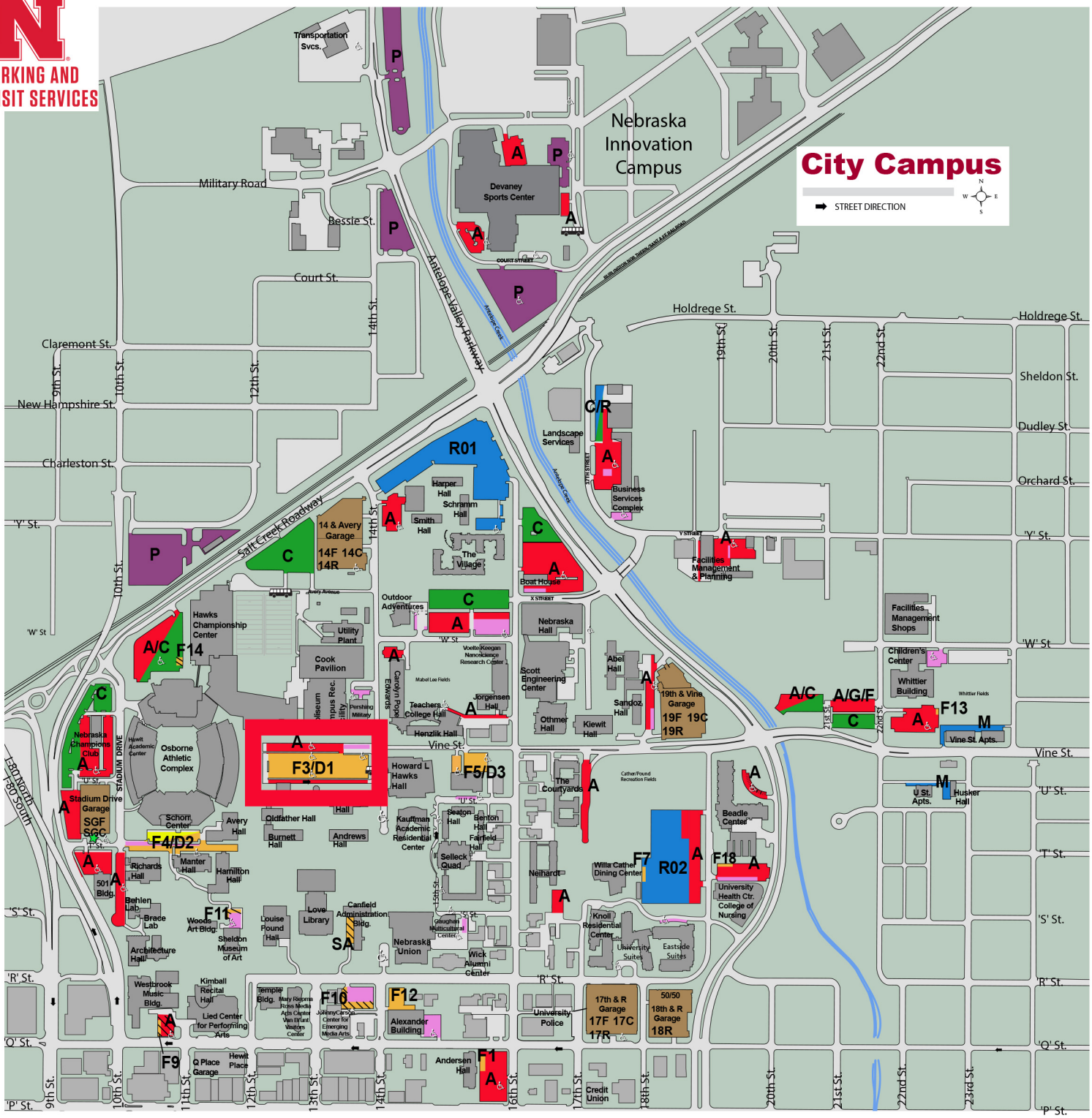
We look forward to seeing you at the 7th Annual Meeting of MSPS!

Sincerely,

Sathish Kumar Natarajan

President of MSPS.

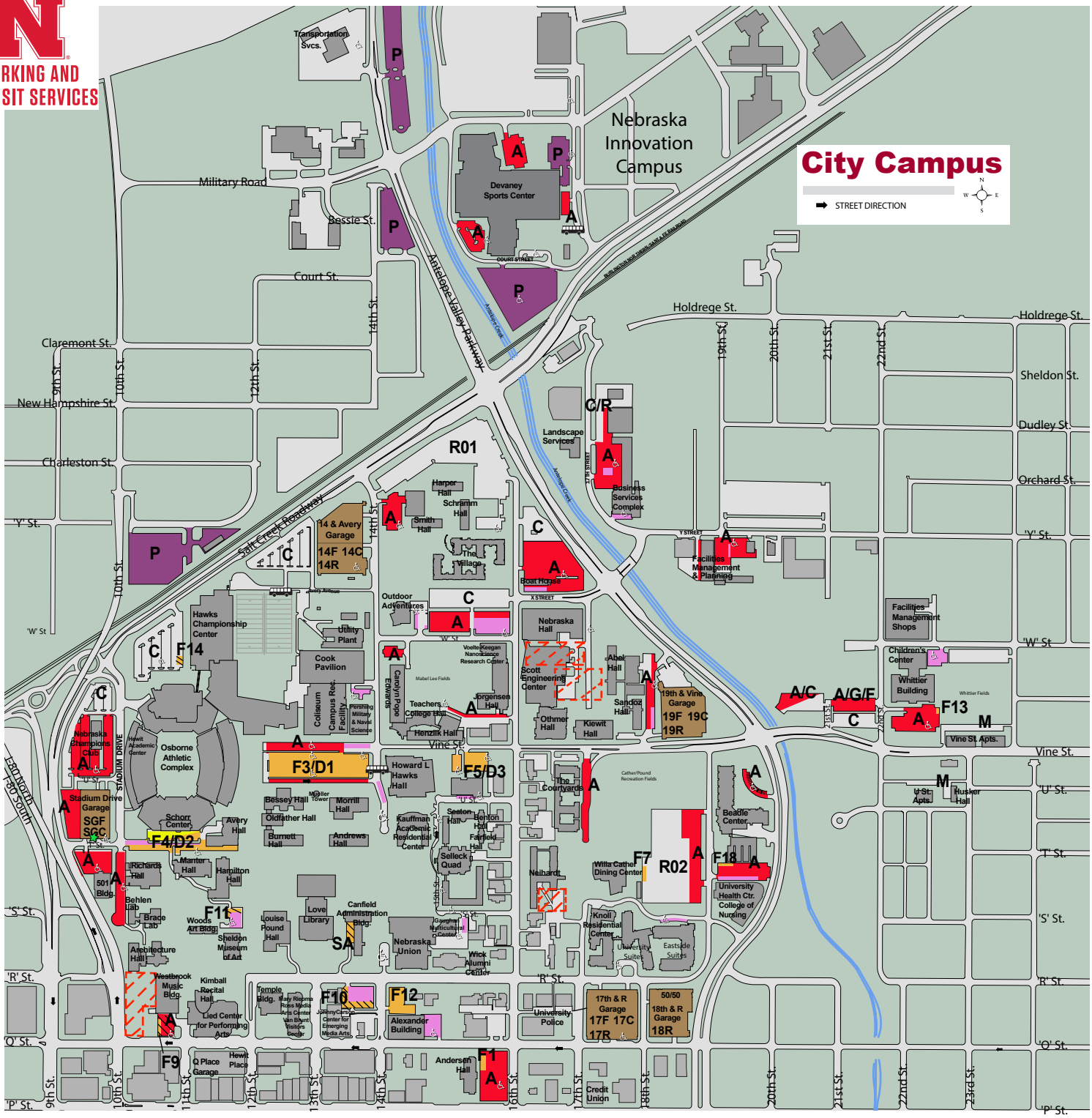
PARKING AND TRANSIT SERVICES



This version of the University of Nebraska parking map is intended for web distribution only. Further information, including parking regulations, permit information, etc.., is available at Parking and Transit Services website, http://parking.unl.edu. For additional questions please contact Parking & Transit Services by email at unlpts@unl.edu or telephone: at 402-472-1800.

Current as of August, 2025

PARKING AND TRANSIT SERVICES



Faculty/Staff Parking (A)

Reserved Faculty/Staff Parking (F#)

Faculty/Staff Parking (time restricted)
See Lot Entrance Sign for Times

Reserved Student Parking (D#)

Resident Student Parking (R, M)

Commuter Student Parking (C)

Visitor/Metered Parking

Perimeter Parking (P)

Garage Parking (SG,14, 17, 18, 19)

Parking & Transit Svcs.

Construction Site

Bus Parking

Accessible Parking

This version of the University of Nebraska parking map is intended for web distribution only. Further information, including parking regulations, permit information, etc.., is available at Parking and Transit Services website, http://parking.unl.edu. For additional questions please contact Parking & Transit Services by email at unlpts@unl.edu or telephone: at 402-472-1800.

Current as of March, 2024

2025 MSPS Annual Meeting

Abstract Judges

Amirhossein Abazarikia

Willam Chen

Surabhi Chandra

Flobater Gawargi

Cassandra Hays

Jee-Yeon Hwang

Sathish Kumar Natarajan

Harold Schultz

Ivan Vechetti

2025 MSPS Annual Meeting

Poster Judges

Amirhossein Abazarikia, Tomasz Bednarski, Surabhi Chandra, William Chen, Uma Maheshwari Deshetty, Bhuvana Gopal, Cassandra Hays, Jee-Yeon Hwang, So-Youn Kim, Sathish Kumar Natarajan, Brian North, Tara Nordgren, Perrie O'Tierney-Ginn, Tapan Patel, Kaushik Patel, Thomas Pisarri, Ankit Shroff, Wonmi So, Harold Schultz Ivan Vechetti, Mohammad Zaidi, Irving Zucker, and Hong Zheng

Our Sponsors

OFFICE OF RESEARCH AND INNOVATION

COLLEGE OF EDUCATION AND HUMAN SCIENCES
cehs.unl.edu

College of Education and Human Sciences
NUTRITION AND HEALTH SCIENCES

University of Nebraska Medical Center℠
College of Medicine

UNIVERSITY OF NEBRASKA MEDICAL CENTER℠
CENTER FOR HEART & VASCULAR RESEARCH

Creighton UNIVERSITY
School of Medicine

DEPARTMENT OF PHARMACOLOGY AND NEUROSCIENCE
SCHOOL OF MEDICINE

Frontiers in Physiology > Integrative Physiology > Research Topics > The Midlands Society of Physiol...

The Midlands Society of Physiological Sciences (2025-2026)

avantor™

https://www.avantorsciences.com/us/en/

Avantor

fisher scientific
part of Thermo Fisher Scientific

https://www.fishersci.com/us/en/home.html

Fisher Scientific

2025 Annual Meeting

Accepted Abstracts

SPEAKERS ABSTRACT

Faculty Presentation in Session 1: Tomasz Bednarski

The effect of T4 and adiponectin receptor agonist (AdipoRon) supplementation on the left ventricular lipid metabolism.

Olichwier A.J.[1], Bińczak A.[2], Duda M.[2], Bednarski T.K.[1]

1. Department of Nutrition and Health Sciences, University of Nebraska Lincoln, USA
2. Centre of Postgraduate Medical Education, Department of Clinical Physiology, Warsaw, Poland

Hypothyroidism is one of the key risk factors for developing cardiovascular diseases (CVDs) such as atherosclerosis, ischemic heart, or heart failure. Hypothyroidism treatment involves constant thyroid hormone (TH) supplementation, usually triiodothyronine (T3) or thyroxine (T4). TH action in the cell is facilitated by TH transporters, such as monocarboxylate transporter 8 (MCT8), and TH receptors (TR). These proteins were also shown to regulate metabolism by controlling the expression of enzymes involved in such processes as lipid trafficking, synthesis, and utilization. Furthermore, obese patients with hypothyroidism have been shown to have lower levels of adiponectin, an adipokine secreted by adipocytes, that regulates insulin sensitivity as well as balances glucose and lipid metabolism. Therefore, the aim of this project is to determine if adiponectin receptors agonist, AdipoRon, is involved in lipid metabolism regulation in hypothyroid cardiomyocytes in TH-dependent manner. To accomplish that, we have used left ventricles from 3-month-old female Wistar-Kyoto rats that underwent thyroidectomies to develop hypothyroidism. Additionally, some animals were given T4 (2μg/100g body weight/day) or simultaneously T4 and AdipoRon (25μg/100g body weight/day) intraperitonially for 4 weeks after thyroidectomy. AdipoRon treatment elevated MCT8 protein level and gene expression, but at the same time decreased TRα and did not affect TRβ protein levels in cardiomyocytes compared to T4 only treated group. No changes in TR-dependent lipogenic genes expression, such as transcription factor sterol regulatory element binding transcription factor 1 (*Srebpf1*) or *Cd36* transporter, were detected, that corresponds with constant TRβ level. On the other hand, AdipoRon supplementation decreased gene expression and protein level of adipose triglyceride lipase (ATGL), a rate-limiting enzyme of triglyceride (TAG) lipolysis, and elevated gene expression of *Gos2*, a protein inhibitor of ATGL. Moreover, gene expression and protein level of diglyceride acyltransferases (DGAT1 and 2), involved in last step of TAG synthesis, were also downregulated by AdipoRon action compared to T4 treatment alone, leading to a conclusion that observed changes are TRα dependent. Taking together, obtained results suggest that AdipoRon, through activation of different TR isoforms, can modulate lipogenesis and lipolysis in cardiomyocytes during hypothyroidism.

***Keynote Presentation* by Dr. O'Tierney-Ginn.**

The placenta-heart connection: impact of the maternal metabolic milieu

Perrie O'Tierney-Ginn PhD, Tufts University.

The risk of cardiovascular disease increases 1.8-2.5 - fold in offspring of mother's who have pregnancies complicated by obesity. The risk of obesity in offspring is also greater even after controlling for genetic and environmental factors. This intergenerational transmission of cardiometabolic risk may underlie the rapidly increasing rates of cardiometabolic disease in the US. Understanding the mechanisms underlying this connection may identify opportunities for intervention to mitigate the vicious cycle of disease. We have shown that the placenta is exquisitely sensitive to the maternal metabolic milieu beginning in early pregnancy. Changes that are initiated in placental pathways in early gestation may

be sustained, impacting nutrient delivery to the fetus and fetal growth patterns that increase offspring risk for future disease. Our group has studied placental lipid metabolism in the context of challenging metabolic environments for over a decade. Lipids are essential for normal cardiovascular and neurological development and the fetal supply of critical polyunsaturated fatty acids is dependent upon placental metabolism and transfer. In her talk, Dr. O'Tierney-Ginn will discuss the connection between placental function and fetal cardiometabolic health and opportunities for improving outcomes.

Faculty Presentation in Session 2 by Dr. Nordgren

Dust and Diet: Environmental Factors Impacting Inflammation and Lung Health

Tara Nordgren * [1], **Abigail Burr**[2], **Edward Dominguez**[2], **Stefanie Sveiven**[2], **Jalene Velazquez**[2], **Alissa Threatt**[3], **Logan Dean**[3], **Emmanuel Oyewole**[3], **Casey McDermott**[3], **Arzu Ulu**[2]

[1] *University of Nebraska Medical Center*
[2] *University of California Riverside*
[3] *Colorado State University*

Inhalation of aerosolized dusts from urban, rural, and agricultural environments provokes airway inflammation and injury, and chronic exposure to these insults can markedly increase the risk of inflammatory lung disease. Yet, while it is well recognized that dust exposures negatively impact lung health, factors contributing to protection versus susceptibility to lung disease following these continual inhalational exposures are unclear. An increasingly recognized class of lipid mediators—specialized pro-resolving mediators (SPMs) derived from omega-3 fatty acids—have been shown to play a critical role in regulating lung inflammation, immunity, and repair. Indeed, our laboratory has demonstrated that the omega-3 fatty acid docosahexaenoic acid (DHA) and its bioactive SPM derivatives can protect against airway inflammation in murine models of acute and repetitive organic dust exposure. We have further determined that these protective effects are driven in part by the regulation of macrophage functioning and the activation of epithelial pro-repair pathways, including amphiregulin and IL-22 signaling. Together, these findings identify omega-3 fatty acids and their SPM derivatives as key modulators of immune responses to inhaled environmental insults, and highlight the potential of dietary interventions to preserve lung health in chronically exposed populations.

Keyword: *omega-3 fatty acids, environmental exposures, lung disease*

Faculty Presentation in Session 3 by Dr. Hays

In Sync with Students: Making Physiology Matter to Modern Medical Trainees

Cassandra Hays PhD, Crieghton University, Omaha, NE

A majority of traditional university students today are aged 18-24 and are stereotyped as "Generation Z," a cohort whose learning styles have been uniquely influenced by ultra-fast technological advancement and real-time exposure to global social events. The context of their adolescence causes them to report unique learning styles, including: preferences for concise audio and visual content rather than text, independent and asynchronous learning environments, discomfort with being wrong, and a pragmatic need for immediate application to real-life situations. This presents a challenge for the teaching of physiology in health professions curricula, as most time for basic sciences is allocated as a lecture period with limited time and infrastructure for incorporating visual or kinesthetic learning modalities. Furthermore, physiology is traditionally taught by a number of different basic scientists with niche expertise, little training in pedagogical skills and no opportunities for input on assessment. Educators in recent years have been investigating ways to improve delivery of content to modern

students in order to promote interest in the topic and subsequent retention of information. Some of these can be employed by basic scientists to pique interest in physiology even during a traditional lecture period. These include ways to enhance the perceived relevance of the topic, and suggesting strategies for incorporation of technology and concise messaging.

UNDERGRADUATE STUDENT CATEGORY

ID: 1 - MAM2025 - Poster Presentation

ORM1 Is Upregulated in the Left Ventricle After Acute Lung Injury in a Bleomycin-Induced Rat Lung Injury Model

Sankar Ramamurthy *, **Grace Nielsen, Michael Lush, Han-Jun Wang**
Department of Anesthesiology at University of Nebraska Medical Center, Omaha, NE 68198

Introduction: Acute lung injury (ALI) and its clinical correlate, the acute respiratory distress syndrome (ARDS), result from disruption of the normal capillary endothelial barrier and invoke perturbations of ventilatory control. Patients suffering from ALI are at a high risk of developing cardiac arrhythmias. Our recent study demonstrated that bleomycin (Bleo)-induced ALI rats exhibited significantly more spontaneous premature ventricular contractions (PVCs) at week 1 post ALI. However, the molecular mechanisms underlying cardiac injury/arrhythmia in this model remain unclear. ORM1 (orosomucoid 1), is an acute phase protein typically synthesized in the liver. It is produced during the acute phase response of inflammation, and it may amplify or reduce this response through feedback loops involving cytokines. Since ALI is associated with systemic inflammatory responses, we hypothesize that the expression of ORM1 in cardiac tissue is elevated in rats with acute ALI, which might be involved in the molecular signaling pathway underlying cardiac injury post ALI.
Method: ALI was induced in male SD rats (200-250g) using a single intra-tracheal injection of Bleo (2.5 mg/kg). 1 week post Bleo (n=6) or saline (sham, n=6), rats were euthanized under deep anesthesia. Cardiac and liver tissues were harvested to perform western blotting analysis. Differences between sham and Bleo rats were determined by one-way ANOVA followed by the Tukey post hoc test and the Mann-Whitney U Test.
Result: Compared to sham rats, there was a significant increase in the protein expression of ORM1 in the left ventricle (LV) and the liver tissues of Bleo rats.
Conclusion: Our data demonstrate that the ORM1 protein was upregulated in the LV and liver tissues 1 week post ALI, indicating a high degree of inflammation in cardiac tissue. ORM1 may also serve as a target for future research.
Keyword: *Acute Lung Injury, ORM1, Bleomycin*

ID: 2 - MA2025 - Poster Presentation

Glioblastoma Subtyping using Internal Gene Expression Ranks, Ensemble Random Projection, and Deep Learning

Eswar Ramamurthy *, **Mengtao Sun, Hanyu Xiao, Lusheng Li, Shibiao Wan**
University of Nebraska Medical Center, Omaha, NE

Glioblastoma is an aggressive brain tumor originating from astrocytes which has morphologically and genetically distinct molecular subtypes. Accurate subtype classification is essential for personalized medicine, but traditional subtyping methods rely on human curation and inefficiently process large-scale data. Instead, deep learning approaches can recognize complex patterns from large amounts of transcriptomic data, improving turnaround for diagnosis. However, conventional deep learning models suffer from the curse of dimensionality and data processing inefficiency.
To address these concerns, we propose a new deep learning approach that leverages gene ranking with Standard Normal Distribution (SND) and Ensemble Random Projection (ERP), facilitating the processing of larger and more diverse datasets for better identification of glioblastoma subtypes.

A combination of two transcriptomic glioblastoma datasets were used to train the models (n=442 samples). Gene ranking with SND was implemented as genes of each sample were ranked and reordered internally from lowest to highest expression, then fitted to a standard normal distribution. ERP was used by applying random projections from the data which were input into a deep convolutional neural network, followed by ensemble learning. Accuracy values were calculated from the results and paired t-tests were performed to evaluate the models.
Model testing with two separate glioblastoma datasets (n=197 per dataset) showed a statistically significant improvement in classification accuracy when using gene ranking with SND in both datasets (from 32.79% to 60.41%; p=1.99×10^-9, from 44.11% to 59.95%; p=2.49×10^-6). ERP also significantly improved accuracy for one of the datasets (from 60.41% to 74.97%; p=6.15×10^-5) and maintained accuracy in the other.
Our proposed approach using gene ranking with SND and ERP significantly improves the classification accuracy for glioblastoma subtyping. Gene ranking with SND also enables sample normalization and ERP reduces computational complexity. We believe our proposed method is promising in improving glioblastoma subtyping, bolstering tailored treatment design.

Keyword: *Glioblastoma, Deep Learning, Bioinformatics*

ID: 3 - MAM2025 - Poster Presentation

Inhibition of Metastasis of Triple Negative Breast Cancer Cells by Black Seed Oil and Thymoquinone

Emily Flowers * **, Surabhi Chandra**
Department of Biology, University of Nebraska-Kearney, Kearney, NE

Patients with breast cancer have a greater chance of cancer metastasis throughout the body, including to bones, lymph nodes, lungs, liver, and brain. Diabetic individuals experience a 10-20% higher risk of breast cancer development and subsequent metastasis. There has been limited success in finding methods to reduce this metastatic behavior of cells. We studied black seed oil (BSO) as well as its active compound, thymoquinone that are over-the-counter health supplements with anti-cancerous properties. Thymoquinone is active as an anticancer agent, and BSO can reduce inflammation and diabetes. Thus, we hypothesized that both might reduce migration and metastasis of breast cancer cells. To test this hypothesis, scratch wound assays were performed with MDA-MB-231 cells (late-stage metastatic triple negative breast cancer cells), treated with thymoquinone or BSO. After 24 hours, the area of the scratch wound was analyzed and compared to a control group. Transwell migration assays were also performed with cells after 24 hours of treatment, and migratory cells were counted and compared to a control group. Normal glucose (5mM) and elevated glucose (25mM) media were used to simulate non-diabetic and diabetic environments. The scratch wound healing assay showed limited inhibition of metastasis using Black Seed Oil at 1µg/ml and 2µg/ml, and 1µM thymoquinone treatment. Total cell death was observed at 2µM Thymoquinone. Findings of the transwell migration assays showed a limited inhibition of metastatic ability of cells with 1µM Thymoquinone in 5G and 25G environments, but further investigation are necessary to determine the extent of inhibition. Although our final results are not conclusive, treatment with black seed oil and thymoquinone has not yet yielded results that they significantly reduce triple negative breast cancer cell metastasis.

Keyword: *Breast Cancer, Diabetes, Black Seed Oil*

ID: 4 - MAM2025 - Poster Presentation

Decoding Mechanisms Contributing to Impaired Feto-Placental and Maternal Outcomes with Chronic HIV Infection

Murali Devanaboyina * **, Victoria Schaal, Amir Elrefaie, Sowmya Yelamanchili, Gurudutt Pendyala,** *UNMC*

HIV infection poses a significant health risk to all infected individuals; however, women are uniquely vulnerable due to potential complications during pregnancy. As of 2024, approximately 40 million

people worldwide are living with HIV, with women comprising 53% of this population. Although combination antiretroviral therapy (cART) has nearly eliminated vertical transmission of HIV from mother to fetus, the virus and its treatment may still contribute to fetal abnormalities. This study aims to investigate feto-placental alterations in HIV/cART-exposed individuals using transgenic (TG) rat model. Rats were euthanized at gestational day 19.5, corresponding to the third trimester in human pregnancy. Placental and fetal tissues were collected for analysis. Morphological assessments of placental and fetal structures were also conducted. RNA was isolated from placental tissue for RT-qPCR, and western blotting was performed on tissue lysates to validate gene expression. Preliminary data revealed that there was a significant reduction in placental and fetal weights in TG rats compared to wild-type (WT) animals. Furthermore, key placental growth factors and nutrient transporters including PIGF, LAT1, LAT2, and FGF2 are markedly downregulated. Analysis of oxidative phosphorylation pathways indicates disrupted mitochondrial function in TG rats, suggesting adverse fetal outcomes. Based on these findings, we hypothesize that while cART effectively prevents HIV transmission, it may negatively impact fetal development through alterations in placental function. Future directions include evaluating inflammatory markers in both placental and fetal tissues and exploring the combined effects of HIV infection with psychostimulants (e.g., methamphetamine) or opioids (e.g., oxycodone) on feto-placental development.

References: Nesheim, S. R., FitzHarris, L. F., Lampe, M. A. & Gray, K. M. Reconsidering the Number of Women With HIV Infection Who Give Birth Annually in the United States. Public Health Rep 133, 637-643, doi:10.1177/0033354918800466 (2018).

Keyword: *HIV*

ID: 5 - MAM2025 - Poster Presentation

Effects of Environmental Chemicals on Adipogenesis in Mouse Mesenchymal Stem Cells

Laura Velasco * **, Yipeng Sui**

University of Nebraska-Kearney

Obesity and obesity-associated metabolic disorders, such as insulin resistance and type 2 diabetes, are rapidly growing public health epidemics, and there is an urgent need to understand the molecular mechanisms underlying these chronic diseases. Increasing evidence has linked exposure to environmental chemicals as contributing to the development of obesity by interfering with lipid metabolism and cellular signaling pathways. This study investigated the effects of two plasticizers, 2-ethylhexyl diphenyl phosphate and alkyl sulphonic phenyl Ester (ASE), and two organophosphate pesticides, malathion and dichlorvos, on adipogenesis in murine mesenchymal stem cell line C3H/10T1/2 cells. Lipid droplet formation was visualized with Oil Red O Staining to evaluate lipid accumulation and differentiation. Our results demonstrated that ASE and EHDPP did not alter adipogenesis. In contrast, malathion and dichlorvos inhibited adipogenesis in C3H/10T1/2 cells. These findings contribute to our understanding of how plasticizers and pesticides may influence obesity and will be valuable for future risk assessments and toxicological studies.

Keyword: *Obesity, Environmental chemicals*

ID: 6 - MAM2025 - Poster Presentation

Increase in stress and opioid abuse post social defeat in HIV+ adolescents

Ria Vootla *

University of Nebraska Medical Center (UNMC)

Adolescents living with HIV face heightened social stress due to stigma and stereotypes, increasing their vulnerability to psychiatric disorders and substance abuse. According to UNICEF, ~145,000 adolescents globally were newly infected with HIV in 2024, underscoring the need to understand the psychosocial impacts during this critical developmental phase. This study investigates how social defeat stress in HIV-1 transgenic (Tg) adolescent rats influences anxiety-like behavior, neuroinflammation,

and susceptibility to opioid use in early adulthood. Using a resident-intruder paradigm, HIV-1 Tg and wild-type (WT) rats were subjected to 10 days of social defeat (SD) to model bullying-related stress. Anxiety behavior was assessed via marble burying, and brain tissue was collected post-defeat for inflammatory marker analysis. Additional groups were also treated with saline or oxycodone to evaluate drug seeking behaviors through infusion counts, motor activity, disc index, and active lever presses. Results showed that Tg rats exhibited more anxiety-like behavior than WT rats, while Tg rats demonstrated elevated neuroinflammation, particularly in TNF-α, Iba-1, and IL-6 levels. Drug-seeking behavior was significantly higher in Tg rats exposed to SD, indicating increased vulnerability to opioid use. These findings suggest that social stress during adolescence exacerbates neuroinflammatory responses and enhances susceptibility to opioid abuse in HIV+ individuals. Future research will focus on identifying the molecular mechanisms underlying synaptic dysregulation contributing to this vulnerability.

Keyword: *social defeat*

ID: 7- MAM2025 –Poster Presentation

Lysophosphatidyl Choline induce Cholangiocyte Lipoapoptosis and Mitochondrial Dysfunction via activation of FOXO3-miR34a

Gabrielle White [*], **Sathish Kumar Natarajan**
University of Nebraska-Lincoln

The progression of Metabolic Associated Steatotic Liver Disease (MASLD) is initially triggered by excess lipid accumulation and insulin resistance in the liver, which increases hepatic de novo lipogenesis. During overnight fasting time points enhances adipocyte lipolysis in individual with metabolic syndrome results in enhanced levels of Saturated free fatty acid release from adipocytes. This leads to elevated levels of saturated free fatty acids (FFAs) in circulation and increased synthesis of lipophosphatidicholine (LPC) causing lipotoxicity and mitochondrial dysfunction in the liver. These processes further contribute to gut microbiome alterations, reactive oxygen species (ROS) production, and endoplasmic reticulum (ER) stress, which collectively result in hepatic steatosis. Chronic inflammation associated with this condition drives liver fibrosis, biliary obstruction, and hepatic immune cell infiltration. Eventually, it can lead to liver cirrhosis and hepatic cellular carcinoma (HCC). While significant research has focused on the impact of MASLD on hepatocytes, the effects on non-parenchymal liver cells, particularly cholangiocytes (biliary epithelial cells), remain underexplored. Our recent studies have shown that exposure of cholangiocytes to palmitate, a saturated free fatty acid, activates FOXO3 and increases miR-34a expression, inducing lipoapoptosis in cholangiocytes. However, the mechanistic role of LPC in promoting cholangiocytes lipoapoptosis during LPC exposure remains unclear

Keyword: *free fatty acids, cellular carcinoma*

ID: 8 - MAM2025 - Poster Presentation

Early-Stage Construction of a Viral Vector for Stabilin-1 Knockout in Mouse Models

Ashley Texel [*] [1], **Edward Harris**[2]
[1] *UNL Biochemistry Department*
[2] *UNL Biochemistry Department Faculty*

Stabilin-1 (Stab1) and Stabilin-2 (Stab2) are scavenger receptors found on liver sinusoidal endothelial cells (LSECs), where they play critical roles in clearing cellular debris and molecular waste. Deficiency in these receptors can result in the accumulation of harmful substances, which has been linked to autoimmune disease, tissue fibrosis, impaired immune clearance of cancer, and liver dysfunction. Studying Stab1 function is therefore important for understanding these disease processes. However, mice lacking Stab1 or both Stab1 and Stab2 exhibit severely reduced reproductive success, limiting their

use as experimental models. To overcome this challenge, we are developing a viral vector containing short hairpin RNA (shRNA) to knock down Stab1 expression postnatally. ShRNA is a synthetic RNA molecule that silences target genes by promoting their degradation. A plasmid with an insertion site was transformed into E. coli and verified by colony PCR, gel electrophoresis, and sequencing. Bioinformatics tools were used to design eight shRNA sequences targeting Stab1, which will be inserted in future steps. This approach offers an alternative to breeding knockout mice and enables further research on Stabilin-1's role in health and disease.

Keyword: *shRNA, Viral Vector, Stabilin-1*

GRADUATE STUDENT CATEGORY:

ID: 9 - MAM2025 - Oral and Poster Presentation

Leg dysfunction and synaptic degradation in a mouse model of peripheral artery disease

Ryan Antony * **, Huiyin Tu, Yulong Li**

University of Nebraska Medical Center

Peripheral artery disease (PAD) is caused by prolonged atherosclerosis which results in pain and numbness of extremities, muscle atrophy, and potential amputation in severe cases. Despite exercise being an effective method to combat early stage PAD, the primary effective treatment for mid-late stage PAD is revascularization surgery; however, this surgery often fails to prevent disease progression and reduce rate of amputation and mortality. To explore the mechanisms and potential interventions for PAD, BALB/c mice were used to induce an animal model to simulate severe pathological manifestations in PAD patients. BALB/c mice underwent femoral artery ligation of the left hindlimb 6 weeks prior to sample collection. First, using laser doppler imaging, we confirmed that mice with femoral artery ligation had significantly lower blood flow to the affected hindlimb, as compared to the control limb ($p<0.05$). Additionally, femoral artery ligation significantly decreased muscle contraction force of ligated hindlimbs, when compared to non-ligated hindlimbs ($p<0.05$). Using H&E and Masson's trichrome staining, we visualized centrally located nuclei and collagen deposition in ligated muscles. Our previous data provided evidence of increased fragmentation of nicotinic acetylcholine receptors (nAChR's) and reduced endplate potentials (EPP's). Consistent with these results, western blot showed that Rapsyn, a critical cytoplasmic nAChR-associated protein in the neuromuscular junction (NMJ), was significantly downregulated in ligated muscles, when compared to control muscles ($p<0.05$). Additionally, Osteopontin, a multifunctional protein involved in immune function, was significantly lower in ligated muscles than in control muscles ($p<0.05$). Interestingly, western blot also showed that macrophage colony-stimulating factor (M-CSF), a promotor of macrophage activation and proliferation, was significantly upregulated in ligated extensor digitorum longus (EDL) muscles compared to control EDL muscles, but was not significantly different between ligated and control soleus muscles. This data suggests that muscle atrophy and NMJ damage may be associated with chronic inflammation-related signaling pathways during PAD.

References: Tu, H., Qian, J., Zhang, D., Barksdale, A. N., Wadman, M. C., Pipinos, I. I., & Li, Y. L. (2022). Different responses of skeletal muscles to femoral artery ligation-induced ischemia identified in BABL/c and C57BL/6 mice. Frontiers in Physiology, 13, 1014744.

Keyword: *Peripheral artery disease, neuromuscular junction, femoral artery ligation*

ID: 10 - MAM2025 - Poster Presentation

Hyperbaric Oxygen Therapy Attenuates Tourniquet-Induced Ischemia-Reperfusion Injury in Muscles of The Lower Extremity

Emmanuel Adu-Agyekum * **, Lauren Whitney, Huiyin Tu, Aaron Barksdale, Michael Wadman, Yu-Long Li**

Department of Emergency Medicine, University of Nebraska Medical Center

Background: Tourniquets control life-threatening extremity bleeding on the battlefield or traumatic civilian accidents. Tourniquet use and subsequent release induce oxidative stress, which causes skeletal muscle damage and delays functional recovery. Hyperbaric oxygen therapy (HBOT) increases bioavailability of oxygen and promotes healing. This study investigated HBOT's potential to restore skeletal muscle function after tourniquet-induced IR injury in mice.
Methods: Unilateral hindlimb ischemia was induced in male C57/BL6 mice with an orthodontic rubber band (ORB) placed around the hip joint for 3 hours, followed by reperfusion. Mice were assigned to sham, IR alone, and IR+HBOT groups. Sham and IR alone mice received room air at normal pressure (21% oxygen and 1 atmosphere absolute, ATA). IR+HBOT mice received 100% oxygen at 2.5 ATA for 1 hour daily during 1-week reperfusion. Skeletal muscle function was assessed in conscious and anaesthetized conditions by rotarod testing and in situ sciatic nerve-stimulated gastrocnemius contraction. Reactive oxygen species (ROS) were measured in harvested gastrocnemius muscle by oxidized DCFDA staining.
Results: In assessing skeletal muscle motor function, rotarod endurance was lower in the IR alone group (95.4 ± 5.8seconds) compared to sham (185±31.2 seconds, p=0.003) but not the IR+HBOT group (121.3 ± 4.2seconds, p=0.61). Sciatic nerve-stimulated muscle contraction was lower in the IR alone group (8.0 ± 1.5grams) compared to both Sham (21.9 ± 0.8grams, p=0.001) and IR+HBOT groups (22.1 ± 2.8grams, p=0.001). Mean DCFDA fluorescence intensity was higher in the IR alone group (922.4 ± 16.2) compared to both Sham (159.1 ± 26.2, p<0.0001) and IR+HBOT groups (462.6 ± 51.7, p<0.0001). Kaplan-Meier analysis showed more stable survival in the IR+HBOT group, with a trend toward significance [$\chi^2(2, N=48) = 9.407$, p=0.009].
These findings demonstrate that HBOT reduces oxidative stress and improves skeletal muscle contraction in mice subjected to tourniquet-induced IR injury and shows promise as therapy for extremity injury.

Keyword: *Hyperbaric oxygen therapy, Ischemia-reperfusion injury, Skeletal muscle*

ID: 11 - MAM2025 - Poster Presentation

Examination of histological characteristics along the murine aorta: consideration of sex as a biological variable

Benjamin Brockmann * [1], **Timothy Sveeggen**[1], **Ray Mitchell**[1], **Trevor Meisinger**[2], **Jason Cook**[2], **Pooneh Bagher**[1]

[1] *Department of Cellular and Integrative Physiology, University of Nebraska Medical Center*
[2] *Department of Surgery, University of Nebraska Medical Center*

The three layers that compose the aorta, from internal to external, are the tunica intima, the tunica media, and the tunica adventitia. The tunica media is composed of vascular smooth muscle cells and elastic fibers, organized into elastic lamellae. The elastic lamellae respond to an increase in pressure within the cardiovascular system, allowing for the aorta to stretch and recoil. When broken, the aorta loses the ability to stretch and recoil, leading to adverse cardiovascular events. The goal of this project is to analyze sex differences in histological parameters in different segments of the murine aorta. This project looks at sex differences in tunica media thickness, layers of elastic fibers, and number of free ends in four segments of C57BL/6J mice aorta (ascending, proximal thoracic, distal thoracic, and infrarenal). Aorta segments were dissected systematically, fixed in formalin, sectioned, and labeled with Verhoeff's stain, allowing for elastin and collagen to be visualized. Imaged histological sections were then analyzed using ImageJ for tunica media thickness, elastic lamellae layers, and free ends per square millimeter for each sample. We observed that the thickness of the tunica media and the number of layers decreased in the order of ascending, proximal thoracic, distal thoracic, and infrarenal aortas. The only sex difference, a greater media thickness in the ascending aorta, was observed in females, while no sex difference was noted for the number of elastic lamellae layers. We observed that the number of free ends in male infrarenal aortas were statistically different than all other segments of the male aorta, and statistically different than the female infrarenal. There were no other differences noted between sexes.

Further work can further analyze the differences in infrarenal arteries, as this was observed to be the most variable aortic segment studied.

Keyword: *Aorta, Histology, Sex as biological variable*

ID: 12 - MAM2025 - Poster Presentation

Smad3 Drives Inner Hair Cell Identity During Cochlear Development

Dominika Rogalska * **, Alyanna Rodelas, Jemma Webber**

Creighton University

The cochlea's ability to detect and amplify sound depnds on the distinct and specialized functions of inner (IHC) and outer hair cells (OHC), which are specified during development and acquire unique structural and molecular features. Recent studies have identified transcription factors, including Tbx2 and Insm1, as critical regulators of IHC and OHC fate. However, the mechanisms by which IHCs acquire and maintain their distinct identity from OHCs remain largely unknown. In the absence of Insm1, approximately half of all OHCs transdifferentiate into IHCs during embryogenesis. These outer-compartment-IHCs (oc-IHCs) express all known IHC markers and display characteristic features, including centrally located nuclei and long, straight stereocilia, unlike basally the located nuclei and V-shaped stereocilia of neighboring OHCs. Intriguingly, Smad3, a TGF-β pathway effector, is upregulated in transdifferentiating cells, suggesting that it may contribute to the OHC-to-IHC fate switch (Wiwatpanit et al., 2018). Using an Insm1 conditional knockout combined with Smad3 loss-of-function, we show that Smad3 deficiency reduces the extent of transdifferentiation, disrupts nuclear morphology, alters stereocilia structure, and leads to ectopic expression of OHC makers in IHCs. Together, these findings demonstrate that Smad3 promotes IHC fate acquistition and is required for the development and/or maintenance of key morphological features essential to IHC identity.

Keyword: *Cochlear development, Auditory system, Cell identity and differentiation*

ID: 13 - MAM2025 - Poster Presentation

Characterization of Phosphoinositide Populations on Lipid Droplet Membranes

Joseph Bernal * **, Ankit Shroff, Micah Schott**

Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center

Dysregulation of lipid droplet (LD) metabolism has been implicated in several disease states, such as cancer, liver disease, and neurodegenerative disorders. Lipid droplets (LD) are energy-storing organelles that exhibit vital functions in ROS and lipotoxicity protection, signaling regulation, organellar trafficking, and membrane growth. Energy and free fatty acids (FFA) can be derived from these organelles via two major catabolic processes, lipolysis and lipophagy. In fact, the unique structure and dynamics of these fatty organelles is essential to their functionality to carry out these processes throughout the cell.

LDs contain a hydrophobic core composed of neutral lipid species, such as triacylglycerols (TAG) and cholesterol esters (CE) and are surrounded by a phospholipid monolayer comprised of phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI), sphingomyelin (SM), and lysophosphatidylcholine (LPC). The dynamic composition of phospholipids of the LD monolayer are implicated in processes like lipophagy and lipid transfer, but the exact mechanisms remain largely unknown. This project will investigate and characterize how phosphoinositides (PIP) modulate LD metabolism.

Keyword: *Lipid Droplet, Lipid Metabolism, Phosphoinositides*

ID: 14 - MAM2025 - Poster Presentation

The role of PKA-mediated phosphoregulation of 26S proteasomes in post-MI cardiac remodeling

Md. Giash Uddin * **, Penglong Wu, Xuejun Wang**

Division of Biomedical and Translational Sciences, University of South Dakota Sanford School of Medicine, Vermillion, SD

Heart failure (HF) is primarily driven by ischemic heart disease (IHD), including myocardial infarction (MI). Phosphorylation of RPN6 at Ser14 (pS14-RPN6) by PKA enhances proteasomes and proteostasis, but its pathophysiological importance in IHD remains unclear. This study investigates the role of pS14-RPN6 in post-MI cardiac remodeling, with emphasis on its influence on proteasome function and HF progression.
Genetic blockade of the activation of 26S proteasomes by PKA was previously validated in $Rpn6^{S14A/S14A}$ knock-in (S14A) mice. MI were surgically induced in wild-type (WT) and S14A mice. Myocardial pS14-Rpn6 and ubiquitinated protein levels were measured by western blot. Serial echocardiography was performed before and after (every two weeks for 12 weeks) surgery.
Following acute MI, pS14-Rpn6 was significantly increased in WT MI mice (1.32±0.25-fold vs. sham, $p<0.05$). Compared to WT MI, S14A MI showed higher levels of total ubiquitinated (1.20±0.13 vs. 1.57±0.25-fold, $p<0.05$) and K48-linked ubiquitinated proteins (1.31±0.14 vs. 1.53±0.21-fold, $p<0.05$), indicating impaired proteasome function. From 4 to 12 weeks post-MI, echocardiography revealed a progressive decline in systolic function in S14A mice compared with WT mice, as reflected by greater reductions in ejection fraction (EF) and fractional shortening (FS), and more progressive chamber dilatation in S14A MI vs. WT MI. At 12-week endpoint, S14A MI mice showed more decreases in EF (19.04±2.62% vs. 12.31±2.18%, $p<0.001$) and FS (8.71±1.19% vs. 5.55±0.93%, $p<0.001$), along with more increases in left ventricular (LV) end-diastolic internal diameter (5.47±0.64 vs. 6.24±0.63, $p<0.01$), LV end-diastolic volume (147.90±37.29 vs. 199.30±44.21, $p<0.001$), and LV mass to body weight ratio (5.84±1.19 vs. 6.99±1.10, $p<0.05$).
These findings demonstrate that pS14-RPN6 is essential for myocardial proteostasis and mitigating post-MI cardiac remodeling, indicating proteasome phosphoregulation by PKA as a key cardioprotective mechanism and potential therapeutic target in IHD.

Keyword: *cardiac remodeling, RPN6 phosphorylation, proteostasis*

ID: 15 - MAM2025 - Poster Presentation

Declines in proteasome proteolytic efficiency in aged mouse myocardium

Yifan Wang * **, Sternburg Jack, Samiksha Giri, Mingqi Cai, Xuejun Wang**
Division of Biomedical and Translational Sciences, University of South Dakota Sanford School of Medicine, Vermillion, SD 57069

Background and Objectives: The proteasome performs the targeted degradation of most cellular proteins. Proteasome malfunction is implicated in the genesis of age-dependent diseases, including a large subset of heart disease, but age-dependent changes in myocardial proteasome abundance, activities, and catalytic efficiency, as well as their sex dependence, remain murky. This study was conducted to help address this critical issue.

Methods: Crude protein extracts from ventricular myocardium of male (n=4, 4, 3) and female (n=4, 5, 3) FVB/N mice at 4-, 12-, and 24-month were subjected to native gel electrophoresis. In-gel chymotrypsin-like activity assays followed by Western blot analyses for the β5 subunit (Psmb5) of the 20S proteasome and Rpt2 of the 19S cap were performed, which allows quantification of the abundance and activities of capped proteasomes (30S, 26S-1, and 26S-2) and the abundance of 20S proteasomes on the same gel. Two-way (age, sex) ANOVA followed by Tukey's post hoc tests were adopted for statistical comparisons.

Results: Age-dependent increases in 20S and 30S abundance (p=0.002, 0.046) were detected in males and females, respectively. The activities of 30S showed age- and sex- dependent changes (p=0.012, 0.027); however, the abundance-adjusted 30S activities showed age-dependent (p=0.005) declines without sex-difference (p=0.162). The abundance of 26S-1 was not altered by age, but its activity showed age-dependent declines (p=0.004), resulting in an age-dependent decline in the abundance-adjusted 26S-1 activities (p=0.021). The 26S-2 showed age-dependent increases in abundance (p=0.048) but decreases in activities (p=0.015); consequently, the abundance-adjusted

26S-2 activities showed a tendency of age-dependent declines (p=0.073). Neither 26S-1 nor 26S-2 showed sex-difference in any of the parameters.

Conclusions: During aging, the trajectory of changes in the myocardial abundance and activities of different proteasome forms vary considerably and sometimes show sex-difference, but age-dependent declines in catalytic efficiency are uniform across all capped proteasomes.

Keyword: *aging, heart, proteasome assembly, enzymatic activities, mouse, sex*

ID: 16 - MAM2025 - Poster Presentation

The role of sympathetic activation - α1 adrenergic receptor axis in diabetes-related cardiac vagal neuronal and myocardial dysfunction

Afia Saabea Owusu Konadu * **, Jaswinder Singh, Huiyin Tu, Boris Shabaltiy, Lauren Whitney, Yu-Long Li**

Department of Emergency Medicine,University of Nebraska Medical Center

Cardiovascular disease is the leading cause of death in type 2 diabetes mellitus (T2DM). T2DM affects cardiac vagal and sympathetic nerve function and further alters cardiac function. We hypothesized that sympathetic activation - α1 adrenergic receptor axis reduces cardiac vagal function in T2DM.

We induced T2DM in male and female Sprague Dawley rats using a high-fat diet for 12 weeks plus a one-time intraperitoneal injection of streptozocin (30milligram/kilogram). We measured the cardiac vagal function by whole-cell patch clamp. T2DM exhibited decreased nAChR currents (213.9 ± 25.6 pA/pF for T2DM, 375.5 ± 44.3 pA/pF for sham) and cell excitability (9.1 ± 0.5 spikes/s for T2DM, 19.9 ± 1.4 spikes/s for sham) of intracardiac (ICG) vagal neurons. We measured cardiomyocyte size using hematoxylin-eosin staining and cardiac function using echocardiography. We also measured the mRNA expression of cardiac hypertrophy marker -atrial natriuretic peptide (ANP) by real-time polymerase chain reaction. T2DM induced cardiac hypertrophy and diastolic dysfunction with preserved ejection fraction, and increased ANP levels as compared to sham (10.1 ± 2.4 for T2DM, 1.1 ± 0.2 for sham). We determined the mRNA and protein expression of α1 adrenergic receptors in the ICG of sham and T2DM by RT-PCR and western blot. We found that T2DM increased mRNA (2 ± 0.1 for T2DM vs. 1.1 ± 0.3 for sham) and protein expression (0.3 ± 0.06 for T2DM vs. 0.1± 0.04 for sham) of α1-adrenergic receptors in the ICG.

Our findings revealed that elevated levels of α1-adrenergic receptors in the ICG of T2DM are associated with cardiac vagal neuronal and myocardial dysfunction. In future experiments, we will downregulate α1-adrenergic receptors via the transfection of α1-adrenergic receptor shRNA into the ICG of T2DM rats to improve cardiac vagal activity and cardiac function, aiming to establish a potential therapeutic target.

Keyword: *diabetic cardiomyopathy, cardiac vagal dysfunction, α1-adrenergic receptor*

ID: 17 - MAM2025 - Poster Presentation

cAMP promotes acute lysosome biogenesis through TFEB nuclear import-export dynamics.

Saumya Bhatt * **[1], Mohammad Ali Abbas Zaidi[1], Nicholas Woods[2], Micah Schott[1]**

[1] Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE 68105

[2] Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, NE 68105

Although cyclic adenosine monophosphate (cAMP) is a well-established regulator of lipid and glucose metabolism[1], its role in lysosomal dynamics remains largely unexplored, despite emerging evidence linking cAMP signaling to lysosomal function. Transcription factor EB (TFEB), a master regulator of lysosomal biogenesis, undergoes phosphorylation-dependent nuclear–cytoplasmic shuttling to control lysosomal function[2]. Dysregulation of TFEB has been implicated in a broad spectrum of human diseases, including neurodegenerative disorders such as Alzheimer's and Parkinson's disease, cancer,

and metabolic disorders[3]. Interestingly, sequence analysis revealed a conserved cAMP-dependent Protein Kinase A (PKA) phosphorylation motif (RRxS) at Ser467 in TFEB, but its regulatory function had not been previously defined.

Here, we uncover a cAMP–PKA–TFEB signaling axis that temporally controls lysosomal gene expression, revealing a new layer of TFEB subcellular regulation. We find that elevating intracellular cAMP using Forskolin (Fsk) and 3-Isobutyl-1-methylxanthine (IBMX) promotes transient TFEB nuclear import via a calcium-dependent mechanism, leading to acute induction of its target genes in HeLa cells. Surprisingly, this nuclear accumulation peaks at 30 minutes and is followed by export back to the cytoplasm within 8 hours, coinciding with TFEB reduced transcriptional activity. High-throughput imaging revealed that PKA inhibition using H89 did not block cAMP-induced TFEB nuclear import but specifically prevented its export, indicating PKA-dependent nuclear export. This observation is further supported by phosphoproteomic analysis, which showed increased phosphorylation of the PKA-specific RRxS motif during the export phase, but not during the import phase. Moreover, mutagenesis experiments demonstrated that phosphorylation of Ser466 and Ser467 within this conserved C-terminal motif is essential for TFEB's cytoplasmic localization.

These findings define novel evidence of a biphasic mechanism: transient calcium-mediated import followed by PKA-dependent export, enabling precise, time-dependent control of lysosomal gene activation. This study provides insights into how cells coordinate nutrient signals with lysosomal function in health and disease.

References: 1. Ravnskjaer, K., Madiraju, A., and Montminy, M. (2016). Role of the cAMP Pathway in Glucose and Lipid Metabolism. Handb Exp Pharmacol 233, 29-49. 10.1007/164_2015_32. 2.Takla, M., Keshri, S. & Rubinsztein, D. C. The post-translational regulation of transcription factor EB (TFEB) in health and disease. EMBO Rep 24, e57574 (2023). https://doi.org:10.15252/embr.202357574 3.Gebrie, A. (2023). Transcription factor EB as a key molecular factor in human health and its implication in diseases. SAGE Ope

Keyword: *cAMP/PKA signaling, TFEB nuclear import/export, lysosomal biogenesis*

ID: 18 - MAM2025 - Poster Presentation

Adolescent Exposure to a Per- and Polyfluoroalkyl Substances (PFAS) Mixture Disrupts Estrous Cyclicity in Mice

Rasha Ghorab * [1], **Kendra Clark**[2], **Jitu George**[3]

[1] *Department of Obstetrics and Gynecology, Olson Center for Women's Health, University of Nebraska Medical Center, Omaha, NE*

[2] *Department of Obstetrics and Gynecology, Olson Center for Women's Health, University of Nebraska Medical Center, Omaha, NE Department of Environmental, Agricultural, and Occupational Health, University of Nebraska Medical Center, Omaha, NE Veterans Affai*

[3] *University of Minnesota Genomics Center, University of Minnesota, Minneapolis, MN*

Per- and polyfluoroalkyl substances (PFAS) are man-made chemicals widely used in consumer products due to their resistance to biodegradation, leading to persistent environmental contamination. Human exposure is associated with reproductive health effects, including delayed menarche, irregular cycles, early menopause, and altered steroid hormone levels. Although many legacy PFAS, such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), have been phased out, they remain detectable in humans and animals, and the use of alternative short-chain PFAS, such as undecafluoro-2-methyl-3-oxahexanoic acid (GenX/HFPO-DA) and perfluorobutanesulfonic acid (PFBS), is increasing. Most prior research has focused on individual compounds, while the effects of PFAS mixtures on ovarian function remain poorly understood. We previously showed that adolescent exposure to a PFAS mixture reduced the ovarian reserve, disrupted endocrine signaling, and increased ovarian fibrosis in adulthood, though the timeline of these effects was unclear. We hypothesized that exposure initiates ovarian dysfunction during adolescence. To test this, postnatal day (PND) 30 female CD-1 mice were orally exposed to vehicle (distilled water with 1% DMSO; n = 10) or a PFAS mixture (0.1 mg/kg each of PFOA, PFOS, GenX, and PFBS; n = 10) for 15 days (until PND45). The dose was

selected based on the lowest observed adverse effect levels reported in juvenile mice. Body weight was recorded weekly, and vaginal cytology was monitored for 10 days before euthanasia at PND45. Serum hormones were analyzed by ELISA. PFAS exposure did not affect body or reproductive organ weights but significantly increased liver weight ($P < 0.05$). Exposed mice showed disrupted estrous cyclicity, with prolonged estrus, reduced metestrus/diestrus, and more frequent cycles compared to controls ($P < 0.05$). There were no changes in progesterone, estradiol, testosterone, or luteinizing hormone. These findings demonstrate that adolescent PFAS mixture exposure disrupts estrous cycle during exposure, highlighting the need for further investigation into the mechanisms driving these changes.

Keyword: *Per- and polyfluoroalkyl substances (PFAS), Adolescent exposure, Estrous cycle, Endocrine disruption, Mouse model (CD-1 mice), SNRNA seq*

ID: 19 - MAM2025 - Poster Presentation

Overexpression of skeletal muscle Nrf2 protects the heart against ischemia-reperfusion injury in mice

Sarah Pribil Pardun * [1], **Spencer Duff**[1], **Payton Ward**[1], **Kanika Sharma**[2], **Sankarasubramanian Jagadesan**[3], **Vikas Kumar**[4], **Chittibabu Guda**[3], **Irving H Zucker**[5], **Lie Gao**[1]

[1] *Department of Anesthesiology University of Nebraska Medical Center Omaha NE*

[2] *Multiomics Mass Spectrometry and Proteomics Core Facility University of Nebraska Medical Center Omaha NE*

[3] *Bioinformatics & Systems Biology Core University of Nebraska Medical Center Omaha NE*

[4] *Department of Biochemistry and Molecular Biotechnology UMass Chan Medical School Shrewsbury MA*

[5] *Department of Cellular and Integrative Physiology University of Nebraska Medical Center Omaha NE*

Physical activity is well-documented to confer cardioprotection against ischemia-reperfusion (I/R) injury; however, the underlying molecular mechanisms remain to be fully understood. We hypothesized that activation of skeletal muscle (SkM) Nrf2 and its downstream antioxidant enzymes play a pivotal role in this protective effect. To test this, we utilized iMS-Keap1$^{flox/flox}$ mice, a transgenic model that enables SkM Nrf2 activation via Keap1 knockout. Compared to wild-type controls, iMS-Keap1$^{flox/flox}$ mice subjected to 60 minutes of myocardial ischemia followed by 60 minutes of reperfusion exhibited significantly reduced infarct size and improved left ventricular (LV) function both during ischemia and throughout reperfusion. Western blot analysis revealed markedly elevated levels of the antioxidant proteins NQO1 and GSTA2 in the soleus muscle, plasma extracellular vesicles/non-vesicular extracellular nanoparticles (EVs/nvENPs), and myocardium of iMS-Keap1$^{flox/flox}$ mice. The highest expression of these antioxidant enzymes was found in the SkM, followed by EVs/nvENPs, and then the heart. This gradient suggests a possible inter-tissue transfer of antioxidant enzymes from SkM to the myocardium via circulating EVs/nvENPs. Furthermore, mass spectrometry-based proteomics and pathway enrichment analyses of LV myocardium revealed additional cardioprotective pathways, including TCA cycle II, integrin-linked kinase signaling, integrin signaling, and paxillin signaling. These findings demonstrate that SkM-specific overexpression of Nrf2 confers significant protection against myocardial I/R injury, potentially through systemic inter-organ antioxidant transfer via circulating EVs/nvENPs. Targeting SkM Nrf2 pathways may offer a novel therapeutic strategy for enhancing cardiac resilience to ischemia/reperfusion injury.

Keyword: *ischemia/reperfusion, extracellular vesicles and non-vesicular extracellular nanoparticles (EVs/nvEPs), Nrf2*

ID: 20 - MAM2025 - Poster Presentation

Developing coacervate nanoparticles for localized and systemic programming of cardiac inflammation

Jing Zhang * [1], **Xiaoping Li**[1], **Chelsea Loh**[2], **Kang Kim**[2], **Yadong Wang**[2], **William Chen**[1]

[1] *Division of Basic Biomedical Sciences, Sanford School of Medicine, University of South Dakota*

[2] *Department of Biomedical Engineering, Cornell University*

Background: Excessive and prolonged inflammation post myocardial infarction (MI) drives cardiac fibrosis and adverse remodeling. Potent biologics like immunomodulatory cytokine interleukin (IL)-10 and lymphangiogenic growth factor vascular endothelial growth factor (VEGF)-C can accelerate immune resolution and lymphatic repair, respectively. However, these cardiotrophic factors are limited by short half-life and potential off-target effects. Coacervate nanoparticles (CoaNPs) enable localized and systemic controlled delivery by protecting labile proteins and precisely sustaining the release of payloads. However, whether controlled delivery of the above biologics can ameliorate cardiac dysfunction post-MI is not clear.
Hypothesis: We hypothesized that CoaNP-based controlled delivery systems—administered locally (intramyocardial) or systemically (IV)—will sustain cardiotrophic signaling (IL-10 ± VEGF-C) by polarizing macrophages (Mφ) toward reparative phenotypes, accelerating inflammation resolution, reducing tissue fibrosis, and improving cardiac function after MI.
Methods: We separately examined the functions of CoaNP-IL-10 and CoaNP-VEGF-C. CoaNP-IL-10 and CoaNP-VEGF-C were prepared by mixing recombinant human IL-10 or VEGF-C with natural polyanion heparin and synthetic polycation PEAD. We then: 1) evaluated the effects of CoaNP-IL-10 on macrophage phenotype and phagocytic function, 2) examined the efficiency of CoaNP-VEGF-C on lymphangiogenesis, and 3) assessed the efficacy of both CoaNPs on cardiac function in a mouse MI model.
Results and conclusion: The CoaNPs effectively prolonged the bioavailability of IL-10 and VEGF-C in vitro and in vivo. Compared to free 100ng IL-10, CoaNP-500ng IL-10 significantly reduced Mφ M1 polarization ($p<0.01$) while enhancing Mφ M2 polarization ($p<0.001$) to benefit tissue repair. CoaNP-100ng IL-10 induced the highest rate of phagocytosis in M2 macrophages compared to free 100ng IL-10 in vitro ($p<0.05$). When administered intramyocardially, CoaNP-500ng IL-10 reduced myocardial fibrosis ($p<0.05$), diminished CD68+ phagocytic cell infiltration ($p<0.001$), and decreased left ventricular dilation ($p<0.05$) at 6 weeks post-MI. When administered systemically post-MI, CoaNP-2000ng VEGF-C appeared to reduce cardiac dysfunction in a pilot study (n=2), warranting future investigation.
Keyword: *Myocardial infarction, Nanoparticle, cytokine*

ID: 21 - MAM2025 - Oral and Poster Presentation

Studying the thoracic aorta using pressure myography: technique and functional characterization

Anna Kosmach * **, Ray Mitchell, Pooneh Bagher**
Department of Cellular & Integrative Physiology, University of Nebraska Medical Center, Omaha, NE 68198

Pressure myography, a technique where isolated blood vessels are cannulated and pressurized, is a widely used, physiologically relevant method to study the function of blood vessels ex vivo, but technical limitations have restricted its use to small vessels. Our group has adapted this method to study large conduit vessels in murine models, specifically the thoracic aorta. In this study, thoracic aorta segments were exteriorized from 15-20-week-old C57BL/6J male and female mice and cannulated onto glass micropipettes in a perfusion chamber. Temperature was maintained at 37°C, and the vessels were superfused with physiological salt solution (PSS). Intercostal arteries branching from the aorta were tied off with surgical suture to allow for pressurization without leaks. The intraluminal pressure of the aortas was set to 100mmHg using gravity-fed pressure lines. Pharmaceuticals were delivered via the PSS superfusion solution. Vessel outer diameter was recorded using AmScope Imaging Software and analyzed post-hoc using VasoTracker software. Isolated, pressurized thoracic aortas from male mice were significantly larger in diameter than aortas from females (outer diameter 1330μm ± 15 (male) vs. 1275μm ± 11 (female)). Male and female mice had similar responses to the vascular smooth muscle cell (vSMC)-dependent vasoconstrictor phenylephrine ($logEC_{50}$= -7.301 ± 0.113 vs. -7.269 ± 0.066 for males and females, respectively), endothelial cell-dependent vasodilator acetylcholine ($logEC_{50}$= -5.995 ± 0.433 vs. -6.043 ± 0.224), and vSMC-dependent vasodilator DEA-NONOate ($logEC_{50}$= -6.354 ± 0.151 vs. -6.403 ± 0.124). Overall, we demonstrate a new approach to studying the vasoreactivity of an ex

vivo, pressurized thoracic aorta that maintains the intercellular connections and allows for probing of molecular mechanisms underlying vascular responses.

Keyword: *Pressure myography, Thoracic aorta, Vasoreactivity*

ID: 22 - MAM2025 - Oral and Poster Presentation

Single-Cell Transcriptomics Reveals Progressive Proximal Tubule Reprogramming in Diabetes

Darshan Jadhav * [1], **Najeong Kim**[2], **Maria Rivoira**[3], **Gastón Repossi**[3], **Nestor Garcia**[3], **William Chen**[4], **Agustin Gonzalez-Vicente**[2]

[1] *Division of Biomedical and Translational Sciences University of South Dakota, Case Western Reserve University School of Medicine*

[2] *Case Western Reserve University School of Medicine*

[3] *Instituto de Investigaciones en Ciencias de la Salud Córdoba*

[4] *Division of Biomedical and Translational Sciences University of South Dakota*

Background: Diabetic Kidney Disease (DKD) is a leading cause of end-stage kidney disease in developed countries. Even though glomerular damage is a key feature of DKD, early damage to proximal tubule (PT) is increasingly being recognized as an independent pathogenic event.

Hypothesis: We hypothesized that diabetic PTs undergo maladaptive metabolic reprograming and that distinct damage-repair cell transcriptional trajectories precede structural damage as evidenced by histology.

Methods: We generated paired kidney single-nucleus (sn)RNASeq data from 17-week-old (n=2 per group) and 25-week-old (n=1 per group) Zucker Diabetic Fatty and Control rats; recovering 36861 PT nuclei, 14950 from controls and 21911 from diabetics. PT nuclei were extracted and annotated into 5 clusters i.e. Subsegments 1&2 (PT-S1/S2), subsegment S3 (PT-S3), De-differentiated, Recovered and Injured (PT-Inj).

Results and conclusion: Diabetic Rats show proteinuria and increased blood urea nitrogen. Trajectory analysis using slingshot showed two recovery trajectories:

1) PT-Inj → De-differentiated → Recovered → PT-S1/S2.

2) PT-Inj → De-differentiated → PT-S3.

Histologic analysis revealed no increase in interstitial fibrosis or tubular atrophy in diabetics, nor evidence of interstitial inflammation or exudative lesion at 17-weeks. However, cell state distribution differed as follows: 1) Diabetics: 72% mature state (PT-S1/S2 and PT-S3), 14.9% De-differentiated, 6.5% Recovered, and 6.7% PT-Inj. 2) Controls: 85.9% mature state, 9.0% De-differentiated, 3.6% Recovered, and 1.4% PT-Inj. Hallmark pathway analysis shows altered metabolism between controls and diabetics as well as increase in inflammatory pathways. The observed shifts in PT cell states indicate that transcriptional alterations precede structural damage, underscoring the sensitivity of snRNAseq in capturing early DKD progression and segment-specific vulnerability and repair responses.

Keyword: *Diabetic Kidney Disease, Metabolic Reprogramming, Single-nucleus RNAseq*

ID: 23 - MAM2025 - Poster Presentation

BubR1 is Indispensable in Maintaining Intestinal Epithelial Homeostasis and Stem Cell Function

Rodaina Hazem Monieb * [1], **Balawant Kumar**[2], **Rizwan Ahmad**[2], **Geoffrey Talmon**[3], **Ishwor Thapa**[4], **Dhundy Bastola**[4], **Anna Hepperlen**[1], **Lily Calisto**[1], **Adrian Black**[3], **Amar Singh**[2]

[1] *Creighton University*

[2] *Kansas University Medical Center*

[3] *University of Nebraska Medical Center*

[4] *University of Nebraska Omaha*

The intestinal epithelium self-renews in an intricate process of balanced proliferation and differentiation, termed intestinal homeostasis. Intestinal stem cells (ISC) are central to this homeostasis under both normal physiologic conditions and during injury. While ISCs and their niche

are necessary for intestinal homeostasis and barrier integrity, a clear molecular understanding is lacking. BubR1 is a critical regulator of organismal aging and a component of the mitotic spindle assembly checkpoint, preventing aneuploidy. However, its role in the intestinal epithelium, and maintaining ISC function, remains unknown. Therefore, we crossed our conditional BubR1 mouse model to Vil1-cre mice to generate intestinal-specific BubR1 knockout (ISBK) mice. ISBK mice were born in expected mendelian ratios but failed to thrive postnatally and had a median survival of 25 days. Histological findings revealed that BubR1 loss leads to epithelial damage, crypt hypertrophy, villous atrophy, and stem cell loss coupled with increased apoptotic cell death. We also found BubR1 loss led to aberrant epithelial differentiation, such as reduced goblet cell numbers and ectopic lysozyme expression. Epithelial barrier breakdown was evident as ISBK mice have dysregulated expression of tight junction proteins and increased intestinal permeability. Organoid cultures using crypts harvested form ISBK mice failed to grow or mature, consistent with a loss of epithelial regenerative capacity. Bulk-RNA sequencing revealed a global downregulation of a-defensins indicating significant Paneth cell dysfunction. Upregulation of pro-inflammatory pathways such as TNF-a and activation of oncogenic pathways such as p53 indicate that these perturbations lead to disrupted epithelial homoeostasis, and poor ISC survival, resulting in barrier breakdown favoring a pro-inflammatory state, ultimately leading to reduced organismal survival. These studies reveal a non-canonical role for BubR1 in regulating intestinal epithelial homeostasis and stem cell function, opening new avenues to explore the molecular underpinnings of ISC niche regulation as well as therapeutic targets for inflammatory bowel disease.
Keyword: *Intestinal Epithelium, BubR1, Intestinal Stem Cells*

ID: 24 - MAM2025 - Poster Presentation

A superoxide dismutase-mimetic (BMX-001) mitigates lipid peroxidation in a mouse model of alcohol-associated liver disease

Delia Omar * [1], **Nourhan Mostafa**[2], **Mohammad Zaidi**[2], **Rebecca Oberley-Deegan**[2], **Micah Schott**[2]

[1] *University of Nebraska Medical Center, Department of Cellular and Integrative Physiology, Department of Biochemistry and Molecular Biology*

[2] *University of Nebraska Medical Center, Department of Biochemistry and Molecular Biology*

Alcohol-associated liver disease (ALD) is a growing health concern. Hepatic steatosis represents the earliest stage of ALD and is marked by accumulation of lipid droplets within hepatocytes. Ethanol (EtOH) metabolism generates reactive oxygen species (ROS), which react with polyunsaturated fatty acids (PUFA) to promote lipid peroxidation. Whether ROS can be therapeutically targeted to improve ALD remains unclear. BMX-001, a superoxide dismutase (SOD)-mimetic currently in Phase 2 clinical trials as a radioprotector, scavenges excess ROS. Pharmacokinetic studies showed that BMX-001 accumulates within mouse liver in a dose-dependent manner; however, its efficacy to mitigate ROS-driven hepatocyte damage in ALD has not been investigated.

A modified chronic-plus-binge ethanol with PUFA supplementation was employed to induce hepatic injury in C57BL6 mice. Mice were assigned into four groups: Control + PBS, Control + BMX-001, EtOH + PBS, EtOH + BMX-001. BMX-001 was administered in four intraperitoneal doses. Liver tissue and serum were collected 7 hours post-binge.

Findings demonstrated that ethanol plus PUFA induced macrovesicular steatosis, elevated hepatic triglycerides, and increased perilipin 2 (PLIN2) expression. BMX-001 treatment significantly reduced lipid peroxidation, evidenced by decreased 4-Hydroxynonenal (4HNE) staining and partial attenuation of malondialdehyde (MDA). Ethanol markedly increased hepatic 4HNE levels, with males exhibiting greater oxidative stress than females; However, BMX-001 abolished these sex-specific differences, with the most pronounced reduction in lipid peroxidation observed in males. Co-localization analyses revealed that lipid peroxidation was primarily associated with lipid droplets. Despite antioxidant effect, BMX-001 did not alter triglyceride accumulation, or PLIN2 expression, indicating that steatosis persisted despite reduced oxidative stress.

Chronic-plus-binge ethanol feeding with PUFA supplementation recapitulates key features of ALD. The SOD-mimetic BMX-001 selectively ameliorates lipid peroxidation but does not improve steatosis,

suggesting that oxidative stress is a significant yet insufficient driver of ALD pathology. These findings highlight the need for combination strategies targeting both lipid metabolism and oxidative damage in ALD therapy.

Keyword: *Alcohol-Associated Liver Disease, Lipid Peroxidation, Superoxide Dismutase-Mimetic*

ID: 25 - MAM2025 - Poster Presentation

Validation of the proteasome-targeted biosensors for cAMP/PKA signaling in cultured cardiomyocytes

Jingyi Lin * **, Samiksha Giri, Ana Tomoson, Xuejun Wang**

Division of Biomedical and Translational Sciences, Sanford School of Medicine, University of South Dakota, Vermillion, SD 57069

Background and Objectives:
Augmenting cAMP/PKA signaling selectively at proteasome nanodomains would enhance proteasome function while minimizing its undesirable effects. Although FRET-based biosensors (like H187/AKAR3) are widely used for studying cAMP/PKA signaling, proteasome-targeted biosensors are not currently available. Hence, we sought to develop and validate such biosensors for in situ detection of cAMP/PKA signaling selectively at proteasome nanodomains in cardiomyocytes.

Methods and Results:
FRET-based Proteasome-targeted (Psm-H187/Psm-AKAR3) and non-targeted (H187/AKAR3) cAMP/PKA signaling biosensors that are validated in non-cardiac cells were delivered to cultured neonatal rat cardiomyocytes (NRCMs) via recombinant adenoviral vectors. Confocal microscopy revealed that Psm-H187/Psm-AKAR3 distributed differently from H187/AKAR3 but consistently with proteasomes including nuclear enrichment. Native gel electrophoresis followed by direct fluorescence imaging and in-gel chymotrypsin-like activity fluorogenic assays showed that Psm-H187/Psm-AKAR3 but not H187/AKAR3 colocalized with functional proteasome assembles. Western blot analyses confirmed the size of all biosensors and the expected reduction of the endogenous counterparts of proteasome-targeting subunits in cells expressing Psm-H187/Psm-AKAR3 but not in those expressing H187/AKAR3. These results demonstrate the incorporation of the Psm-H187 and Psm-AKAR3 biosensors into the proteasome.

cAMP/PKA activation induced changes in FRET were captured in real-time using confocal microscopy. The acceptor to donor fluorescence ratio (YFP/CFP; FRET efficiency) was subsequently derived from the images. Compare to baseline, adenylate cyclase activator forskolin treatment (10mM) increased both cytoplasmic and nuclear YFP/CFP ratios in Psm-AKAR3 expressing cells as well as AKAR3 expressing cells and decreased cytoplasmic and nuclear YFP/CFP ratios in Psm-H187 expressing cells and H187 expressing cells, as expected. These experiments have validated that Psm-H187 and Psm-AKAR3 work as designed as proteasome-targeted biosensors for cAMP and PKA signaling.

Conclusion:
We have successfully created proteasome-targeted biosensors (Psm-H187/Psm-AKAR3) for in situ monitoring cAMP/PKA signaling in cardiomyocytes, providing valuable tools for studying cAMP/PKA signaling compartmentalization at proteasome nanodomains.

Keyword: *FRET-based Proteasome-targeted biosensors, cAMP/PKA signaling, cardiomyocytes*

ID: 26 - MAM2025 - Poster Presentation

Palmitoleate Protects against Zika virus infection-induced Endoplasmic Reticulum Stress and Apoptosis in Neurons

Chandan Krishnamoorthy * [1], **Anthony Delaney**[1], **Devanshi Shukla**[2], **Taija Hahka**[3], **Ann Anderson-Berry**[3], **Sathish Kumar Natarajan**[4]

[1] *Department of Nutrition and Health Sciences, University of Nebraska-Lincoln, Lincoln, NE, USA*
[2] *Graduate Interdisciplinary Program Neuroscience, University of Arizona, Tucson, AZ, USA*
[3] *Department of Pediatrics, University of Nebraska Medical Center, Omaha, NE, USA*
[4] *Department of Nutrition and Health Sciences, University of Nebraska-Lincoln, Lincoln, NE, USA; College of Allied Health Professions Medical Nutrition Education, University of Nebraska Medical Center, Omaha, NE, USA; Department of Biochemistry, University*

Zika virus (ZIKV) infection during pregnancy is associated with the development of fetal complications such as microcephaly. We have recently demonstrated that palmitoleate protects against ZIKV-induced apoptosis in placental trophoblasts. In the present study, we hypothesize that palmitoleate prevents ZIKV infection-induced endoplasmic reticulum (ER) stress and apoptosis in neurons. Neurons were infected with 0.1-1 multiplicity of infection of recombinant MR766 or PRVABC59 strains of ZIKV for an hour followed by treatment of palmitoleate (100 μM-200 μM) for different post-infection time points. Apoptosis was measured by nuclear morphological changes, caspase 3/7 activity, and immunoblot analysis of pro-apoptotic mediators. Activation of ER stress markers and viral envelope levels were detected using qRT-PCR and immunoblot analysis. Infectious virus particles were measured by using plaque assay. ZIKV infection to neuronal cells showed increased levels of pro-apoptotic markers like cleaved-PARP, cleaved caspase-3, Bim, and Puma, whereas decreased levels of anti-apoptotic markers such as Mcl-1, Bcl-1, and Bcl-xL. Further, we observed activation of three arms of ER stress namely: inositol requiring enzyme 1 alpha (IRE1), protein kinase-like ER kinase (PERK), and activating transcription factor (ATF6) pathways with ZIKV infection. Treatment of palmitoleate dramatically decreased ZIKV infection-induced increase in percent apoptotic nuclei and caspase 3/7 activity. Further, treatment of palmitoleate decreased cleaved PARP and PUMA protein expressions. Treatment of palmitoleate reduced ZIKV-induced ER stress activation as evidenced by decreased levels of phosphorylated forms of IRE1 and eukaryotic initiation factor 2 alpha; decreased expressions of cleaved ATF6, spliced X-box associated protein 1 and C/EBP homologous protein compared to ZIKV infection alone. Further, treatment of palmitoleate attenuated ZIKV envelope levels and infectious titer in SH-SY5Y and primary fetal cortical neurons isolated from humanized STAT2 knockin mice. These data suggest that palmitoleate supplementation protects against ZIKV-induced neuronal ER stress, apoptosis and decreases Zika viral load thereby mitigates neuronal damage.

Keyword: *Congenital Zika Syndrome, Microcephaly, ER stress*

ID: 27 - MAM2025 - Poster Presentation

Neutrophil Reprogramming Fuels Immunosuppression in Staphylococcus aureus Biofilm Infections

Adedayo Ogunware * **, Zachary Van Roy, Gunjan Kak, Tammy kielian**
Department of Pathology, Microbiology, and Immunology, University of Nebraska Medical Center, Omaha, Nebraska

Neurological conditions-such as brain tumors, traumatic injuries, epilepsy, hydrocephalus, and intracranial hemorrhage-often require a craniotomy to access the brain. Unfortunately, these essential interventions carry a significant infection risk of up to 15%, predominantly due to Staphylococcus aureus (S. aureus) biofilm formation. Biofilms are notoriously difficult to treat because they tolerate antibiotic therapy and establish immunosuppressive environments that severely impair host immune responses. A key element of biofilm-associated immunosuppression involves granulocytic myeloid-derived suppressor cells (G-MDSCs; $Ly6G^{+}Ly6C^{+}CD11b^{high}$), which accumulate at infection sites alongside neutrophils (PMNs; $Ly6G^{+}Ly6C^{+}CD11b^{low}$). G-MDSCs promote infection persistence by secreting anti-inflammatory molecules, inhibiting T cell proliferation, and diminishing neutrophil antibacterial activity.

Our research demonstrates that bone marrow-derived neutrophils exposed to S. aureus biofilms transition to a G-MDSC-like phenotype, characterized by elevated expression of CD11b, CD14 (a traditional monocyte marker), and programmed death-ligand 1 (PD-L1), an essential inhibitor of T cell responses. Functionally, these transformed neutrophils exhibit diminished bactericidal capacity and

actively suppress T cell proliferation, potentially perpetuating chronic infections. Employing RNA-sequencing and genetic knockout models, we identified Toll-like receptor 2 (TLR2) as a critical mediator driving the transition from neutrophils to G-MDSCs. Biofilm-induced expression of immunosuppressive markers depends on intact TLR2 signaling, as demonstrated by the significant reduction in G-MDSC traits in TLR2-deficient neutrophils. Moreover, complete deletion of MyD88, a key adaptor molecule in TLR signaling pathways, entirely prevented this neutrophil transformation, indicating the involvement of additional MyD88-dependent receptors.
Further insights from a screen of the Nebraska Transposon Mutant Library (NTML)-comprising 1,920 S. aureus mutants- confirmed that specific bacterial-derived TLR2 ligands are essential for driving neutrophil anti-inflammatory reprogramming. Collectively, these findings highlight both pathogen-derived TLR2 ligands and host TLR2 signaling as promising therapeutic targets to impair G-MDSC development and promote immune clearance of biofilms in neurosurgical patients.

Keyword: *Host-pathogen interactions, Toll like receptors, Biofilms*

ID: 28 - MAM2025 - Poster Presentation

Targeting TREM1: A Small Molecule Approach to Mitigate Global Cerebral Ischemia-Induced Neuronal Death and Cognitive Impairment

Prerna Lalan Tiwari [*] [1], **Hyunha Kim**[1], **Rachael Urquhart**[1], **Vanessa Duncan**[2], **Peter Abel**[1], **Gopal Jadhav**[1], **Jee-Yeon Hwang**[1]

[1] *Department of Pharmacology and Neuroscience, Creighton University School of Medicine, Omaha, Nebraska, 68178*

[2] *College of Saint Mary, Omaha, NE*

Global cerebral ischemia, commonly resulting from cardiac arrest, causes widespread oxygen deprivation and selective neuronal death, particularly in the hippocampal CA1 region, leading to severe cognitive deficits. Despite its clinical significance, no effective neuroprotective therapies currently exist. Neuroinflammation plays a central role in post-ischemic neurodegeneration, marked by glial activation, peripheral immune cell infiltration, and elevated proinflammatory cytokines. The triggering receptor expressed on myeloid cells-1 (TREM1), a membrane-bound immune receptor, amplifies inflammatory responses and is implicated in central nervous system disorders. Preliminary studies revealed that global ischemia upregulates TREM1 and its adaptor DAP12 in the hippocampal CA1, and that inhibition of TREM1 via the LR12 peptide confers neuroprotection. However, peptide-based inhibitors including LR12 are limited as viable drug candidates due to poor pharmacokinetics, blood-brain barrier permeability, and the need for intravenous administration. To overcome these limitations, we employed molecular docking of 80,000 compounds to the hTREM1 crystal structure (PDB: 1SMO) and identified an N4-(amino-substituted)-N-substituted-benzene sulfonamide pharmacophore as a hit molecule. Surface Plasmon Resonance (SPR) analysis confirmed its affinity to TREM1 (Kd = 14.3 μM), though solubility was poor. To improve this, we synthesized a para-fluoro analog, GJ079, which demonstrated enhanced solubility and significantly improved affinity (Kd = 4.8 nM). Building on this scaffold, we synthesized 44 analogs across five structural series to optimize pharmacological and pharmacokinetic properties. These included aromatic-acetamido substitutions on thiazolyl and N^4-aromatic amino groups, fluoro substitutions on the terminal phenyl ring, and heteroaromatic replacements of the thiazolyl ring. GJ079 was evaluated under inflammatory and ischemic conditions in vitro and in a rat model of global ischemia in vivo, where it reduced proinflammatory cytokines, attenuated neuroinflammation, and prevented neuronal death, demonstrating neuroprotective efficacy. Ongoing studies are validating the 44 analogs in vitro, with promising candidates advancing to in vivo testing to further assess therapeutic potential.

Keyword: *Global Cerebral Ischemia, TREM1, Small-Molecule Drug Discovery*

ID: 29 - MAM2025 - Poster Presentation

Maternal Macadamia Nut Powder Supplementation Reshapes Gut Microbiota, Enhances Short-Chain Fatty Acid Production in an animal model of Maternal Obesity

Deekshika Sekar * **, prakash Sahoo, Aiswariya Ravi, Chandan krishnamoorthy, Jean Jack Riethoven, Amanda Ramer- Tait, Jennifer Wood, Sathish Kumar Natarajan**

University of Nebraska - Lincoln

Background

Maternal obesity poses significant public health challenges, contributing to adverse fetal programming and increasing the risk of metabolic disorders like insulin resistance and obesity in offspring. These effects are partially driven by alterations in maternal gut microbiota and their metabolic outputs, such as short-chain fatty acids (SCFAs) and folate, which play critical roles in fetal development. Targeting the maternal gut microbiome through dietary interventions could mitigate these risks. Macadamia nuts, rich in bioactive compounds like palmitoleate, have demonstrated protective effects against metabolic dysfunction associated with saturated free fatty acids and offer a promising strategy for maternal obesity.

Hypothesis

This study hypothesized that dietary supplementation with macadamia nuts could attenuate obesity-induced gut dysbiosis, enhance SCFA and folate production, and reduce pro-inflammatory pathways, ultimately improving fetal metabolic outcomes.

Experimental Design. Female C57BL/6 mice were fed a high-fat, high-sucrose, high-cholesterol diet (HFHS) for 16 weeks to induce obesity. Dietary interventions included control, HFHS, and HFHS diets supplemented with macadamia nuts at 2%, 5%, and 10% (wt/wt). Microbial and metabolic changes were assessed using shotgun metagenomic sequencing by COSMOS ID and HUMAnN2 analysis.

Results

Results revealed that maternal macadamia supplementation (5–10%) enriched beneficial gut microbiota such as Akkermansia muciniphila and Bifidobacterium pseudolongum, taxa associated with improved gut barrier integrity and metabolic health. SCFA-producing pathways (e.g., butanoate fermentation) and folate biosynthesis were significantly enhanced, especially by B. pseudolongum. Conversely, HFHS-fed mice exhibited increased Firmicutes/Bacteroidetes ratio, pro-inflammatory pathways, and gut-derived inflammation. Macadamia supplementation reduced circulating lipopolysaccharide (LPS) levels, improved butyrate levels, and supported fetal neurodevelopment through folate-dependent neural tube formation and gut-brain axis modulation.

Conclusion

In conclusion, macadamia-rich diets remodel maternal gut microbiota, improve metabolic outputs, and mitigate adverse fetal programming associated with maternal obesity. Future studies will focus on offspring SCFA levels and long-term metabolic outcomes.

Keyword: *Gut Microbiota, Macadamia Nuts, Maternal Obesity*

ID: 30 - MAM2025 - Oral and Poster Presentation

Chronic In Utero Oxycodone Exposure Alters Placental EV Proteome and Fetal Cardiomyopathy-Linked Pathways

Amin Foroughi-Nezhad * **, Dalia Moore, Vicki Schaal, Tousif Hediyal, Elizabeth Stone, Sree kolli, Pranavi Athota, Omar Shukri, Sowmya Yelamanchili, Gurudutt Pendyala**

Department of Anesthesiology, College of Medicine, University of Nebraska Medical Center (UNMC), Omaha, NE 68198, USA

The rising prevalence of opioid use during pregnancy poses serious public health concerns. The placenta is a critical organ during gestation, and opioid exposure can disrupt its function and fetal development. However, the molecular mechanisms by which opioids such as oxycodone impact feto-placental development remain poorly understood. This study aimed to investigate the effects of chronic in-utero oxycodone exposure on the composition and signaling functions of placenta-derived extracellular vesicles (PEVs) using a rat model. EVs were isolated from placental tissue and

characterized through nanoparticle tracking analysis, transmission electron microscopy, western blotting, and label-free quantitative proteomics. Bioinformatic enrichment analyses were conducted to evaluate changes in EVs biophysical properties and protein cargo. Chronic oxycodone exposure significantly altered PEV characteristics, including particle size distribution and proteomic composition. Among the 456 identified EV proteins, 107 proteins were significantly dysregulated. We found key downregulatory proteins including Atp2a2, Lmna, Tgfb3, Agt, and Sgce, which are crucial for myocardial calcium cycling, nuclear integrity, extracellular matrix remodeling, and blood pressure regulation. These findings indicates disruptions in fetal cardiac programming, particularly hypertrophic and dilated cardiomyopathy pathways. Additionally, enrichment analyses revealed notable perturbations in metabolic processes (e.g., TCA cycle, fatty acid degradation, N-glycan biosynthesis), along with upregulation of vesicle transport and neurodevelopment-related proteins, indicating broader systemic effects on fetal development. While these proteomic findings are robust, further independent validation (e.g., via targeted assays or Western blotting) will be necessary to confirm individual protein-level changes. These results highlight PEVs as sensitive molecular indicators linking maternal oxycodone use to disrupted fetal cardiovascular, metabolic, and neurodevelopmental pathways. This study provides a novel systems-level framework for understanding opioid-induced placental signaling alterations and lays the groundwork for developing EV-based diagnostic biomarkers and targeted interventions.

References: Jeyarajah MJ, Patterson VS, Jaju Bhattad G, Zhao L, Whitehead SN, Renaud SJ. Placental extracellular vesicles promote cardiomyocyte maturation and fetal heart development. Commun Biol 2024;7:1254. [PMID: 39363116 DOI: 10.1038/s42003-024-06938-4].

Keyword: *EV biomarkers, Perinatal exposure, Proteomics*

ID: 31 - MAM2025 - Poster Presentation

Evaluating the Utility and Function of LSMEM2 in Cardiomyocyte Physiology

Joy Adewumi * **, Rebekah Gundry**

University of Nebraska Medical Center

Cardiac surface proteins play critical roles in normal cardiomyocyte (CM) function. A better understanding of this subproteome provides molecular detail necessary to understand cardiac physiology and pathophysiology, paving the way for the discovery of novel therapeutic agents and improving existing treatment modalities for cardiac diseases. Our recent primary human cardiomyocyte surfaceome profiling study identified >1,000 cell surface N-glycoproteins, including a novel cardiac surface protein – leucine-rich single-pass membrane protein 2 (LSMEM2), found exclusively on cardiomyocytes, and only previously identified on human pluripotent stem cell-derived cardiomyocytes. LSMEM2 is a novel CM-restricted protein present in relatively equal abundance across the four myocardial regions of the heart. Surface abundance of LSMEM2 decreases in primary cardiomyocytes isolated from failing hearts compared to non-failing hearts. These preliminary findings highlight LSMEM2 as a never-before-discovered and uncharacterized protein of interest with considerable potential utility for downstream applications, including targeted drug delivery and marker of quality control for both primary and stem cell-derived cardiomyocytes. The goal of this study is to characterize LSMEM2 and further inform its future utility for research applications and its relevance to cardiomyocyte physiology. Ongoing studies are aimed at determining its specific subcellular localization within the plasma membrane, binding partners, evaluating specificity for cardiomyocytes in context of other major organ systems, and determining its functional requirement during stem cell differentiation. Overall, these are the first studies designed to characterize this newly discovered protein, and are expected to contribute to our basic understanding of cardiomyocyte physiology and pathophysiology.

Keyword: *cardiac surface protein, stem cell-derived cardiomyocytes, cardiomyocyte physiology*

ID: 32 - MAM2025 - Oral and Poster Presentation

Duration and Severity of Maternal Hyperglycemia Drive Fetal Endothelial Injury and Newborn Blood Pressure Elevation

Colman Freel * [1], **Kayla Adams**[2], **Corrine Hanson**[3], **Ann Anderson-Berry**[4], **Paras Kumar Mishra**[1]

[1] *Department of Cellular and Integrative Physiology, University of Nebraska Medical Center, Omaha, NE, USA*

[2] *College of Allied Health Professions, University of Nebraska Medical Center, Kearney, NE, USA*

[3] *College of Allied Health Professions, University of Nebraska Medical Center, Omaha, NE, USA*

[4] *Division of Newborn Medicine, Department of Pediatrics, University of Nebraska Medical Center, Omaha, NE, USA*

Background: Maternal diabetes complicates >16% of pregnancies, and its three types (type 1, type 2, gestational) differentially impact newborn blood pressure (BP). A hallmark of diabetes is hyperglycemia, which varies in duration and severity depending on type. Maternal hyperglycemia drives fetal hyperinsulinemia and increases circulating fatty acids. This can damage the fetal endothelium, which regulates newborn BP. However, the relationships between these impacts and the duration/severity of maternal hyperglycemia remain poorly defined. We hypothesized that fetal endothelial damage and newborn BP elevation would be dependent on the duration and severity of maternal hyperglycemia.

Methods: We performed a retrospective analysis of clinical data and complementary experiments in vitro. Patient data were obtained from UNMC deliveries (2012-2025). Associations between newborn BP and maternal HbA1c (n=132) or oral glucose tolerance test (OGTT) results (n=244) were determined using multivariate regression. HUVECs (fetal endothelial model) were exposed to different doses of glucose and/or insulin for 48 or 96 hours before viability (CCK8), cytotoxicity (LDH), and survival assays (crystal violet). To model chronic conditions, HUVECs were co-exposed to glucose, insulin, and palmitate for 96 hours before assessment of oxidative stress (MitoSox), cytoskeletal structure (vimentin immunocytochemistry), and cell death (western blot).

Results: Positive correlations were observed between newborn BP and Maternal HbA1c (all $p<0.05$, $R^2>0.2$) but not OGTT. In vitro, exposing HUVECs to high levels of glucose over time increased cell damage in a dose-dependent fashion. Insulin offered short-term protection which faded with continued glucose exposure. Adding palmitate exacerbated damage by increasing oxidative stress ($p=0.04$), altering cytoskeletal structure ($p=0.01$), and triggering cell death through apoptosis (pro Cas3, $p=0.03$; cleaved Cas3, $p<0.0001$) and ferroptosis (GPX4, $p=0.03$).

Conclusions: Impacts on fetal endothelial damage and newborn BP are dependent on the duration and severity of maternal hyperglycemia. Future studies should compare fetal endothelial damage between different types of maternal diabetes.

References: https://tinyurl.com/5dsakwpa

Keyword: *Maternal Hyperglycemia, Newborn Blood Pressure, Fetal Endothelial Damage*

ID: 33 - MAM2025 - Oral and Poster Presentation

Cardiomyocyte Specific Knockout of p62/SQSTM1 Exacerbates CryABR120G-based Cardiac Proteinopathy in Mice

Md Salim Ahammed * **, Jack Olson Sternburg, Xuejun Wang**

Division of Biomedical & Translational Sciences, University of South Dakota Sanford school of Medicine, Vermillion, SD 57069, USA

Aberrant protein aggregation and impaired proteostasis are suggested as central drivers for cardiac pathogenesis. αB-crystallin (CryAB), a small heat shock protein, functions as a molecular chaperone that binds desmin and cytoplasmic actin, supporting intermediate filament assembly and cytoskeletal

integrity. In the heart, CryAB is essential for proteostasis, and mutations like R120G missense variant cause familial desmin-related cardiomyopathy (DRC) in humans and mice, featured by aberrant protein aggregation, cardiac hypertrophy, heart failure, and mouse premature death.
Sequestosome-1 (p62/SQSTM1) is a multifunctional adaptor protein highly expressed in cardiomyocytes, where it regulates proteostasis by linking polyubiquitinated proteins to both the autophagy–lysosome and ubiquitin–proteasome systems. Although the upregulation of p62 was documented in CryAB^{R120G} hearts in prior studies and cell cultures studies indicated a protective role of p62, its functional role in cardiac proteinopathy remains undefined in animals. We conducted the present study to test in mice the hypothesis that p62 facilitates proteostasis and protects heart from CryAB^{R120G} -induced pathology.
We generated cardiomyocyte-specific p62 knockout mice (p62-cko; p62$^{flox/flox}$::Myh6-Cre) which showed a lifespan similar to Myh6-Cre mice. We then crossed the p62-cko with CryAB^{R120G} transgenic mice to model selective p62 ablation during DRC. Serial M-mode echocardiography was performed on the mice at 4-, 5- and 6-months of age. At 5 months, p62-deficient CryAB^{R120G} mice exhibited significantly reduced fractional shortening (mean±SEM; 24.54±3.72 vs. 40.77±4.84, p=0.014), ejection fraction (47.79±6.08 vs. 70.55±5.32, p=0.013), and stroke volume/body weight ratio (0.93±0.09 vs. 1.28±0.06, p=0.008), compared with control (CryAB^{R120G}::Myh6-Cre) mice. Furthermore, the lifespan of CryAB^{R120G}::Myh6-Cre::p62$^{flox/flox}$ mice was significantly shorter than that of CryAB^{R120G}::Myh6-Cre mice (median: 186 vs. 196 days; p=0.005).
These findings provide the first direct in vivo evidence that p62 is protective in CryAB^{R120G} -induced cardiac proteinopathy. Ongoing studies aim to elucidate the molecular mechanisms by which p62 maintains proteostasis and mitigates proteotoxic cardiomyopathy.
Keyword: *Proteostasis, Heart Failure, p62/SQSTM1*

ID: 34 - MAM2025 - Poster Presentation

Fetal Sex Differences in Circulating Specialized Pro-Resolving Mediators and Associations with Neonatal Intensive Care Admission.

Samuel Owolabi * [1], **Colman Freel**[2], **Corrine Hanson**[3], **Tara Nordgren**[1], **Ann Anderson-Berry**[1]

[1] *Division of Newborn Medicine, Department of Pediatrics, University of Nebraska Medical Center, Omaha, NE, USA.*

[2] *Department of Cellular and Integrative Physiology, University of Nebraska Medical Center, Omaha, NE, USA.*

[3] *College of Allied Health Professions, University of Nebraska Medical Center, Omaha, NE, USA.*

Background: In the US, almost 10% of newborns are admitted to the neonatal intensive care unit (NICU) following delivery. The reasons for NICU admission are multifaceted with inflammatory dysregulation as a central pathologic feature. Specialized pro-resolving mediators (SPM) derived from omega-3 fatty acids, such as resolvin D1 (RvD1) and resolvin D2 (RvD2), are mobilized during inflammatory states and can alleviate inflammatory dysregulation. However, there is limited understanding of their role in maternal-fetal health. Moreover, it remains unclear whether sexual dimorphisms of lipid metabolism impact SPM regulation, a critical gap considering disparities in newborn health outcomes attributable to male sex. We hypothesized that NICU admission would be associated with elevated SPM with increased levels associated with delivery of male sex newborns.
Methods: Maternal-infant dyads (n=99) were recruited at UNMC. Maternal and cord plasma samples were collected at delivery and clinical information was obtained from the electronic health record. Plasma RvD1and RvD2 were quantified by ELISA. Cord, maternal, and the ratio of cord:maternal plasma levels of RvD1 and RvD2 were compared between NICU groups and newborn sexes. Sub-analyses of levels in NICU groups were stratified by newborn sex. Mann-Whitney U tests were used for all statistical analyses (significance set at $p<0.05$).
Results: Cord, maternal, and cord:maternal plasma RvD1 and RvD2 levels did not differ significantly between newborn sexes, although cord RvD2 trended lower in males compared to females (p=0.06). Levels of maternal plasma RvD1 were significantly higher in NICU compared to non-NICU groups (4551

pg/mL vs 1381 pg/mL; p=0.0008); however, sex stratification revealed significant differences only among males (4552 pg/mL vs 1929 pg/mL; p=0.03). **Conclusion:** SPMs are associated with NICU admission in newborns in a sex-dependent manner. This highlights the need for sex consideration in biomarker development and deeper mechanistic investigation of SPM metabolism during inflammatory events.

References:

https://docs.google.com/document/d/16cwpNclHko15DZjIEy9xzbPcJdZPiFX4VWLS9h4YCmc/edit?usp=sharing

Keyword: *Specialized Pro-Resolving Mediators, Sexual Dimorphisms, Newborn Health Outcomes*

ID: 35 - MAM2025 - Poster Presentation

P-REX1 Modulates Polarization And Functions of Tumor-associated Macrophages

Daniel Afolabi * **, Peter Abel, Holly A.F Stessman, Yaping Tu**

Department of Pharmacology and Neuroscience, Creighton University School of Medicine

Background: Tumor-associated macrophages (TAM) are a key subset of macrophages that infiltrate the tumor microenvironment, often adopting an M2-like phenotype. TAMs contribute to tumor progression by promoting angiogenesis and immune suppression, inhibiting cytotoxic CD8+ T-cell activity, and inducing T-cell exhaustion. Aberrant expression of P-Rex1 (Phosphatidylinositol-3,4,5-Triphosphate-dependent Rac exchanger 1) in cancer cells has been implicated in tumor progression by promoting growth and metastasis. Interestingly, while P-Rex1 is highly expressed in macrophages, its role in the polarization and function of TAM remains unclear. **Results**: Tumor-conditioned medium (TCM) from Lewis lung carcinoma (LLC) cells induced TAM-like differentiation in mouse primary bone marrow-derived macrophages (BMDMs) and murine RAW 264.7 macrophage-like cells, characterized by a marked upregulation of ARG1 and vascular endothelial growth factor (VEGF), a key factor in angiogenesis and tumor progression, with minimal changes in M1 marker iNOS expression. TCM treatment also downregulated P-Rex1 expression in BMDMs. Notably, P-Rex1 deletion in BMDMs and P-Rex1 silencing in RAW cells enhanced TCM-induced TAM-like differentiation. Although P-Rex1 deletion did not affect the phagocytic ability of naïve macrophages, it significantly reduced the phagocytic capacity of TAM-like macrophages. P-Rex1 deletion also promoted macrophage migration toward tumor cells, enhanced TAM suppression of CD8+ T-cell migration, and contributed to Tcell exhaustion. **Conclusion**: Our findings suggest that P-Rex1 plays a pivotal role in regulating TAM's polarization and functional plasticity within the tumor microenvironment. Loss of P-Rex1 promotes TAM differentiation, impairs their phagocytic capacity, and exacerbates immune evasion by enhancing TAMmediated suppression of CD8+ T-cell migration and promoting T-cell exhaustion

Keyword: *Tumor Associated Macrophages, P-Rex1, CD8+ T cells*

ID: 36 - MAM2025 - Poster Presentation

Exploring the role of dietary γ-glutamyl valine (γ-EV) in modulating the gut microbiota to mitigate high-fat diet-induced atherosclerosis

Sedoten Ogun * **, Amanda Ramer-Tait, Kaustav Majumder**

Department of Food Science and Technology, University of Nebraska–Lincoln, USA , Nebraska Food for Health Center, Lincoln, Nebraska, USA

Atherosclerosis, marked by plaque buildup and lipid deposition in arteries, is a significant contributor to cardiovascular diseases, the leading cause of global morbidity and mortality. Current treatments primarily focus on addressing the aftermath of atherosclerosis, such as plaque rupture, thrombosis, or heart attacks. However, there are limited preventive strategies available to target the underlying progression of the disease. γ-glutamyl valine (γ-EV), a bioactive peptide predominantly found in fermented foods, has promising anti-atherosclerotic properties. However, only a portion—76%—of this peptide is bioavailable. Thus, we hypothesized that the 24% non-bioavailable γ-EV may modulate the gut microbiome, bringing about the prevention of atherosclerosis. Atherosclerosis-prone APO E-/-

mice were fed either a Low-Fat Diet (LFD; 10% kcal fat), a High-Fat Diet (HFD; 40% kcal fat), or a HFD supplemented with 150 mg/kg γ-EV for 15 weeks. Treatment with γ-EV significantly reduced the lipid accumulation in the aortas of HFD-fed APO E-/- mice compared to mice receiving HFD alone. 16S rRNA bacterial gene sequencing analysis of the microbiome revealed that γ-EV treatment increased Akkermansia compared to HFD feeding, and qPCR testing identified that γ-EV particularly enriched for Akkermansia muciniphila, a beneficial gut bacterium. Correlation analyses revealed a strong, negative association between Akkermansia abundance and reduced lipid deposition and inflammation. Previous studies have shown that A. muciniphila limits chronic inflammation and protects against atherosclerosis. We therefore tested if treatment with γ-EV could directly influence the abundance of A. muciniphila in vitro. Treatment with 5 mM and 10 mM doses of γ-EV both significantly enhanced the growth of A. muciniphila compared to control media. Altogether, the findings from this study suggest that γ-EV may limit the development of atherosclerosis by promoting the growth of A. muciniphila. Future studies will investigate the requirement of A. muciniphila in vivo for mediating the anti-atherosclerotic properties of γ-EV.

Keyword: *Atherosclerosis, Gamma glutamyl peptides, Inflammation*

ID: 37 - MAM2025 - Poster Presentation

Effects of Environmental Enrichment and Sex on the Rewarding and Analgesic Effects of Vaporized Delta-8-Tetrahydracannabinol

Hunter Hess * [1], **Cassandra Cavuoto[1], Elizabeth Mark[1], CeCe Taylor[2], Dustin Stairs[1]**

[1] *Creighton University*

[2] *Central High School, Omaha NE*

Exposing rats to an enriched environment has been shown to alter the sensitivity to behavioral effects of psychostimulants. A majority of research has focused on the enrichment-induced differences in the rewarding effects of stimulant drugs, no research has investigated the effects of environmental enrichment on the behavioral effects of cannabis. This study was designed to determine whether the environmental enrichment paradigm could alter the sensitivity to the rewarding and analgesic effects of vaporized delta-8 THC, and if these effects varied by the sex of the animal. Once male and female (PND 21) Sprague Dawley rats arrived, they were placed into either the enriched condition (EC) or isolated condition (IC), where they remained for the duration of the experiment. The potential rewarding effects of delta-8 THC were measured using a conditioned place preference (CPP) paradigm. Animals were given 10-min vapor exposures to either vehicle (Propylene Glycol (PG)), or 10 mg/.300ml delta-8 THC, then animals were immediately confined to one of the paired chambers of the CPP apparatus for a 30-min conditioning trial. Animals were given eight conditioning trials that alternated between delta-8 THC and PG exposure with associated CPP chamber pairing, followed by a drug-free test day. The test day analyzed the CPP response to the delta-8 THC paired side. The animals were then given another 8 conditioning trials, followed by another test day. Following CPP trials, animals were exposed again to either vehicle or delta-8 THC vapor for 10-min. Directly after exposure, the analgesic effect of delta-8 THC was tested at various times using a warm water tail withdrawal procedure. Results indicate female EC rats showed a stronger CPP effect compared to male rats. Also, EC female rats showed a stronger analgesic effect of delta-8 THC 60-min post vape exposure, whereas males did not show significant analgesic effects.

Keyword: *rat, delta-8 THC, Vapor*

ID: 38 - MAM2025 - Poster Presentation-ABSTRACT WITHDRAWN

~~Epidemiological Insights and Diagnostic Advances for West Nile Virus in Nebraska~~

~~**Sarah Uhm** * [1], **Dylan George[1], Joseph Fauver[2], M. Jana Broadhurst[1]**~~

~~[1] *University of Nebraska Medical Center, College of Medicine, Department of Pathology, Microbiology and Immunology, Omaha, NE 68198, USA*~~

[2] *University of Nebraska Medical Center, College of Public Health, Department of Epidemiology, Omaha, NE 68198, USA*

Background: West Nile virus (WNV) is the most prevalent mosquito-borne disease in the United States, with Nebraska consistently reporting high rates. Although <1% of infections progress to neuroinvasion, the manifestations, including meningitis and encephalitis, are severe and cause lasting damage. Even mild febrile illness can cause long-term neurological sequelae, underscoring the significant physiological impact of WNV. Despite these notable consequences, the burden of WNV, especially subclinical cases, remains unclear. To better characterize this burden, we analyzed Nebraska case data and developed a novel WNV diagnostic assay.

Methods: WNV county-level case data was obtained from the CDC (2002-2023) for Nebraska. Total and neuroinvasive average annual cumulative incidence per 100,000 people were computed. A novel Luminex-based multiplex serologic assay was developed to measure immunoglobulin M and G responses to key WNV antigens (envelope [E] and nonstructural protein 1 [NS1]). We used an indirect immunoassay, forming a stable ester and covalent link between the Luminex beads and His-tagged antigens. Quality control was conducted using antigen-specific monoclonal or polyclonal antibodies and anti his tag antibodies.

Results: The Nebraska WNV average annual cumulative incidence was 23.46 per 100,000, with 4.84 per 100,000 neuroinvasive cases. Nebraska's average annual cumulative incidence was nearly 30-fold higher than the national average (0.79 per 100,000). Cases clustered in western Nebraska, suggesting a disproportionate impact on rural populations. Preliminary Luminex findings demonstrate efficient coupling of WNV E and NS1 antigens to Luminex beads, with reliable detection at 80 pmol of protein per 1,000,000 beads.

Conclusions: The high WNV rates in Nebraska highlight a significant burden in comparison to the overall country. This assay also provides a scalable tool to gain insight into WNV antibody-mediated immunity and seroprevalence in high-risk populations. This work lays the foundation for future studies of long-term immunological outcomes, advancing our understanding of WNV immune responses.

References:
https://docs.google.com/document/d/1pPQH_GazCSml1X5uCgKoQkBzInM6ohrbL4Wseaz2eh0/edit?usp=sharing

Keyword: *West Nile virus (WNV), Epidemiology, Luminex assay*

ID: 39 - MAM2025 - Oral and Poster Presentation

Metabolic and Mitochondrial Dysfunction in Human Granulosa Cells from Poor Responders

Bunmi Owolabi [*] [1], **William Synder**[2], **Micah Schott**[3], **Zeljka Korade**[4], **Jordan Rowley**[5], **Chittibadu Guda**[5], **Elizabeth Constance**[6], **Stephanie Gustin**[6], **Oleh Khalimonchuk**[7], **James Diaz**[8], **John Davis**[2], **Michele Plewes**[2]

[1] *Department of Cellular and Integrative Physiology, University of Nebraska Medical Center*
[2] *Department of Obstetrics and Gynecology, University of Nebraska Medical Center*
[3] *Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center*
[4] *Department of Pediatrics, University of Nebraska Medical Center*
[5] *6Department of Genetics, Cell Biology, and Anatomy, University of Nebraska Medical Center*
[6] *Heartland Center for Reproductive Medicine*
[7] *Department of Biochemistry, University of Nebraska-Lincoln*
[8] *Department of Biomedical Sciences, University at Albany*

Age-related metabolic dysfunction contributes to declining fertility, with women over 37 experiencing reduced oocyte quality and impaired ovarian response during in vitro fertilization (IVF). Granulosa cells, which regulate steroid hormone biosynthesis and follicular development, undergo metabolic changes that may underlie poor ovarian response. This study characterized the metabolic phenotype of granulosa cells from women with varying ovarian responses to identify therapeutic targets for age-related fertility decline. Women undergoing IVF were stratified by age [young (≤30) vs. advanced

maternal age (AMA; ≥37)] and ovarian response [good responders (R; ≥16 oocytes) vs. poor responders (POR; ≤7 oocytes)], with age and elevated progesterone-to-follicle ratios significantly associated with poor ovarian response (P<0.0001). RNA-sequencing of patient granulosa cells followed by Ingenuity Pathway Analysis identified cholesterol biosynthesis and SREBP-mediated gene expression as the most significantly enriched pathways in poor responders, with SREBP1 (z-score 4.95; P=1.66E-22) and SREBP2 (z-score 3.96; P=1.11E-29) emerging as top upstream regulators. Mitochondrial analysis revealed ~10% of mitochondrial-associated genes (MitoCarta3.0) were differentially expressed in AMA poor responders, with nearly half involved in lipid metabolism and SREBP1/2 as predicted upstream regulators. Confocal imaging confirmed reduced mitochondrial DNA nucleoids in poor responder cells (P<0.05), indicating impaired genome maintenance. Functional validation using primary human granulosa cells and a human granulosa cell line (KGN) showed that SREBP1/2 overexpression significantly increased both basal and stimulated progesterone production (P<0.05). In KGN cells, SREBP1/2 overexpression induced dramatic cellular remodeling, including increased lipid droplet number (1.9-fold, P<0.001), size (3.3- and 2.2-fold, respectively, P<0.0001), and cellular hypertrophy (7.0- and 4.7-fold, respectively, P<0.0001 and P<0.001). These findings reveal coordinated SREBP-mediated metabolic dysfunction and mitochondrial impairment in granulosa cells from poor ovarian responders. This work links mitochondrial metabolism to ovarian aging, showing that SREBP dysregulation coordinates sterol biosynthesis with mitochondrial impairment. Targeting this mechanism may improve reproductive outcomes in age-related ovarian dysfunction.

Keyword: *IVF, Granulosa cells, SREBP*

ID: 40 - MAM2025 - Poster Presentation

Navigating the Metabolomics Landscape and Mitochondrial Bioenergetics of LCHAD/MTP Deficiency using human-Derived Fibroblasts

Aiswariya Ravi [*] [1], **Taija Hahka**[2], **Chandan Krishnamoorthy**[1], **Deekshika Sekar**[1], **Javier Seravalli**[3], **Sathish Kumar Natarajan**[1]

[1] *Department of Nutrition and Health Sciences, University of Nebraska-Lincoln, Lincoln, NE 68588*
[2] *School of Natural Sciences, Black Hills State University, Spearfish, SD 57799*
[3] *Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE 68588*

Background:
LCHAD/MTP deficiency, caused by mutations in the HADHA and HADHB genes, is a rare fatty acid oxidation (FAO) disorder with an estimated prevalence of ~1 in 100,000. Our previous data show that HADHA and HADHB variants lead to the accumulation of lipotoxic 3-hydroxy fatty acids, contributing to life-threatening phenotypes including cardiomyopathy, rhabdomyolysis, neuropathy, liver dysfunction, hypoglycemia, and sudden infant death syndrome.
Significance:
In MTP-null mice, defective energy homeostasis causes severe cardiac dysfunction and death within 36 hours of birth. Energy homeostasis requires abundant metabolites to fuel mitochondria; however, the specific roles of cellular metabolites and mitochondrial pathways in LCHAD/MTP deficiency remain undefined.
Hypothesis:
We hypothesize that human-derived HADHA and HADHB mutant fibroblasts (LCHAD/MTP) display altered metabolites and mitochondrial dysfunction compared to controls.
Experimental design and methods:
We obtained control fibroblasts and HADHA/HADHB variants expressing human fibroblasts from the Coriell Institute. Metabolite extraction was performed, followed by untargeted metabolomics using liquid chromatography–mass spectrometry (LC–MS). The resulting metabolic profiles were analyzed with MetaboAnalyst 6.0 to identify differential metabolites. In addition, mitochondrial bioenergetic

parameters were assessed using Seahorse™ technology, and flow cytometry was employed to determine the proportion of functional versus non-functional mitochondria.
Results:
Metabolic profiling revealed clear separation between control and HADHA, HADHB mutant fibroblasts, with ~50 significantly altered metabolites, including taurine, glutathione disulfide, asparagine, S-adenosylhomocysteine, and glyceraldehyde-3-phosphate. Pathway analysis indicated disruptions in mitochondrial pH, redox balance, antioxidant defense, respiration, DNA methylation, and glycolysis. Extracellular flux assays confirmed mitochondrial dysfunction, with reduced spare respiratory capacity and ATP production in mutants. Flow cytometry using MitoTracker™ Red/Green showed increased dysfunctional mitochondria in HADHA and HADHB mutants than control fibroblasts.
Conclusion:
Our findings reveal that HADHA and HADHB mutant cells exhibit broad metabolic alterations and mitochondrial dysfunction compared to control fibroblasts, providing new insights into the metabolic dysregulation underlying LCHAD/MTP deficiency.

Keyword: *Fatty acid oxidation, LCHAD/MTP deficiency, 3-Hydroxy fatty acids*

ID: 41 - MAM2025 - Poster Presentation

Expanding the Cardiac Surface Proteome in Advanced Heart Failure

Dorcas Akinuli * **, Rebekah Gundry**
University of Nebraska Medical Center (UNMC)

Background, Significance, Hypothesis: Cardiomyocyte cell surface glycoproteins are critical for ion transport, signal transduction, cell-cell communication, and molecular recognition. In advanced heart failure, dysregulation of these glycoproteins contributes to cardiomyocyte dysfunction, impaired contractility, and hypertrophy. Despite their importance, current understanding of glycoprotein alterations in failing hearts is limited, in part due to methodological constraints. Current surface proteomics rely on N-glycosylated-based enrichment, overlooking O-glycosylated site, O-glycan composition and even non-glycosylated proteins, thereby limiting proteome coverage. To address this, we propose a cell surface capture strategy targeting lysine residues on surface proteins. Lysine is abundant and surface-accessible to selective chemical labeling. We hypothesize that lysine-specific labeling will capture a broader spectrum of surface proteins, including non-glycosylated proteins thereby providing a more comprehensive view of the cardiomyocyte surface proteome and improving insight into proteomic alterations associated with advanced heart failure.
Experimental Design: Using CIRFESS, online tool that integrates multiple prediction strategies for annotations relevant for the analysis of cell surface proteins by mass spectrometry, we applied it to interrogate the human proteome. Lysine was identified as an optimal target. We employed a membrane-impermeable, cleavable, water-soluble biotinylating reagent specific to lysine. This reagent was validated in B cells and HEK 293T cells. Viability assays confirmed the reagent's non-toxicity, and fluorescence/confocal microscopy demonstrated exclusive surface labeling, verified by co-staining with nuclear (Hoechst), membrane (WGA-Fluor 488), and biotin(Streptavidin-PE) markers.
Data and Results: The method yielded clear, membrane-localized biotin signals without intracellular labeling, confirming specificity. These results validates the lysine-targeted labeling as a viable approach for wider cell surface protein coverage beyond glycosylation-based techniques.
Conclusion: This lysine-targeting strategy proposes a broader surface protein capture, including glycosylated and non-glycosylated proteins, in cardiomyocytes. Integration into mass spectrometry workflows may uncover novel biomarkers and therapeutic targets in advanced heart failure by revealing previously undetectable protein dysregulation at the cardiomyocyte surface.

Keyword: *Cell surface proteins, Lysine labeling chemistry, Advanced heart failure*

ID: 42 - MAM2025 – Poster Presentation

Development and characterization of an autoresuscitation assay for preclinical SUDEP models

Jillian Hinman * **, Shruthi Iyer, Samantha Draves, Stephanie Matthews, Kristina Simeone, Timothy Simeone**

Creighton University, Department of Pharmacology and Neuroscience

Approximately 1:1000 people with epilepsy die of Sudden Unexpected Death in Epilepsy (SUDEP) annually. Cardiorespiratory dysfunction has been implicated in SUDEP, but the underlying mechanisms remain elusive. Terminal events have been reported to result from prolonged apnea suggesting a failure to autoresuscitate, which involves gasping and return to eupnea. To investigate the efficacy of the autoresuscitation reflex and potential contribution to SUDEP, we adapted an anoxia-induced autoresuscitation challenge for use in the SUDEP field. The challenge was optimized to allow majority survival of wild-type mice regardless of age. We tested Kcna1$^{-/-}$ mice, a SUDEP model, at two different ages corresponding to low-risk and high-risk SUDEP susceptibility. As SUDEP risk increased in Kcna1$^{-/-}$ mice, autoresuscitation failure and mortality increased. During the challenge, Kcna1$^{-/-}$ mice had increased breathing frequency, tidal volume, minute ventilation, and peak expiratory flow compared to wild-type mice. Once gasping was initiated, Kcna1$^{-/-}$ mice had reduced gasp frequency and duration. Two distinct phases of gasping- Type I and Type II- were identified during the autoresuscitation response. Kcna1$^{-/-}$ mice exhibited reduced latency, number, frequency, and time spent in Type II gasps, suggesting potential greater importance for this phase. These data indicate that Kcna1$^{-/-}$ mice have enhanced chemosensitivity in response to anoxia, which may contribute to autoresuscitation failure.

Keyword: *Autoresuscitation, Respiration, Chemosensing*

POSTDOC CATEGORY

ID: 43 - MAM2025 - Poster Presentation

Modeling HIV Associated Neuropathology in a Novel Microglia Containing Brain Organoids

Adrian Domene * [1], **Dalia Moore[1], Mattew Van Hook[1], Mengmeng Jin[2], Ava Papetti[2], Peng Jiang[2], Sowmya Yelamanchili[1]**

[1] University of Nebraska Medical Center

[2] Rutgers University

Modeling HIV infection in brain organoids represents a promising approach to studying the impact of virus on the central nervous system (CNS). Cerebral brain organoids are 3D cultures derived from human induced pluripotentant stem cells that mimic the complexity of the brain and offer an excellent in vitro platform for investigating HIV neuroinvasion and neurocognitive disorders associated with chronic infection. This model enables us to explore the dynamics of HIV entry, replication, and the resultant neuroinflammation within a more physiologically relevant context compared to traditional cell cultures or animal models. By utilizing brain organoids, scientists can uncover key mechanisms underlying the virus's effects on neuronal function, identify potential therapeutic targets, and assess the efficacy of antiretroviral and neuroprotective drugs. Additionally, these models provide insights into how HIV may contribute to cognitive decline and other neurological complications in people living with HIV, thus advancing our understanding of the intersection between virology and neurobiology. Here, we have established a novel organoid model that recapitulates the immunotypic human brain mimicking the Trojan horse model thereby facilitating HIV entry. Our results reveal establishing a chronic infection resulting in neuroinflammation and synaptic injury as seen with HAND.

References: Dos Reis, R. S., et al. (2020). "Modeling HIV-1 neuropathogenesis using three-dimensional human brain organoids (hBORGs) with HIV-1 infected microglia." Sci Rep 10(1): 15209.

Keyword: *HIV, Organoids, Brain*

ID: 44 - MAM2025 - Poster Presentation

Impact of PKC-MAPK signaling on cardiac sympathetic overactivation in Type-2 Diabetes Mellitus

Jaswinder Singh * **, Afia Saabea Owusu Konadu, Yu Li, Boris Shabaltiy, Yu Long Li**

UNIVERSITY OF NEBRASKA MEDICAL CENTER

Background: Type-2 Diabetes Mellitus (T2DM) is a pervasive global health issue, with T2DM-related ventricular arrhythmias (VA) being a leading cause of death. The stellate ganglia (SG), part of the sympathetic nervous system, regulates heart function. Stellate glial cells (SGCs) within the SG, though electrically non-excitable, have gap junction channels (Cx43). Increased Cx43 permeability can depolarize SGCs membranes, elevate intracellular Ca^{2+} levels, and activate the PKC-MAPK pathway to release inflammatory cytokines, activating nearby cardiac postganglionic sympathetic neurons in the SG.

Hypothesis: We hypothesize that PKC-MAPK signaling pathway is associated with cardiac sympathetic overactivation through activating cardiac postganglionic sympathetic neurons in T2DM.

Methods: Rat T2DM was induced by a high fat diet plus injection of low dose of streptozotocin (30 mg/kg). Real-time RT-PCR, western blot, and ELISA were used to analyze mRNA, protein and enzyme activity. Single-cell patch-clamp technique was used to measure calcium currents and action potentials of stellate neurons. Electrophysiological recording was used to examine cardiac sympathetic nerve activity.

Results: The protein and gene expression of MAPK14, PKC-α, and ADAM17 were markedly elevated in the SG from T2DM rats. Simultaneously, the enzymatic activities of ADAM17 and PKC significantly increased in the SG from T2DM rats. These data suggested that the PKC-MAPK signaling pathway was upregulated in the SG from T2DM rats, compared to sham rats. Additionally, patch clamp data showed that voltage-gated Ca^{2+} currents and cell excitability of cardiac sympathetic neurons in the SG were higher in T2DM rats. Moreover, T2DM also induced the elevation of cardiac sympathetic nerve activity.

Conclusion: PKC-MAPK signaling pathway is upregulated in SGCs of the SG, which might contribute to cardiac sympathetic overactivation in T2DM rats via enhancing cell excitability of cardiac postganglionic sympathetic neurons. Furthermore, elevated PKC and ADAM17 enzymatic activities highlight therapeutic targets to reduce fatal cardiac events in T2DM.

Keyword: *Cell signaling, Type 2 Diabetes Mellitus, Stellate Ganglia*

ID: 45 - MAM2025 - Poster Presentation

Regional heterogeneity of vascular reactivity along the aorta: consideration of sex as a biological variable

Timothy Sveeggen * **, Ray Mitchell, Pooneh Bagher**

Department of Cellular & Integrative Physiology, University of Nebraska Medical Center

In mammals, the aorta develops from multiple embryonic blood vessels to extend as a single blood vessel from the heart to the abdominal cavity. Due to the heterogenic origins of this vessel, we hypothesized that there are distinct regional differences in vascular function in adults. Indeed, in pathophysiological conditions, such as aortic aneurysm, incidence of aneurysm varies along the length and between sexes. Here, we characterize vasocontractile, vasodilatory, and structural components from four distinct segments of the aorta (ascending, descending, lower thoracic, and infrarenal) in male and female C57BL/6J mice at 15-20 weeks of age. Isolated aortic segments were mounted on a 4-channel wire myograph (Danish Myo Technology) containing physiological salt solution. Once warmed to ~37°C, aortic segments were normalized and isometric force measurements were recorded. Aortic segments were treated with exogenous pharmacological agents (agonists and/or antagonists) for concentration response curves or electrical field stimulation (EFS) to stimulate endogenous neurotransmitter release from sympathetic perivascular nerves. Overall, we observed distinct functional profiles and length-tension relationships for each aortic segment, including differences between vessels from males and females. With exception of the infrarenal aortic segment, we observed enhanced vasoconstriction to the alpha-1 adrenoceptor agonist, phenylephrine, in females compared to males. Additionally, the infrarenal aorta was uniquely resistant to KCl-mediated vasoconstriction relative to the other segments. Nonspecific adrenoceptor stimulation by norepinephrine showed regional differences in vasoconstriction, while the sex differences were less apparent as compared to phenylephrine. Although regional differences in vasoconstriction were observed during stimulation of sympathetic nerves by EFS, there were no sex differences observed. Finally, endothelial-dependent vasodilation differed throughout aortic regions, while smooth muscle-dependent vasodilation was consistent throughout. Altogether, these data highlight the importance of sex as a biological variable in vascular reactivity along the aorta, and demonstrates that no two regions of the aorta are the same.

Keyword: *Aorta, Sex as a biological variable, Vascular function*

ID: 46 - MAM2025 - Oral Presentation

Molecular insights on the impact of chronic HIV infection on male fertility

Tousif Ahmed Hediyal * **, Elizabeth Stone, Omar Shukri, Vicki Schaal, Amin Foroughi, Murali Devanaboyina, Sree Kolli, Sowmya Yelamanchili, Gurudutt Pendyala**

Department of Anesthesiology, University of Nebraska Medical Center (UNMC), Omaha, NE.

Human immunodeficiency virus (HIV) infection remains a major global health concern, impairing immune function and increasing susceptibility to opportunistic infections. Fertility desire among people living with HIV has remained low over the past two decades, primarily due to poor health status, fear of transmission, and restrictive reproductive policies. In HIV-infected men, significant abnormalities in sperm quality have been reported, especially in advanced AIDS, including reduced ejaculate volume, impaired motility, abnormal morphology, and increased risk of sperm aneuploidy, all of which can negatively impact male fertility. This study investigated the molecular mechanisms underlying HIV-associated male infertility using a preclinical HIV transgenic (Tg) rat model. RT-PCR analysis of testes from 3–4-month-old wild-type (WT) and HIV-Tg male rats revealed significant

downregulation of cation channel sperm-associated proteins 1-4 (CatSper 1-4), which are pH-sensitive ion channels essential for sperm motility and hyperactivation. Histological analysis showed structural alterations in the testicular tissue of Tg rats compared to WT controls. Computer-assisted semen analysis (CASA) further demonstrated abnormal sperm morphology and reduced motility in Tg rats. Transcriptomic sequencing of testicular tissue identified novel gene signatures and key molecular pathways significantly disrupted in Tg animals. These findings suggest that HIV-1 infection interferes with sperm production and function, contributing to male infertility. Collectively, this study provides clinically relevant insights into reproductive complications in HIV-infected individuals and may inform future therapeutic strategies.

References: Liu W, Han R, Wu H, Han D. Viral threat to male fertility. Andrologia. 2018 Dec;50(11):e13140.

Keyword: *HIV, Male fertility, Sperm motility, CatSper 1-4, Computer-assisted semen analysis, Sperm morphology*

ID: 47 - MAM2025 - Poster Presentation

The ESCRT-0 protein, HRS, Regulates Hepatocellular Lipid Droplet Catabolism

Mathilda M. Willoughby * **, Ankit Shroff, Micah Schott**

University of Nebraska Medical Center

Lipid droplets (LDs) are highly dynamic organelles that store neutral lipids and protect cells from lipotoxicity. However, excessive LD accumulation contributes to metabolic disorders such as hepatic steatosis, a major driver of metabolic-associated steatohepatitis (MASLD) and alcoholic liver disease (ALD). LD accumulation can result from increased biogenesis or impaired degradation. LD breakdown occurs through two distinct pathways: cytoplasmic lipolysis and lysosome-mediated lipophagy, the latter representing the selective degradation of LDs by lysosomes. Although lipophagy defects are increasingly recognized as contributors to LD accumulation, the molecular mechanisms regulating this process remain poorly defined. In yeast, the endosomal sorting complex required for transport (ESCRT) proteins facilitate the direct engulfment of LDs by lysosomes, a non-canonical form of lipophagy (i.e. microlipophagy). However, whether ESCRT proteins play a comparable role in mammalian cells remains unclear. Here, we identify the ESCRT-0 protein hepatocyte growth factor receptor tyrosine kinase substrate (HRS) as a key regulator of LD homeostasis in mammalian hepatocytes. An RNAi screen in Hep3B hepatoma cells revealed that HRS knockdown led to LD accumulation, a phenotype confirmed in the mouse hepatocyte cell line AML12. Mechanistic analyses showed that the elevated LD levels stemmed from impaired LD breakdown. While lipolysis remained largely unaffected, HRS depletion disrupted lysosomal LD degradation resulting in excessive LD accumulation within lysosomes. Specifically, HRS downregulation reduced LD targeting by microlipophagy while enhancing conventional, autophagosome-dependent macrolipophagy. Further, upregulation of autophagosome-dependent macrolipophagy correlated with decreased mTOR signaling, a central negative regulator of autophagy. Moreover, HRS depletion altered lysosomal pH, thereby impairing lysosomal degradative capacity. Together, these findings establish HRS as a critical regulator of LD catabolism in hepatocytes, coordinating macro- and micro-lipophagy pathways with lysosomal function. Given the strong relationship between lipophagy defects and metabolic liver disease, our results suggest that HRS-dependent pathways may represent promising therapeutic targets for lipid metabolism disorders.

Keyword: *Lipid Droplet, ESCRT, Lipophagy*

ID: 48 - MAM2025 - Poster Presentation

Systemic Neutralization of Granulocyte-Macrophage Colony-Stimulating Factor Attenuates Stellate Ganglion Neuroinflammation and Cardiac Sympathetic Overactivation in Chronic Heart Failure

Sulail Fatima * **, Yu Li, Boris Shabaltiy, Lauren Whitney, Yulong Li**

Emergency Medicine, University of Nebraska Medical Center, Omaha, NE, United States.

Background: Chronic heart failure (CHF) is marked by cardiac sympathetic overactivation, a critical driver for ventricular arrhythmogenesis and sudden cardiac death. Evidence suggests that macrophage-mediated neuroinflammation in stellate ganglia (SG) fuels cardiac sympathetic overdrive in CHF. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a multipotent cytokine involved in M1 (pro inflammatory) macrophage polarization. In patients with CHF, serum levels of GM-CSF correlate with disease severity and neurohormonal activation.
Hypothesis: We hypothesize that systemic perfusion of GM-CSF neutralizing antibody reduces macrophage-induced neuroinflammation and cardiac sympathetic overactivation in SG.
Methods: CHF was induced by surgical ligation of left coronary artery in rats. Echocardiography was conducted 16 to 18 weeks after surgical ligation. Then a mini osmotic pump was subcutaneously implanted, and GM-CSF neutralizing antibody was perfused for two weeks. Serum GM-CSF levels were measured by ELISA. Protein expression of IBA1 (activated macrophage marker), TNF-a, and IL-1b in SG was measured using western blotting. Whole-cell patch-clamp was used to measure action potentials (AP) of SG neurons. Cardiac sympathetic nerve activity and left ventricular pressure were also recorded by general electrophysiological technique and a Millar pressure transducer.
Results: GM-CSF neutralization reduced circulating levels of GM-CSF elevated in CHF rats (8.9±1.6 to 3.3±0.5 pg/ml; $p<0.01$). GM-CSF neutralization also decreased protein expression of IBA1 (0.81±0.12; $p<0.01$), TNF-a (0.74±0.08; $p<0.05$) and Il-1b (0.03±0.02; $p<0.01$), compared to CHF controls (1.98±0.1, 1.13±0.003, and 0.67±0.05, respectively). These changes were accompanied by reduced cell excitability of SG neurons, as evidenced by lower AP spikes in rats with GM-CSF antibody perfusion (17.5±1.7 vs. 25.1±1.4 spikes/sec; $p<0.01$). GM-CSF neutralization significantly attenuated the CHF-induced elevations in cardiac sympathetic nerve activity and left ventricular diastolic pressure ($p<0.05$).
Conclusion: GM-CSF neutralization mitigates CHF-induced sympathetic overactivation, mechanistically linked to a reduction in macrophage-mediated neuroinflammation and neuronal excitability in SG. These data highlight GM-CSF as a critical mediator of cardiac autonomic dysfunction in CHF.

Keyword: *Inflammation, heart failure, arrhythmias*

ID: 49 - MAM2025 - Poster Presentation

Biomarker Discovery in Parkinson's Disease using CNS-derived Blood Exosomes

Beom Seok Kang * [1], **Nick Sande**[1], **Hyunha Kim**[1], **Ji Hye Bahn**[1], **Yujin Chae**[1], **Melinda Burnett**[2], **Jee-Yeon Hwang**[1]

[1] *Department of Pharmacology and Neuroscience, Creighton University School of Medicine, Omaha, NE, USA*

[2] *Department of Neurology, Creighton University School of Medicine, Omaha, NE, USA*

Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide. PD is caused by the progressive loss of nigrostriatal dopaminergic neurons and is characterized by increases in cytoplasmic inclusions of alpha-synuclein. Timely diagnosis of PD is critical to providing the proper treatment and care planning, however, current diagnostic laboratory tests for PD are limited. Therefore, identification of reliable biomarkers is clinically imperative. Exosomes are nanovesicles found throughout the body that carry various proteins, mRNAs, and microRNAs. Central nervous system (CNS)-derived exosomes can cross the blood-brain barrier and have been suggested as potential biomarkers in some pilot studies. Thus, research related to a further validation and a large independent cohort study is needed for relevancy and accuracy. To examine the possibility of using CNS-derived exosomes as biomarkers and identify novel candidates, we isolated CNS-derived exosomes from the peripheral blood of PD patients and age-matched healthy controls. The results from Western blot analysis using antibodies against L1CAM and ATP1A3, markers for CNS exosomes, showed that CNS-derived exosomes are successfully isolated from blood. We then examined whether PD-related pathogenic proteins, such as Parkin, PINK1, DJ-1, and phosphorylated α-synuclein are altered in these CNS-derived exosomes. Our findings indicate that a subset of these proteins significantly differ between

PD patients and healthy controls, suggesting their potential use as diagnostic biomarkers. To further identify novel biomarkers, we performed a proteomic analysis of CNS-derived exosomes and are currently conducting validation experiments. We anticipate that this analysis will enable the discovery of novel biomarker candidates for PD diagnosis.

Keyword: *Parkinson's disease, CNS-derived exosome, Biomarkers*

ID: 50 - MAM2025 - Poster Presentation

The role of BRD4 in neuroinflammation associated with global cerebral ischemia-induced neuronal death.

Hyunha Kim * [1], **Parisa Rafiei[1], Sannidhi Poojary[2], Rachael Urquhart[1], Alekha K Dash[2], Jee-Yeon Hwang[1]**

[1] Department of Pharmacology and Neuroscience, Creighton University School of Medicine, Omaha, NE 68178

[2] Department of Pharmacy, Creighton University School of Pharmacy and Health Professions, Omaha, NE 68178

Global cerebral ischemia, most commonly caused by cardiac arrest, leads to severe cognitive deficits due to the selective and delayed death of hippocampal CA1 neurons. Although this phenomenon is well documented, the underlying molecular mechanisms responsible for neuronal death and cognitive deficits remain unclear. To identify key factors involved in delayed neuronal death, we performed RNA-seq on the hippocampal CA1 at 24 and 48 h after global ischemia. Ingenuity Pathway Analysis (IPA) of differentially expressed genes (DEGs) revealed that inflammatory response-related pathways, including TREM1 signaling, were among the top canonical pathways. In addition, a subset of genes, including BRD4, was identified as predicted upstream transcriptional regulators. BRD4 is an epigenetic reader that recognizes acetylated lysine residues in target gene promoters to activate its expression and is known to regulate TREM1 and other inflammatory factors. Therefore, we investigated **1)** whether global ischemia activates BRD4 and thereby induces neuroinflammation by increasing TREM1 expression, and **2)** whether inhibition of BRD4 protects neurons against ischemic insults. We first validated that BRD4 was increased at 24 and 48 h after global ischemia, and TREM1 mRNA and protein levels were also increased at these time points. Consistent with this, pro-inflammatory cytokines were significantly increased in the hippocampal CA1 at 48 h. We further found that BRD4 co-localized with TREM1-positive cells using immunohistochemistry, and that BRD4 binds to the TREM1 promoter following global ischemia as assessed by ChIP assay. Accordingly, inhibition of BRD4 by siRNA or JQ1 significantly reduced TREM1 expression. Importantly, BRD4 inhibition by siBRD4-loaded nanoparticles attenuated microglia activation, and ultimately prevented neuronal death in the hippocampal CA1 after global ischemia. Taken together, these findings indicate that BRD4 upregulates TREM1 expression, which in turn enhances neuroinflammation and promotes neuronal death in the hippocampal CA1 after global ischemia, implicating BRD4 as a potential therapeutic target.

Keyword: *Global cerebral ischemia, Epigenetic regulation, Neuroinflammation*

ID: 51 - MAM2025 - Poster Presentation

Molecular insights on the impact of chronic HIV infection on male fertility

Omar Shukri * **, Tousif Ahmed Hedyial, Elizabeth Stone, Murali Devanaboyina, Amin Foroughi, Sree Kolli, Vicki Schaal, Sowmya Yelamanchili, Gurudutt Pendyala**

Department of Anesthesiology, University of Nebraska Medical Center (UNMC), Omaha, NE.

Human immunodeficiency virus (HIV) infection poses a continual challenge to global health, affecting the immune system and rendering individuals susceptible to opportunistic infections. The prevalence of fertility desire/intention among people living with HIV has been low for the past two decades due to poor health status, fear of infecting one's spouse or fetus, and discouraging policies in many countries. Gross abnormal sperm quality has been reported in HIV-infected men with more

pronounced changes in AIDS cases, including reduced ejaculate volume, reduced motility, abnormal sperm morphology, and increased risk of sperm aneuploidy. These changes have possible impacts on the fertility potential of males with HIV. One such key regulators in male fertility and hyperactivation of sperm cells are the cation channel sperm-associated proteins (CatSper). The CatSper is a pH-sensitive ion channel protein found on the sperm surface. However, the exact mechanisms exerted by HIV still need to be elucidated. It is necessary to understand the molecular mechanism behind these complications after the HIV infection. We have used RT-PCR, histopathology, and sperm morphological analysis (using CASA and nuclear morphology software) on HIV-1 infected and wild-type rats to understand the molecular mechanism associated with male infertility. The results revealed a downregulation of CatSper 1-4, mitochondrial-associated genes, RNA sequencing analysis, and histological modifications in the testicular tissue, and alterations in sperm morphology and velocity compared with wild-type rats. These results demonstrated that abnormality in sperm production and its motility leads to infertility in rats with HIV-1 infection. These findings may be relevant to the clinical observation of patients with HIV infections and for future investigations.

References: Sun XH, Zhu YY, Wang L, Liu HL, Ling Y, Li ZL, Sun LB. The Catsper channel and its roles in male fertility: a systematic review. Reproductive Biology and Endocrinology. 2017 Aug 15;15(1):65.

Keyword: *infertility, HIV, sperm*

ID: 52 - MAM2025 - Poster Presentation

Different inflammatory responses in the remote organs during tourniquet-induced hindlimb ischemia-reperfusion

Lauren Whitney * **, Huiyin Tu, Anthony Evans, Aaron Barksdale, Michael Wadman, Yu-Long Li**

Department of Emergency Medicine, University of Nebraska Medical Center, Omaha, NE 68198

Background: Tourniquets are a tool used to stop life threatening hemorrhages and are used to create bloodless surgical fields. The military also use this tool, and all deployed personnel are trained and carry tourniquets with them. Despite the tourniquet's usefulness, it causes skeletal muscle injuries and is associated with secondary organ damage. Previous studies on skeletal muscle tourniquet-induced ischemia-reperfusion (IR) showed the involvement of inflammatory cytokines in tissue damage. Objective: this study investigates the inflammatory responses of various organs after the induction of hindlimb IR. We hypothesized that interleukin 1-beta (IL-1β) and TNF-alpha (Tnf-α) protein expression would increase in the organs after skeletal muscle tourniquet-induced IR. Methods: For hindlimb IR induction; a tourniquet was applied to the unilateral hindlimb of male C57BL/6 mice and was on hindlimb at the greater trochanter for 3hrs. After 3hrs the tourniquet was cut, reperfusion occurred for 1, 3, 7, 14, and 28 days. Finally, mice were euthanized, and tissues were collected for Western blot analysis. Results: In the lungs, the expression of IL-1β and TNF-α (two proinflammatory cytokines) increased on 3-day, 7-day, 14-day and 28-day IR compared to control levels. In the liver, IL-1β expression increased at 7 days, 14 days and 28 days as compared to control level, whereas TNF-α in the liver increased at 3 days and 7 days before returning to control levels. In the heart, there were no changes in the expression of TNF-α and IL-1β. In the kidneys there were no changes in IL-1β expression. Conclusion: Based on the data, it is suggested that the protein levels of IL1-β and TNF-α after tourniquet-induced hindlimb IR varies depending on the different organs and IR time-points. Seeing how proinflammatory cytokines vary in the different organs can provide novel therapeutic approaches to prevent secondary organ damage during tourniquet-induced hind limb IR.

Keyword: *Interluekin-1 beta, TNF-Alpha, ischemia-reperfusion*

FACULTY CATEGORY ABSTRACTS:

ID: 53 - MAM2025 - Poster Presentation

Surface Matters: Diacylglycerol buildup on Lipid Droplets

Ankit Shroff * **, Micah Schott**

University of Nebraska Medical Center

Lipid droplet (LD) catabolism occurs through two distinct pathways: cytoplasmic lipolysis and lysosome-mediated lipophagy. These processes are fundamental to cellular energy homeostasis and implicated in metabolic diseases such as obesity, type 2 diabetes, and fatty liver disease. Lipolysis, driven by cytoplasmic enzymes such as adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL), preferentially targets large LDs, whereas lipophagy selectively sequesters small LDs for lysosomal degradation. While the mechanisms governing lipase recruitment to LDs are well defined, the signals directing lipophagy to LDs remain unclear. Here, we show that small LDs exhibit distinct lipid compositions, including enrichment in membrane lipids, consistent with their increased surface-to-volume ratio. Unexpectedly, small LDs also accumulate diacylglycerol (DAG), a lipid not typically associated with LD membranes. We demonstrate that ATGL activity drives DAG enrichment on LD surfaces, which DAG-consuming enzymes such as HSL counteract. Inhibition of other DAG-metabolizing enzymes, including DGAT2, similarly increases DAG levels on LDs. These findings reveal a mechanism by which lipolysis regulates lipophagy and suggest a role for DAG in mediating the selective targeting of small LDs for lysosomal degradation. Given the central roles of lipolysis and lipophagy in metabolic regulation, these insights may also provide a foundation for understanding how their dysregulation contributes to metabolic disorders.

Keyword: *lipid droplet, diacylglycerol, lipolysis*

ID: 54 - MAM2025 - Poster Presentation

Neural enhanced expression of SGLT2 and MAP17 scaffolding complex in the kidneys of rats with congestive heart failure

Tapan Patel * [1]**, Hong Zheng**[2]**, Kaushik Patel**[1]

[1] *Department of Cellular and Integrative Physiology, University of Nebraska Medical Center, Omaha, Nebraska 68198, USA.*

[2] *Basic Biomedical Sciences, Sanford School of Medicine, University of South Dakota, Vermillion, South Dakota 57069, USA.*

Congestive heart failure (CHF) is characterized by the sympathoexcitation with sodium and fluid retention. Recently we demonstrated a relationship between enhanced renal sympathetic nerve activity and overexpression of SGLT2 during CHF, however, the precise underlying molecular mechanisms remains to be examined. MAP17 (membrane associated protein 17), a key scaffolding protein, is required for the functional activation of SGLT2. The aim of this study was to evaluate the role of enhanced renal sympathetic nerve activity on the expression of SGLT2 and MAP17 via activation of extracellular signal-regulated kinases (ERK) and nuclear factor kappa B (NF-KB) in the renal cortex of CHF rats. CHF was induced by coronary artery ligation. Four weeks after surgery, bilateral renal denervation (RDN) was performed. Rats were randomly divided into four groups (Sham, Sham+RDN, CHF and CHF+RDN). Western blot analysis was performed to evaluate the expression of SGLT2, MAP17, pERK/ERK and pNF-KB/NF-KB in the renal cortex. Immunohistochemistry was performed to evaluate the precise location of SGLT2 and MAP17 within the kindey. We determined the direct effect of norepinephrine (NE) on the expression of SGLT2 and MAP17 as well as the activation of ERK/NF-KB pathway using HK2 cells. Rats with CHF exhibited, significantly enhanced expression of SGLT2 and MAP17 and significant activation of ERK/NF-KB compared to SHAM in the renal cortex. Bilateral RDN mitigated enhanced expression of SGLT2, MAP17, as well as activation of ERK/NF-KB pathway in the renal cortex of rats with CHF. Direct action of NE triggered enhanced expression of SGLT2 and MAP17 by activation of the ERK/NF-KB pathway, in HK2 cells. These data demonstrate that enhanced renal sympathetic nerve activity in CHF activates the ERK/NF-KB pathway which in turn facilitates enhanced

expression of SGLT2 and MAP17 which is mitigated by RDN. Consequently, RDN exhibits reduction in sodium and water retention in CHF.

Keyword: *Sodium-glucose cotransporter-2 (SGLT2), Membrane associated protein 17 (MAP17), Congestive heart failure (CHF)*

ID: 55 - MAM2025 - Poster Presentation

Targeting lipid droplet catabolism to inhibit HCC progression.

Mohammad Zaidi * [1], **Kelly Othkhor**[1], **Tatsuya Yamada**[2], **Micah Schott**[1]

[1] *Dept. of Biochemistry and Molecular Biology, UNMC, Omaha, Nebraska*

[2] *Department of Biochemistry, UNL, Lincoln, Nebraska*

Introduction: Hepatocellular carcinoma (HCC) is the sixth most frequently diagnosed cancer and the third leading cause of cancer-related mortality worldwide. Obesity and heavy alcohol consumption are major risk factors that drive HCC development. These factors promote excessive accumulation of lipid droplets (LDs) within hepatocytes, leading to hepatic steatosis, steatohepatitis, and eventually cirrhosis and HCC. While de novo lipogenesis is known to support HCC growth, the role of LD catabolism remains poorly understood. LDs are trafficked to lysosomes for degradation by lysosomal acid lipase (LAL, gene name LIPA). Several LD-trafficking proteins, as well as LAL itself, are overexpressed in HCC and correlate with poor patient survival.
Hypothesis: Inhibiting LD catabolism will suppress HCC progression.
Methods: Immunohistochemistry (IHC) and immunofluorescence (IF); in vitro cell-proliferation, spheroid, and colony-formation assays; oxygen-consumption-rate (OCR) assays; hydrodynamic tail-vein injection (HTVI) mouse model of HCC; in vivo Lipa knockout; and intraperitoneal (IP) administration of the LAL inhibitor Lalistat2.
Results: Genes involved in LD catabolism, including LIPA, were overexpressed in both human and mouse HCC. Treatment of human HCC cells with Lalistat2 significantly reduced proliferation, colony formation, and spheroid growth. In vivo CRISPR/Cas9 KO of Lipa in mouse liver exhibited a markedly reduced tumor burden. Consistently, IP administration of Lalistat2 lowered the liver-to-body-weight ratio, although more targeted delivery to HCC cells may further enhance efficacy while minimizing off-target toxicity.
Conclusion: HCC relies on LD catabolism for tumor growth. Targeting LD catabolism, for example through LAL inhibition, represents a promising therapeutic strategy to slow or prevent HCC progression.

Keyword: *Hepatocellular Carcinoma, Lipid Droplets, Hydrodynamic Tail Vein Injection*

ID: 56 - MAM2025 - Poster Presentation

Impact of NLRP6 inflammasome in the combined effect of HIV Tat and ethanol-mediated neuroinflammation in astrocytes

Seema Singh * **, Shilpa Buch, Palsamy Periyasamy**

Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198, USA

Nearly half of people living with HIV are alcohol abusers, putting them at a 2–3-fold higher risk of developing HIV-associated neurological disorders (HAND), a significant public health concern. HIV Tat-induced neuroinflammation is linked to the activation of brain cells, including astrocytes, which release chemokines and cytokines. This study investigates the molecular mechanisms driving neuroinflammation in the context of NeuroHIV in people living with HIV who consume alcohol. In two independent studies, previously, we identified that NLRP6 (NOD-like receptor family pyrin domain-containing 6) plays a critical role in HIV Tat or ethanol-induced neuroinflammation, and is regulated by microRNAs (miRNAs). Based on the premise that alcohol use exacerbates HIV-associated neuroinflammation, we aim to explore the epigenetic regulation of NLRP6-mediated neuroinflammation in HIV Tat and ethanol-exposed astrocytes. Our findings demonstrated that

exposure of mouse primary astrocytes (mPAs) to HIV Tat (50 ng/mL) and ethanol (50 mM) led to astrocyte activation (GFAP) and the inflammasome, NLRP6 in a cooperative manner. Combined exposure also increased NLRP6 downstream signaling mediator, caspase-1, along with proinflammatory cytokines (IL1β and IL18). Notably, silencing NLRP6 expression inhibited these effects, confirming its critical role in HIV Tat and ethanol-mediated neuroinflammation. Furthermore, a microarray study identified miR-339 as significantly downregulated, which was further validated by qPCR in mPAs exposed to both HIV Tat and ethanol. Argonaute immunoprecipitation assay confirmed that miR-339 targets the 3'UTR of NLRP6 transcripts. In addition, overexpression of miR-339 in mPAs exposed to HIV Tat and ethanol validated its role in the epigenetic regulation of NLRP6 inflammasome signaling. In summary, our findings highlight the involvement of miR-339 in regulating NLRP6 inflammasome activation and associated neuroinflammation in HIV Tat and ethanol-exposed mouse primary astrocytes. These results provide novel insights into the epigenetic mechanisms underlying HAND pathogenesis in the context of alcohol use.

References: Singh, Seema, Muthukumar Kannan, Abiola Oladapo, Uma Maheswari Deshetty, Sudipta Ray, Shilpa Buch, and Palsamy Periyasamy. "Ethanol modulates astrocyte activation and neuroinflammation via miR-339/NLRP6 inflammasome signaling." Free Radical Biology and Medicine 226 (2025): 1-12.

Keyword: *HIV Tat and ethanol, NLRP6 signaling, miR-339*

ID: 57 - MAM2025 - Poster Presentation

Small Animal Ultrasound Core

Bryan Hackfort *

UNMC

The Small Animal Ultrasound Core provides state-of-the-art preclinical ultrasound imaging. The Core houses three high resolution ultrasound machines including a Vevo 3100, Vevo LAZR-X, and a RevvityVega. Imaging frequency ranges from 15 MHz (100 µm axial resolution) to 40 MHz (40 µm axial resolution). Ultrasound imaging is a minimally invasive, cost-effective approach to investigating real-time physiology of animals. Many parameters of cardiac function can be accurately measured including heart size, systolic function, diastolic function, right ventricular function, synchrony of contraction (strain/speckle tracking), etc. Vascular diameters can be measured along with blood flow velocities using color Doppler and pulsed-wave Doppler. Ultrasound is a valuable tool for investigating cancer biology. Chronic measurements of 3D tumor size monitor growth over time and effectiveness of cancer therapeutics. Oxygen saturation of tissues using photoacoustic imaging and vascularity of tumors with Doppler provide information on hypoxic cores and perfusion of tumors. Finally, tissue stiffness can be measured using shear wave elastography.

Assisted and unassisted use and analysis options are offered. Core staff can quickly train individual labs on ultrasound use. For more information, email bryan.hackfort@unmc.edu.

Keyword: *Ultrasound, SWE, Imaging*

ID 58: - MAM2025 - Poster Presentation

Bioassay Core

Director: Bryan T. Hackfort, Ph.D. Manager: Jennifer Schneider, B.S.

The mission of the CHVR Bioassay Core is to provide access to routine and advanced cellular and molecular assays for cardiovascular research. Introduce new analytical capabilities using the instrumentation and computing resources available. Provide a "measure what matters" approach to guiding investigators.

The Core will provide equipment, personnel, and protocols for molecular and cellular assays including multiplex immunoassays, RNA extraction and qPCR, automated cell sorting, automated immunoblot, human cardiomyocyte isolation followed by cellular function assays, advanced specimen homogenization for 'omics, in situ hybridization, and glycobiology arrays. The IonOptix System tests

cardiomyocyte contractility and calcium signaling. Core members will help investigators develop and implement new assays as needed. Please reach out to Jennifer Schneider at jschneider@unmc.edu for more information.

ID: 59 - MAM2025 – Oral and Poster Presentation

Protective effect of a small molecule, C381 in attenuating HIV Tat-induced microglial activation and neuroinflammation

Uma Maheswari Deshetty * **, Athreya Reddy, Stuti Jain, Shilpa Buch, Palsamy Periyasamy**

Department of Pharmacology & Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198, USA

HIV Tat (transactivator of transcription), a viral protein, is known to play a central role in triggering microglial activation, thereby fostering a proinflammatory environment that contributes to both neuroinflammation and neurodegeneration. Prior research has linked this activation to mechanisms such as mitochondrial dysfunction, disrupted mitophagy, and increased cytotoxicity. Targeting these mechanisms could offer new therapeutic avenues for reducing HIV related neuroinflammation. This study explores the lysosome-protective potential of the small molecule C381 in counteracting microglial activation and inflammation triggered by HIV Tat. Mouse primary microglial cells (MPMs) were treated with C381 (30 μM) one hour before exposure to HIV Tat (50 ng/ml) for 24 hours. Western blotting was used to analyze markers of microglial activation, mitophagy/autophagy, and lysosomal function, while qPCR assessed proinflammatory cytokine levels. Mitochondrial functions such as oxygen consumption rate (OCR), extracellular acidification rate (ECAR), membrane potential, and reactive oxygen species (ROS) production was evaluated using Seahorse analysis, JC1 staining, and, respectively. HIV Tat exposure resulted in increased expression of activation markers (CD11B), mitophagy regulators (DLP1, PINK1), and autophagy-related proteins (Beclin1, LC3B-II, p62), alongside clear signs of mitochondrial dysfunction. Specifically, HIV Tat treatment led to reduced membrane potential, elevated mitochondrial ROS, and decreased OCR and ECAR. In contrast, pretreatment with C381 restored mitochondrial parameters including membrane potential, ROS levels, OCR, and ECAR. Hence, C381 effectively reduced microglial activation, normalized mitophagy/autophagy processes, and enhanced mitochondrial health. Additionally, in silico molecular docking studies showed strong binding efficiency of C381 to the lysosomal V-ATPase complex. These results indicate that C381 holds promise as a therapeutic agent for combating HIV Tat-induced microglial activation and neuroinflammation, potentially offering a novel approach to managing HIV-related neurotoxicity.

Keyword: *HIV Tat, microglial activation, small molecule C381*

LATE BREAKING ABSTRACTS: GRADUATE STUDENT CATEGORY

ID: 60 - MAM2025 - Poster Presentation

Multi-Patient Analysis of Steroid-Induced Hyperglycemia in Diabetic Patients Using Continuous Glucose Monitoring

Pravardhan Birthi[1], Mahesh Pattabiraman[2], Akila Ramaswamy[2], Mritunjay Kumar * **[3], Akshay Agrawal[4], Srinivas R. Nalamachu[5], Surabhi Chandra[6]**

[1] Grand Island Pain Relief Center Grand Island, NE 68803; TriCity Research Center, Grand Island, NE 68803

[2] TriCity Research Center, Grand Island, NE 68803,

[3] Grand Island Pain Relief Center, Grand Island, NE 68803

[4] Bryan Health, Grand Island, NE 68803, USA

[5] Analgesic Clinical Research, LLC, Overland Park, KS, USA

[6] University of Nebraska Kearney, Kearney, NE 68845

Steroid-induced hyperglycemia (SIH) is a frequent complication of glucocorticoid therapy, yet most prior studies have relied on sparse glucose measurements that limit understanding of timing and trajectory. Continuous glucose monitoring (CGM) provides high-resolution insights into these fluctuations. In this observational study we have analyzed CGM data from 58 diabetic patients (mean age 64.6 years) who received a single steroid injection for pain relief. Participants were grouped by steroid type (dexamethasone, methylprednisolone, triamcinolone) and stratified by gender and age (<64.6 vs. ≥64.6 years). Glucose levels were recorded every 5 minutes for up to 10 days post-injection. Across all steroids, glucose rose within 2 hours. Dexamethasone produced the most consistent excursion, peaking at ~220–225 mg/dL and returning toward baseline by 24–36 hours. Methylprednisolone caused a more moderate, delayed increase (175–185 mg/dL) with a sustained elevation lasting several days. Triamcinolone showed comparable peak levels (~220 mg/dL) but with marked variability, limiting interpretability. Stratification analyses revealed higher and earlier peaks in females compared to males, particularly after dexamethasone, and a faster recovery trajectory. Younger patients exhibited more dynamic excursions with prolonged elevations and secondary dips, whereas older patients showed flatter and more stable recovery curves.

This study provides one of the most detailed CGM characterizations of SIH, demonstrating steroid-specific and demographic-modified responses. Dexamethasone displayed a reproducible excursion phase, methylprednisolone a delayed pattern, and triamcinolone inconsistent variability. These findings highlight the utility of CGM for identifying temporal SIH features, with the first 48 to 72 hours yielding clinically reliable monitoring window.

References: Saigi, I., and Perez, A. (2010) Management of glucocorticoid induced hyperglycemia, Revista clinica Espanola 210, 397-403.

Keyword: *Steroids, Steroid-Induced Hyperglycemia, Diabetes*

LATE BREAKING ABSTRACTS: POST DOC CATEGORY

ID: 61 - MAM2025 - Poster Presentation

An Animal Model to Decipher Mechanisms Responsible for Increased Sudden Cardiac Death in HIV-1 Infection

Ali Namvaran * [1], **Julian Garcia**[1], **Mahendran Ramasamy**[1], **Bryan Hackfort**[2], **Dash Prasant**[1], **Santhi Gorantla**[1], **Keshore Bidasee**[3]

[1] *Departments of Pharmacology & Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE.*

[2] *Cellular and Integrative Physiology, University of Nebraska Medical Center, Omaha, NE.*

[3] *Departments of Pharmacology & Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE., Nebraska Redox Biology Center, Lincoln, NE*

Background: Sudden cardiac death (SCD) is >4-fold higher in people living with HIV infection (PLWH) compared to the general population. Studies attribute this to an electrical disorder of the heart that predisposes PLWH to tachycardia-induced ventricular arrhythmias and cardiac arrest. To date, the molecular reasons for these changes remain incompletely understood, due to a paucity of relevant animal models.

Hypothesis: NOD.Cg-PrkdcscidIl2rgtm1Wjl/SzJ (NSG) humanized mice (Hu-mice) infected with HIV-1 and treated with antiretroviral therapy (ART) will exhibit electrocardiographic (ECG) changes seen in PLWH.

Aim: To investigate ECG changes in HIV-infected Hu-mice with and without treatment with the WHO-first-line recommended dolutegravir (DTG), tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC).

Methods: Hu-mice were assigned to two groups: uninfected controls (n=8), HIV-1-infected mice (n=16). Baseline ECGs were recorded. One-month post-infection, the infected group was split into two subgroups: one received daily antiretroviral therapy (DTG/TDF/FTC) via feed for two months. ECGs were obtained at 1 and 3 months post-infection. Cardiac tissues were analyzed for expression of calcium-handling proteins RyR2 and SERCA2.

Results: HIV-infected Hu-mice develop a progressive increase in QTc interval, including widening of QRS complex, and prolonged ST-T segments over twelve weeks. Other ECG changes reported in PLWH were also observed. In cardiac tissues from HIV-1-infected Hu-mice, expression of the SR Ca2+-release channel, ryanodine receptor (RyR2) remained unchanged, but its phosphorylation was increased ~3-fold, indicative of a gain-of-function. Expression of SR Ca2+-ATPase (SERCA2) was also 35% lower. Treatment with DTG/TDF/FTC prevented worsening but did not reverse ECG change. Treatment with ART attenuated phosphorylation of RyR2 but not SERCA2 downregulation.
Conclusions: These data show for the first time that HIV-1-infected Hu-mice treated with DTG/TDF/FTC can recapitulate changes in ECG reported in PLWH. This model also shows for the first time changes in cycling proteins involved in cardiac contraction/relaxation, namely RyR2 and SERCA2.

References:

Ramasamy M, Venn ZL, Alomar FA, Namvaran A, Edagwa B, Gorantla S, Bidasee KR. Elevated Methylglyoxal: An Elusive Risk Factor Responsible for Early-Onset Cardiovascular Diseases in People Living with HIV-1 Infection. Viruses. 2025 Apr 8;17(4):547.

Keyword: *HIV-1 infection, Sudden cardiac death, Electrocardiogram*

ID: 62 - MAM2025 - Poster Presentation

Antiretroviral drugs Impairs Nuclear Translocation of HIF1α : Insight into Early onset Heart Failure

Mahendran Ramasamy [*] [1], **Ali Namvaran[1], Zachary L Venn[1], Benson Edagwa[2], Beutline Malgija M[3], Reagan Fisher[1], Keshore R Bidasee[1]**

[1] Departments of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE

[2] Departments of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha

[3] 2Centre for Computational Informatics Research and Innovation, MCC-MRF Innovation Park Madras Christian College, Chennai 600 059, Tamil Nadu , India

Early-onset heart failure remains a critical health concern among people living with HIV-1 (PLWH). Despite effective viral suppression with antiretroviral drugs (ARDs), the molecular mechanisms driving ARD-associated cardiac dysfunction remain unclear. Our recent findings revealed elevated hypoxia-inducible factor-1α (HIF-1α) in cardiac homogenates from HIV-infected humanized mice treated with dolutegravir (DTG), tenofovir disoproxil fumarate (TDF), and emtricitabine (FTC), as well as in H9C2 cardiomyocytes exposed to these drugs under normoxic conditions. These results suggest that DTG, TDF, and FTC upregulate HIF-1α in an oxygen-independent manner.
We hypothesize that DTG/TDF/FTC treatment increases cytoplasmic HIF-1α, leading to impaired mitochondrial function, elevated reactive oxygen species (ROS), and early cardiac injury. Immunofluorescence was used to assess HIF-1α localization in cardiac tissue from HIV-infected humanized mice treated with DTG/TDF/FTC and in H9C2 cells treated with DTG/TDF/FTC (1.0/0.5/1.0 μM, 2 h, normoxia). $CoCl_2$-treated H9C2 cells (400 μM) served as a positive control for HIF-1α nuclear translocation. MitoSOX® and Seahorse XF assays were used to evaluate mitochondrial ROS and basal oxygen consumption, respectively. Molecular docking (Schrödinger Suite) analyzed drug interactions with prolyl hydroxylase domain (PHD), HIF-1α, and importin α. DTG/TDF/FTC treatment significantly increased HIF-1α expression in both in vivo and in vitro models, with over 80% of HIF-1α retained in the cytoplasm. In contrast, $CoCl_2$ treatment induced nuclear translocation of HIF-1α. Drug-treated H9C2 cells exhibited elevated ROS and oxygen consumption. In silico analysis revealed strong DTG and FTC binding to PHD's catalytic domain and the HIF-1α ODD domain, while TDF and FTC showed moderate affinity to importin α. These findings suggest that DTG/TDF/FTC stabilize cytoplasmic HIF-1α by inhibiting PHD-dependent degradation and interfering with importin α–mediated nuclear translocation, contributing to mitochondrial dysfunction and oxidative stress in cardiomyocytes.

Keyword: *Antiretroviral drugs, HIF-1α, Heart Failure*