5th annual
Life Sciences
Symposium

MAY 18-19 2021
9 AM - 1 PM

Sponsors:
PennState
Huck Institutes of the Life Sciences

GPSCA
Graduate & Professional Student Association

Organized by:
HGSCAC
Huck Graduate Student Advisory Committee
Symposium Handbook

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Welcome!

The Life Sciences Symposium is an annual meeting organized by the Huck Institutes of the Life Sciences and the Huck Graduate Student Advisory Committee (HGSAC) at Penn State. Since 2015, this event has brought together hundreds of researchers from diverse scientific fields, promoting unique research collaborations, funding and networking opportunities. The symposium encourages the understanding of the role of scientists in society at local, national, and global scales. In addition, this event increases the visibility of Penn State’s laboratories, centers, and core facilities to maximize engagement and use of on-campus facilities. Due to the challenges and risks presented by the COVID-19 pandemic, the 5th Annual Huck Life Sciences Symposium will assemble with virtual space Gather.town on May 18th and 19th. This year’s conference will be unique and HGSAC is working hard to deliver the same quality and opportunities as an in-person conference.

Event highlights
● Keynote speakers
● Oral & poster presentations
● “My research” video contest
● Cash prizes & gift giveaways

Important Dates
● April 3rd - Abstract submission deadline
● April 12th - Abstracts selected of oral presentation
● April 30th - Posters and videos must be uploaded
● May 1st - Registration deadline

Sponsors: Huck Institutes of Life Sciences, Graduate & Professional Student Association (GPSA)

Supporters: Eberly College of Science, College of Health and Human Development, College of Agricultural Sciences, College of Medicine, College of Nursing, Graduate School American Society for Microbiology

Organizing committee: Huck Graduate Student Advisory Committee (HGSAC)
### The HGSAC

**President**  
Maria Isabel da Silva  
Integrative & Biomedical Physiology

**Vice-President**  
Jocelyn Delgado  
Integrative & Biomedical Physiology

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Bioinformatics & Genomics

**Secretary**  
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Kemin Mu  
Plant Biology

Chris Muriel Mundo  
Biochemistry, Microbiology & Molecular Biology

Alanna Staffin  
Integrative & Biomedical Physiology

**Faculty advisor**  
Troy Ott  
Department of Animal Sciences
Visit the virtual symposium at [https://gather.town/i/1qqKRS7F](https://gather.town/i/1qqKRS7F) | Passcode: hgsac

*** Full access to all virtual areas in the link above opens on May 18-19

You MUST HAVE:
- A desktop/laptop with microphone and camera
- A web browser (Chrome or Firefox recommended)
- A keyboard with arrow keys to move your avatar
- Headphones (to prevent feedback noise)

Joining the virtual symposium

1. Access the link: [https://gather.town/i/1qqKRS7F](https://gather.town/i/1qqKRS7F)
2. Type the password: hgsac
3. Turn-ON your camera and microphone
4. Click on “edit character” and type your first and last name and pronouns
   Example: Maria Silva, she/her
5. Select your avatar, and click “next”
6. Click on “join the gathering”

Setup immediately upon entering

1. In the bottom-screen menu bar, click on your name.
2. In the status text, type your title:
   Undergrad; MS student; PhD student; Post-doc; Faculty; Facility name or other.

Using Gather.town

- **Move your avatar**: use the arrow keys on the keyboard.
- **IMPORTANT Press “x” to access features**: when walking into a room/poster or getting close to an object that shines yellow, you will see a notification “Press x to…” at the bottom of your screen. By pressing “x” (when needed) allows you to enter Zoom calls, visualize posters, videos, websites and use any interactable object.
- **Information and instructions**: if you see the icons move closer and press “x” to see instructions and information about the schedule, presenters, abstracts and others.
- **Video chat**: when you get close to other avatars, a video window will pop up and you can see and talk with other people. Move away to end the interaction.
- **Ghost mode**: hold “g” to walk through other avatars if they are blocking your path.
- **Private spaces**: are areas that bright-up when you walk into. Posters, booths, help desk, and sitting areas in the lobby are all private spaces. These areas allow interaction only between avatars present
inside the space. People walking outside of private spaces will not be able to participate in conversation with people inside the private space.

- **Text-chat**: click on the chat icon on the left side of the screen. You can send text messages to nearby people or everyone.

- **Private text chat**: click on the participants icon on the left side of the screen, click on a participant name, and type your message.

- **Group chat**: click below a video window for a grid view of participants’ videos.

- **Share screen**: use the icon at the bottom-screen menu bar to share your screen during conversations.

- **Mini map**: use the icon at the bottom-screen menu bar to preview the space you’re in.

- **Reactions**: use the icon at the bottom-screen menu to select a reaction.

- **Locating a participant**: to find someone in Gather.town, click on the participants icon on the left side of the screen, click on the participant name, and select “locate on map”. Then, you will see a guiding line connecting your avatar to the other person’s avatar; select “stop locating” at the bottom of the screen to remove the guiding line.

- **Follow a participant**: click on the participants icon on the left side of the screen, click on the participant name, and select “follow”. Then, your avatar will automatically move towards the other person’s avatar. To stop following the person around, go back and click “follow” a second time.

**Technical difficulties:**

- **IMPORTANT HGSAC LIVE help**: for questions/help during the symposium, go to the HELP area (yellow circle in the lobby) to read instructions for Gather.town. Another option is to email HGSAC (psu.hgsac@gmail.com) for help in specific situations.

- **Glitches**: refreshing the page will fix most things! If that doesn’t work, try muting/unmuting your mic and camera and check if your browser allows camera and mic access. Additional troubleshooting at https://gather.town/video-issues

- **Content loading**: if you see a “grapes” icon, that means the content is loading. Give it time.
Schedule TUESDAY MAY 18, 2021

8:30 AM
OPTIONAL
Tutorial and Q&A for the virtual symposium
Link: https://gather.town/i/1qqKRS7F | Passcode: hgsac

9:00-9:10 AM
Opening ceremony: Welcome & tips to make the most out of the symposium
Zoom: https://psu.zoom.us/j/98682456755?pwd=VE5YQmdjTlhqOTdKRkE4cFRGWEVRZz09
Meeting ID: 986 8245 6755 Passcode: 7078

9:10-10:55 AM
Oral presentations - Group A
Zoom: https://psu.zoom.us/j/98682456755?pwd=VE5YQmdjTlhqOTdKRkE4cFRGWEVRZz09
Meeting ID: 986 8245 6755 Passcode: 7078

9:10 AM
ID# 34 | Natalie Yoshioka
Characterizing the role of GRK2 in postnatal skeletal development

9:27 AM
ID# 36 | Radha Dhingra
Physical activity and diet quality in relation with adiposity measures among US adolescents

9:44 AM
ID# 44 | Abriana Cain
The combination of physical activity and energy restriction reduces HIF-1α gene expression in the tumor microenvironment in the 4T1.2 murine breast cancer model

10:01 AM
ID# 53 | Abirami Ravichandran
Relationship between tumor progression and immune response in the 4T1.2-HER2 mouse mammary tumor model

10:18 AM
ID# 18 | Bailey Keller
The effects of chronic fluoxetine treatment on alcohol intake and metabolic functioning in binge drinking/eating mouse model

10:35 AM
ID# 4 | Julia Kelliher
The role of oxylipins in neuroinflammation

11:00-11:55 AM
Keynote presentation
Zoom: https://psu.zoom.us/j/94525504282?pwd=ZUhmWVRLUUF0ZC9FRENxV3q1NTJiUT09
Meeting ID: 945 2550 4282 Passcode: 1669

How fair is our labware? Reducing environmental impact in laboratory practices
Dr. Una Fitzgerald | Professor & Director of Galway Neuroscience Centre, University of Ireland

12:00-1:00 PM
LIVE Poster presentations - Session 1: ID# 1 to ID# 27
Link: https://gather.town/i/1qqKRS7F | Passcode: hgsac
Presenters will be online for Q&A at the poster room.
### Schedule

**WEDNESDAY MAY 19, 2021**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:30 AM</td>
<td>Tutorial and Q&amp;A for the virtual symposium</td>
</tr>
<tr>
<td>Optional</td>
<td>Link: <a href="https://gather.town/i/1qqKRS7F">https://gather.town/i/1qqKRS7F</a></td>
</tr>
<tr>
<td>9:00-10:45 AM</td>
<td>Oral presentations - Group B</td>
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<tr>
<td></td>
<td>Zoom: <a href="https://psu.zoom.us/j/98682456755?pwd=VE5YQmdjTlhgOTdKRkE4cFRGWEVRZz09">https://psu.zoom.us/j/98682456755?pwd=VE5YQmdjTlhgOTdKRkE4cFRGWEVRZz09</a></td>
</tr>
<tr>
<td></td>
<td>Meeting ID: 986 8245 6755 Passcode: 7078</td>
</tr>
</tbody>
</table>
| 9:00 AM       | ID# 8 | Mariam Melkumyan  
Neuroimmune cells play a critical role in mediating the effect of acute alcohol on central amygdala glutamatergic transmission |
| 9:17 AM       | ID# 41 | Maria Isabel Da Silva  
Evidence for Immune tolerance in peripheral blood leukocytes of dairy cattle during early pregnancy |
| 9:34 AM       | ID# 19 | Mingyao Yang  
Deletion of Pramel1 disrupt the retinoic acid-coordinated spermatogenesis |
| 9:51 AM       | ID# 24 | Evelyn Weaver  
Dietary metformin supplementation improves ovarian function in broiler breeder hens |
| 10:08 AM      | ID# 20 | Riëtte van Biljon  
Dissecting the regulatory role of an enriched DNA sequence motif found upstream of Plasmodium falciparum gametocyte-associated genes |
| 10:25 AM      | ID# 3 | Chiara Vanalli  
Interactions between climate change and coinfections: what should we expect from the future? |
| 9:00-10:45 AM | Oral presentations - Group B                                          |
|              | Zoom: [https://psu.zoom.us/j/98682456755?pwd=VE5YQmdjTlhgOTdKRkE4cFRGWEVRZz09](https://psu.zoom.us/j/98682456755?pwd=VE5YQmdjTlhgOTdKRkE4cFRGWEVRZz09) |
|              | Meeting ID: 986 8245 6755 Passcode: 7078                              |
| 10:50-11:50 AM| LIVE Poster presentation - Session 2: ID# 28 to ID# 53               |
|              | Link: [https://gather.town/i/1qqKRS7F](https://gather.town/i/1qqKRS7F) | Passcode: hgsac |
| 12:00-12:55 PM| Keynote presentation                                                 |
|              | Zoom: [https://psu.zoom.us/j/94525504282?pwd=ZUhmWVRLUUF0ZC9FRENxV3g1NTJjUT09](https://psu.zoom.us/j/94525504282?pwd=ZUhmWVRLUUF0ZC9FRENxV3g1NTJjUT09) |
|              | Meeting ID: 945 2550 4282 Passcode: 1669                              |
| 12:55-1:00 PM | Closing ceremony: Acknowledgments, announcement of video contest winners |
|              | Zoom: [https://psu.zoom.us/j/94525504282?pwd=ZUhmWVRLUUF0ZC9FRENxV3g1NTJjUT09](https://psu.zoom.us/j/94525504282?pwd=ZUhmWVRLUUF0ZC9FRENxV3g1NTJjUT09) |
|              | Meeting ID: 945 2550 4282 Passcode: 1669                              |

#### My Research video contest
People’s choice winner is the most "liked" video on YouTube. Vote by May 19 at 1:00 PM.

Link: [https://youtube.com/playlist?list=PLyGR--iUgex7-XmoTXsi8igXp-67Od0r3](https://youtube.com/playlist?list=PLyGR--iUgex7-XmoTXsi8igXp-67Od0r3)

#### Show booths and Poster visualization
Link: [https://gather.town/i/1qqKRS7F](https://gather.town/i/1qqKRS7F)  
Passcode: hgsac

#### Oral presentations - Group B

**ID# 8 | Mariam Melkumyan**  
Neuroimmune cells play a critical role in mediating the effect of acute alcohol on central amygdala glutamatergic transmission

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Dietary metformin supplementation improves ovarian function in broiler breeder hens

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**ID# 3 | Chiara Vanalli**  
Interactions between climate change and coinfections: what should we expect from the future?

#### LIVE Poster presentation - Session 2: ID# 28 to ID# 53

Link: [https://gather.town/i/1qqKRS7F](https://gather.town/i/1qqKRS7F)  
Passcode: hgsac

Presenters will be online for Q&A at the poster room.

#### Keynote presentation

**The role of science in decision making: how interpretation & communication of scientific results impact our personal choices and public policy**

Dr. Adele Turzillo | Professor & Department head of Animal Science, Penn State

#### Closing ceremony:
Acknowledgments, announcement of video contest winners

Link: [https://gather.town/i/1qqKRS7F](https://gather.town/i/1qqKRS7F)  
Passcode: hgsac
Una Fitzgerald | TUESDAY MAY 18, 2021 11:00 - 11:55 AM

How fair is our labware? Reducing environmental impact in laboratory practices

Dr. Una FitzGerald completed her B.S. in Industrial Engineering and MS.c. in Biotechnology at the National University of Ireland, Galway. Later, she worked in the pharmaceutical sector for five years in France and in the UK before embarking on a Ph.D. in Molecular Biology at the University of Strathclyde, Glasgow. After a brief stint researching cancer for Prof. Sue Barnett at Glasgow University, Dr. Fitzgerald discovered her true passion for neurological disorders like multiple sclerosis and Parkinson’s disease. Since returning to the University in Galway, Ireland, Dr. FitzGerald has established a track record in research multiple sclerosis. Currently, she is a funded investigator in CÚRAM and the SFI Centre for Research on Medical Devices, and was the previous director of the Galway Neuroscience Centre (2019-2020). Dr. Fitzgerald is the lead PI and Coordinator of a €3.9M E.U.-funded Innovative Training Network that trains 15 Ph.D. students across Europe. This training network aims to develop a novel device for treating the progressive phase of multiple sclerosis. In 2019, she led the initiative that earned CÚRAM lab a Green Lab Certification from My Green Lab, making it the first in Europe. She is now the chair of a national working group on sustainable public sector labs.

Adele Turzillo | WEDNESDAY MAY 19, 2021 12:00-12:55 PM

The role of science in decision making: How interpretation and communication of scientific results impact our personal choices and public policy

Dr. Turzillo earned her B.S. in biological sciences and a Ph.D. in physiology, both from Cornell University. She continued on to become a postdoctoral fellow at Colorado State University. As an assistant professor of physiology and animal science at the University of Arizona from 1998-2004 she studied ovarian function in dairy cattle. Dr. Turzillo went on to work as a physiologist at the U.S. Food and Drug Administration’s Center for Veterinary Medicine for the next several years. She then began her work with the U.S. Department of Agriculture’s (USDA) National Institute of Food and Agriculture (NIFA) from 2008 to 2013. As the national program leader for animal production systems, she developed priorities and led the peer review process for several USDA-NIFA’s Agriculture and Food Research Initiative grants. For more than six years, Dr. Turzillo provided leadership, planning, and oversight for programs in animal health and production with approximately $100 million annual budgets. In 2019, Dr. Turzillo became senior adviser for animal health, production, and animal products in the USDA’s Office of the Chief Scientist. Currently, Dr. Turzillo resides as the vice president for animal agriculture systems at the World Wildlife Fund and has been named head of the Department of Animal Science in Penn State’s College of Agricultural Sciences.
Visit the poster area at any time!

To see the poster, press “x” when your avatar walks in each poster space.

Use the zoom icon on the right of the screen to better visualize posters details.

Presenters will be LIVE for Q&A by their poster at the following times:

**Session 1**
Posters ID# 1 to ID# 27
Tuesday, May 18 at 12:00-1:00 PM

**Session 2**
Posters ID# 28 to ID# 53
Wednesday, May 19 at 10:50-11:50 AM

During LIVE poster presentation, when your avatar walks in each poster space, you will automatically join the video-chat with the presenter and other participants present in each poster area.
Human Health, Nutrition & Physiology

# 4  The role of oxylipins in neuroinflammation  
Julia Kelliher

#11 Red and Processed Meat Consumption and Food Insecurity in Relation with High Blood Pressure: A Population-Based Study  
Djibril M. Ba

#14 Aryl Hydrocarbon Receptor is a key mediator of intestinal epithelial homeostasis  
Debopriya Chakraborty

# 18 The Effects of Chronic Fluoxetine Treatment on Alcohol Intake and Metabolic Functioning in Binge Drinking/Eating Mouse Model  
Bailey Keller

# 19 Deletion of Pramel1 disrupt the retinoic acid-coordinated spermatogenesis  
Mingyao Yang

# 22 Docosopentaenoic Acid (Dha) is Superior to Race in Predicting an Individual's Risk for Myocardial Infarction (Mi)  
Carmen Annevelink

# 25 Interrogation of the Role of Gpr44 in Acute Myeloid Leukemia  
Fenghua Qian

# 32 Alzheimer’s disease risk factor APOE4 alters cytokine secretion patterns in the frontal cortex of mice  
Rebecca Fleeman

# 34 Characterizing the role of GRK2 in postnatal skeletal development  
Natalie Yoshioka

# 35 Cardiovascular Disease Burden: Comparison of a Psychiatric Outpatient Population and a US General Population Sample  
Radha Dhingra

# 36 Physical Activity and Diet Quality in Relation with Adiposity Measures among US Adolescents  
Radha Dhingra

# 37 A randomized controlled trial of dietary supplementation with dried plums on inflammatory markers in postmenopausal women  
Janhavi Damani

# 44 The combination of physical activity and energy restriction reduces HIF-1α gene expression in the tumor microenvironment in the 4T1.2 murine breast cancer model  
Abriana Cain
# 45  Food additive guar gum exacerbates colonic inflammation in murine models of inflammatory bowel disease  
Divek V T Nair

# 52  Associations between multiple physiological mechanisms within an individual  
Elyse McMahon

# 53  Relationship between tumor progression and immune response in the 4T1.2-HER2 mouse mammary tumor model  
Abirami Ravichandran

Molecular Biology, Genetics, & Chemistry

# 7  The feasibility of strong acid-free hairy nanocellulose production from lignocellulosic sources through periodate and chlorite oxidation  
Mica Pitcher

# 9  Understanding Adenylosuccinate Lyase Deficiency locomotion deficit using C. elegans as a model  
Latisha Franklin

# 12  A kinetic dissection of the fast and superprocessive kinesin-3 KIF1A reveals a predominant one-head-bound state during its chemomechanical cycle  
Taylor Zaniewski

# 16  Novel Cofilactin Bundling in Neuronal Growth Cone Filopodia  
Ryan Hylton

# 17  Sequence analysis of the Petunia inflata S-locus region containing 17 S-Locus F-Box genes and the S-RNase gene involved in self-incompatibility  
Lihua Wu

# 20  Dissecting the regulatory role of an enriched DNA sequence motif found upstream of Plasmodium falciparum gametocyte-associated genes  
Riëtte van Biljon

# 21  The genome-wide role of NusA on RNA polymerase pausing in Bacillus subtilis  
Oshadhi Jayasinghe

# 23  Molecular and Morphological Correlates of Terminal Differentiation of Chandelier cells  
Matthew Dickinson

# 30  Mechanisms of allostERIC regulation in the Farnesoid X receptor  
Tracy Yu

# 31  Elucidating Driver Genes in PIK3CA-mutated Mammary Carcinogenesis and Relapse Using an Inducible and Mammary-Specific Transposon Mutagenesis System  
Maryknoll Palisoc

# 38  Transforming Growth Factor β modulates IRE1α-mediated ER stress response in keratinocytes expressing oncogenic H-Ras  
Saie Mogre
Paramyxovirus-like Particles: a novel strategy for proteins delivery
Santosh Panthi

Elucidating the roles of 2’, 3’-cyclic nucleotide monophosphates in bacterial signaling and stress response
Shikha Chauhan

Defining DNA sequence and chromatin features that influence binding specificity of transcription factors in Plasmodium falciparum
Victoria Bonnell

Plant & Agricultural Sciences, Zoology & Ecology

Exploring the potential of in silico tools to enhance agricultural resilience to climate change
Ele Saltmarsh

Dietary metformin supplementation improves ovarian function in broiler breeder hens
Evelyn Weaver

FRO3 Plays an Integral Role in Sub-Cellular and Whole Plant Iron Homeostasis in Arabidopsis
Brendon Juengst

Role of the bovine PRAMEY protein in sperm function during in vitro fertilization (IVF)
Chandlar Kern

Impacts of One-time Tillage Compared to No-tillage on Soil Health in a Diverse Rotational Cropping System
Devyn McPheeters

Evidence for Immune tolerance in peripheral blood leukocytes of dairy cattle during early pregnancy
Maria Isabel da Silva

Generation of novel alleles of rice extra-large G proteins (XLGs) via CRISPR/Cpf1 gene editing system
Christian Cantos

Computational Biology & Statistics

Interactions between climate change and coinfections: what should we expect from the future?
Chiara Vanalli

Impact of Solvent Interactions upon Interfacial Phenomena
Varun Mandalaparthy

Towards Synthetic Microbiota Transplants: Insights for C. difficile Treatment From Meta-analysis
Susan Tian

Effect of a kinked trachea on the dose distribution of reactive air pollutants in proximal airways of the human lung
Minyoung Kim
Effect of metformin treatment on the gut mycobiome of type 2 diabetes patients: A meta-analysis.
Emily Bean Van Syoc

Volatile Organic Compounds Produced by Bacteria Associated with Decomposition
Veronica Cappas

Neuroimmune cells play a critical role in mediating the effect of acute alcohol on central amygdala glutamatergic transmission.
Mariam Melkumyan

Structure and function of an unusual flavodoxin from the domain Archaea
Divya Prakash

Characterizing new vitamin D targets in the immune system using novel vitamin D receptor (VDR) reporter mouse.
Juhi Arora

Short- and Long-Term Effect of Persistent Organic Pollutants in Gut Microbiota Community and Function in Immature Mice
Bipin Rimal
Human Health, Nutrition, & Physiology

# 4 | The Role of Oxylipins in Neuroinflammation
Julia Kelliher, Ivana Maric, Karolina Skibicka, Ph.D., & Gregory Shearer, Ph.D.
Integrative and Biomedical Physiology

Neuroinflammation plays a key role in the risk and development of diseases like Alzheimer’s Disease (AD), a progressive neurodegenerative disease that affects 1 in 10 adults over 65 years old and is the sixth leading cause of death in the United States. Understanding the molecular basis of neuroinflammation could help identify novel therapeutic targets and prevention strategies for diseases like AD. Neuroinflammation may be linked to oxylipins, which regulate intracellular inflammatory responses via phospholipase A2-mediated synthesis and recycling between non-esterified and esterified forms. To investigate this, we measured CSF and plasma oxylipin concentrations in response to a centrally- or peripherally-administered acute inflammatory stimulus – interleukin-1β (IL-1β). Specifically, we measured 60 non-esterified and esterified oxylipins in cerebrospinal fluid (CSF), plasma apolipoprotein (apo) A-I high-density lipoprotein (HDL), and apo E HDL of male and female Sprague Dawley rats that received an intracerebroventricular or intraperitoneal injection of control or IL-1β. Highly pro-inflammatory esterified CSF hydroxy-eicosatetraenoic acids (HETEs) were three times higher in centrally IL-1β-treated rats compared to controls (36.0 nanomolar [nM] versus 12.5 nM, respectively; p = 0.02). Highly anti-inflammatory esterified CSF epoxy-eicosatrienoic acids (EpETEs) were double the concentration in centrally IL-1β-treated rats compared to controls (4.2 nM versus 2.2 nM, respectively; p = 0.04). Peripheral apo A-I HDL esterified EpETEs were similarly double the concentration in centrally IL-1β-treated rats compared to controls (1.9 nM versus 0.9 nM, respectively; p = 0.04), emphasizing the potential for plasma oxylipins to serve as markers of central inflammation. Interestingly, the opposite response was seen in non-esterified apo A-I EpETEs, where centrally IL-1β-treated rats had half the concentration of non-esterified apo A-I EpETEs compared to controls (0.8 nM versus 1.4 nM, respectively; p = 0.03), underscoring the importance of measuring both non-esterified and esterified oxylipins. Rats treated with peripheral IL-1β had 4.5 nM esterified apo A-I HDL HETEs, twice the 2.3 nM concentration in controls (p = 0.002). Peripherally IL-1β-treated rats had half the concentration of non-esterified CSF hydroxy-eicosapentaenoic acids (HEPEs) compared to controls (0.9 nM versus 1.8 nM, respectively; p = 0.04), providing support for CSF oxylipins responding to peripheral inflammation. Sex differences were also detected, with oxylipins in males generally responding to a greater extent to inflammation than oxylipins in females. Overall, this study shows compartment-specific oxylipin responses to inflammation, identifies plasma oxylipins that could be useful as markers for central inflammation, and supports a role for oxylipins in the development of neuroinflammation.

# 11 | Red and Processed Meat Consumption and Food Insecurity in Relation with High Blood Pressure: A Population-Based Study
Djibril M. Ba, Xiang Gao, Duanping Liao, John P. Richie, & Jr., Laila Al-Shaar
Penn State College of Medicine

Background: Greater consumption of red and processed meat has been associated with higher risk of mortality and major chronic diseases including hypertension. Food insecurity has been also associated with worse health outcomes. The magnitude of the association between red and processed intake and high blood pressure may be more pronounced among food insecure individuals. This study aimed to examine the joint
association of red/processed meat intake and food insecurity with high blood pressure (HBP) among US adults. Methods: A total of 31,519 adult participants (mean (SE) age of 46.5 (0.3) years) of the National Health and Nutrition Examination Survey (NHANES) 2003-2016 were included. Total red meat intake included red meat and processed meat items such as beef, veal, pork, lamb, and cured meat and was estimated using 24-hour dietary recalls. Food insecurity status was assessed using the Food Security Survey Module developed by the US Department of Agriculture and was defined as having three or more affirmative responses. HBP was defined using the average of the second and third readings (mean systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg) or use of anti-hypertensive drugs in the past 30 days prior to the survey interview. Multivariable survey logistic regression models were used to examine the independent and joint associations of total red meat and food insecurity with HBP, adjusting for potential confounders such as demographics, lifestyle, total energy, and other dietary factors. Results were presented as adjusted odds ratio (aOR) and 95% confidence interval (CI). Results: More than half of participants (69%) were Non-Hispanic White, and 14% were food insecure. In a multivariate analysis, total red meat consumption and food insecurity were independently associated with higher odds of HBP. Compared to the first quintile of total red meat intake, participants in the third to fifth quintiles of total red meat intake had 16% to 45% higher odds of having HBP. These associations were stronger among food insecure participants as compared to food secure participants. Food insecure adults in the 3rd to 5th quintiles of total red meat consumption had 40% to 63% higher odds of HBP as compared to food secure adults in the lowest total red meat intake quintile (p-value for interaction < 0.001). Further adjustment for other dietary factors and protein sources such as seafood, poultry, eggs, dairy products, nuts, beans, and legumes did not change the significant association between total red meat intake, food insecurity, and HBP. Similar trends of associations were observed with processed meat intake, where participants in the fourth and fifth quintiles had 13% to 28% higher odds of HBP compared with the first quintile. Conclusions: This study provides more evidence about the health hazards of total red meat consumption in relation with HBP. This association is more pronounced among food insecure US adults.

# 14 | Aryl Hydrocarbon Receptor is a key mediator of intestinal epithelial homeostasis
Debopriya Chakraborty, Xiaoliang Zhou, Iain Murray, Denise Coslo and Gary Perdew
Department of Veterinary and Biomedical Sciences

Dioxins provoke profound disruption of intestinal integrity, effects of which are mediated by persistent activation of the transcription factor Aryl hydrocarbon Receptor (AHR). AHR is an environmental sensor which detects a wide variety of exogenous (TCDD and polycyclic aromatic hydrocarbons), endogenous (kynurenine), dietary (indole-3-carbinol, Indolo[3,2-b]carbazole) and microbial metabolites (indole-3-aldehyde). It activates a signaling cascade that causes expression of well-characterized xenobiotic-metabolizing enzymes cytochrome P450 1A1 (CYP1A1) and 1B1 (CYP1B1), among other proteins. In the gastrointestinal tract, AHR ligands modulate the development and function of intestinal epithelial cells as well as mucosal immune cells such as Th17 and ILC3s (innate lymphoid cell 3), necessitating a better understanding of its role in the maintenance of intestinal homeostasis. In contrast, much less is known about the role of the AHR in the intestinal epithelium. We have demonstrated that AHR activity can be stimulated by dietary and microbial metabolites and that such activation is likely protective against intestinal challenges. Gene expression analyses reveal a decreasing, proximal to distal, AHR gradient within the intestine, consistent with a concentration gradient of dietary AHR ligands. Exposure to broccoli diet (rich in AHR ligands), and dietary ligands e.g. indolo[3,2b] carbazole (ICZ) induces expression of AHR target gene Cyp1a1, which isn’t observed with semi-purified diet. Cyp1a1 expression, a marker of AHR activity, positively correlates with biomarkers of enterocyte differentiation (Klf4, Krt20, Math1, Tff3) and with tight junction proteins that maintain intestinal integrity (Ocln, Cldn, Tjp). AHR activation further enhances expression of antimicrobial factors (Reg3g, Lyz),
goblet cell numbers and mucin production, and decreases epithelial stem cell proliferation. The chemotherapeutic drug doxorubicin causes intestinal side effects, which might be ameliorated with pre-exposure to dietary AHR ligands. Thus, dietary manipulation of AHR activity confers protection against various intestinal insults that affect normal physiology and can provide a cost-effective and readily available route to increase or restore intestinal resilience.

# 18 | The Effects of Chronic Fluoxetine Treatment on Alcohol Intake and Metabolic Functioning in Binge Drinking/Eating Mouse Model
Bailey Keller, Angela Snyder, Mariam Melkumyan, Yuval Silberman
Department of Neural and Behavioral Sciences, Pennsylvania State University College of Medicine

Alcohol use disorders (AUDs) are intersectional and causal to many other disease states, including depression, metabolic disorders, and Type II diabetes. Binge eating disorder (BED) is the most prevalent eating disorder and, like AUD, is also co-morbid with depression and metabolic disorders. BED and AUD co-morbidity may increase susceptibility to metabolic dysfunction and Type II diabetes. Our lab has developed a novel mouse model of co-morbid BED+AUD that mimics aspects of binge high-fat diet (HFD) and alcohol (EtOH) intake and presents with hyperglycemia and insulin/glucose intolerance as a result of their binging behaviors. Fluoxetine has shown some effectiveness in the treatment of AUD and is used clinically for BED; however, the mechanisms involved in these fluoxetine effects remain elusive. We aim to directly examine the effects of chronic fluoxetine treatment on EtOH intake behaviors and metabolic functioning in our BED+AUD mouse model. Adult male C57Bl/6J mice received 15-20 mg/kg fluoxetine p.o. or water for two weeks before undergoing our BED+AUD time course followed by metabolic testing. During the BED+AUD time course, all mice were exposed to an increasing percentage of EtOH through limited access two-bottle choice and received either HFD (1 day per week with control chow the remaining days) or control chow for 8 weeks. Glucose tolerance tests were performed at the end of the study. Our preliminary data indicate that chronic fluoxetine treatment reduces binge intake behaviors toward both HFD and EtOH, resulting in improvements in glucose tolerance in these mice. Further elucidation of fluoxetine’s therapeutic effects in our BED+AUD mouse model may lead to improved targeted treatments of BED and AUD.

# 19 | Deletion of Pramel1 disrupt the retinoic acid-coordinated spermatogenesis
Yang, M.Y., Ma, W.Z., Oatley, J., and Liu, W.-S.
Department of Animal Science

The PRAME (PRAME nuclear receptor transcriptional regulator) is one of cancer/testis antigens that is expressed only in tumors and the germ line. It was reported that PRAME inhibits the retinoic acid (RA)-induced differentiation, growth arrest, and apoptosis in tumors. However, the roles of PRAME in testis are barely known. Periodic production of RA coordinates four key transitions of spermatogenesis: spermatogonial differentiation, meiotic initiation, initiation of spermatid elongation and release of spermatozoa, and then propels successive rounds of spermatogenesis to continuously produce spermatozoa in the testis. To initiate spermatogenesis, the first RA pulse at high concentration occurs at Postnatal day 3 (P3). In the rigid time-scale spermatogenic cycle, the four RA-coordinated transitions of spermatogenesis are precisely organized in stage VII-VIII when RA pulses arise. PRAME constitutes a large gene family in mammals. Pramel1, known to be a PRAME family member, is expressed predominately in the testis. Given that PRAME acts as a dominant repressor for RAR signaling in tumors, we hypothesized that Pramel1 is involved in the organization of RA-coordinated spermatogenesis. The objective of this study was to determine the roles of Pramel1 in RA-coordinated spermatogenic program using Pramel1 global knockout (KO) mice model which was generated by CRISPR/Cas9. We firstly characterized their reproductive phenotypes, such as testis size,
sperm count, pregnancy rate, and the litter size, etc. The young KO males (≤2-months-old) had a larger testis and a higher reproductive ability than wild type (WT) males. In contrast, the mature KO males (>2-months-old) had a significant reduction in testis size and sperm count, and thus, were subfertile. Then, we detected the apoptotic rate in the testis through TUNEL assay and found less apoptotic cells in the young KO mice but more in the mature KO males than WT. To further understand the subfertile phenotypes, we used histological methods to identify the germ cells and spermatogenic stages after RA pulses. Less germ cells were calculated in the KO mice than control after the first RA pulse at P3. Besides, germ cell arrest was observed after the RA pulse during seminiferous cycle, at stage IX-X, in the young KO males. The percentage of normal seminiferous tubules at stage VII-VIII was increased, but decreased at stage IX-X for the P35 KO mice. In mature KO mice, spermatogenesis was jeopardized by absence of leptotene spermatocytes or spermatogonia at stage IX. The defected spermatogenesis in the mature KO males was confirmed by heat-shock treatment. The KO males had a significantly slow recovery than WT after heat-shock treatment. We concluded that loss of Pramel1 disorganize RA-coordinated spermatogenic program and then change germ cells number in different rounds of spermatogenesis. Our results suggested that deletion of Pramel1 may relieve the restrictions on RA and increase germ cell to process the first round of spermatogenesis. Thus, more sperm production will be observed in the young KO males. However, the subsequent rounds of spermatogenesis were impaired after RA pulses in the KO mice. Overall, this study shed a light on that Pramel1 participated in RA-coordinated spermatogenesis.

# 22 | Docosopentaenoic Acid (DHA) is Superior to Race in Predicting an Individual's Risk for Myocardial Infarction (MI)
Carmen Annevelink and Gregory Shearer
PSU Nutritional Sciences

Background: Algorithms identifying who is at risk for MI include the use of race, but using race to encompass potential differences in metabolism and as well as potential differences in diet and lifestyle generalizes specific biological processes which can be more specifically described by fatty acid metabolism. Fatty acids can be used to define race-independent subpopulations using their specific enzymatic activities in the fatty acid synthesis pathway and their diet and lifestyle. These subpopulations can then be specifically targeted to mitigate risk for MI. Methods and Setting: A total of 6,564 participants aged 44-84 years old from the Multi-Ethnic Study of Atherosclerosis (MESA) dataset had their fatty acids measured at baseline. We used generalized regression and proportional hazard modeling to identify the presence of any significant plasma fatty acids involved in the polyunsaturated fatty acid synthesis pathway for the outcome of interest, MI. After identification of significant factors, distribution analyses and regression equations were used to describe subpopulations and determine the associated risk for each subpopulation. Primary outcome: Superiority in risk prediction for MI of DHA-defined populations compared to populations defined by race. Results: After a median follow-up of 13.9 years, 164 MIs were documented. In the generalized regression, DHA was the only significant fatty acid for development MI and, in the proportional hazard model, DHA was more descriptive of risk for MI (p=0.0002) compared to race (p=0.35). After outlier exclusion and log transformation to improve normality, the levels of DHA could be described by a normal multimodal distribution (mean1 = 0.35, mean2 = 0.55, mean3 = 0.74) accounting for 15.6%, 54.2%, and 30.2% of the population, respectively. Using these values in normal Gaussian regression equations, each individual found to have greater than or equal to 70% likelihood for component membership was assigned to the corresponding group. Proportional hazard modeling found individuals assigned to component 1 (lower DHA) of the DHA distribution to be at 2.65 times higher risk for MI than individuals in component 3 (higher DHA) (X2<0.0001). Conclusion: The three DHA subpopulations, or components, have significant differences in their risk for MI and are superior to race
classification in predicting risk for MI. Allocation to these subpopulations has greater specificity than allocating individuals based on their reported race.

# 25 | Interrogation of the Role of Gpr44 in Acute Myeloid Leukemia  
Fenghua Qian, Brooke Arner, Jiayan Zhou, Shaneice Nettleford, Molly Hall, Robert Paulson, Sandeep Prabhu  
Department of Veterinary and Biomedical Sciences

Purpose: To investigate the activation of a membrane bound G-protein coupled receptor 44 (Gpr44) as a new modality for treatment of acute myeloid leukemia (AML) by cyclopentenone prostaglandin J2 (CyPGs) ligands that are produced endogenously upon dietary selenium (Se) supplementation, or their exogenous administration.  

Experimental Design: To investigate the effect of Se supplementation on the outcome of AML, CD45.2 mice were maintained under Se adequate (Se-A; 0.08 ppm Se) or supplemented (Se-S; 0.4 ppm Se) diets for four weeks prior to transplantation. CD45.1 hematopoietic stem cells (HSCs) isolated from donor mice were transduced with MLL-AF9 retrovirus and then transplanted into above primary recipient mice on specific diets, which were sublethally irradiated. Secondary transplantation was done with leukemic splenocytes from primary recipient mice into experimental mice on diets. Prognosis was analyzed by comparing 1) complete blood cell counts, 2) leukemic cell infiltration and leukemia-initiating cell (LIC) numbers in bone marrow and spleen, and 3) normal hematopoiesis measured to determine if Se supplementation had any toxic effects. To determine the role of Gpr44 activation in the prognosis of AML, CD45.1 primary AML splenocytes were transplanted into CD45.2 secondary recipient under Se-S diet for four weeks. Daily treatments of Gpr44 agonists (CyPGs, 0.2 mg/kg) were administered intraperitoneally (i.p.) for two weeks starting one week post transplantation. Mice were euthanized following treatment. At endpoint, disease progression was followed as described earlier. To investigate the mechanism of Gpr44 activation in regulating the apoptosis of LSCs, Gpr44-/- HSCs were transduced with oncogene (MLL-AF9) expressing retrovirus and then transplanted into sublethally irradiated TdTomato mice. Secondary transplantation was done with primary Gpr44-/- leukemic splenocytes into TdTomato mice and compared with mice transplanted with Gpr44+/+ leukemic splenocytes on Se-S diet. To investigate the mechanism of Gpr44 activation in AML, isolated Gpr44/- and Gpr44+/+ LICs were examined by RNA sequencing. Further analyses were done using differential expression analyses, Gene Set Enrichment Analysis (GSEA), and Ingenuity Pathway Analysis (IPA). Results: 1. Se supplementation at supraphysiological levels eliminates LICs in murine model of AML without affecting the normal HSCs. 2. Exogenous and endogenous CyPGs induced by Se supplementation induce apoptosis in LICs via the activation of Gpr44 in LICs. 3. Gpr44 activation on LSCs interferes with MAPK signaling pathway to affect the outcome of LICs. Conclusions: This study suggests that Se-induced endogenous production of CyPGs contributes to the activation of apoptotic pathways via Gpr44 in AML LSCs.  

Impact: These findings provide a rationale for the development of novel treatments for AML. A combination therapy with Gpr44 agonists and Se could effectively eliminate the leukemic cells and alleviate the disease.

# 32 | Alzheimer’s disease risk factor APOE4 alters cytokine secretion patterns in the frontal cortex of mice  
Rebecca Fleeman, Amanda Snyder, Madison Kuhn, & Elizabeth Proctor  
Departments of Neurosurgery and Pharmacology, Pennsylvania State College of Medicine

Alzheimer’s disease (AD) is a progressive neurodegenerative disease characterized by amyloid-β plaques, neurofibrillary tau tangles, and neuroinflammation that currently affects more than 6 million Americans. The ε4 variant of apolipoprotein E (APOE) is the strongest and most common genetic risk factor for AD, increasing risk for AD by 4-14-fold and decreasing the age of onset by 8-12 years. APOE has three common isoforms in the population, ε2, ε3, and ε4, which are found in 8%, 78%, and 14% of the population, respectively. The
difference between APOE3, the most common isoform, and APOE4, the AD risk isoform, is a single amino acid change that alters lipid binding, receptor affinity, and APOE expression level, however, the mechanisms by which APOE4 confers greater risk for AD is not fully understood. Neuroinflammation has increasingly garnered attention for exacerbating AD pathology, and we hypothesize that APOE genotype affects the neuroinflammation cascade in the brain to increase AD risk. Our goal was to measure whether there are discernible differences in cytokine signaling between the frontal cortex of APOE3 and APOE4 mice across the lifespan. Differences that appear with age, that also correspond with pathways known to be upregulated in AD will elucidate potential AD-promoting mechanisms of APOE4. Importantly, cytokine signaling is extremely complex, with many interacting pathways, and thus, multivariate analysis is necessary to take into account the covariation and dependence between levels of cytokines. Using partial least squares discriminant analysis, we found that cytokine signatures differ greatly between genotypes in the frontal cortex of APOE3 and APOE4 mice. Interestingly, even when comparing mice of various ages, diets, and sexes, the cytokine profile differences between APOE3 and APOE4 remained. Specifically, the frontal cortex of APOE4 mice had higher levels of interleukin (IL)-2, macrophage inflammatory protein (MIP)-1a, and IL-1a, while the frontal cortex cells of APOE3 mice secreted more IL-5, MIP-1b and MIP-2. Our results highlight the importance of understanding the impact of genetic risk factors on neuroinflammation. In our future studies, we plan to define the downstream neural mechanisms underlying the protective and detrimental effects of the cytokine signatures found in APOE4 and APOE3 systems. Discovering the mechanisms of APOE4 conferring greater AD risk will allow us to design better biomarker tests for early diagnosis of AD and preventative medicine regiments for APOE4-carrying individuals.

# 34 | Characterizing the role of GRK2 in postnatal skeletal development
Natalie Yoshioka, Vengadeshprabhu Karuppa Gounder, William Pinamont, Gina Deiter, & Fadia Kamal
The Pennsylvania State University College of Medicine

Background: Most bones develop by a process called endochondral ossification (EO), in which a cartilaginous precursor gradually ossifies. In postnatal life, a cartilaginous growth plate (GP) persists, allowing for continued bone elongation. The chondrocytes in the GP undergo several phenotypic shifts, observed as three distinct histological zones: resting, proliferative, and hypertrophic. Resting chondrocytes first enter a proliferative state and rapidly divide to push the ends of the bone forward, then become hypertrophic to create room for new bone formation. Significance: The balance and timing of chondrocyte proliferation and hypertrophy is essential for proper bone development. Imbalances result in a variety of chondrodysplasias, characterized by premature GP closure and decreased skeletal length. As chondrodysplasias are still incompletely understood, studies of EO molecular regulation offer insight and potential for development of innovative therapeutic approaches. Aim: EO is regulated by the Indian Hedgehog (Ihh)/Parathyroid hormone related protein (PTHrP) feedback loop, which are ligands for membrane receptors patched-1 (Ptch1) and parathyroid hormone receptor-1 (PTH1R) respectively. Both receptors are known to interact with the G-protein receptor kinase 2 (GRK2). Recently, our lab demonstrated a role for GRK2 as a major regulator of chondrocyte hypertrophy (CH) in adult cartilage with osteoarthritis. Therefore, we hypothesize that GRK2 is integral in bone development as a regulator of CH in the GP. Methods: We utilized the Cre-Lox system to generate transgenic mice with inducible -conditional-knockout of GRK2 (GRK2-icKO) in chondrocytes. As GRK2 expression peaks in hypertrophic chondrocytes during early postnatal development, we induced deletion postnatally in 3-day-old mice pups (PN3) and harvested at both PN10 and PN28. We measured bone length and performed whole-mount-skeletal staining to explore short- and long-term effects of GRK2 deletion on skeletal development. Then, we performed Hematoxylin-Eosin staining to analyze changes in GP structure and cellular composition. Finally, we performed immunofluorescence (IF) staining to quantify CH markers and molecular changes; and TUNEL and BrdU staining to quantify apoptosis and proliferation, respectively.
Results: GRK2-icKO mice (both short- and long-term) had decreased bone length. Their GPs were longer with increased proliferative zone and decreased hypertrophic zone lengths, indicating increased chondrocyte proliferation and decelerated hypertrophy, leading to delayed EO and shorter bones. This was confirmed by reduced apoptosis (TUNEL-positive cells) and increased proliferation (BrdU-positive cells) of GP chondrocytes. Furthermore, we found decreased expression of CH markers (MMP13, ColX), and PTHrP and Ihh in GRK2-icKO mice. Interestingly, expression of receptors PTH1R and Ptch1 were increased. Together, this suggests that GRK2 regulates the IHH/PTHrP feedback loop to promote CH in EO. We replicated our studies in chondrocyte primary cultures in vitro, where GRK2 knockdown reduced CH and matrix mineralization. RT-qPCR showed a direct inhibitory effect of GRK2 knockdown on CH in these cells through IHH/PTHrP regulation. Conclusion: Our data establish an original role for GRK2 in EO as a regulator of CH in postnatal skeletal development. Mechanisms of EO regulation remain incompletely understood, and dysregulation leads to a variety of chondrodysplasias. Here, we present GRK2 as a novel molecular target for therapeutic approaches to skeletal diseases characterized by dysregulated CH.

# 35 | Cardiovascular Disease Burden: Comparison of a Psychiatric Outpatient Population and a US General Population Sample
Radha Dhingra, Fan He, Erika F.H. Saunders, Daniel A. Waschbusch, Amanda M. Pearl, Dahlia Mukherjee, Edward O. Bixler, Jody L. Greaney, Duanping Liao
Department of Public Health Sciences, Penn State College of Medicine

Objective: Depression and heart disease are the two major causes of disability worldwide. Examining the relationship between psychiatric and cardiac conditions is important, as cardiovascular diseases (CVDs) are the leading contributor of the premature mortality among persons with mental illness. We aimed to determine the burden of CVDs in a psychiatric outpatient population in comparison to the US general population. Methods: Electronic health record data of 3,556 adults with mental illness, enrolled between 2015-2020 in the ongoing Penn State Psychiatry Clinical Assessment and Rating Evaluation System (PCARES) Registry, are included in this report. We compared the prevalence of CVDs and mean levels of major CVD risk factors in our sample with that reported in the 2013-2016 National Health and Nutrition Examination Survey (NHANES). NHANES is well-representative of the US general population. To enhance the validity of the comparisons, proportions and means of CVD risk factors for NHANES were adjusted to the mean age and proportions of race and sex of PCARES. Results: PCARES participants were 42.36 ± 16.97 years of age, with 63% females and 85% Non-Hispanic Caucasians. The most common psychiatric disorders in the PCARES population were Major Depressive Disorder (41%), Generalized Anxiety Disorder (37%), and Bipolar Disorder (10%). In PCARES compared to NHANES, the prevalence (%) of CVD was 9.6 vs. 4.6; type 2 diabetes: 20.7 vs. 11.0; hypertension (HTN): 50.1 vs. 50.4; dyslipidemia: 47.5 vs. 61.9; and smoking: 21.9 vs. 36.5. No difference was observed in HTN prevalence (p=0.69), and dyslipidemia and smoking prevalence were higher in NHANES compared to PCARES (p<0.0001). All other CVDs were significantly higher in the PCARES population compared to NHANES (p<0.0001). Although glucose levels were not different in PCARES and NHANES populations (111.4 vs. 112.0, p=0.48), PCARES population had significantly higher HbA1C (6.1% vs. 5.6%, p<0.0001). In addition, lipid labs (mg/dl) in PCARES compared to NHANES populations were: total cholesterol (C) 185.4 vs. 181.7; LDL-C: 107.6 vs. 111.4; HDL-C: 48.5 vs. 41.3; and triglycerides: 153.0 vs. 135.6 (all significantly higher in PCARES at p<0.0001, except LDL-C, which was higher in NHANES (p<0.0001)). Lastly, proportions of individuals with prescriptions for anti-hypertensives (95.0 vs. 76.1); antidiabetics (86.3 vs. 61.1); and lipid-lowering medications (47.81 vs. 43.22) were all significantly higher in PCARES at p<0.0001. Conclusions: Cardiovascular disease burden, for most CVD risk factors and comorbidities, is significantly higher among persons with mental illness compared to the US general Population.
population. Our data support attention to integration of mental and physical health care services in both mental health and primary care settings to maximize the overall health of this population.

# 36 | Physical Activity and Diet Quality in Relation with Adiposity Measures among US Adolescents
Radha Dhingra, Laila Al-Shaar, Fan He, Alexandros N. Vgontzas, Edward O. Bixler, Duanping Liao, Julio Fernandez-Mendoza
Department of Public Health Sciences, Penn State College of Medicine

OBJECTIVE: Unhealthy eating patterns and low physical activity (PA) are major contributors to the persisting obesity epidemic in adults. Adolescent adiposity is a strong predictor of adulthood obesity, thus, there is a greater need to better understand the impact of diet quality (DQ) and PA on adipose tissue (AT) distribution and composition in adolescents. Our study aimed to examine DQ and PA in relation to AT in a sample of US adolescents. METHODS: Data obtained from 384 adolescents participating in the Penn State Child Cohort first follow-up examination were analyzed. DQ was estimated with the Youth/Adolescent Food Frequency Questionnaire. A modified Alternate Healthy Eating Index (AHEI), excluding the alcohol component, was derived to assess adherence to dietary guidelines, ranging from 0 (non-adherence) to 100 (perfect adherence). PA was assessed with the Youth/Adolescent Activity Questionnaire. A weekly energy expenditure score for moderate-to-vigorous intensity PA (MVPA) was computed after summing up the metabolic equivalent task (MET) scores associated with each reported activity. Dual-energy x-ray absorptiometry (DEXA) scans assessed AT distribution (android, gynoid, and total fat mass in Kg) and composition [subcutaneous (SAT) and visceral (VAT) adiposity in cm2). Multivariable-adjusted linear regression models examined the association between AHEI and MVPA with AT distribution and composition, while adjusting for age, race, sex, tobacco use, and total caloric intake. RESULTS: Participants were 16.5 ± 2.26 years of age, 54% were males, 21% belonged to a racial/ethnic minority, and 15% were obese as per body mass index percentile criteria. Low adherence to dietary guidelines was observed among participants [mean (SD) of AHEI: 39 (8), range: 20 to 70], and 75% were physically active for ≥ 60 minutes/day with no sex differences (p=0.27). Compared to the lowest MVPA tertile, the highest MVPA tertile was associated with lower SAT (β= -58.44, p<0.001), VAT (β= -12.69, p=0.01), android to gynoid fat mass ratio (β= -0.043, p=0.002), android fat mass (β= -0.42, p<0.001), gynoid fat mass (β= -0.50, p=0.021), and total fat mass (β= -3.80, p<0.001). No independent association was observed between AHEI and AT, except for VAT to SAT ratio. Compared to the lowest AHEI tertile and after simultaneous adjustment for all covariates as well as SAT and MVPA, adolescents in the highest AHEI tertile had a lower VAT to SAT ratio (β= -0.04, p=0.01). CONCLUSIONS: Low adherence to dietary guidelines was observed among US adolescents. Higher physical activity levels were associated with lower total and localized adiposity. Developing effective, multi-component interventions tailored to promote healthful dietary choices, food literacy, and/or MVPA among adolescents may target specific body compositions and limit the increasing rates of adolescent obesity and its associated cardio-metabolic risk.
A randomized controlled trial of dietary supplementation with dried plums on inflammatory markers in postmenopausal women

Janhavi Damani, Mary Jane DeSouza, Connie J Rogers
Intercollege Graduate Degree Program in Integrative and Biomedical Physiology

Significance and Purpose: The prevalence of osteoporosis among women aged ≥50 years is expected to reach 13.6 million by 2030. Osteoporosis is characterized by a reduction in bone mineral density (BMD) and represents a major public health issue that necessitates effective treatment regimens that are safe, cost-effective, and associated with fewer adverse effects than pharmaceutical agents. Alternative therapies for osteoporosis are becoming increasingly popular, of which dried plum has been extensively studied as a dietary intervention to mitigate bone loss in preclinical models of osteoporosis. In postmenopausal women, estrogen deficiency triggers upregulation of inflammatory pathways, which may promote bone loss, putting them at a high risk for fracture. Our understanding of the effect of dried plum consumption on inflammatory markers in humans is limited. This study aimed to evaluate the effects of 12 months of dried plum consumption on immune and inflammatory mediators.

Methodology: Postmenopausal women (n=95, 55-75 years old) with low BMD were randomized to three treatment groups: control, 6 dried plums/day (50 g/day), or 12 dried plum/day (100 g/day). Fasting blood samples were collected at baseline and after 12 months on study. Peripheral blood mononuclear cells (PBMCs) were isolated to quantify 1) the number and activation status (HLA-DR and TLR-2 expression) of circulating monocytes, an immune cell type that mediates inflammatory responses, and 2) inflammatory cytokine levels following in vitro stimulation with lipopolysaccharide (LPS).

Results: No significant differences in baseline characteristics, including age, age at menopause, time since menopause, body mass index, and age at menarche, were found among the control (n=34), 6 DP (n=37), and 12 DP (n=24) treatment groups. Dried plum consumption did not alter the number of circulating monocytes or their activation status. Dried plum consumption did not significantly impact inflammatory cytokine secretion from cultured PBMCs when all women were included in the analyses. However, a significant time × treatment effect was found for MCP-1 (F(2,26)=5.43, p=0.011), IL1-β (F(2,26)=5.30, p=0.012), and IL-8 (F(2,26)=10.52, p<0.001) in women with overweight and obesity (n=29, body mass index < 25 kg/m2). Dunnett’s post-hoc test for multiple comparisons revealed a significant reduction in IL1-β in the 6 dried plum group (P<0.05) and in IL-8 in both the 6 and 12 dried plum groups compared to the control group (P<0.05) after 12 months on treatment. Conclusion: Our findings suggest that chronic consumption of 6 and 12 dried plums suppresses inflammatory markers in postmenopausal women with an elevated BMI. Future analyses are underway to evaluate the association between inflammatory markers and bone outcomes.

The combination of physical activity and energy restriction reduces HIF-1α gene expression in the tumor microenvironment in the 4T1.2 murine breast cancer model

Abriana Cain, Yitong Xu, William J. Turbitt, & Connie Rogers
Department of Nutritional Sciences

Significance and Purpose: Breast cancer is the second leading cause of cancer death in women in America. The average five-year survival rate for women with invasive breast cancer localized in the breast is 98.9% but is considerably reduced to 28.1% for women diagnosed with metastatic breast cancer. Halting the metastatic spread of breast cancer would dramatically improve survival rates in women. Several biological mechanisms have been proposed to explain the beneficial effect of physical activity and energy restriction on tumor growth including systemic changes in metabolic, and immune mediators, as well as changes in the tumor microenvironment (TME), including vascularization of the tumor. Tumors have excessive but dysfunctional
vasculature which typically feature immature and leaky vessel structure characterized by pockets of hypoxia. Hypoxia inducible factor-1 alpha (HIF-1α) becomes activated in hypoxic cells. Altered HIF-1α expression in women is associated with increased metastases and poor clinical outcomes. Several preclinical studies demonstrate that physical activity suppresses HIF-1 or markers of vascularization (expression of vascular endothelial growth factor, platelet-derived growth factor) and that energy restriction alters tumor vessel maturity. Thus, both physical activity and energy restriction may alter oxygenation or vascularization in tumors, which may influence tumor growth and metastasis. Thus, the purpose of the current study was to determine whether physical activity, energy restriction, or the combination reduces HIF-1α gene expression in the TME. Methodology: Female BALB/c mice were randomized to sedentary (SED) or activity wheel physical activity (PA) cages and fed ad libitum (AL) or 90% of control food intake (ER). After 8 weeks on the interventions, mice were inoculated with 5x104 4T1.2Luc cells into the 4th mammary fat pad and continued on their respective intervention. Results: The combination of PA+ER significantly delayed primary tumor growth (p<0.001), reduced metastatic burden in the lungs (p=0.054) and increased survival (p=0.043). The fold change in HIF-1 gene expression in the TME in each treatment group was 0.83 (0.62-1.08, 95% CI) in PA+AL, 0.089 (0.66-1.13, 95% CI) in SED+ER and 0.75 (0.55-0.95, 95% CI) in PA+ER, respectively compared to the SED+AL group. Thus, only the combination of PA+ER significantly reduced gene expression of HIF-1 in the TME. Conclusion: These data suggest that both PA and the prevention of weight gain via ER are needed to reduce primary tumor growth, delay metastatic progression, and improve survival, and that this protection is associated with a reduction in HIF-1 in the TME. Future studies are underway to evaluate additional markers of hypoxia and vascularization in the PA+ER treatment group.

# 45 | Food additive guar gum exacerbates colonic inflammation in murine models of inflammatory bowel disease
Divek V T Nair, Devendra Paudel, Vishal Singh
Department of Nutritional Sciences

The role of fermentable dietary fibers in patients with inflammatory bowel disease (IBD) is not understood. Unlike healthy individuals, a subgroup of IBD patients develops clinical complications upon consuming soluble fibers, including fermentable oligo-saccharides, di-saccharides, monosaccharides, polyols (FODMAPs). Guar gum, a soluble fiber, is commonly used as a thickener and stabilizer in processed foods. The health benefits linked with the consumption of naturally occurring guar gum encouraged the food industry to fortify packaged foods with refined guar gum. However, we have limited knowledge on whether refined guar gum holds physiological effects similar to its naturally occurring counterpart, particularly in patients with IBD. Herein, we employed three different experimental mouse models of IBD to examine the effect of guar gum on colonic inflammation. Use of three different experimental models—1) immune hyperactivity [IL-10 receptor (IL-10R) neutralization], 2) epithelial injury [dextran sulfate sodium (DSS)], and 3) infection [Citrobacter rodentium (CR)]-mediated inflammation—allowed us to elucidate the effect of guar gum on IBD comprehensively. Wild-type (WT) mice receiving guar gum (7.5% w/w) containing diet (GuD) along with -IL-10R mAb displayed severe colonic inflammation—as characterized by an enlarged spleen, thickening of the colon, and elevated systemic [serum amyloid A (SAA) and lipocalin 2 (Lcn2)] and colonic [Lcn2 and interleukin (IL)-1β] markers of inflammation—when compared to mice fed control (cellulose) diet. Inline, GuD-fed mice maintained on DSS (1.4% w/v) exhibited relatively more worsened colitis. In contrast to what we observed with immune hyperactivation and epithelial injury models, GuD did not exacerbate the CR-induced infectious colitis; however, no sign of protection was evident in the GuD-fed group. This study collectively demonstrates that refined guar gum may aggravate intestinal inflammation in patients with IBD.

# 52 | Associations between multiple physiological mechanisms within an individual
Elyse McMahon, Elizabeth Youatt, & Sonia Cavigelli
Physiological responses of multiple systems (e.g., endocrine, immune, autonomic) are key for determining how animals respond to their environment. Understanding how multiple physiological mechanisms function together provides further insight into how individuals function. The objective of this study was to determine if there are reliable relationships among different physiological systems within an individual. We measured several physiological responses within the same individuals and used correlational analysis to identify related processes. We measured hormonal stress response, innate and adaptive immune function, and sympathetic reactivity in 54 adult male Sprague-Dawley rats. To measure hormonal stress responses, we conducted an acute restraint test and measured glucocorticoid (GC) responses. Innate immunity and basal GCs were measured during an 8-hour period after lipopolysaccharide injection. Adaptive immunity was measured with relative hind foot swelling after keyhole limpet hemocyanin (KLH) re-exposure. And heart rate was measured non-invasively during an acute restraint stress to determine fluctuations in sympathetic activity. We found that GCs were associated with most other physiological measures; specifically, elevated GCs during the innate immune challenge were associated with elevated pro-inflammatory cytokine responses (TNF-alpha and IL-6). Additionally, elevated cell-mediated immune responses were associated with elevated circulating GCs during KLH exposure and elevated circulating TNF-alpha levels during the innate immune challenge. Heart rate in response to restraint was not associated with any physiological measures. These results are the first step in understanding how different physiological systems interact to support organismal responses to complex environmental challenges.

# 53 | Relationship between tumor progression and immune response in the 4T1.2-HER2 mouse mammary tumor model
Abirami Ravichandran, Yitong Xu, & Connie J. Rogers
Intercollege Graduate Degree Program in Physiology, Department of Nutritional Sciences

Significance and Purpose: Breast cancer (BC) is the most diagnosed cancer worldwide and is the leading cause of cancer-related deaths in women. The immune system plays a key role in the control of many tumor types, including breast cancer. Preclinical cancer models are valuable tools to study cancer immune responses and to develop novel therapies. The murine 4T1.2 cell line is a mammary tumor model of triple-negative breast cancer (TNBC) that metastasizes to the lung and bone. However, this model is poorly immunogenic with no defined tumor-associated antigens, which limits its use in immunological studies of breast cancer. A modified 4T1.2 model has been developed that stably expresses a surrogate tumor antigen, human epidermal growth factor receptor-2 (HER2). The goal of the current study was to characterize tumor growth, lung metastasis, and host immune responses in the 4T1.2-HER2 tumor model with the aim of using this tumor model to test novel prevention and therapeutic strategies. Methodology: Female BALB/c mice (n=60) were orthotopically injected with 2x10^6 4T1.2-HER2 tumor cells. Primary tumor growth was measured thrice weekly. The mice were sacrificed at day 35 post tumor inoculation. Immune cells in the spleen and tumor were quantified by flow cytometry. To determine lung metastases, gene expression of gp70 was quantified using qPCR. Antigen-specific IFNg responses against HER2 were assessed following in vitro stimulation of splenocytes and tumor-infiltrating immune cells with the MHC class I restricted HER2 peptide (TYLPTNASL) by ELISA. Results: Tumor growth in the 4T1.2-HER2 model was characterized by initial tumor growth followed by spontaneous tumor regression by day 20 post tumor inoculation. Following tumor regression, mice demonstrated a second tumor growth phase that was heterogeneous, with some mice developing small and others large tumors. In the spleen, final tumor volume was negatively correlated with percentage of dendritic cells (p=0.001) and positively correlated with percentage of myeloid-derived suppressor cells (MDSC) (p=0.0042) and monocytic MDSC (p=0.005). In the tumor, final tumor volume was
negatively correlated with the percentage of CD4+ T cells (p=0.009) and NK cells (p=0.018) and positively associated with the percentage of MDSC (p=0.011). Lung metastasis was observed in all the mice. Significant HER2-specific IFNg response was observed from the tumor infiltrating immune cells (p=0.006) but not from the splenocytes. When IFNg responses were stratified based on high and low tumor volumes, significant HER2-specific IFNg secretion from tumor-infiltrating immune cells was only observed in smaller tumors (p=0.026). Conclusion: The addition of HER2 to the 4T1.2 tumor cell line significantly changes the original growth trajectory of the parental cell line by altering the tumor cell’s immunogenicity. Tumor volume outcomes were associated with immune cell infiltration in the spleen and tumor. In the tumors, antigen-specific immune responses were induced against HER2. The lung metastatic property was retained despite the slow tumor growth. These features are ideal in a mammary tumor model for preclinical immunological studies of breast cancer. Future studies are planned using this tumor model to study the effects of physical activity and energy restriction on antigen-specific immune responses in breast cancer.

**Molecular Biology, Genetics & Chemistry**

# 7 | The feasibility of strong acid-free hairy nanocellulose production from lignocellulosic sources through periodate and chlorite oxidation
Mica Pitcher, Breanna Huntington
Department of Chemistry

Nanocelluloses are an emerging naturally-derived class of materials with interesting properties and potential for applications in a vast range of fields. Produced from cellulose, the most common forms of nanoscale cellulose structures include cellulose nanocrystals (CNCs), cellulose nanofibrils (CNFs), and hairy cellulose nanocrystals (HCNCs). HCNCs are a novel type of nanocelluloses, which are synthesized by partially preserving the amorphous regions of cellulose, yielding a crystalline body resembling conventional CNCs sandwiched between two layers of highly functional amorphous cellulose chains. The amorphous cellulose chains impart a very high functional group density to HCNCs, up to one order of magnitude higher than conventional nanocelluloses. HCNCs can be synthesized as neutral, cationic, or anionic nanocelluloses. The large charge density on HCNCs enables them for many water treatment applications, including the removal of antibiotics, organic dyes, and heavy metal ions from wastewater. HCNCs are also a great candidate for 3D printing of bio-based inks for various applications. There is an abundance of sources of which to isolate cellulose, however, the differences of the natural sources lead to varying properties in the production of all types of nanocelluloses. Even when narrowed down just to plant cellulose, changes in the composition, cellulose content, and properties such as crystallinity of the plant have a considerable effect on the nanocelluloses formed. In order to fully understand how the cellulose source affects the synthesis of HCNCs, the variations in cellulose pulp sources must be investigated. This work studies the structure-property relationships of four cellulose sources (softwood kraft pulp, Whatman™ grade 1 filter paper from cotton linters, corncob, and tomato peel), and how the different sources alter the synthesis and properties of the three products of the HCNC synthesis, including hairy nanoparticles, solubilized polymers, and un fibrillated products. This will lead to a better understanding of this new and interesting nanomaterial for further use of HCNCs in many environmental applications.
# 9 | Understanding Adenylosuccinate Lyase Deficiency locomotion deficit using C. elegans as a model
Latisha Franklin, Wendy Hanna-Rose
Biochemistry, Microbiology, and Molecular Biology

Adenylosuccinate Lyase Deficiency (ASLD) is a disorder at the intersection of genetic diseases, metabolic disorders, and nervous system diseases. ASLD is caused by decreased function of adenylosuccinate lyase (ADSL), which converts SAICAR to AICAR and S-AMP to AMP in purine metabolism. Symptoms range from mild, such as slower intellectual development to severe, such as neonatal fatality. Severe motor delay and low muscle tone are common symptoms of this disorder with SAICAR accumulation being suggested as a key player in muscle dysfunction. I use C. elegans to model ASLD. adsl-1 loss of function mutant C. elegans are slower and uncoordinated when moving. I hypothesize abnormal crawling and swimming locomotion in adsl-1 deficient C. elegans has distinct pathogenesis due to usage of adsl-1 in neuronal and muscular tissues. We use knockout allele engineered tissues specific knockdowns and overexpressing animals to study the movement of normal and adsl-1 deficit animals. Using WormLab software we were then able to quantify speed, bending angle, and thrashing of these animals during crawling and swimming locomotion. Crawling data from whole-body loss of adsl-1 results in an increased preference to commit tighter bending patterns and slower moving speed. Knockout of adsl-1 in neuronal tissue results in similar crawling patterns as whole-body loss of function animals which is not ameliorated when adsl-1 is overexpressed in the muscle alone and adsl-1 is not expressed in the neuron. Swimming data from whole-body loss of adsl-1 results in loss of a bending pattern and decreased thrashing. Knockout of adsl-1 in muscular tissue results in altered control of bending angle while swimming, whereas neuronal knockout of adsl-1 with expression remaining in muscle is unimpacted. In addition to using mutant animals, we use RNAi of adsl-1 for metabolic studies. Liquid chromatography paired with mass spectrometry allows for the quantification and identification of metabolites within purine metabolism as well as other pathways of interest. Metabolic analysis shows adsl-1 RNAi animals have a distinct metabolic profile. SAICAR is increased, while S-AMP and other metabolites remain relatively unchanged. Further studies of muscle dysfunction amelioration and metabolic restoration using drug treatments are to be completed. In conclusion, our work suggests adsl-1 has specific phenotypic outcomes depending on location of expression.

# 12 | A kinetic dissection of the fast and superprocessive kinesin-3 KIF1A reveals a predominant one-head-bound state during its chemomechanical cycle
Taylor Zaniewski, William Hancock
Department of Chemistry

Kinesin-3 are the fastest and most processive motors of the three neuronal transport kinesin families, yet the sequence of states and rates of kinetic transitions that comprise the chemomechanical cycle are poorly understood. We used stopped-flow fluorescence spectroscopy and single-molecule motility assays to delineate the chemomechanical cycle of the kinesin-3, KIF1A. Our bacterially expressed KIF1A construct, dimerized via a kinesin-1 coiled-coil, exhibits fast velocity and superprocessivity behavior similar to wild-type KIF1A in BRB80. We established that the KIF1A forward step is triggered by hydrolysis of ATP and not by ATP binding, meaning that KIF1A follows the same chemomechanical cycle as established for kinesin-1 and-2. The ATP-triggered half-site release rate of KIF1A was similar to the stepping rate, indicating that during stepping, rear-head detachment is an order of magnitude faster than in kinesin-1 and kinesin-2. Thus, KIF1A spends the majority of its hydrolysis cycle in a one-head-bound state. Both the ADP off-rate and the ATP on-rate at physiological ATP concentration were fast, eliminating these steps as possible rate limiting transitions. Based on the measured run length and the relatively slow off-rate in ADP, we conclude that
attachment of the tethered head is the rate limiting transition in the KIF1A stepping cycle. The fast speed, superprocessivity and load sensitivity of KIF1A can be explained by a fast rear head detachment rate, a rate-limiting step of tethered head attachment that follows ATP hydrolysis, and a relatively strong microtubule interaction in the post-hydrolysis state.

# 16 | Novel Cofilactin Bundling in Neuronal Growth Cone Filopodia
Ryan Hylton, Michael Grillo, Jessica Heebner, & Matthew Swulius
Biochemistry and Molecular Biology Department, Penn State College of Medicine

In the brain, proper neural circuits are built by the directed growth of nascent axons and dendrites towards their eventual synaptic partners through a process termed neurite guidance. At the distal tip of these neurites exists a subcellular compartment termed the growth cone. The growth cone integrates environmental cues, both chemical and mechanical, and converts them into mechanical force to move the developing neurite towards its target. Translocation of the growth cone is largely facilitated by the combined polymerization and depolymerization of actin filaments (F-actin) in its peripheral (or “P”) domain. The P-domain contains two actin-based structures: filopodia and lamellipodia. Filopodia are finger-like protrusions shaped by tightly bundled, unipolar actin filaments and serve as antennae for the growth cone, finding and responding to extracellular cues. Lamellipodia consist of networks of branched actin filaments between filopodia at the leading edge of the cell. Actin polymerization here propels the plasma membrane, and the growth cone itself, forward. During neurite guidance, a delicate balance of actin dynamics must be maintained for proper growth cone motility. F-actin possesses the ability to grow, shrink, sever, branch, and bundle when assisted by the right actin binding proteins. In fact, a number of these proteins have been shown to be crucial for growth cone outgrowth and/or guidance. Here, we focus predominantly on two such proteins. First, fascin is a crosslinking protein whose primary function is the bundling of F-actin in the filopodia of motile cells, including the growth cone. Second, cofilin binds to old, ADP-bound actin filaments (forming a structure called cofilactin), where it facilitates their severing and depolymerization. To date, surprisingly little investigation has been done on the detailed ultrastructure of the growth cone cytoskeleton resulting from the activity of actin binding proteins. To resolve this, we use a multi-faceted approach to examine the structural effects fascin and cofilin have on actin in the growth cone, specifically in filopodia. First, immunofluorescence microscopy on fixed cells enables us to better understand the proteins’ spatial distribution. Second, we use live cell fluorescence microscopy to study their impact on filopodial dynamics. Finally, we utilize cryo-electron tomography and, subsequently, subtomogram averaging and AI-based tomographic segmentation to investigate the molecular structure of actin filaments and the architecture of filament networks, respectively. Using these techniques, we have found that the base of growth cone filopodia consist of tightly-packed cofilactin filaments. This is in stark contrast to the more distal tips of filopodia, which lack cofilin and are made up of ‘normal’, undecorated F-actin. In addition, we show evidence that these cofilactin filaments are crosslinked via a mechanism independent of fascin, which is responsible for the bundling of ‘normal’ F-actin in the filopodial tips. Further details of what we’ve seen and the implications of this switch between filament states in cytoskeletal remodeling and filopodial function will be discussed.

# 17 | Sequence analysis of the Petunia inflata S-locus region containing 17 S-Locus F-Box genes and the S-RNase gene involved in self-incompatibility
Lihua Wu, Justin Williams, Linhan Sun, Teh-hui Kao
Plant Biology

Self-incompatibility in Petunia is controlled by the polymorphic S-locus, which contains the S-RNase gene encoding the pistil determinant and 16 to 20 S-locus F-box (SLF) genes collectively encoding the pollen
determinant. Here we determined ~3.1 Mb sequence of the S2-locus of P. inflata using BAC (Bacterial Artificial Chromosome) clones collectively containing all 17 SLF genes, SLFLike1, and S-RNase. Two SLF pseudogenes and 28 potential protein-coding genes were identified, 20 of which were also found at both S6a-locus of P. inflata and SN-locus of self-compatible P. axillaris, but not in S-locus remnants of self-compatible potato and tomato. Comparative analyses of these three S-loci revealed potential genetic exchange in the flanking non-coding regions of SLF genes, resulting in highly similar flanking regions, both between different types of SLF and between alleles of the same type of SLF of different S-loci. The high degree of sequence similarity in the SLF flanking regions could often be explained by the presence of similar Long Terminal Repeat (LTR) retroelements, which were enriched in all three S-loci and in the flanking regions of all S-locus genes examined. We also found evidence of the association of transposable elements with SLF pseudogenes. Finally, based on the hypothesis that SLF genes were derived from retrotransposition, we identified 10 F-box genes as putative SLF parent genes. Our results shed light on the importance of non-coding sequences in the evolution of the S-locus, and on possible evolutionary mechanisms of generation, proliferation and deletion of SLF genes.

# 20 | Dissecting the regulatory role of an enriched DNA sequence motif found upstream of Plasmodium falciparum gametocyte-associated genes
Riëtte van Biljon, Guoyue Xu, Abhai Tripathi, Victoria Bonnell, Timothy J Russell, Photini Sinnis, Manuel Llinás
Department of Biochemistry & Molecular Biology

Plasmodium falciparum parasites cause the disease malaria, killing hundreds of thousands of children across the world each year and are one of the most pernicious plagues of poverty-stricken nations, particularly in Sub-saharan Africa. The parasite can rapidly produce millions of parasites, causing cyclical fevers and anemia, through its asexual development, dividing one parasite into ~32 new parasites every 48 h before rupturing and newly infecting red blood cells. However, only gametocytes, a minor population of once asexually dividing cells (<10%), terminally differentiate into mature pre-sexual forms that can be taken up by feeding female Anopheles mosquitoes. These gametocytes rapidly form sexually dimorphic gametes that proceed through several steps of sexual reproduction before resting in the mosquito salivary glands as infectious sporozoites that can be transmitted to new human hosts. The formation of mature Plasmodium gametocytes in human red blood cells is therefore a critical stage in ensuring malaria transmission. Two specific DNA binding proteins have been shown to play an integral role in P. falciparum gametocyte development, AP2-G and AP2-G2. However, we have recently identified an enriched DNA sequence motif (AGACA) upstream of genes whose mRNA abundance peak in gametocytes. Although this motif has been associated with P. falciparum sexual development by several transcriptome studies there has been no link to a specific trans-acting regulatory factor associated with this motif. The aim of this study is to identify and characterize a candidate trans-acting factor associated with this motif and determine the role of this DNA/protein complex during parasite development. Our preliminary studies support that AGACA is a gametocyte-specific motif, since it is bound in nuclear lysate extracted from P. falciparum gametocytes but not from asexual parasites. DNA pull-down assays using biotinylated oligomers containing the AGACA motif and gametocyte lysate have consistently enriched for an uncharacterized protein that contains a CCCH zinc finger nucleic-acid binding domain (PF3D7_0315600). We produced a genetic knockout of this gene using CRISPR-Cas9 in a P. falciparum parasite line which shows a difference in growth phenotype during asexual and sexual blood-stage development as well as a more pronounced phenotype which negatively affects mosquito stage development. In the mosquito, the production of oocysts is severely reduced, showing a loss in reproductive fitness, although these oocysts go on to form sporozoites. In addition, we are currently investigating whether this protein directly binds the AGACA motif in vitro using electrophoretic mobility shift assays and protein binding microarrays and in vivo using a 3xHA tagged parasite line for chromatin
immunoprecipitation coupled with next generation sequencing (ChIP-seq) experiments. Together, we assert that the AGACA motif is bound by one or more trans-acting factors during gametocyte development, one of which could be PF3D7_0315600 and that this protein is important for the transmission stages of malaria parasite development. Further investigations will shed light on the relevance of this putative interaction between PF3D7_0315600 and the AGACA motif in vivo and establish whether this is the mechanism for the reduction in reproductive fitness in parasites lacking pf3d7_0315600.

# 21 | The genome-wide role of NusA on RNA polymerase pausing in Bacillus subtilis

Oshadhi Jayasinghe, Zachary Mandell, Paul Babitzke
Department of Biochemistry & Molecular Biology

Transcription elongation is punctuated by ubiquitous as well as finely regulated pausing events. Isomerization of RNA polymerase to enter into the elemental paused state results in a reversible inhibition of elongation. Stabilization of these elemental pauses at regulatory sites could occur owing to folding of the nascent RNA into hairpin structures, interactions with different factors and/or the presence of downstream RNA and DNA sequences, with pausing occurring ~11 nucleotides downstream from the hairpin. Such long-lived pauses can provide time for diverse regulatory events to occur, which play a significant role in modulating gene expression. Transcription elongation factors dramatically affect RNAP pausing in vitro, but the genome-wide role of such factors has only been explored for Bacillus subtilis NusG. NusA is another general elongation factor, known to induce RNAP pausing in vitro. To investigate the in vivo role of NusA in this process, we performed RNET-seq in isogenic wild type (WT), nusA depletion (dA), nusG deletion (∆G), and nusA depletion nusG deletion (dA∆G) B. subtilis strains. Through mapping of the RNET-seq reads to the genome and exerting thresholding criteria to the 3’ end counts, we identified pause peaks in the four strains. Differential analysis of RNET-seq data of WT and ∆G strains previously revealed an extensive role of NusG in pausing, with an upstream consensus TTNTTTT pause motif. Although NusA was known to be a general pausing factor, our differential analysis of WT and dA strains revealed only modest effects on genome-wide RNAP pausing by NusA in vivo. Through modified thresholding criteria for pause peak identification and pause strength differential analysis, we identified 234 NusA-stimulated pauses and 117 NusA- suppressed pauses genome-wide. RNA folding of sequences upstream of the pause sites revealed putative pause hairpins for several NusA-stimulated pauses. In contrast to NusG, we did not identify a NusA-stimulated pause motif through sequence enrichment analysis. Thus, a consensus sequence motif as an intrinsic sequence signal may not be necessary for NusA-stimulated pausing. NusA-stimulated pausing was confirmed for some sites in vitro, but not at others, perhaps due to the relatively weak nature of these pauses. The in vitro pause efficiencies and half-lives were relatively weaker compared to previously validated NusG-dependent pauses, suggesting that NusA has mild effects on RNAP pausing.

# 23 | Molecular and Morphological Correlates of Terminal Differentiation of Chandelier cells

Matthew Dickinson, Anirban Paul
Neural & Behavioral Sciences Department, Penn State College of Medicine

Altered neurodevelopment is a common pathoetiology of complex brain disorders like schizophrenia, autism, depression and bipolar disorder. After cell fate is determined during embryonic stages, precursor neurons continue to terminally differentiate during a protracted post-natal development phase as they migrate to cortex, commit to distinct mature identities and form functional circuits. Our overarching goal is to study the trajectory of longitudinal biological changes associated with terminal neuronal differentiation and its impact on complex brain disorders. However, as neuronal precursors remain molecularly heterogeneous each with different developmental trajectories, studying neuronal subtype formation has proven to be difficult. Hence,
when and how one specific terminally differentiated neuron-type identity emerges from their precursors is largely unknown. To address the molecular basis of the emergence of terminal identity during post-natal differentiation we are using a highly stereotypic cardinal GABAergic interneuron (IN) type, the Chandelier cell (CHC), as a model. CHCs are developmentally the last IN-type to be generated, with unique stereotypic morphology, connectivity and physiological properties. These are perhaps the most powerful INs, poised to control cortical output streams by innervating hundreds of neighboring axon initial segments of pyramidal neurons via a dense cartridge of buttons. CHCs have also been implicated in neuropsychiatric disorders. To unravel the molecular mechanism of terminal differentiation, we are performing longitudinal high-throughput single-cell RNA sequencing, whole brain developmental mapping and single cell morphometry on only CHCs through specific genetic labelling. This will allow us to identify the underlying molecular players on the terminal differentiation process. This study will reveal the highly-discriminant gene families and phase locked transcriptional factors at each stage of post-natal development and correlate with the stereotypic developmental changes in CHC. Fundamental knowledge gained from the study of terminal development of CHC will be vital for understanding the genetic and environmental cues needed for neuronal subtype specification during mammalian neurodevelopment.

# 30 | **Mechanisms of allosteric regulation in the Farnesoid X receptor**
Tracy Yu, Noriko Mikeasky, Emily Meinert, Namita Dube, & C. Denise Okafor
Biochemistry, Microbiology, and Molecular Biology

Farnesoid X receptor (FXR) is a bile acid-induced transcription factor that belongs to the superfamily of nuclear receptors (NRs). Ligand activated-FXR regulates transcription of genes related to bile acid, lipid, and glucose metabolism. Similar to other nuclear receptors, FXR consists of two allosterically linked domains: a ligand binding domain (LBD) that binds small lipophilic ligands, and a DNA binding domain (DBD) that targets specific DNA sequences called FXR response elements (FXREs). However, the underlying mechanisms of allosteric regulation of Farnesoid X receptor (FXR) remain unclear. My project utilizes a variety of biochemical, biophysical, and computational methods to study how ligand binding, promoter specificity and coregulator recruitment contribute to allosteric signaling in FXR. To study the correlation between ligand structure and promoter specificity, I am performing dual luciferase assays to quantify the transcriptional activity of different ligands in a set of FXRE-driven promoters. To gain a structural understanding of how FXR achieves promoter specificity, I am purifying FXR-DBD in preparation for crystallization studies in collaboration with the Boal lab. Finally, I am performing molecular dynamics (MD) simulations to study how changes in the ligand structure modulate allosteric signaling between the ligand-binding pocket and the AF-2 surface, the binding site for coregulators on the FXR-LBD. The integration of these experimental and computational studies will allow me to generate a robust profile of allosteric signaling in FXR transactivation.

# 31 | **Elucidating Driver Genes in PIK3CA-mutated Mammary Carcinogenesis and Relapse Using an Inducible and Mammary-Specific Transposon Mutagenesis System**
Maryknoll Palisoc & Edward Gunther, MD
Penn State College of Medicine, Medical Scientist Training Program and Jake Gittlen Laboratories for Cancer Research

Mutant PIK3CA is a validated and FDA-approved target in human breast cancer. Approximately 40% of hormone receptor (HR)-positive and human epidermal growth factor receptor 2 (HER2)-negative breast cancer acquire PIK3CA mutations, encoding for an overactive catalytic p110α subunit of PI3K (phosphatidylinositol 3-kinase). Enhanced PI3K signaling promotes tumorigenesis through uncontrolled cell proliferation, survival, and growth. Nevertheless, altered PIK3CA is not sufficient for carcinogenesis, as
demonstrated in patients with PIK3CA-related overgrowth spectrum, who do not possess increased susceptibility for breast cancer despite harboring PIK3CA mutation(s). Instead, PIK3CA mutations require additional genomic aberrations to transform normal mammary cells to malignant foci. While large-scale genomic efforts provide an impressive catalog of mutations from human cancer genomes, the functional relevance of most genetic mutations is seldom clear, and distinguishing driver events from passenger mutations remains a complicated task. Moreover, preclinical studies validating the interaction between mutant PIK3CA and other gene mutations primarily focus on only a few and often well-established driver genes. The goal of this study is to uncover novel driver genes by using an inducible version of the mutagenic Sleeping Beauty (SB) transposition system, which enables timed and mammary-specific mutagenesis through regulated transposon mobilization. Mutagenic transposon concatemers (T2/Onc) are randomly mobilized by the expression of Sleeping Beauty (SB11) transposase under the control of tet operator (Tet-O-SB11). Combining the Tet-O-SB11 transgene with both a mammary epithelial cell (MEC)-specific transactivator (MMTV-rtTA), T2/Onc, and Tet-O-PIK3CAH1047R enables concurrent PIK3CAH1047R activation and transposition upon doxycycline treatment (iSBM/iPIK mouse model). Mice containing four transgenes (iSBM/iPIK) are administered with doxycycline-impregnated chow indefinitely and monitored for tumor onset. The ability of iSBM/iPIK to augment baseline mammary cancer predisposition are examined using the following three endpoints: 1) acceleration of tumor onset, 2) increase of primary tumor multiplicity, and/or 3) increase of incidence/multiplicity of metastases. Our preliminary data suggests no significant difference in tumor latency, multiplicity, and metastasis in iSBM/iPIK and iPIK only. However, success of tumor explantation in syngeneic hosts using iSBM/iPIK primary tumors is significantly different when compared to engraftment using iPIK primary tumors (chi-square statistic with Yates correction = 11.32; p-value = 0.000765). This suggests that additional genetic changes have been induced through transposition mutagenesis, resulting to enhanced invasion in the hosts. Future directions include next generation sequencing in order to identify genes with common insertion sites (CIS) in iSBM/iPIK3CAH1047R tumors. Finally, to validate candidate genes, we propose to query CIS genes for mutations using The Cancer Genome Atlas (TCGA) and Catalog of Somatic Mutations in Cancer (COSMIC) and copy number variation using TCGA and RNA-seq Analysis of Molecular Abundance (RoMA) datasets. Complementing this high-throughput cancer gene discovery platform, our bioinformatics pipeline program will facilitate the validation of putative driver genes. Doing so will uncover how additional genetic mutation(s) drive transformation of PIK3CA-mutated mammary cells to malignant lesions (Aim 1) and how these changes promote treatment relapse (Aim 2) in mutant PIK3CA breast cancer.

# 38 | Transforming Growth Factor β modulates IRE1α-mediated ER stress response in keratinocytes expressing oncogenic H-Ras

Saie Mogre, Adam Glick
Veterinary and Biomedical Sciences

Transforming Growth Factor β (TGF-β) is a critical regulator of oncogenic progression in response to mutant H-Ras. In primary mouse keratinocytes, activation of mutant H-Ras can induce the unfolded protein response (UPR), predominantly by triggering the adaptive splicing of XBP1 by Inositol-requiring enzyme 1α (IRE1α) or by activation of the Regulated IRE1-dependent mRNA decay (RIDD) to induce premature senescence. Here, we show an anti-oncogenic role of the UPR sensor proteins IRE1α and (PKR)-like endoplasmic reticulum kinase (PERK) to mediate the tumor-suppressive roles of TGF-β in keratinocytes expressing mutant H-Ras. In primary and immortalized mouse keratinocytes, TGF-β significantly blocked H-Ras induced phosphorylation of IRE1α, XBP1 splicing, and expression of the ER chaperone Grp78 (or BiP) but activated PERK signaling. While decreased XBP1 splicing is associated with a dampened ER stress response, direct visualization by Thioflavin T (ThT), a fluorescent marker for ER stress, showed a significant increase in unfolded proteins in the mutant H-Ras keratinocytes 48h after treatment with TGF-β versus H-Ras alone. TGF-β-dependent
dephosphorylation of IRE1α was reversed in keratinocytes treated with GSK2606414, a potent inhibitor of PERK activity as well as with PERK knockdown. Furthermore, inhibition of PERK rescued a decreased rate of proliferation in TGF-β treated mutant H-Ras keratinocytes as measured by BrdU incorporation and MTT assays. Pharmacological inhibition of PERK also showed a marked decrease in unfolded proteins in these cells. Quantitative real-time PCR revealed that the mRNA for RPAP2, a PERK-dependent IRE1α phosphatase, was upregulated in TGF-β treated mutant H-Ras keratinocytes whereas another IRE1α phosphatase, PPP2CA, remained unchanged. Silencing of RPAP2 using RNAi approach in immortalized mouse keratinocytes notably reversed the IRE1α phosphorylation status and cell proliferation suggesting its role in TGF-β-dependent phenotypic responses in mutant H-Ras keratinocytes. Taken together, our results suggest a cross-talk between UPR proteins as one of the mechanisms to suppress tumorigenesis by TGF-β in cells expressing oncogenic H-Ras.

# 39 | Paramyxovirus-like Particles: a novel strategy for proteins delivery
Santosh Panthi, Phuong Schmitt, Anthony Schmitt
Department of Veterinary and Biomedical Sciences

Virus particles are biological delivery vehicles meant to transport viral genomes into infectable cells. Virus-like particles (VLPs) are nanocages that mimic the conformation of authentic native viruses but lack viral genetic materials, which makes them noninfectious. VLP-based delivery vehicles can potentially provide a highly flexible and safe platform for the therapeutic delivery of functional proteins to cells. However, foreign proteins do not naturally package into VLPs. Viruses assemble in a way that is meant to maximize the packaging of viral components and prevent the packaging of irrelevant proteins. The Schmitt lab recently discovered that paramyxovirus NP proteins harbor short packaging sequences near their C-terminal ends that can be fused with foreign proteins, allowing the foreign proteins to participate in viral assembly and package efficiently into VLPs. These VLPs in turn are naturally capable of binding to the target cells and delivering the foreign cargo proteins to the cell interior. Using this approach, we have generated paramyxovirus VLPs loaded with different cargos, including Renilla Luciferase (RLuc), superoxide dismutase (SOD), and green fluorescent protein (GFP). Cargos were efficiently delivered to target cells. Also, we show that nuclear targeting cargos such as Cre recombinase can be efficiently packaged into paramyxovirus VLPs and subsequently delivered into the nuclei of target cells. Overall, these studies demonstrate the high degree to which paramyxovirus particles can be manipulated to create a flexible platform for protein delivery. Advantages of our approach compared to other delivery technologies include 1) Direct delivery of cargo into target cell interiors, which avoids the limitations of endosomal entrapment; 2) Absence of exogenous nucleic acid potentially avoids the risk of target cell genome modification and oncogenic mutation; 3) Valuable in a situation where the extended activity of the delivered cargo molecule develops unwanted effects; 4) Efficient incorporation and release of the biologically active cargo proteins.

# 42 | Elucidating the roles of 2', 3'-cyclic nucleotide monophosphates in bacterial signaling and stress response
Shikha Chauhan and Emily Weinert
Department of Biochemistry and Molecular Biology

To survive extreme and rapidly changing conditions, bacteria sense environmental changes and then respond with appropriate alterations in gene expression and protein activity. Bacterial adaptation to environmental signals plays important roles in human health, including how bacteria respond to anti-bacterial treatments and how bacteria switch from free-living to invading a host. Therefore, an important scientific challenge is to identify mechanisms that allow bacterial to sense external cues and translate those cues into internal signals.
that maximize survival. Novel intracellular small molecule signals, 2', 3'-cyclic nucleotide monophosphates (2', 3'-cNMPs), have been recently discovered in eukaryotes and prokaryotes. Within plants and mammals, wounding has been found to cause increased levels of 2', 3'-cNMPs, suggesting an intriguing connection between 2',3'-cNMPs and cellular stress [1-3]. However, not much is known about these unusual nucleotides in bacteria, even though 2',3'-cNMPs were originally detected in Escherichia coli over five decades ago. My research focus on determining the functions of 2, 3-cyclic nucleotide monophosphates (2', 3′-cNMPs) in bacterial signaling, as well as the enzymes responsible for 2',3'-cNMP production and degradation. 2', 3'-cNMP levels in E. coli are generated specifically from RNase I-catalyzed RNA degradation and RNase I and 2', 3'-cNMP levels play important roles in controlling biofilm formation [4]. I am investigating the proteins and pathways involved in prokaryotic 2', 3-cNMP production, degradation, and stress response pathways, with the aim to develop a mechanistic understanding of 2',3'-cNMP signaling. To identify 2',3'-cNMP-sensing proteins, I synthesize 2',3'-cNMP based molecules and use it as a bait in affinity-based chromatography to capture the proteins and validate their role in signal transduction. My recent experiments have revealed multiple protein hits, that are involved in RNA salvage, among them ribosomal proteins are most abundant. Therefore, I am currently investigating the role of 2',3'-cNMP in the “central dogma” of bacterial cells. By elucidating the role(s) of 2',3'-cNMPs in bacterial signaling pathways and stress responses, this research has the potential to methods to control bacterial phenotypes and potentially discover new targets for antibacterial therapies. A successful completion of this goal has the potential to provide new treatments for antibiotic resistant bacteria, decreasing the mortality and cost associated with infections.

# 46 | Defining DNA sequence and chromatin features that influence binding specificity of transcription factors in Plasmodium falciparum

Victoria Bonnell, Yuning Zhang, Alan Brown, Tsu-Pei Chiu, John Horton, Remo Rohs, Shaun Mahony, Raluca Gordân, & Manuel Llinás

Biochemistry, Microbiology, and Molecular Biology

Malaria is a major global health and economic burden impacting billions of people worldwide with an estimated 229 million cases and 409,000 deaths in 2019 alone. Malaria is caused by a unicellular parasitic eukaryote from the genus Plasmodium, with the species Plasmodium falciparum causing a majority of the annual deaths. The life cycle of P. falciparum is complex with developmental stages in the mosquito, human hepatocytes, and human erythrocytes. The characteristic clinical symptoms of periodic fevers and anemia are caused by the rupture of parasites from human erythrocytes every 48-hours. One of the most unique aspects of this cycle is the tight temporal regulation of gene transcription. The transcriptome of the malaria parasite during asexual blood stage development forms a cascade of gene expression where each protein-coding gene is transcribed in a periodic, “just-in-time” manner. This distinctive pattern is believed to be controlled by a family of sequence-specific transcription factors (TFs), called the Apicomplexan AP2 (ApiAP2) proteins, which have homology to plant-lineage TFs. While most of these ApiAP2 proteins recognize divergent DNA motifs, a subset binds similar DNA motifs. This is intriguing since functional gene redundancy is not often evolutionarily conserved in pathogens. In higher eukaryotes, TFs with similar binding preferences (i.e. paralogous TFs) can carry out divergent regulatory functions in a given cell type, work synergistically or antagonistically, perform similar functions in different cell types, or can be functionally redundant. Therefore, despite the similar binding specificities of ApiAP2 proteins, we predict that they carry out distinct regulatory functions. There are several established features that modulate binding specificity of a TF, including: DNA sequence context, local DNA topology, interactions with cofactors, and the chromatin environment. In this study, we use a variety of in vitro, in vivo, and in silico approaches to identify how sequence preferences are established during parasite development by probing the effects of cis- and trans- regulation on TF binding. To examine the role of sequence context on binding specificity of each paralogous ApiAP2 TF, we used a uniquely designed, in vitro
high-throughput genomic context protein binding microarray (gcPBM). Our results indicate that sequence context and DNA topology play a significant role on binding site recognition. Additionally, complementary in vivo approaches such as chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq) and protein immunoprecipitation followed by mass spectrometry (IP-MS), were applied to investigate temporal association with specific genomic loci and interactions with cofactors/protein complexes, respectively. Interestingly, results from our ChIP-seq experiments demonstrate that only a small subset are selected in vivo out of 1000s of possible genome-wide binding sites. We also used computational modeling to predict which unique features, or in combination, can explain the binding seen in vivo and find that chromatin accessibility is a major contributor to why only a limited number of sites are accessible in vivo. Our results highlight that paralogous TFs determine which genomic regions to bind based on a combination of factors, thereby contributing to a better understanding of the complex gene regulatory network governing P. falciparum pathogenesis.

Plant & Agricultural Sciences, Zoology, & Ecology

# 15 | Exploring the potential of in silico tools to enhance agricultural resilience to climate change
Ele Saltmarsh, Armen Kemanian
Department of Plant Science

Climate change presents a significant threat to global food security, with moderate increases in temperature and elevated CO2 unlikely to enhance yields enough to counterbalance losses caused by extreme temperatures and reduced precipitation. Effects will be felt hardest in rainfed, equatorial areas, many of which already suffer from widespread degradation of marginal agricultural soils. To ensure access to sufficient calories and nutrition for a growing population, we must not only increase yields but do so in a way that is viable in low-input, marginal systems. Through novel use of an evolutionary algorithm, we introduce a tool which allows rapid identification of root distributions that produce resilient plants across variable and uncertain conditions. Eventually, this framework may be used more broadly to determine the potential of in silico optimized crops to improve food security in climate and soil challenged areas. Evaluation of the benefits of deep roots in situ is costly and time-consuming due to the volumes of soil involved, and the reliance on pre-existing phenotypes limits the possibilities that can be explored. Modelling frees us to explore the phenotypic space, including traits and trait combinations that have not previously been imagined, all of which can be tested under different climates and in different soils. A soil-plant-atmosphere continuum model was coupled to an evolutionary algorithm to create an optimisation tool, the soil-plant-atmosphere continuum evolutionary model, or SPACE. The model can predict harvest yields for a specific plant, based on user-specified soil and climatic conditions. Initially, the algorithm generates random and novel traits, allowing a complete exploration of phenotypic space, creating artificial equivalents to ‘chromosomes’ and ‘genotypes’ which are assessed for survival and yield across multiple years. As the algorithm progresses, it iteratively selects on successful trait values to converge on those that maximise yield and survival under the specified conditions. Our initial focus has been on root architecture, using the model to assess the effect of changing root density over depth by balancing the costs of root growth with their effect on water uptake and yield. Deeper roots have been hypothesised to be beneficial to plant survival and crop yield under drought conditions, since subsoils are not subject to evaporation and therefore roots in the subsoil should have access to a reliable source of water during dry periods. Preliminary results support this hypothesis, with optimised root distributions in many test locations showing slight increases in root density in the lowest layer of soil. This increase in density is more pronounced in drier years. Comparative yield benefits range from <0.05 kg/m2 to approximately 0.2 kg/m2. These results indicate that the combination of in silico plant growth and optimisation models has the potential to increase yield and reliability of yield in drought-prone regions. This provides a
novel approach to problem-solving in ecological systems and may act to support further integration of computing science in biological research- a key step towards overcoming many of the most rate limiting elements to research.

# 24 | Dietary metformin supplementation improves ovarian function in broiler breeder hens
Evelyn Weaver and Ramesh Ramachandran
Department of Animal Science

The broiler industry's focus on genetic selection for efficient and rapid weight gain has made broiler chickens highly efficient and approximately 6-fold heavier than those raised five decades ago. Consequently, broiler breeder hens, the parent stock of commercial broiler chickens, have poor reproductive efficiency due to the selection pressure for superior growth-related traits in broiler progeny. Broiler breeder hens require strict feed restriction to avoid obesity-related disorders, such as lameness, reproductive dysfunction, and increased mortality. However, even when restricted fed, we still observe increased reproductive and metabolic issues due to their propensity for rapid somatic growth and increased adiposity. The hyper-recruitment of prehierarchical follicles and a deranged preovulatory follicular hierarchy often leads to decreased egg production, lower percentages of fertility and hatchability of eggs, and decreased viability of embryos. Remarkably, in a preliminary study conducted in our laboratory, the supplementation of metformin into the diet of broiler breeder hens appeared to prolong egg production, however a more in-depth study had yet to be conducted. In the present study, we hypothesize that supplementation of metformin in the diet of broiler breeder hens will alter ovarian function and thus, increase reproductive efficiency. A commercial strain of broiler breeder hens (Cobb 500) and roosters (Hubbard M99) were reared according to the Cobb 500 and Hubbard M99 breeder management guidelines. Supplementation of metformin in the diet (0, 25, 50 or 75 mg/kg body weight) began at 25 weeks of age and continued through the end of the study at 65 weeks of age (n=45 hens/treatment group). Males received no metformin supplementation in their diets. Several parameters related to reproduction were measured over the course of the study including daily egg production, fertility and hatchability, and hen weights. At the end of the study, a subset of hens (n=12/treatment group) were euthanized, and ovaries analyzed for follicle count and ovarian stroma weight. Hens that received the highest dose of metformin (75 mg/kg) had significantly lower body weights at 40-, 50-, 60- and 65-weeks of age when compared to the other treatment groups (P<0.01). Hens in this treatment group also had significantly smaller visceral fat pads when compared to the 0- and 25-mg/kg treatment groups (P<0.05), but there was no difference when compared to the 50 mg/kg group. Ovarian stroma weight, number of preovulatory follicles and number of prehierarchical follicles did not differ between the treatment groups. Interestingly, both hen-day egg production and hen-house egg production was significantly higher across several time points in the hens that received the highest dose of metformin (75 mg/kg) when compared to the hens that received no metformin. This is of particular interest because it is typical for egg production to decline around 50 weeks of age in commercial breeder hens, but metformin is able to recover this loss and prolong egg production. Metformin supplementation had no effect on the fertility or hatchability in all treatment groups. Overall, our data suggest that dietary metformin supplementation improves reproductive efficiency in broiler breeder hens.
Iron deficiency is a major nutrition problem in the developing world. Additionally, iron limiting conditions due to soil pH effects about 30% of all arable crop lands. Understanding how plants uptake, and sense internal iron levels can help mitigate both issues. We have identified an Arabidopsis mitochondrial iron reductase mutant with an altered iron deficiency response, that may give insights on whole plant iron homeostasis. The gene is called FERRIC REDUCTASE OXIDASE 3 (FRO3), is primarily expressed in the vasculature, and is induced during iron deficiency. Loss of FRO3 causes plants to accumulate 1.2X the amount of whole plant iron as WT. However, mutants contain only 50% as much mitochondrial iron as WT. Despite over accumulating iron, fro3 plants display an increased root ferric reductase response compared to WT. RNA-seq and qPCR studies also indicate that fro3 plants display an increased iron deficiency response compared to WT. Additionally, fro3 lines may allocate extra iron to the chloroplast. fro3 plants contain more chlorophyll, have higher expression of photosynthetic genes, and have increased expression of the chloroplast iron storage protein FERRITIN, compared to WT. These findings indicate that vasculature mitochondria may play a role in whole plant iron sensing. And also suggest that disrupting mitochondrial iron levels can alter subcellular iron allocation.

# 27 | Role of the bovine PRAMEY protein in sperm function during in vitro fertilization (IVF)

Chandlar Kern, Chen Lu, Weiwei Wu, Jianbin Zhang, Yaqi Zhao, Olga Ocon-Grove, Francisco Diaz, Wan-sheng Liu

Department of Animal Science

Subfertility/infertility is a common problem in human, cattle and other mammalian species, however the molecular mechanisms underlying infertility/subfertility is largely unknown. Previous studies on human Y-linked genes indicated that they are the most important known genetic factors involved in male infertility/subfertility, which provides a strong incentive for studying the bovine PRAMEY (PRAME, Y-linked) gene family. Therefore, the purpose of this study was to determine whether the PRAMEY protein plays a role during bovine sperm capacitation, acrosome reaction (AR), and fertilization. For the capacitation and AR study, freshly ejaculated sperm was collected from Holstein bulls (n=5) at 3 different time points. PRAMEY localization was observed by western blot (WB) and immunofluorescent (IF) staining on sperm samples with the following treatments: A. 0 hr. control, B. 5 hr. control, C. capacitated and AR, D. capacitated and non-AR, E. capacitated and AR with PRAMEY antibody, F. capacitated and AR with Rabbit IgG, G. capacitated and non-AR with PRAMEY antibody, and H. capacitated and non-AR with Rabbit IgG. Sperm (1x108) were incubated at 37°C with 5% CO2 and humidity to induce capacitation and the acrosome reaction for 4 hrs. and 1 hr., respectively. WB analysis indicated that three PRAMEY isoforms (58, 30, and 13 kDa) were detected. The 30 kDa isoform was moderately to highly expressed in all treatments except in the AR sperm. The 13 kDa isoform was detected in the 5 hr. control and non-AR samples, but not in the 0 hr. control, suggesting that the 13 kDa isoform appears after sperm have went through the hyperactivation process of capacitation, and that the 13 kDa isoform could be the active PRAMEY isoform for sperm motility. Furthermore, the PRAMEY isoforms (58, 30, and 13 kDa) were not detected in the AR sperm (C, E, and F treatments) except for treatment (E) where the 58 kDa isoform is rescued due to the addition of PRAMEY antibody during the AR process. These results prompt us to hypothesize that PRAMEY is released during the acrosome reaction. To test our hypothesis, IF staining with PRAMEY-specific antibody was performed on fixed sperm from all eight treatments. A typical acrosome-enriched PRAMEY staining pattern was observed in sperm from all non-AR treatments (A, B, D, G, H), whereas little to no PRAMEY staining was observed in the acrosome region of the AR sperm (C, E, F treatments), supporting our hypothesis. In addition, an in vitro fertilization (IVF) study was
performed using bovine caudal epididymal sperm and aspirated oocytes from ovaries of mature cows. Approximately 135 matured oocytes were inseminated with either control sperm (treated with rabbit IgG) or PRAMEY antibody-treated sperm. Zygotes and early embryos (mainly 2- and 4-cell) were examined 45 h post IVF. Polyspermy rate in the antibody-treated group (18.91%) was 3-fold greater than the negative control (5.97%), suggesting PRAMEY functions in anti-polyspermy defense. The percentage of embryos reaching 4-cell stage was greater in the PRAMEY-treated sperm, signifying a potential role of PRAMEY in early embryo cleavage and development.

# 29 | Impacts of One-time Tillage Compared to No-tillage on Soil Health in a Diverse Rotational Cropping System
Devyn McPheeters, Mary Ann Bruns, Heather Karsten
Ecology and Ecosystem Science and Management

Soil health refers to a soils' ability to sustain biological life into the future while maintaining water and air quality. No-till agriculture is a strategy often used to improve soil health and growers show concern about the effects of any physical disturbance on the health of their soil. This study aimed to answer the question: Can soil health indicators be used to assess impact of one-time tillage events? This question was approached using three soil health indicators, aggregate stability, labile carbon, and total carbon, to determine the impacts of tillage once in a six-year crop rotation on soil health. We studied soil in the Pennsylvania State Dairy Cropping Systems project in Rock Springs, PA that was initiated in 2010 as a full crop entry experiment, with the 6 phases of the crop rotation planted every year in a randomized complete block design, replicated four times. Crop phases were the main plot and weed control treatments were split-plots. Soil was sampled in spring 2010 prior to the start of the experiment and in 2013 and 2016 at two depths: 0-5 and 5-15 cm for labile and total carbon and to 15 cm for aggregate stability. The cropping system features cover crops and perennials and compares one-time tillage as a strategy for herbicide reduction to no-tillage with standard herbicide application. By the end of the six-year rotation, plots that received one-time tillage had recovered to the same soil health levels as the no-till plots in all three indicators, but only after three years of perennial cover. Plots that had not had perennial cover since the tillage event or had only been under perennial cover for one or two years, had significantly lower soil health indicator scores than the no-till plots. Results from this analysis indicate that soil health can return to no-till levels despite a tillage event if they receive adequate perennial crop cover.

# 41 | Evidence for Immune tolerance in peripheral blood leukocytes of dairy cattle during early pregnancy
Maria da Silva, Francesca Gambonini, Neha Oli, Joy Pate & Troy Ott
Integrative & Biomedical Physiology

Reproductive success in dairy cattle directly effects milk production, efficiency and profitability of farms. Despite ongoing improvements in genetic selection and breeding protocols, 50-70% of embryos are loss during early pregnancy. Within the first 25 days, the embryo induces changes in maternal physiology that support its further development, however, the maternal immune system is a critical challenge to embryo survival stemming from the paternal genes in the embryo. Little is known about how the embryo avoids immune attack and regulates uterine and peripheral blood immunity. In previous studies, we observed an increase in the number of macrophages and natural killer cells as well as abundant expression of immune tolerance molecules in the uterus of pregnant heifers. We hypothesize that pregnancy upregulates expression of tolerogenic molecules in circulating immune cells in both heifers and cows. Due to their high fertility, heifers (N=6) were used in a repeated measure design; animals were first heat-synchronized and later
heat-synchronized and inseminated for blood collection at days 14, 17, and 20 of the estrous cycle and days 14, 17, 20 and 23 of pregnancy, respectively. Due to their low fertility, lactating cows (60 days in milk) were randomly assigned to be either heat-synchronized (N=6) or heat-synchronized and inseminated (N=8) with same blood collection times described above. Blood leukocytes were isolated from heifers and cows and protein and mRNA abundance of the tolerogenic molecules indoleamine 2,3-dioxygenase (IDO), aryl hydrocarbon receptor (AHR) and peroxisome proliferator-activated receptor-gamma (PPAR\(\gamma\)) were measured using flow cytometry and qPCR. Data were analyzed using the MIXED procedure of SAS to compare the cyclic and pregnant animals and to assess changes over days in pregnancy. In heifers, protein abundance of AHR and PPAR\(\gamma\) was greater (\(P<0.05\)) in pregnant compared to cyclic animals and IDO increased (\(P<0.05\)) over days in both statuses. AHR mRNA tended to increase (\(P=0.06\)) over time during pregnancy and PPAR\(\gamma\) mRNA increased (\(P<0.05\)) over days in both statuses. In cows, no differences in AHR, PPAR\(\gamma\) and IDO protein abundance were observed between the estrous cycle and pregnancy. However, IDO mRNA was lower (\(P<0.05\)) in pregnant animals and increased (\(P<0.05\)) over days of pregnancy while AHR mRNA decreased (\(P<0.05\)) over days of pregnancy. Interestingly, in heifers, \(\sim80\%\) of leukocytes were AHR+ and IDO+, but only \(\sim20\%\) leukocytes were PPAR\(\gamma\)+. In cows, we observed that \(\sim60\%\) and \(\sim80\%\) of leukocytes were AHR+ and IDO+ respectively. In addition, \(\sim80\%-60\%\) of leukocytes were PPAR\(\gamma\)+ with a decrease in number of positive cells over days of pregnancy. Overall, our results support an increase in tolerance in circulating immune cells during early pregnancy that was greatest in heifers, which exhibit higher fertility. However, over days of pregnancy PPAR\(\gamma\) protein abundance and %PPAR\(\gamma\)+ cells decreased (\(P<0.05\)) in circulating leukocytes of cows. These findings add to our understanding of differences in immunity during the estrous cycle and early pregnancy between heifers and lactating cows and have potential implications not only in animal agriculture but also in the comparative physiology of the wild species and human reproduction.

# 50 | Generation of novel alleles of rice extra-large G proteins (XLGs) via CRISPR/Cpf1 gene editing system

Christian Cantos & Sarah Assmann
Department of Biology

Heterotrimeric G-proteins, (G proteins hereafter), are signal transducers that are present in all eukaryotes. G proteins are comprised of three core subunits G\(\alpha\), G\(\beta\), G\(\gamma\) that transmit extracellular signals to downstream effectors. In rice (Oryza sativa L.), G proteins are composed of one canonical G\(\alpha\) (RGA1) and four non-canonical extra-large G protein (OsXLG3a, OsXLG3b, OsXLG3c, and OsXLG1) genes, one G\(\beta\) (RGB1), and five G\(\gamma\) (RGG1, RGG2, DEP1, GS3, GGC2) genes. A null mutant of RGA1, also known as d1 mutant, shown dwarf phenotype, erect stature and round seeds. d1 plants also shown improved drought tolerance, increased photo avoidance and decreased photoinhibition (Ferrero-Serrano and Assmann, 2016; Ferrero-Serrano et al., 2018). On the other hand, xlg plants shown different phenotypes suggesting specific functions (Cui et al., 2020). To determine the specific functions of rice XLGs in plant architecture and drought stress response, generation of knockout (KO) lines are needed. In this study, novel alleles of rice XLGs were generated using the CRISPR/Cpf1 gene editing system. The CRISPR/Cpf1 allows targeted cleavage of genomic DNA guided by a customizable small noncoding RNA, resulting in modifications of the gene of interest (GOI) of the organism. By next generation, the CRISPR DNA can be segregated out resulting into an organism with desired GOI mutation and can be considered as non-GMO crop. Here, we transformed the Nipponbare wild type and d1/rga1 background with the CRISPR/Cpf1 system (Wang et al., 2017) targeting OsXLG3a (LOC_Os11g10050), OsXLG3b (LOC_Os06g02130), OsXLG3c (LOC_Os10g02814) and OsXLG1 (LOC_Os10g02814). Based on the transgenic rice produced, we successfully transformed the CRISPR/Cpf1 system into the Nipponbare wild type and d1/rga1 background with 100% transformation efficiency. Based on the DNA sequences analyzed, we successfully targeted and generated novel alleles of the four rice XLG
genes with 36-100% efficiency in Nipponbare wild type and 35-100% efficiency in d1/rga1 background. Currently, we are analyzing the candidate crispr-xlg lines for improved plant architecture and drought stress response.

Computational Biology & Statistics

# 3 | Interactions between climate change and coinfections: what should we expect from the future?
Chiara Vanalli, Lorenzo Mari, Renato Casagrandi, Marino Gatto, Brian Boag, Isabella Cattadori
Department of Biology

Climate changes have been predicted to impact the distribution and severity of infectious diseases worldwide. For soil-transmitted parasites, climate is expected to alter the abundance and viability of free-living stages in the environment and, ultimately, the intensity of infection in the host. Within-host processes, such as a strong immune response, can mitigate these climatic effects by controlling the intensity of infection, however, if the host carries more than one parasite species, the immune response might not be as effective as the response of hosts with a single parasite infection. Here, we use a modeling approach and investigate how climate changes affect the dynamics of infection in hosts with single and dual infections. As study case, we selected two common gastrointestinal helminths of the European rabbits (Oryctolagus cuniculus), with contrasting immune responses: Graphidium strigosum, which shows no immune control, and Trichostrongylus retortaeformis, which instead is more clearly controlled. We used laboratory studies to quantify parasite-specific climatic functions of egg hatching, larval development and mortality in the environment. We found that T. retortaeformis survival on pasture is mainly influenced by temperature, while G. strigosum is primarily affected by humid conditions. We then developed an immune-epidemiological model that expletively considers the effect of climate and immune variables on the dynamics of infection in rabbits with one or both helminths; the model was calibrated and validated against populations of rabbits in the UK. Our findings indicate that by carrying a higher parasite burden and by shedding a greater number of eggs on the pasture, dual infected hosts contribute more heavily to the dynamics of infection of both helminths than single infected rabbits. Across the UK, we find higher environmental risk in the humid west-central Scotland for G. strigosum and in the warmer south-east England for T. retortaeformis. Future projections of temperature and humidity indicate a faster hatching and development time, together with a decreased mortality for both helminths. This higher risk of infection will impact the intensity of infection of the two helminths in a different way: G. strigosum intensity will increase in rabbit populations, while the immune response will effectively control T. retortaeformis intensity. Last, we evaluate if our UK findings are representative for the rest of Europe. We examine future infection changes for the Mediterranean, temperate and boreal European regions. Although temperature warming will be experienced in the whole continent, our long-term projections indicate that the climatic differences will determine divergent trends of infections. For both helminths, we expect a decrease in the lifespan of free-living stages in the Mediterranean regions while an increase in survival in the boreal areas. Our study provides novel insights to foresee climate change impacts on the dynamics of infection of two parasites with contrasting reactions to the host immune response and in hosts with single and dual infections. Our findings provide fundamental knowledge that can be used to advise intervention and control strategies in areas where helminthiases are endemic and commonly co-circulate with host populations.
Towards Synthetic Microbiota Transplants: Insights for C. difficile Treatment From Meta-analysis
Susan Tian, Jordan Bisanz
Department of Biochemistry, Microbiology, and Molecular Biology

C. difficile is the most deadly “superbug” in the United States and has been estimated to cost the health care system 4.8 billion dollars annually. C. difficile is frequently found in the healthy gut microbiome, but its pathogenicity is restricted through ecological interactions with other community members which have yet to be comprehensively determined. Antibiotic therapy is ironically both the typical cause and treatment for C. difficile infection (CDI), and the disruption of these complex communities by broad spectrum antibiotics leads to poor long-term efficacy and CDI frequently reoccurs. Fecal microbiota transplants (FMT), the administration of fecal material from a healthy donor to restore the microbiota and constrain C. difficile, have shown great promise. Unfortunately, the reliance on a human donor makes FMTs intrinsically undefined and variable, and they may contain undetected pathogens, traits that are not desirable for a widely-used therapeutic. We hypothesize that synthetic FMT (sFMT) will address these insufficiencies while simultaneously providing insight into the microbe-microbe interactions that regulate C. difficile pathogenicity. To rationally construct a sFMT, we are conducting a sample-level meta-analysis of human studies of CDI. Using a comprehensive search term, we uncovered 370 possible studies of which 90 had relevant designs. Of these 90, 10 contained all necessary data for further study. Analysis of community structure across studies revealed a significant correlation between community composition and C. difficile colonization (R2=0.03, P=0.001, ADONIS PhiLR euclidean). We found that microbiome composition could predict C. difficile status independent of C. difficile abundance (AUROC=0.902) and analysis of the most predictive features revealed new unreported correlations. Our future work will reconstruct communities of strains which anti-correlate with C. difficile and conduct mechanistic in vitro and gnotobiotic experiments to determine their inhibitory activity. These studies may provide new prognostic approaches, therapies, and insights into new microbe-microbe interactions in the gut microbiota.

Impact of Solvent Interactions upon Interfacial Phenomena
Varun Mandalaparthy, Pho Bui, Paul Cremer, Will Noid
Department of Chemistry

Osmolytes are small, neutral organic molecules that can have significant effects on the thermodynamic stability of interfaces and proteins. Introducing osmolytes to an air-water interface changes the surface tension and this change depends on the complicated interaction patterns of all the species in solution. The folding free energy of a polymer in water is analogous to the surface tension of the air/water interface and studying the surface tension therefore provides valuable insight into factors that keep proteins stable. In this study, we develop a thermodynamic theory for understanding the variation of surface tension as a function of concentration for binary and ternary solutions of osmolytes in water. This theory introduces phenomenological fitting parameters ε, a, and b, based on which, trends in the surface tension and preferential interaction coefficients can be understood. We have developed a microscopic theory based on McMillan-Mayer theory and Hill’s Constant Pressure Solution theory that predicts these parameters in terms of molecular structure and interactions. We validate this theory by carrying out Monte Carlo (MC) simulations of a lattice model as well as Molecular Dynamics (MD) simulations of a Lennard-Jones fluid. These simulations also allow us to see how various interfacial macroscopic quantities, such as surface tension, change as a function of microscopic interactions between the species in solution. We find good agreement between the predictions of the theory and the behavior observed via simulations. We have applied our methods to the experimentally well-studied ternary system of urea, water, and trimethylamine oxide (TMAO) and we make predictions about the interfacial behavior of these osmolytes. In particular, our model predicts that TMAO prefers to be...
accumulated at the air/water interface and urea is depleted from that interface. In urea-TMAO-water solutions, however, the presence of urea lowers the propensity of TMAO to accumulate at the interface, pointing at a possible energetic mechanism for the counteraction effect of the two osmolytes observed in many studies.

# 28 | Effect of a kinked trachea on the dose distribution of reactive air pollutants in proximal airways of the human lung
Minyoung Kim, Mary Vogt, Jane Bourke, Simon Royce, Rebecca Bascom, Ali Borhan
Department of Chemical Engineering, Penn State University

The inhalation of reactive air pollutants in the human respiratory tract is associated with serious health problems such as coughing, shortness of the breath, airway irritation and inflammation and even permanent lung injury from long-term exposure. Site-specific tissue injury in the respiratory tract is mainly due to the local dose of toxicant delivered to the tissue on the airway wall, which has recently been related to the deformed geometry of proximal airways. We have developed a patient-specific model by performing computational fluid dynamics simulations of reactive gas transport and uptake in anatomically-accurate airway structures of consented lung disease patients at the Penn State Hershey Medical Center (Borhan et al., Appl. Math. Model. 2021). We characterize the tracheal geometry by its curvature and tortuosity, and present a qualitative and quantitative analysis of the effect of tracheal shape on the delivered dose of reactive air pollutants. Our results show that a kinked trachea characterized by larger curvature and torsion leads to greater uptake and local dose of toxicants, due to the onset of secondary flow downstream of the tracheal bend. Disease-modified proximal airways also show a broader distribution of hotspots of increased toxicant flux.

# 48 | Effect of metformin treatment on the gut mycobiome of type 2 diabetes patients: A meta-analysis.
Emily Bean Van Syoc, Connie J. Rogers, & Erika Ganda
Integrative & Biomedical Physiology and Clinical & Translational Sciences

Often under-studied and poorly comprehended, the fungal component of the gut microbiota (mycobiome) may play an important role in chronic disease risk and progression. Recent work has shown that in diseases characterized by gut bacterial dysbiosis, e.g., Crohn’s Disease and colitis, the gut mycobiome is also perturbed. Correcting gut dysbiosis is hypothesized to be a potential therapeutic target for obesity and some inflammatory diseases, however, little data is available regarding the effects of microbiome-modulating medications on the gut mycobiome and associated host-bacterial-fungi interactions. Metformin is globally the most prescribed drug for type 2 diabetes mellitus (T2D). Metformin reduces T2D-induced hyperglycemia by decreasing hepatic gluconeogenesis and consequently decreasing circulating blood glucose. Metformin also impacts the gut bacterial community, lowering circulating lipopolysaccharide and therefore reducing toll-like-receptor-mediated inflammation. However, the potential effects of metformin on the gut mycobiome are unknown. Therefore, this study aims to characterize the impact of metformin on the gut mycobiome of healthy, pre-diabetic, and T2D individuals. We searched for published research articles with archived, publicly accessible shotgun metagenomics data. The data was re-processed from raw files with one uniform bioinformatics pipeline and fungal taxa were characterized. We analyzed data from 920 individuals from six studies which included two randomized controlled trials on newly diagnosed, treatment-naïve individuals, three cross-sectional cohort studies, and one prospective cohort study. Our objectives were to (1) characterize the impact of metformin treatment on the gut mycobiome in healthy, pre-diabetic, and T2D subjects; (2) correlate changes in fungal community composition and diversity to available clinical metadata, such as body mass index (BMI) and fasting blood glucose; and (3) determine changes in fungal genes (functional capacity). The fungal community was analyzed by comparing alpha (Shannon’s Index; SI and Observed Taxa; OT), beta
diversity, and differential abundance analysis. Fungal alpha diversity was affected by metformin treatment in one cohort study (S1 chi-squared = 18.45; P < 0.001, OT chi-squared = 29.738; P < 0.001) and by diabetes status in a different cohort study (S1 chi-squared = 19.111; P < 0.001, OT chi-squared = 15.735; P = 0.001). Fungal beta diversity differed between treatment-naïve T2D individuals before and 3 days following metformin treatment (adonis F = 3.577; P = 0.002) and in a cohort study between diabetic, pre-diabetic, and healthy individuals (adonis F = 3.152; P = 0.001). Notably, the potentially pathogenic fungi Candida dubliniensis was significantly more abundant in subjects that had taken metformin for three day but was less abundant in subjects that had taken metformin for 2-6 months and was less abundant in the cross-sectional cohort in T2D metformin treated subjects; these findings indicate that the duration of metformin treatment may be a contributing factor to mycobiome shifts. Our findings demonstrate that metformin treatment might influence the gut mycobiome, and these shifts may result in host-bacteria-fungi interactions that have been previously unknown. This project provides novel insight into the interactions between human hosts, the gut microbiome, and the gut mycobiome in response to metformin, a widely used drug to treat T2D.

Microbiology, Pharmacology & Immunology

# 6 | Volatile Organic Compounds Produced by Bacteria Associated with Decomposition
Veronica Cappas, Reena Roy, & Dan Sykes
Forensic Science

Microorganisms play an important role in decomposition and are known to produce volatile organic compounds (VOCs) that contribute to the odor of decomposition. Although microorganisms and VOCs have been studied independently regarding decomposition, not many studies have linked the two subject matters. Microbial communities were sampled at various points of decomposition from a swine placed in an indoor enclosure and sequenced using NextGen Illumina sequencing. Significant bacteria species will be identified based on relative abundance in each stage of decomposition. Currently, the results of this portion are pending. However, the expected results of this phase will most likely be similar to past studies looking at the microbiome of a decomposing body. There will be a noticeable change of community composition as decomposition progresses (1-8). In the abdominal area, there will most likely be an increase in aerobic bacteria after the bloating stage due to an increased amount of oxygen (1). Also, more diversity and higher abundance levels may be present in earlier stages of decomposition (2). Common bacterial phyla that may be present include Firmicutes, Proteobacteria, Bacteroidetes, Gammaproteobacteria, and Actinobacteria (1,3). Because this project’s swine was placed in a closed container indoors, there may be differences in communities due to limited exposure to environmental microbiomes. These selected bacteria will be cultured on human tissue samples in headspace vials. Solid Phase Microextraction (SPME) fibers will be used to sample the VOCs produced by the selected bacteria species. The VOC results produced by each bacteria species will be compared with overall VOC data collected from the same decomposing swine, which is a part of a related study and other decomposition studies. This will help distinguish the origin of VOCs associated with decomposition like compounds produced by bacteria versus produced by the general break-down process. The bacteria VOC results are expected to be in the database, mVOC 2.0, although the results may vary because the bacteria will be cultured on human tissue (9). In addition, temperature, oxygen, or humidity level will be assessed by culturing the bacteria in different ranges of such variables, simulating different seasons, weather conditions, or areas of the body. Depending on results from the initial phase, certain species of bacteria will be cultured together to determine how interactions affect VOC production. Not all decomposition-associated bacteria VOCs have been identified, and it is not necessarily known why or how certain VOCs are produced throughout decomposition. The purpose of the study is to determine what VOCs are produced by bacteria associated with decomposition and how factors such as bacterial communities, the
conditions of a decomposing body, among other factors, can affect this production. VOC research can be useful for improving techniques used for searching bodies in missing person cases, in natural disasters such as Hurricane Katrina, or in cases where cadaver-detection canines or where VOC detection apparatus are used. Overall, researching the VOCs produced by bacteria will improve the understanding of the complex process of decomposition.

# 8 | Neuroimmune cells play a critical role in mediating the effect of acute alcohol on central amygdala glutamatergic transmission.
Mariam Melkumyan, Angela Snyder, & Yuval Silberman
Neural and Behavioral Sciences Department, Penn State College of Medicine

Alcohol Use Disorder (AUD) affects around 15 million individuals annually in the United States. The central amygdala (CeA) has been implicated in AUD, with alcohol (EtOH) exposure inducing neuroadaptive changes resulting in increased neuronal excitability and enhancing further EtOH intake. EtOH also increases neuroinflammation in this brain region which may be causative to AUD, but whether neuroinflammatory signaling contributes to enhanced CeA neuronal activity by EtOH has not been fully elucidated. To that end, we sought to test the hypothesis that acute EtOH exposure may increase excitatory neurotransmission in the CeA via modulation of neuroimmune cells. Whole cell patch clamp recordings of spontaneous excitatory postsynaptic currents in CeA neurons (lateral subdivision) from adult male and female C57Bl6/J mice showed that 10 minutes of bath applied 20mM EtOH significantly increased glutamatergic transmission in the CeA. This effect was significantly attenuated by 100uM fluorocitrate, an astrocyte inhibitor, or by 100uM minocycline, a microglia inhibitor, suggesting that EtOH increases glutamatergic transmission in the CeA by acting on microglia and astrocytes. Effects of 100mM EtOH on glutamatergic transmission were only blocked by fluorocitrate but not minocycline, suggesting that astrocytes may be the primary target of higher EtOH concentrations. Bath application of 500ng/mL lipopolysaccharide (LPS), the endotoxin portion of the gram-negative bacterial cell wall, also significantly enhanced CeA glutamate transmission, an effect that was blocked by fluorocitrate or minocycline pretreatment. These findings suggest that EtOH induces neuroinflammation through a similar pathway as LPS. We next utilized chemogenetics to selectively inhibit CeA astrocyte function and found that the effect of EtOH on CeA glutamatergic transmission was blocked, further confirming that astrocytes are a primary mediator of EtOH enhancement of glutamatergic transmission in the CeA. The exact mechanism of acute EtOH effect on astrocytes and the role that microglia may have in this pathway in the CeA needs to be further explored, and the effect of chronic vs acute EtOH exposure needs to be examined to provide insight into potential therapeutics for treatment of AUD.

# 40 | Structure and function of an unusual flavodoxin from the domain Archaea
Divya Prakash, Prashanti R. Iyer, Suharti Suhartia, Karim A. Walters, John H. Golbeck, Katsuhiro S. Murakami, & James G. Ferry
Department of Biochemistry & Molecular Biology

Flavodoxins, electron transfer proteins essential for diverse metabolisms in microbes from the domain Bacteria, are extensively characterized. Remarkably, although genomic annotations of flavodoxins are widespread in microbes from the domain Archaea, none have been isolated and characterized. Herein is described the structural, biochemical, and physiological characterization of an unusual flavodoxin (FldA) from Methanosarcina acetivorans, an acetate-utilizing methane-producing microbe of the domain Archaea. In contrast to all flavodoxins, FldA is homodimeric, markedly less acidic, and stabilizes an anionic semiquinone. The crystal structure reveals an flavin mononucleotide (FMN) binding site unique from all other flavodoxins that provides a rationale for stabilization of the anionic semiquinone and a remarkably low reduction potentials
for both the oxidized/semiquinone (~301 mV) and semiquinone/ hydroquinone couples (~464 mV). FldA is up-regulated in acetate grown versus methanol-grown cells and shown here to substitute for ferredoxin in mediating the transfer of low potential electrons from the carbonyl of acetate to the membrane-bound electron transport chain that generates ion gradients driving ATP synthesis. FldA offers potential advantages over ferredoxin by (i) sparing iron for abundant iron-sulfur proteins essential for acetotrophic growth and (ii) resilience to oxidative damage.

# 47 | Characterizing new vitamin D targets in the immune system using novel vitamin D receptor (VDR) reporter mouse.
Juhi Arora & Margherita Cantorna
Department of Veterinary and Biomedical Sciences

The vitamin D receptor (VDR) is a ligand-activated nuclear protein that regulates gene transcription. Vitamin D target cells are identified by the expression of VDR. Kidney and colon epithelial cells constitutively express high levels of the VDR. Immune cells also express the VDR; however, at significantly lower levels. Western blots demonstrate that spleen, and lymph nodes have significantly lower expression of the VDR. Gene expression studies in macrophage, B cells and T cells showed that activation for 24-48 hours increased the VDR mRNA levels. We have developed transgenic mice that express Cre recombinase within the VDR locus (VDRcre) and crossed these with a reporter line (Tdtomato/fl). Cre expression in the VDR locus results in a VDR +/- mice. The frequencies of immune cells were similar between VDR-/- and VDR +/- mice. The VDRcre expressing offspring had pink skin. Kidneys, small intestine and colon were also distinctly pink suggesting high expression of the Tdtomato-VDR reporter. Innate lymphoid cells (ILCs) are important for mediating immunity at the barrier surfaces such as lung, gastrointestinal tract, skin. ILCs develop in the bone marrow and migrate to mucosal tissues. Three types of ILCs develop from a common precursor in the bone marrow and can be identified by transcription factor expression: Tbet+ ILC1, GATA3+ ILC2 and RORyt+ ILC3 cells. We used flow cytometry to identify tdtomato-VDR expression in lymphoid precursors in bone marrow and ILC2 & ILC3 cells at the periphery tissues; 80% of the precursors for the ILCs express the VDR. In the periphery, however ILC1 and ILC3 cells expressed the VDR but ILC2 cells did not. We have identified a unique and previously undefined VDR- precursor in the bone marrow for the ILC2 lineage. The data further suggest that ILC3 and ILC1 cells are vitamin D targets in the periphery but that ILC2 cells are not. Future work will determine the effect of vitamin D, expression of the VDR and host defense in the lung.

# 51 | Short- and Long-Term Effect of Persistent Organic Pollutants in Gut Microbiota Community and Function in Immature Mice
Bipin Rimal, Yuan Tian, Erik L. Allman, Imhoi Koo, Wei Gui, Philip B. Smith, & Andrew D. Patterson
Department of Veterinary and Biomedical Sciences

Persistent organic pollutants (POPs) have been implicated in numerous metabolic diseases including cancer, obesity, and diabetes. However, the specific mechanisms of these diseases are still to be elucidated. Emerging evidence suggests exposure to POPs could alter gut microbiota composition and function, which can then affect host health. In this study, the effect on the gut microbiota structure and function of mice following exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDF) was investigated using 16S rRNA gene sequencing and metagenomics. Four weeks old male C57BL/6J wild type mice were treated through the diet with 24 µg/kg TCDF for 5 days. The mice were sacrificed on the day after the last TCDF exposure (short-term) or at 13th week after TCDF exposure (long-term) when all TCDF had cleared the mouse. Cecal contents of sacrificed mice were processed for 16S rRNA and metagenomics. Permanova analysis using weighted distance matrices of 16S rRNA sequencing data showed that long term exposure to TCDF
significantly altered the community structure in mice [vehicle vs TCDF: p<0.1]. Additionally, there was a significant decrease in genus Akkermansia: 0.004±0.001 [vehicle] to 0.001±0.001 [TCDF] after long-term exposure of both POPs. Functional analysis using metagenomics showed significant decrease in abundance of Lipid IVA biosynthesis 25.04±1.3 [Vehicle] to 10.65±2.78 [TCDF], peptidoglycan biosynthesis 61.87±3.75 [Vehicle] to 37.16±9.50 [TCDF] and peptidoglycan maturation 22.39±1.07 [Vehicle] to 11.83±2.12 [TCDF] following long term exposure. Similarly, a significant increase was seen in purine ribonucleosides degradation 411.8±24.39 [Vehicle] to 544.8±23.66 [TCDF]. However, the changes were not significant during short-term exposure. These sequence-based approaches to investigate the microbiome changes show how these pollutants modulate gut microbiota structure and function in the long-term which can affect host health and metabolism.