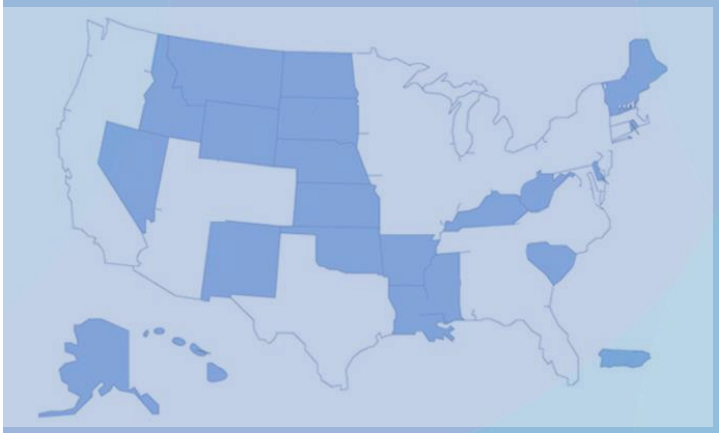
2nd NIH Symposium on Women's Health Research in the Institutional Development Award (IDeA) States

September 16, 2025

1 p.m. – 4 p.m. ET



OVERVIEW

2ND NIH Symposium on Women's Health Research in the Institutional Development (IDeA) States

Symposium Purpose

Continue to facilitate networking, knowledge exchange, and recognition of the substantial contributions made by investigators to women's health research within the IDeA States.

Goals

- Engage IDeA investigators who received an administrative supplement for research on women's health in a conversation to share information on their research and research outcomes.
- Bolster the profiles of the administrative supplement awardees and their research on women's health within their institutions and throughout the IDeA programs.
- Highlight program achievements by showcasing the research outcomes reported by IDeA States investigators who have been awarded administrative supplements.

The IDeA States Women's Health Supplement Program

Since 2020, the Office of Research on Women's Health (ORWH) has partnered with the National Institute of General Medical Sciences (NIGMS), along with other participating NIH institutes, centers, and offices (ICOs), to advance women's health by expanding research and research capacity in IDeA States.

IDeA Program and Background

IDeA States are United States and territories eligible to receive funding from the Institutional Development Award (IDeA) Program. Established in 1993 by congressional mandate, the IDeA Program aims to increase research capacity in states with historically low levels of NIH funding. Managed by NIGMS, the IDeA Program offers multiple types of funding mechanisms that enable institutions in eligible states to jumpstart or bolster their ability to conduct competitive basic, clinical, and translational research. These awards provide research funding as well as support for faculty development and infrastructure improvements that strengthen an institution's biomedical research capacity. Ultimately, the IDeA Program aims to equip institutions to conduct research that addresses the needs of their local communities, particularly those in medically underserved and rural areas.

ORWH-NIGMS IDeA Program Partnership

In 2020, ORWH and NIGMS with support from other ICOs partnered to create the first IDeA funding opportunities with a specific focus on women's health and health disparities experienced by women. Together with 14 other NIH ICOs, NIGMS and ORWH issued multiple Notices of Special Interest (NOT-GM-20-017, NOT-GM-21-018, and NOT-GM-22-005) for administrative supplemental funding, which allowed existing IDeA-funded institutions to apply for 1 year of additional funding to focus on women's health. These opportunities encouraged a broad range of research that addresses important issues of women's health across the lifespan, including maternal and infant morbidity and mortality and their underlying causes.

Women's Health COBRE Institutional Development Award

Encouraged by the positive response to the administrative supplements, ORWH and NIGMS partnered again to create a longer-term funding opportunity focused on women's health. In October 2022, they released a new opportunity using the IDeA Program's Centers of Biomedical Research Excellence (COBRE) Phase 1 P20

funding mechanism (NOT-GM-23-012). Through this mechanism, awardees receive funding for up to three sequential 5-year phases to support the establishment and development of an innovative biomedical research center. The COBRE is designed to help institutions develop a critical mass of investigators who can compete effectively for independent research funding and to improve the infrastructure for biomedical research. This long-term model also provides funded institutions with the necessary time and resources to establish and strengthen collaborations with local community organizations so that they can meet the specific needs of the community.

This effort expanded the distribution of NIH funding for women's health research across the country and advanced ORWH's vision for a world in which every woman receives evidence-based disease prevention and treatment tailored to her own needs, circumstances, and goals.

IDeA States Symposium Planning Team

Crina Frincu, Ph.D.
Program Officer, NIGMS
Symposium Co-lead

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Mercedes Rubio, PhD
Division Director, DRCB
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Cindy L. Caughman, MPH
Acting Deputy Director, ORWH
NIH Office of the Director

AGENDA—VIRTUAL EVENT

Welcome & Opening Remarks:

1:00 PM Welcome (Crina Frincu, PhD, NIGMS co-lead)

1:05 PM Introduction – Opening Remarks

- Mercedes Rubio, PhD, Division Director, Division for Research Capacity Building, NIGMS
- Cindy Caughman, MPH, Acting Deputy Director, ORWH

Scientific Session 1:

1:15 – 2:15 PM Scientific Presentations (Session Chair: Dr. Crina Frincu, NIGMS co-lead)

- Jodi Hadden-Perilla (UNIVERSITY OF DELAWARE) – "Molecular Dynamics of the Human Papillomavirus Type 16 Capsid to Identify Novel Drug Targets"
- Joseph McQuail (UNIVERSITY OF SOUTH CAROLINA AT COLUMBIA) – "Targeting NMDA Receptors and Brain Estradiol to Rescue Memory in Aging Females"
- Filipa Godoy-Vitorino (UNIVERSITY OF PUERTO RICO MEDICAL SCIENCES) – "Strain-level pangenomic analyses of vaginal Lactobacillus in a gradient of dysplasia in Hispanic women living in Puerto Rico"
- Lisette Jacobson (UNIVERSITY OF KANSAS MEDICAL CENTER) – "Advancing Maternal-child Wellness through Nutrition, Physical Activity, and Lactation"

2:15 – 2:30 PM Q&A (Moderator: Dr. Balkissa Ouattara, ORWH)

Break (10 minutes)

Scientific Session 2:

2:40 – 3:40 PM Scientific Presentations (Session Chair: Dr. Regine Douthard, ORWH co-lead)

- Anamaria Zavala (BOISE STATE UNIVERSITY) – "Role of Mechanical Stress in Mitigating Chemotherapy-associated Bone Loss"
- Johann Urschitz (UNIVERSITY OF HAWAII AT MANOA) – "Investigating the Effects of Microplastics Accumulation on Women's Health during Pregnancy"
- Lisa Schwarzburg (UNIVERSITY OF ALASKA) – "Gathering, Learning & Initiating: Setting the Stage for Community-engagement in Women's Health in SW Alaska"
- Anna Strahm (SANFORD RESEARCH/UNIVERSITY OF SOUTH DAKOTA) – "Midwest Birth Outcomes and American Indian Pregnancy"

3:40 – 3:55 PM Q&A (Moderator: Dr. Balkissa Ouattara, ORWH)

Closing Remarks:

3:55 – 4:00 PM (Dr. Mercedes Rubio, NIGMS)

PRESENTER ABSTRACTS

Title: Molecular Dynamics of the Human Papillomavirus to Identify Novel Drug Targets
Presenter: Jodi A. Hadden-Perilla, PhD
Institution: University of Delaware
IDeA Program: COBRE Discovery of Chemical Probes and Therapeutic Leads (COBRE PI: FOX, JOSEPH; 3P20GM104316-09S1)
Outcome: 1R35GM157125-01

Abstract

Background: Cervical cancer, predominantly caused by the human papillomavirus (HPV), remains a significant global health concern despite the availability of vaccines and early-detection screening programs. The HPV capsid—a protein shell that encases the virus—is both the primary immunogen used in vaccines and a promising antiviral target. However, structural studies of the intact capsid are limited by the resolution constraints of cryo-electron microscopy (cryo-EM), which cannot resolve flexible or transient features.

Aims: This project seeks to: (1) identify and characterize cryptic druggable pockets on the HPV capsid surface, and (2) establish the early stages of capsid disruption by heparan sulfate (HS), a known host receptor. Cryptic pockets are not readily apparent in experimentally-derived structures, but raise the possibility of targeting proteins otherwise considered undruggable. The detailed HPV-HS binding interaction has never been resolved by experimental structural biology methods.

Methods: We constructed a complete atomistic model of the HPV type 16 capsid using integrative modeling and performed all-atom molecular dynamics (MD) simulations in explicit solvent on the NSF leadership-class supercomputer Frontera. This >16-million-atom simulation is the largest yet reported for an icosahedral capsid for a human-infective virus. The conformational ensemble afforded by this simulation provides a novel structural characterization of the capsid, enabling the first glimpse into its detailed motions and their role in viral function.

Outcomes: The MD-derived ensemble reveals the complex conformational heterogeneity of the capsid that reduces resolution in cryo-EM reconstructions. We observed spontaneous folding of the disordered C-terminal tail of the L1 capsid protein—implicated in DNA binding, nuclear localization, and host attachment—marking the first structural insights into this domain, which has never been resolved experimentally. The ensemble also enabled reinterpretation of ambiguous cryo-EM densities and revealed chloride ion localization patterns consistent with the location of HS binding grooves reported by cryo-EM. These results allowed us to construct HS-bound complex models to investigate the capsid's dynamical response to receptor binding. Although cryptic pockets have not yet been confirmed, the HS binding groove and multiple antibody epitopes are now characterized at atomistic detail. These insights are essential for future screening efforts against early-entry intermediates and for understanding capsid immunogenicity.

Conclusions: Our simulations uncover functionally relevant dynamics of the HPV capsid, inaccessible to cryo-EM, and provide a foundational framework for identifying new anti-capsid drug targets. This work builds computational virology capacity in Delaware and positions our team to pursue long-term studies on HPV structural biology and therapeutic design, particularly in support of women's health.

About the Presenter:

Dr. Jodi Hadden-Perilla is an Associate Professor of Chemistry & Biochemistry and C. Eugene Bennett Early Career Chair at University of Delaware. The Hadden Lab leverages the computational microscope to study biological machines, including viruses and molecular motors. Her research aims to identify strategies to inhibit undesired functions (e.g., in viral infection) and prevent dysfunction (e.g. in essential cellular processes), ultimately to treat disease. Currently, her team is focused on the hepatitis B virus capsid, the cytoplasmic dynein motor domain, and the role of carbohydrates and glycoconjugates in immunity and disease.

Title: Targeting D-Serine Metabolism and NMDAR Signaling to Improve Cognition in Aging Females
Presenter: Joseph McQuail, PhD
Institution: University of South Carolina School of Medicine
IDeA Program: COBRE (COBRE PI: KIARIS, HIPPOKRATIS)
Outcome: https://pubmed.ncbi.nlm.nih.gov/38259638/
1R01AG091677-01A1

Abstract

Scientific Background: Age-related cognitive decline affects both sexes, but women experience a disproportionate burden, particularly in the context of Alzheimer's disease. D-serine, a co-agonist of synaptic NMDA receptors (NMDARs), is essential for learning and memory. Its availability is regulated by serine racemase (SRR), which synthesizes D-serine, and D-amino acid oxidase (DAO), which degrades it. Disruption of this pathway may impair NMDAR signaling and contribute to cognitive vulnerability in aging. Understanding how D-serine metabolism changes with age—and how these changes relate to cognitive outcomes between the sexes—is critical for developing targeted interventions that support brain health in aging populations.

Aims: This project was designed to investigate age-related changes in D-serine metabolic enzymes in both male and female F344 rats, using cohorts powered to detect sex differences. We aimed to determine whether expression of SRR and DAO in corticolimbic brain regions correlates with individual differences in spatial memory performance. Additionally, we tested whether pharmacological inhibition of DAO could restore cognitive function and synaptic plasticity in aging, and whether treatment effects differed by sex.

Methods: Naturally aging male and female F344 rats were assessed using hippocampus-dependent spatial learning and working memory tasks. Western blotting quantified SRR and DAO expression in the prefrontal cortex and hippocampus. Aged rats received systemic administration of 3-MPCA, a DAO inhibitor, prior to testing on a delayed match-to-place task. Learning-induced ERK phosphorylation was measured as a marker of synaptic plasticity. Statistical analyses were designed to detect main effects of age, sex, and treatment, as well as interactions.

Outcomes: Aging was associated with decreased SRR and increased DAO expression, consistent with reduced D-serine availability. These changes correlated with memory impairment but did not differ by sex or sex-by-age interaction. However, aged females showed greater behavioral responsiveness to DAO inhibition, suggesting enhanced sensitivity to treatment. DAO inhibition improved memory performance and restored ERK phosphorylation during learning, indicating enhanced synaptic plasticity. These findings support DAO inhibition as a promising therapeutic strategy for cognitive aging in both sexes, with particular relevance to women's brain health. Forthcoming experiments will investigate the role of hormone signaling in modulating treatment response in females, as well as interactions with memantine and efficacy in the TgF344-AD model of Alzheimer's disease.

About the Presenter:

Dr. Joseph McQuail is an Assistant Professor in the Department of Pharmacology, Physiology, and Neuroscience at the University of South Carolina School of Medicine-Columbia. The McQuail Lab investigates how aging alters brain function and increases vulnerability to memory loss and Alzheimer's disease (AD). Dr. McQuail's research aims to identify the neural, molecular, and network-level mechanisms that drive cognitive decline, as well as those that support resilience. McQuail uses behavioral neuroscience as a core approach, leveraging preclinical rodent models to study both normal aging and AD-related pathology. By integrating cellular and synaptic analyses with whole-brain imaging and computational modeling, we seek to uncover targetable mechanisms, discover biomarkers for early detection, and build a systems-level understanding of cognitive aging. His work spans multiple domains—from stress biology and synaptic signaling to executive function and brain-wide network dynamics—providing a foundation for future interventions and translational insight.

Title: Strain-level pangenomic analyses of vaginal Lactobacillus in a gradient of dysplasia in Hispanic women living in Puerto Rico
Presenter: Filipa Godoy-Vitorino, PhD
Institution: University of Puerto Rico School of Medicine
IDeA Program: University of Puerto Rico IDeA-CTR (CTR PI: LUCIANO, CARLOS; 3U54GM133807-04S1)
Outcome: https://pubmed.ncbi.nlm.nih.gov/38064478/

Abstract

The Women's Health Supplement Award supported research on the cervicovaginal microbiome in Hispanic women living in Puerto Rico, with particular emphasis on its role in HPV infection and cervical dysplasia. The project sought to isolate and characterize vaginal bacteria, namely Lactobacillus expand knowledge to include multi-kingdom biota, and develop a biobank of isolates to advance future studies and probiotic discovery. The supplement also provided resources to continue participant recruitment, enhance microbiome research capacity, and train students in laboratory techniques and data analysis. This effort has contributed to building one of the largest biorepositories in the Caribbean region with annotated data through 16S sequencing, HPV genotyping, and clinical and cytological data, and new isolates which can be used to develop new probiotic therapeutics. The first aim focused on the isolation of Lactobacillus strains from cervical lavages collected from women in Puerto Rico (Streamlyne IRB ##2290033153). Strains including Lactobacillus iners, L. crispatus, and L. jensenii were being isolated using standard facultative anaerobic and microaerophilic methods. DNA from pure cultures was extracted and sequenced. As shotgun sequencing is ongoing the second aim of meta-pangenome construction for major cervicovaginal species identified in the cohort is still ongoing. In the meanwhile, we developed new protocols for yeast isolation and sequencing and characterized the samples used in the isolates for microbiome at the multi-kingdom level (using patients on a gradient of cervical disease). Methods employed included bacterial culture and isolation, 16S rRNA identification, and the development of protocols for DNA extraction and PCR and bioinformatics analyses. Metadata such as age, BMI, HPV status, and pathology were curated to enable integrative analyses. Future work will extend to whole-genome sequencing and pangenome construction.
Key outcomes include the establishment of a laboratory dedicated to bacterial isolation, the development of a biobank with 168 isolates from 102 women across diverse HPV and cytology groups HGSIL (High-Grade Squamous Intraepithelial Lesion, N=37), LGSIL (Low-Grade Squamous Intraepithelial Lesion, N=31), and NILM (Negative for Intraepithelial Lesion or Malignancy, N=100). Isolates include mixed species as well as pure isolates representing potentially L. crispatus, L. jensenii, L. gasseri, and L. iners. The distribution of Lactobacillus species was assessed through PCR-based bacterial isolation, yielding ten pure isolates that included L. crispatus, L. jensenii, L. gasseri, and L. iners, with several appearing in mixed cultures. Three L. crispatus isolates from NILM controls were prioritized as potential probiotic candidates for shotgun analyses. Future work will focus on genetic characterization and isolation of key species, linking genomic traits to cervical status, and testing colonization and host responses in humanized models, while ongoing metagenomic analyses will clarify microbial community differences by disease state and highlight potential probiotic strains. The project has contributed to three peer-reviewed publications linking cervicovaginal microbiota with inflammation and cervical disease and the training of graduate and undergraduate students. The scope has also been expanded to include fungal biota in addition to the bacterial isolate collection, broadening the ecological perspective. Funding reinforced participant recruitment and research infrastructure, building the foundation for probiotic development and microbiome-based interventions in cervical cancer prevention. This project is the first systematic effort to isolate vaginal probiotics in Puerto Rican women, advancing microbiome insights and cancer prevention strategies.

About the Presenter: Dr. Godoy-Vitorino is a tenured Professor at the University of Puerto Rico, School of Medicine, and the Chair of the Department of Microbiology. She has developed her career studying biodiversity associated with animal and human microbiomes, investigating an eclectic collection of topics, including evolution, community dynamics, biofilm succession and dysbiosis in different systems. She pioneered the use of metagenomics and bioinformatic integration analyses in Puerto Rico in human microbiome and tropical ecosystems. Her laboratory investigates microbiomes in a variety of contexts using various Omic techniques to understand the co-evolution, transmission, and functions of microbial-host symbioses. Her group 's mission is to translate microbial ecology to improve human health and conservation, while empowering education in the microbial sciences.

Title: eMOMSTM – A Feasibility RCT to Improve Weight and Breastfeeding Among Women with Elevated BMI Using a Mobile Health App
Presenter: Lisette Jacobson, PhD
Institution: University of Kansas School of Medicine-Wichita
IDeA Program: COBRE (COBRE PI: WEINMAN, STEVEN A)
Outcome: https://pubmed.ncbi.nlm.nih.gov/39588730/
1R21DK143519-01

Abstract

Co-investigators: Okut H, Zackula R, Wolfe M, Farley D, Brost B, Grainger DA.
Background: Rising trends in obesity contribute to maternal and neonatal morbidity and mortality with people in under-resourced locations at particular risk of pregnancy-related death. Nearly 3 in 10 U.S. women have pre-pregnancy obesity associated with an increased likelihood to develop diabetes and heart disease during/after pregnancy. Lifestyle modifications and breastfeeding longevity may reverse this effect, though few studies have combined these into one intervention that is delivered using a mobile health application (mHealth app).
Research Aims: To quantify interest in use of the eMOMSTM mHealth app and to measure weight retention and breastfeeding duration through 3 months postpartum among women with pre-pregnancy BMI ≥25 and <35.
Methods: The eMOMSTM study was a feasibility, two-arm, randomized controlled trial (NCT06372860) that included an intervention modelled after the national Diabetes Prevention Program (DPP) and the Office on Women's Health "Your Guide to Breastfeeding" supporting pregnant women with postpartum weight and breastfeeding. The curriculum consisted of 34 short, pre-recorded videos delivered pre- and postnatally, using the eMOMSTM mHealth app with weekly/bi-weekly cellphone follow-up by a certified DPP health coach who was also a Certified Breastfeeding Specialist. Intervention duration was 6 months (i.e., 3 months during and 3 months after pregnancy) with a total of 8 intervention contact hours. The study included two groups: DPP+Breastfeeding+Health Coach vs. care as usual. Outcomes included feasibility of recruitment, retention, and estimates of postpartum weight change and breastfeeding duration.
Results: Between May 1 and December 31, 2024, 125 individuals were screened, 73 were assessed for eligibility, and 49 consented to participate in the study. Of those, 43 eligible participants were randomized to the study: 22 to the intervention group (IG) and 21 to the control group (CG). A total of 6 participants (13.9%) were lost to follow-up and 37 study participants completed the study by July 15, 2025. Preliminary findings indicate that 90% of all videos were watched, the average duration of each phone call was 10 minutes, and most participants interacted with their coach via cellphone calls followed by text messages. At study entry, the mean (SD) age of randomized participants was 31.7 ± 6.5 (IG) vs. 28.1 ± 5.5 (CG); and pre-pregnancy BMI was 30.4 ± 3.4 (IG) vs. 30.3 ± 3.1 (CG). Of those randomized, 13.6% (IG) vs. 47.6% (CG) resided in a rural region; 68.2% (IG) self-identified with being non-Hispanic White followed by 22.7% non-Hispanic Black vs. 90.5% (CG) self-identified with being non-Hispanic White followed by 9.5% as "other;" 28.6% (IG) vs. 33.3% (CG) self-identified with being Hispanic; 40.9% (IG) reported having a high school/GED diploma or some college followed by 22.7% with a Bachelor's degree vs. 47.6% (CG) followed by 33.3% respectively; 45.5% (IG) vs. 23.8% (CG) reported participating in the Special Supplemental Nutrition Program for Women, Infants, and Children (WIC); 50% (IG) vs. 42.9% (CG) reported being a first-time mother; and 45.5% (IG) vs. 23.8% (CG) reported having an immediate biological family member diagnosed with/treated for diabetes. Emerging findings show that IG participants had reduced postpartum weight retention vs. those in control; no differences were noted in breastfeeding duration.
Conclusion: Study findings inform intervention programming; use of a health coach appears to have a positive impact on women's overall health. Future work should focus on integrating mHealth apps into the continuum of health care services. This will allow enhanced access to women with pre-pregnancy overweight/obesity and provide strategies to prevent diabetes and heart disease.

About the Presenter:

Dr. Lisette Jacobson is an Associate Professor in the Department of Population Health at the University of Kansas School of Medicine-Wichita. Dr. Jacobson's research interests include translational and behavioral research in women's health focusing on diabetes education and prevention, breastfeeding, pre- and post-natal health using a life-course approach within community settings. Dr. Jacobson and her team have designed, implemented and evaluated digital public health programs targeting medically underserved pregnant women in rural locations. She is the recipient of several grants and contracts including the National Institute for Diabetes and Digestive and Kidney Diseases (NIDDK), National Institute of General Medical Sciences (NIGMS), Health Resources and Services Administration (HRSA), and the Kansas Department of Health and Environment leading to peer-reviewed publications and presentations. She is also the principal investigator on several ongoing clinical trials. Dr. Jacobson is an active member of NIH's Environmental influences on Child Health Outcomes (ECHO) IDeA States Pediatric Clinical Trials Network (ISPCTN).

Title: Role of Mechanical Stress in Mitigating Chemotherapy-associated Bone Loss
Presenter: Anamaria G. Zavala
Institution: Boise State University
IDeA Program: COBRE (COBRE PI: OXFORD, JULIA)
Outcome: 1R15CA293993-01

Abstract

Scientific focus: First-line platinum-based chemotherapies, such as cisplatin, which are crucial for treatment of breast, ovarian, cervical, and testicular cancers, work through the formation of bulky DNA adducts. However, off-target DNA damage in noncancerous cells leads to debilitating short- and long-term side effects, including bone wasting. Exercise can decrease bone loss; however, cancer related fatigue poses an insurmountable challenge.
Chemotherapy-associated DNA-damage leads to stem cell senescence and, ultimately, to bone wasting. Entry of bone progenitor stem cells into senescence means they will not differentiate into osteoblast cells which are responsible for bone deposition during the normal bone remodeling process. Senescent cells secrete a modified Extracellular Matrix (ECM) which negatively impacts stem cell differentiation capacity and osteogenic potential. We hypothesized that the exercise mimetic Low Intensity Vibration (LIV) could enhance cisplatin-associated DNA damage repair and maintain Mesenchymal Stem Cells (MSC) differentiation capacity.
Aims: 1) Quantify the effects of LIV-induced increases in cisplatin adduct removal on the cell potency and senescence of stromal cells. 2) Determine the effect of LIV-associated changes in osteoblast ECM deposition on the cell potency and senescence of stromal cells.
Methods: LIV requires intact Linker of Nucleoskeleton and Cytoskeleton (LINC) complex, to transduce signals into the nucleus of the cell. Critically, endogenous LINC complex downregulation is associated with breast cancer progression and poorer prognosis, potentially allowing us to specifically target noncancerous cell populations while maintaining chemotherapeutic efficacy. We damaged MSCs with cisplatin (10ìM) and exposed them to LIV (90Hz, 0.7g).
Aim 1) We examined whether LIV alters the kinetics of cisplatin-induced DNA adduct repair in cultured MSCs with intact or disrupted LINC complex. Cells were collected at 0, 24, and 48 hours post cisplatin damage.
Aim 2) After seven days of LIV exposure in osteogenic media we decellularized the ECM and reseeded with MSCs. Reseeded cells were cultured in osteogenic media for 14 days before determining mineralized bone nodule formation.
Outcomes: Aim 1) We show that LINC-mediated mechanotransduction is critical for efficient Nucleotide Excision Repair of bulky DNA adducts. DNA damage removal increases by 20% in LIV treated MSCs compared to non-vibrated samples at 24 hours ($P<0.005$). However, LINC complex disruption significantly reduced removal of cisplatin-DNA adducts ($P<0.001$).
Aim 2) We show that ECM deposited by MSCs +LIV +cisplatin enhances mineralized nodule formation compared to ECM from either MSCs +LIV -cisplatin and -LIV -cisplatin MSCs, which we consider our control set. We found that average MSC nodule area increases by 2-fold on +LIV -cisplatin ECM compared to control ECM (-LIV -cisplatin) ($P<.01$). This recapitulates previous findings that LIV enhances MSC osteogenesis. Average nodule area formed on ECM deposited by MSCs +LIV +cisplatin increases 6-fold compared to MSCs seeded onto control ECM (-LIV -cisplatin) ($P<0.001$) and a 2-fold increase over MSCs seeded onto +LIV -cisplatin ECM ($P<0.01$).
Conclusions: These findings suggest a novel interaction between mechanical stimulation, the DNA damage response, and ECM homeostasis. LIV may be sufficient to preserve stem cell differentiation capacity after cisplatin exposure and prevent chemotherapy-associated bone wasting. We suggest that LINC-mediated mechanoregulation of stem cell senescence may lead to novel nonpharmacological therapeutic approaches to combat deleterious musculoskeletal conditions associated with off-target effects during anti-cancer treatment.

About the Presenter:

Dr. Anamaria Zavala is an Assistant Research Professor in the Mechanical Adaptations Lab, Department of Mechanical and Biomedical Engineering at Boise State University. She has an extensive background studying DNA damage and repair in both healthy and disease systems. Dr. Zavala developed a novel immunoprecipitation protocol to map UV-induced DNA adducts across the human genome revealing sequence features and dynamic chromatin changes associated with adduct hotspots and initiation of Nucleotide Excision Repair (NER).

Title: The Contribution of Microplastics to Pregnancy Complications in Women of Hawaii
Presenter: Johann Urschitz, PhD
Institution: University of Hawaii, Honolulu
IDeA Program: COBRE (COBRE PI: WARD, WILLIAM)
Outcome: https://pubmed.ncbi.nlm.nih.gov/39634179/

Abstract

Microplastics (MPs) entering the bloodstream of pregnant women can accumulate in placental and umbilical cord tissue and potentially reach the developing fetus. Fetal development is a crucial window of susceptibility in which exposure may lead to detrimental health outcomes at birth and later in life. We investigated the presence, accumulation, and biological effects of MPs and micropollutants in human placentas, focusing on pregnancies affected by fetal growth disorders as well as normal gestation. In a comprehensive study of 150 term placentas from the Hawaii Reproductive Biospecimen Repository, we collected samples from three groups: appropriate for gestational age (AGA) controls, fetal growth restriction (FGR), and macrosomia. Using a controlled digestion and filtration protocol paired with micro Raman spectroscopy, we identified multiple MPs and micropollutants in the examined placentas. While overall microplastic concentrations did not differ significantly between groups, polymer-associated dyes were markedly elevated in cases of macrosomia, whereas iron oxide particle accumulation was highest in placentas from fetuses with fetal growth restriction (FGR). These findings suggest a potential association between differential micropollutant accumulation and disrupted fetal growth trajectories, implicating obesogenic effects of synthetic dyes in macrosomic outcomes and toxicant-related placental dysfunction in FGR. We observed variations in microparticle burden over time and across demographic factors such as maternal residential zip code. These findings suggest that MPs and associated chemical additives may contribute to adverse placental and pregnancy outcomes in women delivering in Hawaii. We further examined the capacity of polystyrene MPs (PS-MPs) to cross the placental barrier using term chorionic villi explants. The explants were exposed to 100 µg/mL of 5 µm-size polystyrene (PS)-MPs for 72 h, and their internalization was analyzed by optical microscopy, confocal atomic force microscopy (CAFM) and fluorescence confocal imaging. Syncytiotrophoblasts internalized PS-MPs, which translocated into the deeper villous compartments without compromising epithelial barrier integrity. Confocal microscopy revealed PS-MPs enveloped by actin filaments, indicative of active cytoskeletal remodeling and suggesting uptake via macropinocytosis or phagocytic pathways. To evaluate the biological impact of microplastics, cytotoxicity was assessed using the lactate dehydrogenase (LDH) release assay. Reactive oxygen species formation and antioxidant activity were evaluated using biochemical assays. Metabolomic profiling was performed using proton nuclear magnetic resonance. Our analysis of cytotoxicity, oxidative stress, and metabolic changes revealed that PS-MP exposure induced significant time-dependent cytotoxicity and elevated reactive oxygen species (ROS), including mitochondrial superoxide and hydrogen peroxide. This exposure also disrupted antioxidant defenses, decreasing superoxide dismutase and catalase activities while increasing glutathione peroxidase activity. Additionally, we observed a significant rise in markers of oxidative damage, specifically malondialdehyde and carbonylated proteins. Furthermore, metabolomic profiling highlighted broad metabolic dysregulation, with disruptions in the tricarboxylic acid (TCA) cycle and pathways related to folate, amino acid, and energy metabolism. Together, our findings provide strong evidence of MPs, micropollutant, and associated chemicals accumulation in human placentas, their ability to cross the placental barrier, and their capacity to induce oxidative stress and metabolic disturbances. These results highlight potential mechanisms by which environmental MPs and other micropollutants could adversely affect placental function and fetal development, emphasizing the urgent need for further investigation into their health implications during pregnancy.

About the Presenter:

Dr. Johann Urschitz is an Associate Professor at the Yanagimachi Institute for Biogenesis Research, John A. Burns School of Medicine at University of Hawaii, Honolulu. The Urschitz laboratory works towards understanding the molecular mechanisms mediating the aberrant programming of the fetus, which can impact an individual's health into adulthood. The placenta, facilitating nutrient exchange between maternal and fetal circulation, plays a pivotal role in regulating fetal growth and development. Perturbations to fetal growth may lead to poor neonatal outcomes and long-term health issues such as obesity, type 2 diabetes, cardiovascular disease, and neurodevelopmental disorders in the offspring. However, the mechanisms linking in utero nutrient disturbances, fetal growth and disease development later in life are poorly understood and specific clinical treatments are lacking. Dr. Urschitz initiated the investigation of microplastic (MP) particles within the placenta and related implications for pregnancy complications. An initial groundbreaking study of the placental microenvironment of women in Hawaii aimed to uncover how MP pollution affects cellular and molecular pathways, potentially contributing to complications such as fetal growth abnormalities or preeclampsia. These studies aim to enhance our knowledge of placental function, fetal development, and the effects of environmental factors on maternal and child health.

Title: Communities "setting the stage" for improved maternal health in southwest Alaska
Presenter: Lisa Schwarzburg, PhD
Institution: University of Alaska Anchorage
IDeA Program: INBRE (PI: BARNES, BRIAN)
Outcome: 1R16GM159729-01

Abstract

Scientific Focus: As in the U.S., Alaska's increased maternal mortality (63% between 2014-2018) has been associated with substance use disorders (SUD) at disproportionally higher levels in Alaska's rural southwest communities. To address the underlying connections between women with SUD and maternal health services, research and preventative care designs must be tailored to the local community. Development of locally appropriate research and intervention strategies requires engaging the local communities and health care agencies as local stakeholders—particularly with sensitive and unexplored issues of intersectional populations. With these aims in mind, this presentation will present the '*Setting the Stage'* project through the following sections:
Objectives: Designing locally-appropriate health research requires engaging community stakeholders. This INBRE sponsored project funded through 2023-24 NIH Women's Health Administrative Supplemental award laid the groundwork for maternal health study in southwest Alaska with local women in Maternal Health Community Advisory Boards (MH-CABs).
Methods: MH-CABs were created in five communities - Bethel, Dillingham, Kodiak, Sand Point, and Unalaska – using adapted NIH-based toolkit. The university team facilitated meetings as MH-CABs provided insight for research design. Local-based student knowledge-gatherers were also recruited/trained for further community involvement.
Outcomes: Facilitating effective MH-CAB meetings proved challenging with competing time interests of MH-CAB members, seasonal weather delays, student recruitment, and navigating university infrastructure. However, each MH-CAB identified specific areas of maternal health concerns to include in planned study.
Conclusions: MH-CABs allow for meaningful community engagement to facilitate more comprehensive understanding of local drivers of maternal health. While still in the preliminary stages, the insights gained through community-led engagement have been integral to culturally-appropriate study design for future CBPR and plans for the next phase: developing strategies.

About the Presenter:

Dr. Schwarzburg is an assistant professor of Health Sciences specializing in circumpolar, environmental and Alaska Native health research—and among courses taught in the undergraduate program. With a background in medical anthropology and health policy, she seeks to facilitate community-based decisions on environment, food ways and birth ways, while exploring varied ways of knowing.

Title: Midwest Birth Outcomes and American Indian Pregnancy
Presenter: Anna Strahm, PhD
Institution: University of South Dakota/Sanford research
IDeA Program: COBRE (COBRE PI: WEIMER, JILL)
Outcome: https://pubmed.ncbi.nlm.nih.gov/38330371/

Abstract

The U.S. Healthy People 2030 Objectives include reducing rates of infant mortality from 5.4 to 5.0 live births per 1000, and preterm births from 10.1% to 9.6%. However Indigenous American (IA) women residing in the Midwest continue to experience disproportionately high rates of infant mortality (8.8 per 1000 live births) and preterm birth (12.8%). Identified risk factors, such as diet and access to healthcare, do not fully explain these persistent perinatal-health disparities. Therefore, studies have begun to focus on culturally-relevant psychosocial stressors such as past trauma to try to understand the occurrence of adverse pregnancy outcomes. Although reduced resources may have long reaching impact, affecting new generations of IA women and their pregnancies, there is a paucity of psychosocial research on IA perinatal health. It is imperative that associations among IA trauma, proximal stressors, and psychophysiological stress responses during pregnancy are better understood to develop more effective interventions that can improve IA perinatal health. Past trauma may contribute to IA perinatal health disparities by increasing the frequency and intensity of proximal stressors and by influencing psychophysiological responses to stress. To examine these interactions, we are continuing to recruit 100 pregnant IA women from Sanford Health prenatal care providers to complete a lab visit between 23-28 weeks of pregnancy. While at the lab they 1) participate in a standardized laboratory psychophysiological challenge task while blood pressure and heart rate are recorded and saliva is collected to assess cortisol, immunoglobulin A, and c-reactive protein responses; 2) complete standardized surveys on past trauma, and proximal stress, mental and general health, and wellness behaviors; and 3) provide consent to access medical records pertaining to prior and current pregnancy health and perinatal outcomes.
During the funded period we experienced delays in the supply chain, receiving funding, IRB approval, and research contract and approval at the sub-contract location. With these issues we were able to recruit 50 participants across Fargo North Dakota and Sioux Falls South Dakota so far. At the end of the NIH funded period we did not have the minimum data needed for testing our specific aims via a series of hierarchical and interaction regression analyses: 1) to determine if past trauma is associated with IA women's psychophysiological responses to acute stress during pregnancy; 2) to identify the degree to which trauma is associated with increased risks of adverse pregnancy health and perinatal outcomes in IA pregnancies; and 3) to examine how past trauma, psychophysiological responses to stress, and proximal maternal stress may interact to predict adverse perinatal outcomes in IA pregnancies. The PI opted to continue with the study, leveraging additional recruitment mechanisms and approaches derived from the experiences of the funded period. These include direct invitation via MyChart messaging and posting flyers for the study at prenatal service providers outside of Sanford Health in the Fargo and Sioux Falls regions. To date the planned manuscripts are awaiting data, however, information from this project was used as part of a presented symposium.

About the Presenter:

Dr. Anna Strahm is an Assistant Professor in OBGYN & Pediatrics at Sanford School of Medicine, University of South Dakota, and an Assistant Scientist at Sanford Research. She specializes in psychophysiology during pregnancy, with special attention to blood glucose regulation. Dr. Strahm's passion for research in human reproduction and health outcomes began in 2013 and has included projects examining interactions between stress and health to discern the pathways by which stress affects health, with emphasis on reproductive health and health disparities. She earned her Health Psychology PhD from North Dakota State University in 2020 before working on a NIDCR T32 at The Ohio State University. Since 2022, Dr. Strahm has been leading research in psychophysiology during pregnancy across the Sanford Health service area, with lab space in Sioux Falls, SD, and Fargo, ND. The Strahm Lab focuses on the interactions between stress and health to discern the pathways by which stress affects health, with emphasis on reproductive health and health disparities. Current projects are designed to assess the associations between maternal psychosocial experiences and blood glucose metabolization during pregnancy and how these factors influence pregnancy and postpartum health, as well as the developmental outcomes of their children.

ADDITIONAL ABSTRACTS

TITLE: STRETCH-ACTIVATION BASED COMBINATION TOCOLYSIS TO PREVENT PRETERM BIRTH
PROJECT LEAD (SUPPLEMENT): IAIN BUXTON, PHARM.D. (3P20GM103440-22S2)
INSTITUTION: UNIVERSITY OF NEVADA RENO SCHOOL OF MEDICINE
IDEA PROGRAM: INBRE (PI: BAKER, JONATHAN)

GOAL: We seek to discover safe and effective drug-like small molecules for obstetric patients in risk of delivering preterm by gathering preclinical evidence in human cells and tissues and employing an animal model of prematurity. There is a need for tailored treatment of preterm labor to meet the unmet needs of pregnant patients.
THE PROBLEM: Preterm Birth (PTB) is a global problem with preemies at risk for major disability. PTB occurs in 10-15% of US pregnancies depending on geographic location and maternal ethnicity, with Black mothers disproportionally affected. Knowledge of the regulation of birth timing is incomplete. We posit that non-infectious idiopathic (e.g., spontaneous) preterm labor (sPTL) involves dysfunctional myometrial relaxation signaling. Tocolytics currently in use are ineffective for either acute or maintenance tocolysis, have unwanted effects on mother and fetus that limit dosing, and none are FDA approved. Tocolytics to prevent preterm birth must be based on myometrial relaxation mechanisms unique to preterm tissue. Effective tocolysis is an urgent and unmet need exacerbated because the regulation of uterine relaxation is disparate from that of other smooth muscles.
BACKGROUND: In a fascinating exception to the dogma surrounding the action of nitric oxide (NO) in smooth muscle, NO-mediated relaxation of human myometrium is cGMP-independent and instead, is the result of S-nitrosation of contractile-associated proteins. In women delivering spontaneously preterm, NO-mediated relaxation is severely blunted suggesting dysfunctional relaxation mechanisms. Proteomic analysis of the S-nitroso proteome in human preterm myometrium reveals the presence of distinct S-nitrosation differences. We previously described for the first time, the expression of two stretch-activated ion channels in the human uterine myocyte. TREK-1, an outwardly rectifying potassium channel that is activated by the phenmethyl-phenylethyl-piperidinecarboxylic acid compound ML335. And Piezo1, an inwardly rectifying calcium channel activated by the thiadiazole derivative Yoda 1.
HYPOTHESIS: Piezo1-mediated NO generation in the microvascular endothelial cell compartment of human myometrium establishes and maintains human uterine quiescence. NO, acting as S-nitrosoglutathione (GSNO) in the myocyte, S-nitrosates the TREK-1 potassium channel, resulting in membrane hyperpolarization below the threshold for contraction. A Piezo1 agonist, together with blockade of GSNOR and small molecule activation of TREK-1, will reverse the dysfunctional relaxation of human sPTL tissues to NO. In a mouse model of parturition, the combined effect of Piezo1 activation, GSNOR inhibition, and TREK-1 activation will provide preclinical evidence of effective combination tocolysis to prevent PTB.
APPROACH and RESULTS: In electrophysiological patch-clamp studies with freshly isolated CD31 negative human uterine smooth muscle cells, we demonstrate that the outwardly rectifying potassium channel TREK-1 is directly activated by S-nitrosation. Activation of TREK-1 by the small molecule agonist ML335 is potentiated by S-nitrosation. Paradoxically, activation of the inwardly rectifying calcium channel Piezo1 with yoda1 relaxes oxytocin-stimulated human myometrial tissue in organ bath experiments. Blockade of S-nitroso glutathione reductase (GSNOR) by the cyanobenzyl-oxothieno-benzoic acid derivative SPL334 that reduces the availability of NO by metabolism of GSNO, lowers the IC50 for Piezo1-mediated relaxation. GSNO-mediated relaxation of human myometrium is potentiated by SPL334 together with the activation of TREK-1 by ML335. Addition of Yoda 1, ML335 and SPL334 as a triple cocktail profoundly relaxes human myometrium suggesting the possibility of synergism. Treatment of C57BL/6j mice in the RU486 model of preterm birth significantly prolongs gestation.
IMPACT: The impact of this research has justified the development of effective tocolytics to prevent PTB. This has been accomplished by establishing Piezo1-mediated activation of myometrial TREK-1 by S-nitrosation, and that small molecule activators of the channel potentiate TREK-1, and that GSNOR blockers promote S-nitrosation of TREK-1. Our approach provides a rational basis for combination tocolysis with Yoda1, ML335, and GSNOR blockade. The potential of this research to fill gaps in our understanding of stretch-activated relaxation signaling in human myometrium, further characterized in an animal model of PTB, is significant. If administration of triple cocktail can reverse the dysfunctional relaxation of sPTL myometrium to NO, it supports the continued support of our research toward development of a therapeutic alternative for the prevention of preterm birth.
OUTCOME: This Women's Health Research Supplement funding has allowed us to gather the necessary preliminary data to support the submission of an R01 application (HD119793) recently reviewed by the ATB study section. The project received a 21-percentile priority score.

TITLE: AN UPDATE ON THE DELAWARE INBRE WOMEN'S HEALTH SUPPLEMENT: DETECTING EPIGENETIC SIGNATURES TO IMPROVE THE PREVENTION OF TRIPLE-NEGATIVE BREAST CANCER
PROJECT LEAD (SUPPLEMENT): JENNIFER SIMS-MOURTADA (3P20GM103446-23S3)
INSTITUTION: UNIVERSITY OF DELAWARE
IDEA PROGRAM: INBRE (PI: DUNCAN, MELINDA)

Jennifer Sims-Mourtada, Ross Budziszewski, Lisa Frerichs, Ashley Stewart, Caitlin Mbuakoto, Madolyn L. MacDonald, Shawn W. Polson, Yuchen Zhang, Scott D. Siegel.

Background:
Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer with limited treatment options that is more difficult to detect at early stages. Delaware is among the states with the highest incidence of TNBC in the US. We recently identified geographic "hotspots" within the state that have significantly elevated TNBC incidence. Subsequent research provided preliminary evidence that neighborhood effects, or the context within which the patients resided, contributes to the elevated TNBC risk. More specifically, neighborhood characteristics that promote greater exposure to potentially modifiable risk factors such as obesity, limited breastfeeding, alcohol use, and environmental toxins was associated with TNBC risk even after adjusting for patient characteristics. We hypothesize that the cumulative effect of these exposures drives epigenetic reprogramming of circulating immune cells, creating a pro-inflammatory and immunosuppressive microenvironment that promotes TNBC development. This pilot study aimed to evaluate whether breast cancer patients living in TNBC hotspot regions exhibit distinct epigenetic changes in circulating immune cells compared to those from non-hotspot areas.
Methods:
Peripheral blood mononuclear cells (PBMCs) were collected from 32 treatment-naïve breast cancer patients enrolled at the Helen F. Graham Cancer Center & Research Institute and stratified by residential address into TNBC hotspot (n=16) and non-hotspot (n=16) groups, based on geospatial clustering of TNBC incidence in Delaware. Genomic DNA was extracted and profiled using the Twist Human Methylome Panel, targeting over 3.2 million CpG sites across the genome. Methylation data were analyzed using the nf-core methylseq v2.6.0 pipeline and methylKit v1.25 in R.
Results:
A total of 12,200 CpG sites showed significant differences in methylation load, with 5,202 hypomethylated and 6,998 hypermethylated sites in patients from hotspot regions compared to those in non-hotspot regions. Principal component analysis (PCA) of differentially methylated genes and promoters (FDR < 0.05; ≥3% methylation difference) revealed clear clustering by geographic hotspot status. Differentially methylated genes included those involved in immunosuppression, inflammation, and cytokine signaling.
Conclusions:
This pilot study found epigenetic changes in immune-related genes among women residing in TNBC hotspot neighborhoods, supporting a potential link between cumulative exposure and immune reprogramming. Further research is needed to determine the clinical significance of these changes and their role in driving breast cancer outcomes.

TITLE: NON-PUNGENT CAPSAICIN ANALOGS IN OVARIAN CANCER THERAPY.
PROJECT LEAD (SUPPLEMENT): DASGUPTA, PIYALI (3P20GM103434-23S1)
INSTITUTION: MARSHALL UNIVERSITY
IDEA PROGRAM: INBRE (PI: RANKIN, GARY)

BACKGROUND:
Ovarian cancer is the leading cause of death in women diagnosed with gynecological cancers. Despite the improvements of treatment of ovarian cancer, the prognosis and overall survival rates remain dismal. Such sobering statistics define the arena where novel molecular targets and therapies are urgently required to treat this lethal malignancy. The long-term objective of my laboratory is to investigate the anti-tumor activity of capsaicin (the spicy pungent component of chili peppers) in human cancers. The clinical application of capsaicin as a viable anti-cancer drug remain problematic due to its adverse side effect profile. Such drawbacks may be circumvented by the identification of non-pungent capsaicin analogs, which display potent anti-cancer activity. Structure-activity relationship (SAR) studies have revealed that the addition of long chain unsaturated fatty acyl groups to the C-terminus of capsaicin yields non-pungent analogs with potent pain-relieving activity. These capsaicin analogs are referred to as N-acetyl vanillylamide capsaicin analogs (hereby referred to as N-AVAMs). The central hypothesis of our grant application was that non-pungent N-AVAMs will display anti-tumor activity in ovarian cancer *in vivo* and sensitize ovarian cancer cells to gemcitabine-induced apoptosis. We proposed three specific aims to test our hypothesis:
Specific Aim 1. To determine the anti-cancer and chemosensitization activity of N-AVAMs in chicken CAM models of ovarian cancer

Specific Aim 2. To investigate the anti-tumor activity of the top two N-AVAMs in PDX models of ovarian cancer.
Specific Aim 3. To investigate the combinatorial anti-tumor activity of cisplatin with the top two N-AVAMs in PDX models of ovarian cancer.
RESULTS: We screened six N-AVAMs (at a concentration of 10μM) in three ovarian cancer cell lines OVCAR-3, A2780 and SKOV3 (Figure 1). We observed that Dohevanil produced the maximal decrease in cell viability in OVCAR-3, A2780 and SKOV3 human ovarian cancer cell lines over 24 hours. An interesting observation was that Dohevanil had no impact on the viability of normal human lung epithelial cells, kidney epithelial cells and hepatocytes. Based on these results, Dohevanil was selected as our "HIT COMPOUND". Dohevanil displayed robust anti-tumor activity in chicken chorioallantoic membrane (CAM) models. The experiments using CAM models were performed by Dr. Chen's laboratory (Bluefield State University). Currently, we are investigating the anti-cancer activity of Dohevanil in human A2780 tumors xenografted on SCID mice. Dr. Chen's laboratory also showed that dohevanil sensitize A2780-cisplatin resistant human ovarian cancer tumors towards the pro-apoptotic activity of the chemotherapeutic drug gemcitabine, using chicken CAM models. Our future studies will examine the combinatorial anti-cancer activity of gemcitabine and dohevanil in cisplatin-resistant ovarian cancer tumors, using SCID models.
PUBLICATIONS:

1) Brown, K.C., Sugrue, A.M., Modi, K.J., Light, R.S., Conley, K.B., Cox, A.J., Bender, C.R., Miles, S. L., Valentovic, M.A., Dasgupta, P. (2024) An Experimental Protocol for the Boyden Chamber Invasion Assay with Absorbance Readout. *Bio Protoc.*14, e5040.
2) Brown, K.C., Sugrue, A.M., Conley, K.B., Modi, K.J., Light, R.S., Cox, A.J., Bender, C.R., Miles, S.L., Denning, K.L., Finch, P.T., Hess, J.A., Tirona, M.T, Valentovic, M.A., Dasgupta, P. (2024) Anti-cancer activity of capsaicin and its analogs in gynecological cancers. *Adv. Cancer Res*. 164, 241-2

TITLE: LIFE-COURSE BIOLOGICAL AND LIFESTYLE FACTORS IN WOMEN'S EXCESS RISK OF HEART FAILURE IN TYPE 2 DIABETES
PROJECT LEAD (SUPPLEMENT): YILIN YOSHIDA, PHD, MPH (3U54GM104940-08)
INSTITUTION: LSU PENNINGTON BIOMEDICAL RESEARCH CENTER
IDEA PROGRAM: IDEA-CTR (PI: KIRWAN, JOHN)

Scientific focus of the project:
Heart failure (HF) is a prevalent and serious complication of type 2 diabetes (T2D), increasingly affecting individuals under the age of 65. Notably, women with T2D face a disproportionately greater excess risk of HF compared to their male counterparts—a disparity that remains inadequately understood. One potential contributor to this sex difference is the higher prevalence and incidence of early-onset T2D (diagnosed before age 40) among women. The cumulative exposure to hyperglycemia and metabolic dysfunction may accelerate cardiac remodeling and dysfunction during midlife–key precursors to HF. Despite this, there is limited research examining how the life-course burden of T2D contributes to HF risk, particularly through a sex-specific lens. Additionally, although the cardiovascular benefits of physical activity are well established, most studies rely on a single time-point measurement of physical activity, typically assessed in mid- or later adulthood when cardiovascular disease may already be present. There is a critical need for studies that capture the dynamic nature of physical activity and other risk factors across the life course. Furthermore, sex-specific patterns in physical activity trajectories and their influence on HF risk among individuals with T2D are largely understudied.
To address these important gaps, we propose the following aims. Aim 1. Characterize sex differences in the longitudinal burden of early-onset T2D on cardiac dysfunction and remodeling. Aim 2. Characterize sex differences in the role of physical activity in cardiac dysfunction and remodeling in T2D. Additional Aim (not included in the original proposal). To enhance understanding of the molecular mechanisms underlying the HF risk in men and women with T2D, we explored sex differences in the metabolomics signature of cardiac dysfunction and remodeling among individuals with T2D.
Methods:
We primarily used data from the Coronary Artery Risk Development in Young Adults (CARDIA) study, including ~3,500 young Black and White men and women with indicators for T2D onset, repeated measures of metabolic risk factors, physical activity, and echocardiographic assessments over three decades. For the additional aim, we included the metabolomics and echocardiographic data from the ARIC study (Atherosclerosis Risk in Communities) and the Hispanic Community Health Study/Study of Latinos (HCHS/SOL). We employed longitudinal analyses, including mixed linear modeling and survival analysis for the proposed aims, accounting for traditional cardiovascular risk factors and female-specific risk factors, such as parity, pregnancy complications (e.g., gestational diabetes, preeclampsia/hypertension), and menopause related factors (e.g., age at menopause, hormone therapy use).
Results:
Based on the CARDIA data, we found that cumulative exposure to cardiometabolic risk factors, such as sustained high glucose, insulin resistance, dyslipidemia, and hypertension, significantly contributes to midlife cardiac remodeling and dysfunction among

young adults with or at risk for T2D, but the results did not differ between sexes.[1] In a separate analysis, we observed that young adults with prediabetes who progressed to overt T2D had the poorest trajectories of cardiovascular health, as measured by the American Heart Association's Life's Essential 8 (LE8) metrics, including physical activity, compared to those who remained prediabetic or reverted to normoglycemia over 25 years of follow-up. The unfavorable trajectories of physical activity and other LE8 components were particularly pronounced among prediabetic women who progressed to T2D compared to their male counterparts. Moreover, we found that maintaining an optimal physical activity level was significantly associated with a lower risk of progression from prediabetes to T2D in both young women and men.[2] These findings highlight the need for earlier and more proactive interventions, particularly among young women with or at risk for T2D, focused on maintaining optimal physical activity levels to prevent progression of T2D and complications. Analyses from the ARIC and HCHS/SOL cohorts revealed that circulating metabolites associated with microvascular complications (e.g., retinopathy and kidney disease) were significantly linked to cardiac remodeling, dysfunction, and subsequent HF risk in both women and men with T2D. These results support the hypothesis that microvascular dysfunction plays a key role in the pathogenesis of HF across sexes in individuals with T2D.[3]
*Indicating corresponding author.

1. *Yoshida Y, Zu Y, Fan BB, Li S, Yoshida T, Harville E, Zhang T, Bae S, Shikany J, Fonseca V. Cumulative Effect of Risk Factors and Left Ventricular Geometry and Function in Men and Women with Early-Onset Diabetes. Diabetes Obes Metab. 2024. doi: 10.1111/dom.15681. PMID: 38837542.
2. Lovre D, Zu Y, and *Yoshida Y, *Abstract MP39: Life Essential 8 Is Associated with A Lower Risk Of Progression To Type 2 Diabetes.* Circulation, 2025. 151(Suppl_1).
3. *Yoshida Y, Nguyen NQH, Moon EH, Mauvais-Jarvis F, Fonseca V, Yoshida T, Rebholz C, Skali H, Arthur V, Echouffo-Tcheugui J, Ballantyne C, Selvin E, Shah A, and *Yu B. A Metabolomic Study of Cardiac Dysfunction and Remodeling in Diabetes. Diabetes Care. 2025 Jul 29:dc250730. doi: 10.2337/dc25-0730. Epub ahead of print. PMID: 40729824.

TITLE: AGE-INDUCED REPROGRAMMING OF THE TRANSCRIPTIONAL, SIGNALING, AND METABOLIC LANDSCAPES IN MURINE BONE MARROW MESENCHYMAL STEM CELLS
PROJECT LEAD (SUPPLEMENT): ANJA NOHE, PHD (3P20GM139760-04S3)
INSTITUTION: UNIVERSITY OF DELAWARE
IDEA PROGRAM: COBRE (PI: ELLIOTT, DAWN)

Osteoporosis is a progressive skeletal disease marked by reduced bone mass, deterioration of bone microarchitecture, and increased fracture risk. It disproportionately affects women, particularly after menopause, when hormonal changes accelerate bone loss. A major driver of this condition is an age-associated imbalance in bone marrow mesenchymal stem cell (BMSC) differentiation. In youthful bone, BMSCs predominantly generate osteoblasts, supporting bone formation and structural integrity. With aging, however, this balance shifts toward adipogenesis, leading to reduced osteoblastogenesis, impaired bone formation, and an altered marrow microenvironment that further accelerates skeletal degeneration. Aim 1 focused on age-related changes in the spatial distribution and ligand-binding behavior of bone morphogenetic protein receptor type Ia (BMPRIa) in BMSCs and bone tissue. To directly visualize receptor–ligand interactions, we conjugated recombinant BMP2 to quantum dots (QDs) and performed high-resolution colocalization analysis in young (6-month) and aged (15-month) C57B/L6 mice. In aged mice—representative of the postmenopausal stage in women—BMP2 retained its ability to bind BMPRIa, but this occurred in plasma membrane regions lacking mineralization, revealing a misalignment between receptor localization and osteogenic microdomains. Disrupting cholesterol-rich lipid raft domains with β-cyclodextran restored BMP2-mediated signaling, as indicated by Smad1/5/8 phosphorylation, demonstrating that BMPRIa mislocalization within altered membrane architecture, rather than loss of ligand-binding capacity, is a key contributor to impaired BMP2 signaling in aging bone. Aim 2 investigated the downstream molecular effects of BMP2 pathway modulation. Young and aged mice were injected with BMP2, the CK2.3 peptide, or the control peptide CK2.1. Femurs were harvested, sectioned, and analyzed via spatial transcriptomics using the Xenium platform. BMSCs were isolated and stimulated with BMP2, CK2.3, or CK2.1 for phosphoproteomic profiling. Tandem-mass-tag LC-MS/MS analysis of BMSCs revealed 256 proteins upregulated and 118 downregulated in aged cells (FDR < 0.05). Functional enrichment indicated elevated PPAR signaling and fatty acid metabolism, with reduced activity in proteasomal degradation, cell cycle control, and Runx2-driven osteogenesis. Collectively, these findings identify membrane microdomain organization and receptor localization as novel regulatory mechanisms underlying impaired osteogenesis, and pinpoint molecular targets for therapeutic intervention. Given that osteoporosis poses a significant public health challenge for women—especially in the postmenopausal period—this work provides critical mechanistic insights that could inform the development of targeted strategies to preserve skeletal health and reduce fracture risk in aging women.

TITLE: PERSONALIZING DISORDERED EATING TREATMENT USING MOBILE TECHNOLOGY: SELF-GUIDED, PERSONALIZED TREATMENT FOR WOMEN
PROJECT LEAD (SUPPLEMENT): CHERI LEVINSON, PHD (3P20GM103436-23S1)
INSTITUTION: UNIVERSITY OF LOUISVILLE
IDEA PROGRAM: INBRE (PI: BICKFORD, MARTHA)

Scientific Focus: Eating disorders (EDs) are serious mental illnesses, with the second highest mortality rate of any psychiatric disorder. EDs disproportionally impact women, with estimates suggesting that up to 95% of people diagnosed with anorexia nervosa (AN) and atypical AN (A-AN) are women. Despite the high prevalence, impairment, and personal and societal costs, treatments for EDs and DE are subpar, with most treatments only relieving symptoms for 50% of individuals. These response rates are likely low due to substantial variability in ED symptoms and their underlying causes. Further, these response rates are complicated by the fact that treatments for DE are often hard to access, providers specialized in DE are in shortage, and insurance will often not cover this type of specialized care. The current project aims to fill these gaps by creating and implementing a self-guided mobile intervention that is personalized to the individual and delivered directly to the patient.
Aims: Our project has two aims: First, to collect preliminary data on the feasibility, acceptability, and user uptake of a personalized self-guided mobile intervention for DE. Second, to test the initial clinical efficacy of a personalized self-guided DE digital intervention. We hypothesized that our digital intervention will be rated as highly feasible and acceptable, that we will have high retention, and that there will be high uptake (i.e., module completion; time spent on the application). In addition, we hypothesize that our personalized self-guided digital intervention for DE will decrease symptoms of DE, anxiety, depression, and clinical impairment and increase quality of life.
Methods: A total of 50 women with moderate to high DE are participating in the study. All aspects of the study are being conducted remotely across the US. First, we are testing the initial acceptability and feasibility of our four digital modules (n = 5 each). We will measure user uptake by percent of women who engage with and complete modules, assessment of time spent on each module (automatically tracked through our digital system), and time reported spent on each exercise. Then, we are testing the initial clinical efficacy (n = 30) of the four digital modules using measures to assess change in DE (Eating Disorder Examination Questionnaire – 6), related mental health problems (Beck Depression Inventory-2 and Penn State Worry Questionnaire), quality of life (Quality of Life Scale), and impairment (Clinical Impairment Assessment). Finally, we will randomly select 10 participants to complete an online focus group in which we will assess for acceptability, satisfaction, and helpfulness of the digitally-delivered modules.
Outcomes: Our project is currently being tested. Recruitment for Aim 1 is currently underway and is expected to be completed by October 2025. Recruitment for Aim 2 is set to begin in November 2025, with data collection complete by February 2026. Results for the study are anticipated in early Spring 2026.

TITLE: BEHAVIORAL, METABOLIC, AND MOLECULAR EFFECTS OF CONGENITAL AND ADULT-ONSET ESTROGEN DEFICIENCY IN FEMALE MICE: INSIGHTS FOR THERAPEUTIC TARGETING
PROJECT LEAD (SUPPLEMENT): REILLY T. ENOS, PHD (3P20GM103641-10S1)
INSTITUTION: UNIVERSITY OF SOUTH CAROLINA-SCHOOL OF MEDICINE, COLUMBIA, SC
IDEA PROGRAM: COBRE (PI: NAGARKATTI, PRAKASH)

Objective: To compare the behavioral, metabolic, and molecular consequences of congenital/complete (aromatase knockout, AROM KO) and adult-onset/partial (ovariectomy, OVX) estrogen deficiency in female mice, and to validate common phenotypes to guide model selection and therapeutic target identification.
Methods: Female AROM KO, OVX, and wild-type (WT) C57BL/6 littermates were fed a low-fat (LFD) or high-fat diet (HFD) for 17 weeks. Body composition, energy expenditure, physical activity, respiratory exchange ratio (RER), glucose metabolism, and gene expression (microarray and qRT-PCR) in adipose tissue and skeletal muscle were assessed.
Results: Both AROM KO and OVX mice exhibited increased adiposity, reduced physical activity (>40% reduction in ambulatory movement, ≥70% reduction in wheel running), and elevated respiratory exchange ratio, indicating decreased fat oxidation. AROM KO mice displayed higher baseline body weight as well as hyperglycemia and hyperinsulinemia compared to OVX mice, reflecting more severe effects of complete estrogen loss. Transcriptomic analyses revealed downregulated metabolic pathways (e.g., TCA cycle, fatty acid metabolism) and upregulated inflammatory pathways in adipose tissue of AROM KO mice, with similar but less pronounced changes in OVX mice upon qRT-PCR confirmation. Skeletal muscle showed downregulation of exercise-responsive (NR4A3), insulin-signaling (IRS1), metabolic (PCX), and antioxidant (GPX3) genes with estrogen deficiency, implicating impaired energy metabolism and increased oxidative stress in metabolic dysfunction. Unexpectedly, total energy expenditure was comparable across groups despite reduced activity.

Conclusion: Congenital and adult-onset estrogen deficiency share common phenotypes (increased adiposity, reduced physical activity, decreased RER), but congenital deficiency induces more severe metabolic impairments. These findings validate both models for studying estrogen deficiency and highlight potential therapeutic targets (NR4A3, GPX3, PCX, and IRS1) for mitigating estrogen-deficient-related metabolic dysfunction.

TITLE: ESTROGEN DISRUPTS AN ADHERENS JUNCTION - ASSOCIATED RNAI MACHINERY TO PROMOTE PRO-FIBROTIC AND PRO-INFLAMMATORY PHENOTYPES
PROJECT LEAD (SUPPLEMENT): ANTONIS KOURTIDIS, PHD (3P20GM130457-04S1)
INSTITUTION: MEDICAL UNIVERSITY SOUTH CAROLINA, CHARLESTON, SC
IDEA PROGRAM: COBRE (PI: DUNCAN, STEPHEN)

Fibrotic and inflammatory conditions with severe clinical manifestations in the gastrointestinal tract, such as Scleroderma (SSc) and Crohn's Disease (CD), are significantly more prevalent in women, posing substantial challenges to their health and well-being. However, the mechanisms explaining the higher incidence and severity of these diseases in women are still not well-understood. A common feature of these conditions is compromised epithelial integrity. The adherens junctions are E-cadherin – based cell-cell adhesion complexes that are essential for epithelial integrity. In addition to their well-documented structural significance, we have identified a new role of E-cadherin – based junctions, whereby they recruit the core components of the RNAi machinery, including the microprocessor, the DICER, and the RNAi-induced silencing complex (RISC), as well as a specific set of miRNAs in colon epithelial cells to suppress mRNA expression. We have particularly shown that this interaction occurs through PLEKHA7, a member of the E-cadherin cell-cell adhesion complex. PLEKHA7 loss results in compromised epithelial integrity, in decreased levels and silencing activity of a set of miRNAs, and in increased mRNA expression primarily of extracellular matrix (ECM) and pro-inflammatory regulators, in this way promoting ECM remodeling, both in vitro and in vivo. Interestingly, it has been shown that estrogens may disrupt E-cadherin clustering and therefore adherens junction integrity, through a non-canonical pathway involving EGFR and Src activation. This led us to hypothesize that estrogen may be promoting disruption of the junctional - RNAi interaction and promote expression of pro-fibrotic markers, exacerbating the related diseases in women. To test our hypothesis, we conditioned colon epithelial Caco2 cells in hormone – free medium and treated them with estradiol. We then performed immunofluorescence and confocal microscopy to examine junctional localization of RNAi markers, immunoblotting to examine protein expression and phosphorylation, as well as qRT-PCR to examine miRNA levels. Our results show that estradiol treatment of Caco2 cells disrupts junctional localization particularly of DICER, a key component of the RNAi machinery that catalyzes the last step of miRNA biogenesis and delivers mature miRNAs to RISC and its main catalytic component AGO2. Along these lines, we also found that estradiol results in AGO2 phosphorylation at the Y393 site, which would indicate disruption of its interaction with DICER and inhibition of miRNA binding and activity. Indeed, estradiol also results in downregulation of miRNAs that we have found to be associated and regulated by the junctional RNAi machinery. Examination of *Plekha7* knockout mice reveals that the mice exhibit fibrotic phenotypes in the colon, which are exacerbated by age and are more prevalent in females in aged mice. Together, our data demonstrate that estrogen may indeed be promoting pro-fibrotic phenotypes through disruption of the junctional RNAi machinery. These findings link epithelial integrity with colon homeostasis and raise the possibility that disruption of the junction - associated RNAi machinery is a culprit of the higher SSc and CD prevalence in women, which we will also be investigating through examination of SSc and CD colon patient samples that we are currently collecting. The impact of the study is that it will advance our understanding of the underlying mechanistic causes of diseases that pose significant burden on women's health, offering opportunities for therapeutic intervention. Since this involves miRNA regulation as the focal point of this mechanism, the study can lead to future development of RNA-based therapeutics.

TITLE: MECHANISM OF TUMOR SUPPRESSOR FUNCTION OF PROGESTERONE RECEPTOR IN BREAST CANCER
PROJECT LEAD (SUPPLEMENT): MOTOKI TAKAKU, PHD (3P20GM104360-07S1)
INSTITUTION: UNIVERSITY OF NORTH DAKOTA SCHOOL OF MEDICINE AND HEALTH SCIENCES, GRAND FORKS, ND
IDEA PROGRAM: COBRE (PI: VAUGHAN, ROXANNE)

Background (Scientific focus and aims):
Breast cancer development is closely linked to the activity of hormone receptors, including the estrogen receptor (ER) and progesterone receptor (PR). While ER's role in breast cancer and its therapeutic targeting are well understood and established in clinical practice, PR's function remains comparatively underexplored, despite its presence in approximately 70% of breast cancers and the promising inhibitory effects of progesterone treatment. This knowledge gap, along with the dual role of progesterone in both breast cancer progression and suppression, underscores the urgent need to elucidate PR's molecular mechanisms. We

recently identified GATA3 as an upstream regulator of PR. Through this Women's Health in IDeA States program, we aimed to identify the molecular mechanisms underlying the tumor suppressor function of PR.

Methods:

Preliminary data indicate that GATA3 regulates PR expression, and that GATA3 mutations lead to reduced PR levels. We also identified several miRNAs upregulated upon progesterone treatment, presumably through PR chromatin binding near the miRNA coding regions. To investigate the roles of these progesterone-regulated miRNAs and the effects of GATA3 mutations, we designed a CRISPR knockout screen.

Results:

The CRISPR screen revealed that gRNAs targeting specific miRNAs were enriched following progesterone exposure. These miRNAs were upregulated by progesterone treatment, and PR binding near these miRNA loci was observed. Depletion of these miRNAs rendered T47D cells resistant to progesterone treatment. One of the key hits, miR-30a, is known to be involved in breast cancer and shows a significant correlation with PR expression in clinical breast cancer transcriptomic datasets. In addition, we generated a panel of GATA3-mutant breast cancer cell clones using CRISPR and found that various GATA3 mutations differentially affect PR expression levels.

Conclusion and outcomes:

This study identified miRNAs regulated by progesterone and demonstrated that their combined activity can suppress the growth of ER+ and PR+ luminal breast cancer cells. We further confirmed that GATA3 functions upstream of PR, and that GATA3 mutations reduce PR expression. These findings provide new mechanistic insights into how progesterone and PR regulate tumor growth in breast cancer. The project has led to two publications (one published and one preprint), with a third anticipated this fall. In terms of training outcomes, one undergraduate student supported by this program successfully entered the University of North Dakota Medical School, another entered the PhD program and is currently working in my laboratory, and a supported postdoctoral fellow secured an Associate Research Scientist position at Yale University.

TITLE: INTEGRATED MULTI-OMICS DATA PLATFORM TO ADVANCE PRECISION WOMEN'S HEALTH RESEARCH
PROJECT LEAD (SUPPLEMENT): COLIN KAY, PHD (5P20GM103429-23S1)
INSTITUTION: UNIVERSITY OF ARKANSAS FOR MEDICAL SCIENCES
IDEA PROGRAM: INBRE (PI: CORNETT, LAWRENCE)

Background:

An Arkansas INBRE program supplement supported a collaborative effort between the University of Arkansas for Medical Sciences, University of Arkansas–Fayetteville, and the Arkansas Children's Research Institute to make decades of maternal health research readily accessible to future investigators. The goal was to improve access to high-quality data for understanding how dietary and lifestyle factors during pregnancy influence maternal and child health.

Methods:

A centralized knowledgebase was developed integrating multi-omics datasets, including metabolomics, microbiome, genomics, and proteomics, from women's and child health studies. Participant and intervention datasets were standardized, chemically annotated, and cross-linked to public resources (e.g., PubChem, HMDB, DrugBank, KEGG, ChEBI, CTD). Key steps included: (a) normalizing intervention records and mapping them to chemical identifiers and drug classes; (b) curating metabolomics data with validated InChIKeys and linking to pathway and disease databases; and (c) matching USDA polyphenol datasets to FNDDS food descriptors using text similarity metrics (cosine similarity, Jaro–Winkler distance, Jaccard index).

Results:

The platform enables rapid retrieval and integration of clinical, dietary, and molecular data with disease and pathway associations. Back-end pipelines perform dataset standardization, chemical annotation, and bidirectional linking to public databases. A front-end interactive dashboard supports database searching, visualization, analytics, and AI/ML-driven insights into relationships among pregnancy, diet, and health outcomes.

Conclusion:

This unified data and analytics infrastructure accelerates discovery in precision women's health research by connecting clinical, phenotypic, and multi-omics data. The platform supports real-time exploration of molecules, foods, and drug classes, providing a foundation to design targeted interventions to improve health outcomes in diverse populations.

TITLE: THE MOUNTAIN MAMA & BABY COHORT STUDY: RATIONALE, OBJECTIVES, AND INITIAL RESULTS
PROJECT LEAD (SUPPLEMENT): UMER, AMNA (3U54GM104942-09S1)
INSTITUTION: WEST VIRGINIA UNIVERSITY
IDEA PROGRAM: IDEA-CTR (PI: HODDER, SALLY)

Background:
The Mountain Mama & Baby (MMB) Cohort Study was funded as a women's health administrative supplement to the West Virginia (WV) Clinical and Translational Sciences Institute (3U54GM104942-09S1) to address the need for rigorous research investigating associations between perinatal e-cigarette and cannabis use with intergenerational health. Use of e-cigarettes or 'vape' and cannabis during pregnancy has increased, with current U.S. prevalence estimates as high as 5-9%. This is concerning, as data from preclinical and some human studies preliminarily indicate that these exposures may result in harm to both mother and offspring. Yet, rigorous confirmation of previous observations and estimation of the magnitude of risks to mother and child from these exposures is critically needed. Key limitations of published studies include exposure measurement issues such as unknown validity of self-report, lack of dose, type, and timing information, small study sizes, and few prospective studies that begin early in pregnancy and capture maternal outcomes. These limitations are addressed by our prospective study that aimed to recruit a large, representative, and contemporary cohort of pregnant women, to comprehensively assess e-cigarette and cannabis use patterns throughout pregnancy, and to estimate associations of these exposures with maternal-fetal health. The main objectives of the MMB study are to 1) Demonstrate the feasibility of integrating recruitment into the first prenatal care visit of WVU Medicine obstetric clinic patients and the generalizability of the enrolled sample, 2) Establish first and third trimester exposure rates using adaptations of validated instruments and describe the epidemiology of prenatal e-cigarette and cannabis use in WV and surrounding areas, 3) Explore associations of e-cigarette and cannabis exposure with adverse maternal and infant outcomes using electronic medical record (EMR) data abstracted from the first prenatal visit through labor and delivery. This abstract focuses on the first objective of the MMB cohort study.
Methods:
We trained three nurse navigators to introduce the study during their initial telehealth visit with each pregnant patient seeking prenatal care at WVU. Research staff then followed up with interested participants via email and text to obtain e-consent and survey responses. Interest in the study and enrollment outcomes were summarized to estimate the feasibility and yield of enrollment. Deidentified demographic information was abstracted from the EMR for all patients seen by the prenatal nurse navigators, regardless of enrollment, and characteristics were compared between those who enrolled vs. did not enroll to inform the generalizability of the study sample.
Results:
Recruitment was conducted between January 20, 2025, and June 27, 2025, during which time 417 participants were enrolled, representing 40.3% of eligible first trimester pregnant patients (Table 1). The enrollment rate was ~18 participants/week. Comparison of demographic characteristics for those enrolled vs. not enrolled indicated no meaningful differences. Small but statistically significant differences were observed between the two groups for other variables, with enrolled individuals likely to have higher education, non-public health insurance, and planned pregnancies.
Conclusion: The MMB Cohort study's enrollment strategy yielded high rates of recruitment and a study sample that was remarkably representative of the target population of first trimester pregnant women seeking prenatal care with WVU Medicine. Follow-up continues to address additional aims regarding the prevalence and maternal-fetal health impacts of e-cigarette and cannabis use in pregnancy.

TITLE: TARGETING STAT3 IN OVARIAN CANCER
PROJECT LEAD (SUPPLEMENT): SARAH WALKER, PHD (3P20GM113131-05S2)
INSTITUTION: UNIVERSITY OF NEW HAMPSHIRE
IDEA PROGRAM: COBRE (PI: COTE, RICK)

Background:
Ovarian cancer metastasis remains a major health issue for women. Ovarian cancer metastasizes when clusters of cells from the primary tumor interact with the mesothelial layer lining the peritoneum, clearing the mesothelial cells, and invading underlying tissue. We previously identified STAT3 as important for mesothelial clearance. We sought to identify drugs that could inhibit STAT3, hypothesizing this could reduce mesothelial clearance and potentially treat or prevent metastatic disease. We previously identified several statins as potential inhibitors of STAT3 using a drug repurposing hub. Recognizing that several studies demonstrated reduced ovarian cancer incidence in patients that took statins, we wanted to determine the effects of statins on cell survival and mesothelial clearance. As endometriosis shares some similarity with ovarian cancer, we also wanted to

determine if endometriosis cells could undergo mesothelial clearance and if statins could be effective in modulating endometriosis cells.

Methods:

We utilized the mesothelial clearance assay to assess the ability of ovarian and endometriosis spheroids to clear the mesothelial cells. We also carried out genomic analysis on bulk RNA and single-cell RNA samples from ovarian cancer and endometriosis patients.

Results:

We found that statins reduce the viability of both ovarian cancer cells and endometriosis cells. In addition, we have found that statins reduce the ability of ovarian cancer cells to clear mesothelial cells. We have also found a link between the cholesterol pathway and STAT3 in ovarian cancer patient samples. Statins reduce mesothelial clearance of ovarian cancer cells, and this can be partially rescued by geranylgeranyl pyrophosphate, a metabolite downstream of HMGCR in the cholesterol pathway, suggesting that geranylgeranylation is important for mesothelial clearance. Moreover, we have shown that endometriosis spheroids also clear mesothelial cells. In addition, we have found that STAT3 signatures are enriched in endometriosis patient samples compared to normal endometrial tissue and that STAT3 is necessary for full mesothelial clearance by endometriosis cells.

Conclusions:

Our work suggests that statins may be beneficial as prevention or treatment for ovarian cancer. In addition, our work suggests that targeting STAT3 may also be a beneficial treatment strategy for endometriosis and ovarian cancer.

TITLE: HETEROZYGOUS LOSS OF *BRCA1* IN MICE PREDISPOSES TO HEPATIC STEATOSIS
PROJECT LEAD (SUPPLEMENT): KRISTY A. BROWN (3P20GM144269-03S1)
INSTITUTION: UNIVERSITY OF KANSAS MEDICAL CENTER, KANSAS CITY
IDEA PROGRAM: COBRE (PI: WEINMAN, STEVEN)

BRCA1 is a tumor suppressor best characterized for its role in the repair of DNA double-strand breaks. Germline mutations in *BRCA1* increase the risk of breast and ovarian cancer. In the general population, obesity is known to increase the risk of 13 types of cancer. Recently, we demonstrated that obesity and poor metabolic health are associated with more DNA damage in the normal breast tissue of *BRCA1* mutation carriers and an increased likelihood of developing mammary tumors in *Brca1* heterozygous KO (*Brca1* het KO) C57Bl/6 mice. Through these studies, we discovered that female *Brca1* het KO mice on a high-fat diet developed glucose intolerance. We hypothesized that heterozygous loss of *Brca1* predisposes to metabolic dysfunction and aimed to evaluate impact on different metabolic tissue. 4-week-old female mice were fed either a low-fat diet (LFD; 10% calories from fat) or a high-fat diet (HFD; 60% calories from fat) for 22 weeks. Our results showed that HFD-fed *Brca1*het KO mice tended to gain more body weight than wt mice, had greater accumulation of visceral fat with enhanced inflammation, and developed liver steatosis, also known as metabolic dysfunction-associated fatty liver disease (MASLD). Female mice are usually protected from diet-induced MASLD, so these data point towards a genotype-specific effect. To test the liver-intrinsic effect of heterozygous loss of *Brca1*, we performed studies using liver slices from 5-week old female mice, before any signs of overt steatosis, under control conditions or in the presence of palmitate for 24h. We observed more fat accumulation in the liver explants from *Brca1* het KO mice compared to those from wt mice, both in control conditions and in the presence of palmitate, suggesting potentially increased *de novo* lipogenesis, and changes in lipid import, metabolism and/or export. These findings provide novel insights into a role of Brca1 in hepatic lipid metabolism and storage and suggest that loss of function mutations in *Brca1* may predispose to metabolic dysfunction. These changes could also contribute to increase the risk of multiple cancers.

TITLE: IDENTIFICATION OF KEY SUBSTRATES OF RNA-BINDING PROTEINS IMPORTANT FOR FERTILITY AND EARLY EMBRYO DEVELOPMENT
PROJECT LEAD (SUPPLEMENT): SONI LACEFIELD, PHD (3P20GM113132-09S2)
INSTITUTION: GEISEL SCHOOL OF MEDICINE AT DARTMOUTH, HANOVER, NH
IDEA PROGRAM: COBRE (PI: MADDEN, DEAN)

Fertility is a major component of women's reproductive health. In the United States, approximately 19% of married women aged 15-49 with no prior births experience infertility, which affects their physical and emotional well-being. Deleterious variants of the *PATL2* gene are found in 44% of women with oocyte maturation defect (OOMD), in which an oocyte in unable to complete the cell division process of meiosis to produce an egg. Furthermore, other pathogenic variants of *PATL2* can result in an egg that is not competent for embryo development after fertilization. Despite the prevalence of pathogenic *PATL2* variants and their impact on women's reproductive health, PatL2 remains critically understudied.

PatL2 is an RNA-binding protein that serves as a translational repressor in oocytes with an important role during the meiotic divisions. During oogenesis, the oocyte progresses through meiosis to produce an egg with half the number of chromosomes as the progenitor cell. Transcription is silenced from prophase I through the meiotic divisions. New protein production relies on RNA-binding proteins, including PATL2, to regulate the timing of translation. PatL2 is thought to release mRNAs at specific meiotic stages to ensure the presence of proteins required at each stage. However, the mRNAs bound by PatL2 and how their release is regulated is currently unknown.

We aimed to identify the mRNAs bound by PATL2 using the mouse as a mammalian model organism. Our goal was to determine the mRNA targets important for meiotic progression and the formation of a competent egg. A major challenge with working with oocytes is the availability of material for study. Typically, the identification of substrates of RNA-binding proteins is assessed through immunoprecipitation of the RNA-binding protein and sequencing of the bound mRNAs. This experiment typically requires millions of cells, which is not possible in the mammalian system. To overcome this obstacle, we adapted the use of the HyperTRIBE (target of RNA-binding proteins identified by editing) technique to use in mouse oocytes. HyperTRIBE refers to the use of a fusion protein between the RNA-binding protein of interest and a hyperactive mutant version of the catalytic domain of the ADAR (adenosine deaminases acting on RNA) editing enzyme. The mRNAs bound to the RNA-binding protein undergo adenosine-to-inosine editing due to the proximity of the ADAR enzyme. RNA sequencing reveals those mRNAs edited by the enzyme, suggesting that they are direct targets of the RNA-binding protein.

To adapt HyperTRIBE for use in mouse oocytes, we made constructs to in vitro transcribe the *PATL2-ADARcd* gene. We then injected the mRNA into oocytes for translation. As controls, we performed sham injections and injected a construct containing a dead ADARcd enzyme to control for effects of PATL2 overexpression, while giving a comparison of un-edited RNA. We performed three replicates collecting 30 oocytes each and used bulk RNA sequencing. We then used variant calling analysis to identify the Adenosine-to-Inosine (A-I) edits present in mRNA transcripts with >20 reads and >10% editing. This thresholding yielded a list of approximately 110 targets edited by ADAR as an indication of PATL2 binding. Some of the targets include kinetochore proteins, mitochondrial proteins, and proteins involved in DNA methylation specifically in the oocyte. We are currently further studying these targets to verify their binding to PATL2 and to determine how their regulated translation affects meiotic progression. Overall, our results are likely to reveal why pathogenic PATL2 variants cause meiotic arrest. These findings should also lay the groundwork for personalized assisted reproduction therapies for women with PATL2 variants.

Title: Identification and pre-clinical validation of diagnostic biomarkers for ovarian cancer
Project Lead (SUPPLEMENT): Aditya Dutta
Institution: University of Delaware

Background:

Ovarian cancer (OVC) is the deadliest gynecological cancer. It is the 11th most common cancer among women and the fifth leading cause of cancer-related death. A key caveat in ovarian cancer management is the absence of early detection. The five-year survival rate drops from ~90% to ~46% if ovarian cancer is not diagnosed at an early stage. A key modulator of insults to the ovary and the reproductive tract is the local microbiome, which has the potential to both act in a protective role and as an enabler of such insults. Microbiome compositions have been shown to differ significantly between ovarian cancer patients and those with benign gynecologic conditions. The CA125 (cancer antigen 125) blood test, the most accurate available test for detecting ovarian cancer, is not specific to ovarian cancer. Thus, understanding and defining the early diagnostic biomarkers of ovarian cancer initiation, especially in the context of a dynamic reproductive tract microbiome, will drastically change the ovarian cancer management landscape.

Methods:

We employ the hen (female chicken) as an animal model for spontaneous ovarian cancer initiation. Mouse models do not develop spontaneous ovarian cancer, and as such cannot be used as models to study spontaneous ovarian cancer initiation. The hen reproductive system experiences the same process of repetitive ovulation and disease presentation as in humans. We proposed longitudinal analysis of microbial diversity and plasma metabolites from hens to identify microbial-derived metabolites associated with OVC onset. We further proposed analysis of plasma metabolites from human patients with untreated ovarian cancer, benign disease, and age-matched controls, in context of microbial-derived metabolites associated with OVC onset.

Results:

We completed microbial diversity and plasma metabolite analysis from hens but not human samples because of delays in sample procurement. Although we did not see the presence or absence of unique bacterial groups in the OVC and benign groups, we identified differential levels of microbiota across the groups. The overall diversity of microbes was significantly higher in benign samples versus those with OVC. More importantly, we identified significant association across OVC stages and microbial profiles. Moreover, we also identified altered linoleic acid and branched-chain amino acid levels in the birds that developed OVC

versus those that did not.
Conclusions: Significant microbial associations across OVC stages and altered linoleic acid and branched-chain amino acid levels in diseased animals provides new insights into ovarian cancer physiology. These results in conjunction with proposed studies of human plasma samples may provide opportunities for targeted modulation of microbial and metabolite factors associated with early detection and treatment response.

Title: Hyper-IL6 Promotes Inflammatory Gene Expression in Primary Uterine Myocytes
Project Lead (SUPPLEMENT): Heather Burkin
Institution: University of Nevada, Reno School of Medicine

Introduction:
Abnormal uterine distension and inflammatory cytokine expression are associated with an increased risk of preterm delivery. Local cytokine levels are elevated in animal models of uterine strain, and in uterine myometrial tissues from women delivering twins preterm; however, the molecular mechanisms by which excess mechanical strain leads to inflammatory activation and preterm uterine contractions are poorly understood. Our preliminary data indicated mechanical strain induced dramatic increases in IL6 and other cytokine gene transcription in immortalized human uterine myocytes. IL6 serves as a key local mediator of the transition to term and preterm labor and can signal through both classical and "trans" mechanisms. Hyper-IL6 is a synthetic fusion protein developed to mimic IL6+sIL-6R (trans) activity.
Aim:
We performed experiments to test the hypothesis that IL6 and/or Hyper-IL6 promote transcription of genes associated with inflammation in primary uterine smooth muscle cells.
Methods:
Uterine biopsies were obtained from women undergoing elective Cesarean section at term. Primary uterine myocytes were grown to confluence on collagen peptide-coated silicone membranes and allowed to differentiate for 7 days in DMEM containing 1% insulin, transferrin, and selenium 60 nM estrogen and 600 nM progesterone. Differentiated uterine myocytes were treated with vehicle control, 10 ng/mL IL-6, or 10 ng/mL Hyper-IL-6 for 8 h in triplicate. Cellular RNA was extracted with Trizol and RNA sequencing was performed by the Nevada Genomics Center using an Illumina NextSeq 500 High Output v2 flow cell and 2x75 base-pair, paired-end sequencing.
Outcomes:
We identified 33 differentially expressed genes in response to Hyper-IL6 treatment with a threshold p-value of 0.01 and a log fold change of expression with absolute value >0.6. iPathway Guide analysis predicted the top perturbed signaling pathways were TNF signaling and cytokine-cytokine receptor interaction pathways (SOCS3, CCL2, BCL3, IL6, JUNB, and ICAM1).
Conclusions:
Our data did suggest IL6 produced in response to mechanical strain acts in a paracrine manner to contribute to the production of additional pro-inflammatory cytokines by myometrial cells in the presence of progesterone. These data point to IL6 trans signaling as one potential molecular mechanism by which mechanical strain precipitates the transition to labor.

Title: Progesterone as a treatment option for superimposed PE
Project Lead (SUPPLEMENT): Lorena Amaral, PhD, FAHA
Institution: University of Mississippi Medical Center

Scientific Focus:
Preeclampsia (PE) is characterized by new onset of hypertension after 20 weeks of gestation. It affects 5-7% of all pregnancies in the U.S., and is associated with reduced fetal weight, inflammation and hypertension (HTN). Importantly, 30% of HTN disorders in pregnancy are caused by chronic HTN that is present prior to pregnancy which increases the risk of superimposed PE (SIPE). The mechanisms responsible for the pathogenesis of PE and SIPE are not fully understood. We have previously shown that either progesterone supplementation with 17-hydroxyprogesterone caproate (17-OHPC) or Progesterone induced blocking factor (PIBF) improves inflammation, fetal weight and blood pressure in the preclinical reduced uterine perfusion pressure (RUPP) rat mimic model of PE. In addition, our clinical preliminary data show that PE patients have reduced PIBF, and supplementation with 17-OHPC improves maternal outcomes. Although PIBF improves maternal outcomes during pregnancy disorders, whether 17-OHPC stimulates PIBF to protect against PE or SIPE is unknown. Therefore, we hypothesized that an increase in uterine artery resistance index leads to a decrease in PIBF and IL-4 leading to increased TH1, NK and AT1-AA, causing changes in vasoactive factors

(increased sFlt-1, ET-1; decreased nitric oxide), thus contributing to SIPE with exacerbation of HTN in the mother and IUGR in the offspring.
Aim 1: To test the hypothesis that 17-OHPC supplementation in the pregnant Dahl salt-sensitive (DS) rat, a model of SIPE, reduces BP and stimulates PIBF, reduces inflammation, AT1-AA, sFlt-1, ET-1, improves endothelial-dependent relaxation, and prevents development of IUGR in offspring.
Aim 2: To test the hypothesis that PIBF supplementation in pregnant DS rat, a model of SIPE, reduces BP, stimulates IL-4/TH2, reduces cytolytic NK cells, inflammation, AT1-AA, and reduces sFlt-1, ET-1, improves endothelial dependent relaxation, and prevents the development of IUGR in offspring.
Methods:
A subset of pregnant control Sprague-Dawley (SD) and pregnant Dahl S (DS) rats will be injected with 17-OHPC (3.32mg/kg), PIBF (2.0 µg/mL) or vehicle (saline) on day 15 (GD15) of gestation. On GD 18 Uterine Artery Resistance Index (UARI) will be measured by Doppler Ultrasound and carotid catheters will be inserted for mean arterial blood pressure (MAP) measurement on GD19. Circulating and placental populations of CD4+ T, B cells and NK cells will be quantified by flow cytometry. TNF-alpha and IL-6 were measured by ELISA and AT1-AA were quantified by the chronotropic responses to angiotensin II type 1 receptor-mediated stimulation of cultured neonatal rat cardiomyocytes.
Outcomes/Results:
Our preliminary data indicate that blood pressure increases in DS+vehicle rat group compared to SD+ vehicle rat group. 17-OHPC treatment reduces blood pressure, but has no effect on either placenta or pup weights. Importantly, 17-OHPC reduces uterine artery resistance index, sFlt-1 and TNF-levels in DS+17-OHPC rat group. Moreover, 17-OHPC reduces circulating and placental CD4+ T cells, and AT1-AA levels. 17-OHPC has no effect on pup/placental ratio, IL-6 levels and placental PPET-1. PIBF treatment reduces blood pressure and increases put weight in DS+ PIBF rat group. In summary, 17-OHPC and PIBF improve the clinical signs of PE in pregnant Dahl salt-sensitive rat.

Title: Novel Dual-Modality Treatment of Breast Cancer-Induced Osteolysis
Project Leads (SUPPLEMENT): Liyun Wang, Emily S. Day
Institution: UNIVERSITY OF DELAWARE
IDeA Program: COBRE (COBRE PI: ELLIOTT, DAWN; 3P20GM139760-03S1)

Scientific Focus:
Approximately 1 in 8 women will develop invasive breast cancer during their life. Breast cancers not only inflict a significant socioeconomic burden but also reduce patients' quality of life. Osteolytic bone lesions, often found in metastatic breast cancer patients, result in painful skeletal-related events that are difficult to treat or even fatal and significantly reduce patient wellbeing. Thus, it is of high clinical significance to improve the treatment of bone metastases. Chemotherapy drugs like doxorubicin (DOX) are the standard care for metastatic breast cancer but the administration of free drug can be extremely damaging to the body due to systemic drug toxicity. Biomimetic nanodelivery systems consisting of cancer cell membrane wrapped nanoparticles (CCNPs) have been proven to provide homotypic targeting of homologous tumor cells to deliver therapeutic reagents to tumor regions of interest while limiting off targeting toxicity. This research investigated CCNPs as a carrier to increase the targeted delivery of DOX to metastatic breast cancer tumors in the bone, resulting in improved disease remission. We further hypothesized that combining this treatment with vibration therapy or exercise regimens (a dual-modality approach) could mitigate osteolysis associated with breast cancer that might lead to improved patient outcomes and quality of life.
Methods:
DOX-loaded poly(ethylene glycol)-poly(lactic-co-glycolic acid) (PEG-PLGA) nanoparticles (NPs) were synthesized by a single emulsion procedure. To extract plasma membranes, 4T1 murine TNBC cells were subject to lysis, mechanical homogenization, and differential centrifugation. The membranes and unwrapped NPs were co-extruded to form CCNPs. The NPs' size, zeta potential, and concentration were measured by nanoparticle tracking analysis and dynamic light scattering, and DOX loading was measured by dissolving DOX-loaded NPs in dimethyl sulfoxide and reading fluorescence against a standard curve. To assess NP tumor delivery in vivo, luciferase-expressing 4T1 cells were injected in the proximal tibiae of immune-competent female Balb/c mice. After 2 weeks of tumor growth, Cy5-labeled CCNPs and unwrapped bare Cy5 NPs were administered intravenously. After 8-10 hours, the tibiae and major organs were excised and imaged ex vivo to assess the relative biodistribution. To assess therapeutic efficacy, the same tumor inoculation methods were used as described above. After 2 weeks of tumor development, DOX CCNPs or saline were administered weekly for 4 weeks. Additional mice with inoculated tumors were also treated with vibration (0.3g, 30 min) or treadmill running (10-12 m/min, 40 min) for 5 days/week separately or in combination with weekly DOX CCNP injections. Tumor growth was monitored weekly by tracking luminescence with an in vivo imaging system (IVIS). MicroCT was also utilized to track lesion progression and bone integrity.

Results:
Biodistribution data revealed significantly greater accumulation of CCNPs than uncoated NPs in tumor-bearing bones, with lower accumulation of CCNPs in clearance organs (liver, spleen, kidneys). Therapeutic data showed a reduction in tumor radiant efficiency for DOX CCNP treated groups compared to tumor control by ~20X. These results suggest that CCNPs enhance tumor targeting and delivery of DOX to the bone. MicroCT confirmed this result, as the number of slices with full thickness tumor lesion was reduced in mice treated with DOX CCNPs compared to tumor controls. The effects of exercise were inconclusive. While vibration showed similar reduction of the # microCT slices with full thickness lesion as CCNPs, running did not inhibit the cancer-induced osteolysis. Dual-modality treatments of CCNPs with two types of exercise did not show significant additive inhibition of osteolysis relative to CCNPs alone, suggesting a dominate effect from the DOX-loaded CCNPs.
Outcomes:
These promising findings suggest that CCNPs can be used as an effective delivery platform to improve the efficacy of encapsulated drugs against bone metastases. The outcomes from this project justify further study of the dose responses of CCNPs alone and in combination of bone protective regimens to provide holistic treatments for breast cancer-induced osteolysis.

Title: Identifying novel inhibitors of HSV-2
Project Lead (SUPPLEMENT): David Davido
Institution: University of Kansas, Lawrence, KS
IDeA Program: COBRE (COBRE PI: HEFTY, P SCOTT)

Scientific background and focus: Herpes simplex virus 2 (HSV-2) infections can result in genital and orofacial sores, which can recur throughout life. Mothers with primary HSV-2 genital infections can transmit virus to feti in utero and both primary infection or recurrences can transmit virus to neonates during birth, leading to disseminated disease that is often fatal even with aggressive treatment and causes significant sequelae in survivors. There are no vaccines, and current anti-virals, such as acyclovir and its derivatives, reduce the frequency and severity of symptoms but are not highly effective in suppressing HSV-2 shedding. Consequently, there is an urgent need to identify novel anti-viral therapies for HSV-2. The specific events that dictate HSV-host/cell interactions critically affect the outcome of lytic infection. The viral protein, infected cell protein 0 (ICP0), regulates productive infection. ICP0 is an E3 ubiquitin ligase that activates transcription of all HSV gene classes. As HSV-2 is an obligate intracellular pathogen that requires a host to replicate, viral and host cell factors play important roles in stimulating HSV gene expression. Our long-term research goal is to elucidate the HSV-2-host molecular interactions that modulate its life cycle and use this knowledge to ultimately develop therapeutic interventions for treating patients with HSV-2 diseases, especially related to women's and neonatal health. The objective of our research is to use a chemical biology approach to identify novel mechanisms by which HSV and ICP0 facilitates viral gene expression, stimulating HSV-2 productive infection. Our aim: to identify novel inhibitors of HSV-2 and ICP0 using chemical libraries that include compounds that recognize viral and cellular targets/pathways. Our approach: we developed a robust, reproducible high throughput assay to monitor ICP0's activation of viral gene expression using a recombinant virus that expresses beta-galactosidase from an HSV promoter. Cells were infected with this virus in a 384-well plate format, performing a primary screen with ~80,000 compounds. Results from this primary screen identified ~2,500 potential hits, with 294 compounds being further analyzed for dose-dependent responses for beta-galactosidase activity (viral gene expression) and cytotoxicity (cell viability). Future experiments will access the efficacy of select compounds from these screens to impair ICP0's transactivating activity and HSV-2 lytic replication in cell culture.

Title: Identification of cell type profiles and DNA methylation alterations in endometriosis
Project Lead (SUPPLEMENT): Christensen, Brock
Institution: Geisel School of Medicine, Dartmouth

Abstract
Endometriosis affects 6.5M women in the U.S. and >190M worldwide. Endometriosis is marked by endometrial-like tissue deposits outside of the uterine cavity, symptoms include chronic pelvic pain, irregular periods, painful intercourse, and infertility. There is an average delay of approximately seven years between symptom onset and diagnosis which requires laparoscopic surgery, pelvic examination, and imaging. There is an urgent clinical need to identify noninvasive biomarkers for endometriosis. Recent work has identified evidence of epigenetic alterations such as altered DNA methylation (DNAm) associated with endometriosis, indicating the potential utility of DNAm measures to identify disease associated changes, and contribute to development of diagnostic biomarkers. However, cell-type-specific alterations remain to be elucidated, and studies to date have focused on tissue obtained with invasive biopsy procedures. We hypothesize that alterations in DNAm are associated with endometriosis, and cell-type adjusted modeling will identify clinically relevant biomarkers for assessment and diagnosis of

endometriosis. DNAm is an epigenetic modification involved in cell differentiation, and cell-specific DNAm patterns can be leveraged to quantify cell type proportions in DNAm data from bulk biospecimens. We pioneered the development of DNA-based cell typing approaches known as methylation cytometry in blood, tissue, and tumor specimens. To date, there has been limited application of modeling approaches that account for confounding by cell-type in endometriosis. To identify alterations in DNAm and characterize cell type heterogeneity in endometriosis we will apply cell deconvolution methods to data from endometriosis cases and non-endometriosis normal control DNAm data from endometrial tissue samples and from menstrual effluent. Our overarching goal is to identify cell type profiles and DNAm alterations associated with endometriosis in both endometrial tissue and menstrual effluent.
Aim 1. Identify cell type profiles and DNA methylation alterations associated with endometriosis in endometriosis tissue.
Aim 2. Identify cell type profiles and DNA methylation alterations associated with endometriosis in menstrual effluent.

Methods

We will use a hierarchical methylation cytometry cell typing approach to identify cell types present in tissue and menstrual effluent, with a specific focus on comparison of immune cell proportions between endometriosis cases and controls to assess the role of immune cells and immune cell type in endometriosis. By identifying cell proportions present in disease-free endometrium, endometriosis lesion tissue, and matched menstrual effluent, we can assess cell types that are driving pathogenesis and are associated with endometriosis. Additionally, we focus on menstrual effluent as a viable biological specimen for molecular analysis, with potential application for future, non-invasively collected clinically relevant biomarkers for the assessment and diagnosis of endometriosis.

Outcomes

Aim 1. DNAm data from endometriosis cases and controls has been accessed from public sources. Data analyses incorporating cell type adjustment and cell-specific analyses with interaction testing are underway. In addition, a retrospective study at Dartmouth has resulted in the collection of 105 endometriosis case tissues and control tissues. DNAm measures are pending for the retrospective study.
Aim 2. Methods development for DNAm deconvolution of cell types in menstrual effluent has been published. Multiple IRB protocols have been approved and studies launched to collect menstrual effluent in clinical and community settings. For example, see menstrualmarkers.org.

Impact and future work

Identification of cellular profiles and DNAm alterations in endometriosis tissues and menstrual effluent in this proposal will be foundational for expanded work to prospectively validate the clinical utility of cellular and molecular markers of endometriosis in menstrual effluent. Further development and validation of endometriosis biomarkers in menstrual effluent will allow patients and physicians to reduce delays to definitive diagnosis and improve management of endometriosis.

Title: Attenuation of maternal obesity in BPH/5 preeclamptic mice prevents cardiometabolic risk in female offspring
Project Lead (SUPPLEMENT): Jenny L. Sones
Institution: Louisiana State University Pennington Biomedical Research Center COBRE (PI: Jacqueline M. Stephens)

Preeclampsia (PE) is a hypertensive disorder of pregnancy with pre-conception maternal obesity as a risk factor. The PE condition is mirrored in the blood pressure high (BPH)/5 mouse with females exhibiting obesity before pregnancy. There is a sexual dimorphism between BPH/5 males and females, where males are not obese, and despite these differences, both male and female BPH/5 mice have cardiovascular disease (hypertension and heart enlargement). Previous observational studies in BPH/5 have shown blunted hypertension in females and improved offspring outcomes with attenuation of maternal obesity via reduction in food intake. Because maternal adiposity and cardiometabolic offspring health may be correlated, identification and validation of a genetic cause is essential. Recent genome wide association studies (GWAS) performed on hypertensive obese BPH/5 females revealed 69 genetic mutations mapped to the X chromosome. These mutations may contribute to the obese phenotype seen in BPH/5 females, but not males.
We hypothesized that BPH/5 female offspring from ad libitum fed dams will have associated genomic alterations in X-linked genes that predispose them to obesity in conjunction with hypertension. Whole genome bisulfite sequencing (WGBS) of visceral reproductive white adipose tissue (rWAT) from adult female and male BPH/5 littermates was utilized to understand X chromosome silencing (Xist: non-coding RNA [ncRNA] responsible for X silencing) and genetic contributions from the X chromosome (e.g. androgen receptor [AR] expression) to phenotypic differences between male and female BPH/5 mice (n=6). Our WGBS allowed for comparison of methylation levels between male and female BPH/5 mice as well as identification of gene regions on the X chromosome. The two highest region percentages in the genome are intergenic and promoter, 41% and 38%, respectively. Alternatively, the regions making up the lowest percentages were ncRNA and 3UTR with both making up 1% each of the genome. The relative mRNA Xist expression in visceral rWAT is 5-fold higher levels in BPH/5 females when compared to males

and consistent with levels detected in control female mice. This suggests adequate X-inactivation in BPH/5 females. The relative AR mRNA expression in visceral rWAT revealed 2-fold higher levels in BPH/5 males compared to females. Disgenet pathway analysis of obesity-related genes discovered hyper/hypomethylation of differentially methylated regions (DMRs). Brown adipose tissue (BAT) differentiation pathways were implicated with Hoxc10 hypomethylated and thus potentially suppressing beiging of WAT in BPH/5 females. Interestingly, when BPH/5 pregnant females have PE signs/symptoms attenuated by reducing food intake in early gestation, several notable changes are observed in BPH/5 female offspring: 1) reversal of hypertension, 2) reduced rWAT and leptin, and 3) increased rWAT uncoupling protein complex 1, a marker of beiging. In summary, BPH/5 female offspring are more affected by maternal obesity in pregnancy. This may be due to X chromosome methylation status and is a potential marker for genetic testing of cardiometabolic disease and PE risk in women.
Future investigations focusing on testing genetic risk of health and disease in high-risk women before pregnancy may lead to novel interventions to prevent the generational cycle of health and disease in people.

Title: Determining the metabolic capacity of Sneathia vaginalis
Project Lead (SUPPLEMENT): Jeffrey L Bose
Institution: University of Kansas School Medical Center

Sneathia vaginalis is a member of the human normal flora of the vagina but is a human pathogen that is associated with preterm birth and amniotic fluid infections. It is also the most common bacterial species associated with HPV infection and is associated with cervical cancer. The identification of S. vaginalis as a human pathogen is recent. This is believed to largely be the result of detection of the bacteria as opposed to a recent emergence of the organism, since clinical laboratories do not test for the bacterium. One reason for this is due to fastidious growth of this bacterium and a lack of sufficient growth media. Indeed, it is normally grown in BHI supplemented with serum, anaerobically. S. vaginalis has a reduced genome and limited metabolic capacity However, these assertions are based primarily on bioinformatic analysis of the genome with little experimental confirmation. To addresses this, we took a multi-omics approach of transcriptomic and metabolomics. We identified several central metabolic pathways utilized by S. vaginalis under these growth conditions in addition to high-level expression of its only known cytolysin. In addition to reduced energy-generating metabolic pathways, S. vaginalis lacks the enzymes to synthesize fatty acids and would therefore be expected to harvest those essential molecules from the host. In support of this, we found that S. vaginalis cannot grow in delipidated media and is resistant to triclosan, consistent with the need to scavenge host fatty acids. Next, we use lipidomics to define what fatty acids can be used by S. vaginalis and how this bacterium assembles its lipids. These studies also revealed that S. vaginalis can harvest fatty acids from human lipoproteins. Lastly, we identified a novel fatty acid transporter that would be essential for cell viability. These studies define the metabolic capacity of S. vaginalis for the first time and is the first step towards our goal of developing a synthetic media for clinical lab testing and identification of proteins to be used for diagnostics.

Title: Developing a Sleep Intervention for Perinatal Women for Delivery by Direct Care Workers
Project Lead (SUPPLEMENT): Katherine Sharkey
Institution: Brown University
IDeA Program: COBRE (3P20GM139743-03S1; PI: Carskadon, Mary)

Scientific Focus:
Insufficient and disrupted sleep are rarely addressed in expectant and new parents, despite evidence that disturbed sleep is a modifiable risk factor for negative health outcomes in mothers and infants. We are using a community-engaged approach to develop and refine a behavioral sleep intervention to be delivered by direct care workers who serve pregnant and postpartum patients.
Aims:
Evaluate our planned implementation strategy by performing a needs assessment and measuring dimensions related to effectiveness and implementation during focus groups with direct care workers.
Methods:
Using a structured agenda, we performed 90-minute focus groups over Zoom with direct care workers who serve perinatal women. Participants provided input on topics relevant to our planned intervention including: (1) Participants' current knowledge about sleep during the perinatal period; (2) Types of sleep issues that are reported to home direct care workers by their clients; (3) Challenges workers experience when addressing their clients' sleep problems; (4) Preparedness to serve as an educator,

problem-solver, and validator about sleep. Focus group recordings were transcribed and reviewed by the study team to identify themes that emerged during the interviews.

Outcomes:

We conducted 5 focus groups from August-December 2024. All participants (n=14) were women. They ranged in age from 23-68 years (mean (SD) = 50.9 (14.4) years and held a variety of community health roles: 6 were certified community health workers; 2 were RNs, 3 were certified lactation counselors; 3 were certified doulas, 1 was a licensed mental health counselor, and 1 was a certified interpreter.

Future Directions:

In the next phase of this project, we will invite direct care workers to utilize the training materials with patients who have sleep concerns, and we will assess the reach, effectiveness, adoption, implementation, and maintenance of this scalable, efficient intervention to improve sleep.

Title: Marginal iron depletion: Micronutrient partitioning and outcomes in the mitochondria
Project Lead (SUPPLEMENT): Joanna L. Fiddler
Institution: University of Arkansas-Fayetteville

Iron deficiency is the single most common nutrient deficiency in the world, impacting one-third of the world's population. Recent analysis of the US National Health and Nutrition Examination Survey (NHANES) and Supplemental Nutrition Program for Women, Infants, and Children (WIC) data established dietary iron intakes are decreasing in the US population and the decline parallels increasing rates of anemia. Increases in iron deficiency may be caused by declining diet quality and/or high rates of nutrition and food insecurity. Iron deficiency is the leading cause of anemia which severely impacts physical and cognitive development, work capacity, and leads to poor health outcomes. The WHO categorizes iron-deficiency anemia as hemoglobin levels <120 mg/dL (non-pregnant females) and iron deficiency as ferritin levels <15 ng/mL in individuals over 5 years old and <12 ng/mL in children under 5 years old. These ferritin levels, which are used clinically, are not agreed upon. First, a level of 30 ng/mL has been suggested to have 92% sensitivity and 98% specificity for correlating iron deficiency with the absence of iron stores in the bone marrow. Second, marginal iron depletion (normal hemoglobin levels with inadequate ferritin stores, defined as ferritin levels between 15-30 ng/mL) is suggested to impair cellular functions. In human studies, individuals with marginal iron depletion have reduced physical work capacity, yet their ability to transport oxygen is not impaired. This indicates other pathways involved in physical work capacity may be compromised, yet these cellular pathways and their mechanisms have yet to be determined. Furthermore, the correlation between iron-dependent tissues and cellular compartments, blood biomarkers of iron levels, and the cellular pathways that lead to reduced work capacity have not been determined. The aims in this study were to 1) establish the relationship between blood values of ferritin and hemoglobin with tissue and cellular compartment levels of iron, and 2) determine the relationship between iron status and iron-dependent mitochondrial and cellular functional declines. Two models of iron-adequate, marginally iron-depleted, and severe iron-depletion were established: first, weanling C57Bl/6 male and female mice were exposed to varying levels of iron defined diets (<5, 10, 15, 20, 35, and 50 mg iron/kg diet) for 3-weeks, and second, mouse myoblast and liver cells were exposed to iron-adequate to low-dose iron chelation to induce marginal iron depletion. Results from our preliminary data indicate that impairing iron status from mild to severe depletion in C57Bl/6 male and female mice leads to sex-specific responses in serum ferritin but not hemoglobin. Hemoglobin levels saturate in both sexes on diets containing 15 mg iron/kg diet. Serum ferritin levels saturate in male mice near 20 mg iron/kg diet whereas in female mice, serum ferritin levels linearly increase in a dose dependent response. Furthermore, extremely low levels of iron depletion in mouse myoblast and liver cells reduce cellular respiration, ATP production, and individual ETC complex capacity before the cellular biomarker (transferrin receptor) responds. Our results indicate the "control" rodent diet (35-50 mg iron/kg diet) may not be optimized to sex-specific iron biomarkers.

Title: Female Long-term Adverse Metabolic Effects (FLAME): Inflammatory health outcomes of gestational and type 2 diabetes in reproductive-age women
Project Lead (SUPPLEMENT): Cara Frankenfeld
Institution: MaineHealth Institute for Research

Scientific focus:

Metabolic health is complex, and considerable shifts can occur in women's metabolic health throughout life. The overall hypothesis for this work is that immune-disrupting conditions, such as gestational diabetes (GDM) or type 2 diabetes (T2DM), increase risk for chronic inflammatory conditions of the gastrointestinal system.

Aims:
Primary aims of the work are to: (1) Evaluate risk of IBD in relation to GDM in a cohort of reproductive-age women, and (2) Evaluate risk of IBD in relation to T2DM in a cohort of reproductive-age women. A secondary aim of the proposed work is to evaluate colorectal cancer risk in relation to GDM and T2DM in a subset of women who have at least ten years of follow-up data available.
Methods:
This work is using MerativeTM MarketScan claims data to address research aims. MerativeTM MarketScan is one of the largest and longest running proprietary US claims databases used for healthcare research and includes a family of databases that consist of core claims databases linked with other databases. The research databases provide de-identified, longitudinal, patient-level data for >273 million unique patients. For this analysis, we are using a cohort subset of five million reproductive-age women (15-49 years of age) with at least one live birth or stillbirth and no exclusions (prior IBD, colorectal cancer, or type 1 diabetes) and who have claims for live birth or stillbirth data plus one year prior and three years subsequent of the live birth or stillbirth in a 15-year span of time from 2007-2021. A retrospective cohort design will be used to address specific and secondary aims. Pooled logistic regression models will be used to estimate risks of IBD and CRC in relation to GDM and T2DM.
Outcomes:
A cohort of reproductive-age adult women (15-49 years of age) was identified in MarketScan commercial and Medicaid longitudinal claims. Cohort selection is complete, and identification of post-partum diagnoses of interest is in progress. Statistical modeling will be conducted once outcome identification is complete.