# 2023 UAB NATHAN SHOCK CENTER SYMPOSIUM

## *Emerging Fields in Aging Research*

**Hilton Birmingham at UAB**

**February 28, 2023**

UAB NATHAN SHOCK CENTER
The University of Alabama at Birmingham

NATHAN SHOCK CENTERS OF EXCELLENCE IN THE BASIC BIOLOGY OF AGING

# AGENDA

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|---|---|
| 7:30-8:30 AM | **Registration and Continental Breakfast** |
| 8:30-8:40 AM | **Welcome**<br>**Steven Austad, PhD**<br>Distinguished Professor<br>Protective Life Endowed Chair in Healthy Aging Research<br>Co-Director, UAB Nathan Shock Center of Excellence in the Basic Biology of Aging<br>University of Alabama at Birmingham |
| 8:40 – 9:40 AM | **KEYNOTE SPEAKER, Stephen Kritchevsky, Ph.D.**<br>Toby R. Alligood, MD Endowed Professor in Geroscience<br>Wake Forest University School of Medicine<br>Director, Research Centers Collaborative Network of the NIA<br>***"Emerging Fields in Aging Research"*** |
| **SESSION I:** | ***Chair, Steven Austad, PhD*** |
| 9:45-10:05 AM | **Corinna Ross, Ph.D.**<br>Associate Director of Research, Southwest National Primate Research Center<br>Texas Biomedical Research Institute<br>***"Marmosets as Models of Aging and Healthspan"*** |
| 10:05--10:25 AM | **Maria Grant, M.D.**<br>Eivor and Alston Callahan, M.D., Endowed Chair in Ophthalmology<br>University of Alabama at Birmingham<br>**"*Multidimensional Role of Enteral ACE2 in Maintenance of Gut Barrier Integrity and Glucose Homeostasis*"** |
| 10:25-11:00 AM | **BREAK** |
| 11:00-11:20 AM | **Toren Finkel, M.D., Ph.D.**<br>Director, Aging Institute<br>University of Pittsburgh School of Medicine<br>***"Lysosomes in Aging Biology"*** |
| 11:20-11:40 AM | **Melissa Harris, Ph.D.**<br>Associate Professor, Department of Biology<br>University of Alabama at Birmingham<br>***"Targeting PD-L1 to Rejuvenate Somatic Stem Cells"*** |
| 11:40 AM – 1:40 PM | **LUNCH with POSTER SESSION** |

**SESSION II:** ***Chair, Thomas Buford, PhD***
Professor and Endowed Scholar
Co-Director, UAB Nathan Shock Center of Excellence in the Basic Biology of Aging
Director, UAB Center for Exercise Medicine
University of Alabama at Birmingham

1:40-2:20 PM **Richard Holden, Ph.D.**
Professor, Dean's Eminent Scholar, and Chair, Department of Health & Wellness Design
Indiana University School of Public Health-Bloomington
***"Anticholinergic Deprescribing Interventions from The Brain Health Learning Laboratory"***

2:20-2:40 PM **Arthur Arnold, Ph.D.**
Distinguished Professor
Department of Integrative Biology & Physiology
University of California, Los Angeles
***"X and Y Genes That Cause Sex Differences in Physiology and Disease"***

2:40-3:00 PM **Daniel Tyrrell, Ph.D.**
Assistant Professor, Department of Pathology
University of Alabama at Birmingham
***"Exploring CD8⁺ T Cells in Aging and Disease"***

3:00-3:20 PM **BREAK**

3:20-3:40 PM **Jonathan Wanagat, M.D., Ph.D.**
Associate Clinical Professor
University of California, Los Angeles
***"Mitochondrial Genetics in Aging"***

3:40-4:00 PM **Anna Thalacker-Mercer, Ph.D.**
Associate Professor
Department of Cell, Developmental and Integrative Biology
University of Alabama at Birmingham
***"Nutrient and Metabolic Determinants of Skeletal Muscle Regeneration in Older Adults"***

4:00-4:05 PM **Closing Remarks**
**Thomas Buford, PhD**

# KEYNOTE SPEAKER

# Emerging Fields in Aging Research

## Stephen B. Kritchevsky, Ph.D.

Toby R. Alligood, MD Endowed Professor in Geroscience
Wake Forest University School of Medicine
Director, Research Centers Collaborative Network of the NIA

**Dr. Stephen B. Kritchevsky** is the Toby R. Alligood, MD Endowed Professor in Geroscience and the Associate Dean for Research Development. He co-Directs of the Sticht Center for Healthy Aging and Alzheimer's Prevention at Wake Forest School of Medicine where he leads Wake Forest's NIA-funded Claude D. Pepper Older Americans Independence Center (OAIC). Dr. Kritchevsky studies nutritional influences that affect trajectories of health and disability in older adults including vitamins, protein, energy balance, obesity and exercise. Dr. Kritchevsky has held leadership positions in notable aging studies including the on-going Study of Muscle Mobility and Aging. He was the Editor-in-Chief of the *Journal of Gerontology: Medical Sciences* from 2012 - 2016. He leads the Research Centers Collaborative Network (RCCN) with the American Federation for Aging Research. Its goal is to build research collaborations among the 6 NIA-supported center programs through workshops, pilot awards, and educational activities. He is also a leader of the NIA-funded Translational Geroscience Network, the goal of which is to establish an infrastructure for the efficient clinical evaluation of interventions targeting the biology of aging to improve human health.

# SESSION I: FEATURED SPEAKERS

**Dr. Corinna Ross** earned her degrees in biological sciences from Cornell University (BS 1997), the University of Nebraska Omaha (MA 1999) and the University of Nebraska Lincoln (PhD 2005). After completing her PhD, she became a postdoctoral Biology of Aging fellow at UT Health San Antonio. She is currently the Associate Director for Research at the Southwest National Primate Research Center, Texas Biomedical Research Institute. Her research focuses on marmosets and has covered translational modeling topics such as developmental programming, reproductive physiology and the development of obesity. Her research is currently focused on exploring interventions that may protect health while aging.

**Corinna N. Ross, Ph.D.**
Associate Director
Southwest National Primate Research Center
Texas Biomedical Research Institute

**Marmosets as Models of Aging and Healthspan**

Research in geriatric sciences has recently shifted its focus from centering on human longevity to improving human health span and quality of life. The common marmoset, a small nonhuman primate, offers a number of advantages for aging and healthspan research. They have a fast maturation and short life span compared with more commonly used larger nonhuman primate models. Marmosets can be rapidly bred, and they can be maintained in a barrier environment. The development of the model over the last decade has resulted in functional phenotyping relevant to aging, such as metabolic health, homeostatic functioning, immune health, mobility, and cognition. These characterizations now allow us to evaluate potential interventions that may modulate the aging process.

# SESSION I: FEATURED SPEAKERS

**Toren Finkel, M.D., Ph.D.**
Director,
Aging Institute
University of Pittsburgh School of Medicine

**Dr. Toren Finkel** received his undergraduate degree in Physics and his MD and PhD degree from Harvard Medical School. Following a residency in Internal Medicine at the Massachusetts General Hospital, he completed a fellowship in Cardiology at Johns Hopkins Medical School. In 1992, after completing his clinical training, he came to the NIH as an Investigator within the Intramural Research Program of the National Heart, Lung and Blood Institute (NHLBI). Over the next 25 years at the NIH, he held various positions including Chief of the Cardiology Branch and Chief of the Center for Molecular Medicine within the NHLBI. He is a member of the American Society for Clinical Investigation (ASCI) and the Association of American Physicians (AAP). He has also been inducted as a Fellow of the American Association for the Advancement of Science (AAAS) and is an elected member of the National Academy of Medicine. He serves on numerous editorial boards including currently serving on the Board of Reviewing Editors for *Science*. As of Sept 1st 2017, Dr. Finkel assumed the role of the Director of the Aging Institute, and the G. Nicholas Beckwith III and Dorothy B. Beckwith Endowed Chair of Translational Medicine at the University of Pittsburgh/UPMC. Over the last three decades, his laboratory has made fundamental contributions in our understanding of the role of reactive oxygen species and mitochondrial function in aging and age-related diseases. He is also the co-founder of Generian Pharmaceuticals, and with his colleagues, he has co-developed several small molecules which are anticipated to be in Phase I testing within 12 months and may be of potential benefit for a range of age-related disorders.

**Lysosomes in Aging Biology**

I plan to review the general role of lysosomal dysfunction in aging biology. I will review our recent identification of a new pathway that mediates the repair of damaged lysosomes, which we have termed the PITT pathway. This pathway involves the generation of a novel lipid species on the surface of the damaged lysosome and the generation of intra-organelle contacts between the damaged lysosome and the endoplasmic reticulum. Finally, I will discuss our initial work on therapeutics that boost lysosomal activity and how they might be useful for a range of age-related diseases.

# SESSION I: UAB SPEAKERS


**Maria Grant, M.D.**
Eivor and Alston Callahan Endowed Chair in Ophthalmology
University of Alabama at Birmingham


**Dr. Maria Grant** is the Eivor and Alston Callahan Endowed Chair and Professor of Department of Ophthalmology and Visual Science. Dr. Grant completed her Medical Degree and Internship, residency and fellowship in Endocrinology and Metabolism at the University of Florida. She completed a Research Fellowship in the Department of Ophthalmology at Johns Hopkins University. Dr. Grant's laboratory is interested in understanding the role of bone marrow cells and vascular wall cells in vascular repair in diseases such as in aging and diabetes. Dr. Grant's research focus on understanding the biology of hematopoietic and endothelial stem and progenitor cell emergence, maintenance, and their regenerative properties. More recently she has examined the role of the renin angiotensin system in the intestine and how that impacts glucose homeostasis and immune function in aging and diabetes. She has served as principle/co-investigator on more than thirty-five extramural foundation/NIH grants that have led to more than 260 peer reviewed publications.

**Multidimensional Role of Enteral ACE2 in Maintenance of Gut Barrier Integrity and Glucose Homeostasis**

The pathophysiology of diabetes is often considered as "premature or accelerated aging" as diabetic experience activation of key biological and metabolic pathways associated with aged individuals. The renin-angiotensin system (RAS) participates in the regulation of vasoconstriction, fluid homeostasis, cell growth, fibrosis, inflammation, and oxidative stress. In recent years, unprecedented advancement has been made in the RAS, particularly with understanding of how this system has a deleterious (Ang II type 1 receptor – $AT_1R$) arm and a protective arm (angiotensin-converting enzyme 2 (ACE2)/Ang 1-7/MasR). In both aging and diabetes there is a decline in the protective arm of RAS that leads to increased gut barrier permeability, systemic inflammation, and endothelial dysfunction. We showed that the process of "inflammaging" is seen in human subjects with either Type 1 (T1D) and Type 2 diabetes (T2D) and this is due to a dysregulated systemic and intestinal RAS. We showed that T1D and T2D subjects exhibit elevations in gut-derived circulating immune cells (ILC1 cells) and higher gut leakage markers, which were positively correlated with plasma angiotensin II and severity of vascular complications. We also utilized probiotic *Latobacillus paracasei* expressing ACE2 (LP-ACE2) in Akita mice (model of T1D) as well as generating mice with genetic overexpression of *humanAce2* by small intestine epithelial cells (Vil-Cre.hAce2KI-Akita). Both strategies preserved gut barrier integrity, reduced inflammatory response, and delayed development of vascular complications. These studies emphasize the multifaceted role of the intestinal renin-angiotensin system in aging and in diabetes and vascular complications.

# SESSION I: UAB SPEAKERS

**Dr. Melissa Harris** is an Associate Professor in the Department of Biology at UAB. Dr. Harris' research program focuses on understanding how stem cell aging occurs and therapeutic approaches for preventing or reversing stem cell aging. The Harris Lab (aka Team Hair-Us) uses melanocyte stem cells and gray hair as a model stem cell system. The long-term mission of the lab is to identify real biological solutions to combat tissue aging. Dr. Harris is alumni of the University of California, Davis (BS, PhD) and the National Human Genome Research Institute (Postdoc).

**Melissa Harris, Ph.D.**
Associate Professor, Department of Biology
University of Alabama at Birmingham

**Targeting PD-L1 to rejuvenate Somatic Stem Cells**

Quiescence is a key aspect of slow cycling stem cells, yet how the quiescence program is affected by age in a pool of stem cells is not well-defined. Using quiescent melanocyte stem cells (qMcSC) as a model, we find that the pool of qMcSCS is quite heterogenous and this heterogeneity changes with age. One protein that is upregulated in a subpopulation of qMcSCs is the immune checkpoint protein PD-L1. PD-L1+ qMcSCs also represent a higher proportion of the aged qMcSC pool. We anticipate that PD-L1+ qMcSCS may be good targets for replenishing the McSC pool in the context of aging if they can be reactivated. In vivo blockade of PD-L1 in an acute hair graying mouse model can reduce gray hair intensity suggesting that anti-PD-L1 therapeutics to prevent or reverse this aging phenotype may be a real possibility.

# SESSION II: FEATURED SPEAKERS

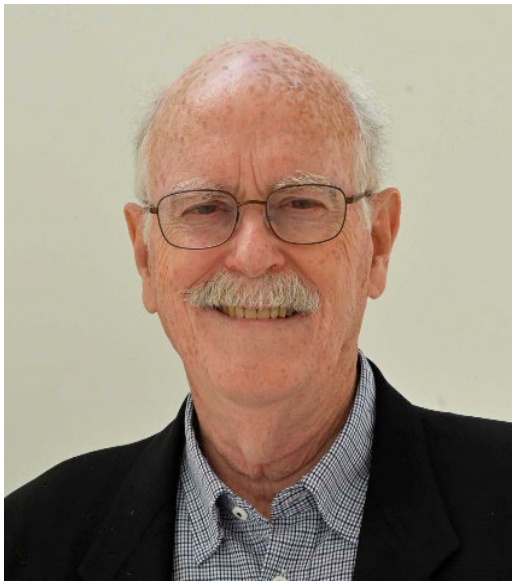
**Richard Holden, Ph.D.**
Professor, Dean's Eminent Scholar, and Chair
Department of Health & Wellness Design
Indiana University School of Public Health – Bloomington

**Dr. Richard Holden** is Professor, Dean's Eminent Scholar, and the inaugural Chair of the Department of Health & Wellness Design at the Indiana University School of Public Health, the Chief Healthcare Engineer of the IU Center for Health Innovation and Implementation Science, and a Scientist and Venture Fellow in the Regenstrief Institute. Research interests include aging and chronic disease, health information technology, health behavior interventions, social determinants of health, and methods development. His work has been published in over 150 books and articles and supported by over $95M in grants and contracts from NIH, AHRQ, PCORI, NIST, CMS, and other federal agencies. He received the Jack A. Kraft Innovator award for his role as co-founder of the subdiscipline of patient ergonomics, the subject of his 2-volume handbook, *The Patient Factor*.

**Anticholinergic Deprescribing Interventions from the Brain Health Learning Laboratory**

This talk will introduce a multidisciplinary systems approach to developing and evaluating deprescribing interventions for brain health. Anticholinergic medications in particular are believed to increase acute risk and long term cognitive decline and pathology among older adults. Evidence from animal experiments and human epidemiological studies suggests that anticholinergics, by blocking cholinergic receptors, over time increase beta-amyloid plaques and neurofibrillary tangles, leading to adverse functional and structural brain damage, lower cognitive performance, and ultimately neuropathology (e.g., dementia). Based on this evidence, the Brain Health Learning Laboratory (the "Brain Safety Lab," P30 HS24384) at Indiana University develops and tests interventions to deprescribe and reduce older adult use of anticholinergics. Dr. Holden, who co-directs the Brain Safety Lab, will present on its unique multidisciplinary team- and systems-based approach, demonstrated in a recent cluster randomized trial of a multicomponent anticholinergic deprescribing intervention and two ongoing randomized clinical trials of pharmacist-led ("R2D2," R01 AG061452) and patient-initiated technology-based ("Brain Safe," R01AG056926) deprescribing interventions.

# SESSION II: FEATURED SPEAKERS



**Arthur Arnold, Ph.D.**
Distinguished Professor
Department of Integrative Biology & Physiology
University of California, Los Angeles

**Dr. Arthur P. Arnold** studies mechanisms causing sex differences in physiology and disease. His research has included the discovery of large structural sexual dimorphisms in the CNS, development of several animal models for studying sex differences, and studies of mechanisms by which sex-biasing factors operate, including sex chromosome effects. He is Distinguished Research Professor in the Department of Integrative Biology & Physiology at UCLA, and a fellow of the AAAS and the John Simon Guggenheim Memorial Foundation. Dr. Arnold was founding President of the Society of Behavioral Neuroendocrinology, and received its Lehrman Lifetime Achievement Award in 2010. He was co-founder of the Organization for the Study of Sex Differences and was founding Editor-in-Chief of OSSD's journal, *Biology of Sex Differences.*

**X and Y genes That Cause Sex Differences in Physiology and Disease**

Sex differences in physiology and disease have long be attributed to the different effects of testicular or ovarian hormones, especially estrogens and androgens. In the last two decades, considerable evidence has emerged that sex chromosome genes also cause intrinsic sex differences in XX and XY cells. Most of this evidence comes from study of mice with modified sex chromosomes, the Four Core Genotypes (FCG) and XY* models. The FCG model allows the comparison of XX and XY mice that have the same type of gonad, to determine if a sex difference in phenotype is caused by X or Y genes acting outside of the gonad. The XY* model compares mice with one or two X chromosomes, but with the same type of gonads, to measure the effects of X gene dose. These studies have led to the identification of specific X and Y genes that cause sex differences in mouse models of metabolism, autoimmune disease, Alzheimer's disease and pulmonary hypertension.

# SESSION II: FEATURED SPEAKERS

**Jonathan Wanagat, M.D., Ph.D.**
Associate Clinical Professor
Department of Medicine, Division of Geriatrics
University of California, Los Angeles

**Dr. Jonathan Wanagat** is a physician-scientist at UCLA who is board certified in internal and geriatric medicine. He provides cares for older Veterans at the Greater Los Angeles VA Healthcare System and focuses his research on the causes of aging and treatments to slow or reverse aging. He earned his medical and doctorate degrees from the University of Wisconsin – Madison with Judd Aiken and Rick Weindruch where he also completed his internal medicine residency. He then completed a fellowship in geriatric medicine and post-doctoral work with Peter Rabinovitch at the University of Washington. Dr. Wanagat has received numerous awards in aging research, including the Paul B. Beeson Career Development Award in Aging from the American Federation for Aging Research and the National Institute on Aging; the Paul F. Glenn Award from the American Aging Association; and the Ellison Medical Foundation New Scholar Award. He is the co-director for the UCLA Medical Student Training in Aging Research (MSTAR) T35 program.

**Mitochondrial Genetics in Aging**

Mitochondrial DNA (mtDNA) deletion mutations cause many incurable human diseases and are linked to age-induced mitochondrial dysfunction. Mapping the mutation spectrum and quantifying mtDNA deletion mutation frequency is challenging with next generation sequencing methods. We hypothesized that long-read sequencing of human mtDNA across the lifespan would detect a broader spectrum of mtDNA rearrangements and provide a more accurate measurement of their frequency. We employed nanopore Cas9-targed sequencing (nCATS) to map and quantitate mtDNA deletion mutations and develop analyses that are fit-for-purpose. We analyzed total DNA from vastus lateralis muscle in 15 males ranging from 20 to 81 years of age and substantia nigra from three 20-year-old and three 79-year-old men. We found that mtDNA deletion mutations detected by nCATS increased exponentially with age and mapped to a wider region of the mitochondrial genome than previously reported. The identified mtDNA deletion frequency measured by nCATS correlates strongly with chronological age and predicts the deletion frequency as measured by orthoganol approaches. In substantia nigra, we observed a similar frequency of age-related mtDNA deletions to those observed in muscle samples but noted a distinct spectrum of deletion breakpoints. NCATS-mtDNA sequencing allows identification of mtDNA deletions on a single molecule level, characterizing the strong relationship between mtDNA deletion frequency and chronological aging.

# SESSION II: UAB SPEAKERS

**Dan Tyrrell, Ph.D.**
Assistant Professor, Department of Pathology
University of Alabama at Birmingham

**Dr. Dan Tyrrell** started out at Washtenaw Community College in Ann Arbor, MI before transferring to the University of Michigan where he earned his Bachelor's degree in 2010. He earned his Ph.D. in Integrated Physiology and Pharmacology from Wake Forest School of Medicine in Winston-Salem, North Carolina, in 2016 before completing postdoctoral training as a research fellow in the University of Michigan's Department of Cardiology. During graduate and postdoctoral training, Dan has done research on mitochondrial dysfunction and inflammation in aging using human blood samples and mouse models. Dr. Tyrrell was on an institutional T32, received a post-doctoral NRSA-F32 award, and a K99/R00 award while at the University of Michigan.

Dr. Tyrrell's research interests include aging, immunology, and neurodegeneration. Dr. Tyrrell was recruited to UAB's Department of Pathology, Division of Molecular and Cellular Pathology in 2022. Dr. Tyrrell's lab uses molecular biology, immunology, and genetic approaches including single-cell RNA sequencing, flow cytometry, and imaging to understand how neurodegeneration and cardiovascular diseases occur during aging. The lab uses a variety of murine model systems to understand two critical aspects of disease pathogenesis. The first explores how the blood vessels are altered by aging and what the implications of this are on cardiovascular diseases and neurodegeneration. The second examines how T cells change with age and contribute to cardiovascular diseases and neurodegeneration by taking advantage of a leakier blood-brain barrier during aging to infiltrate the brain and contribute to disease.

### Exploring CD8+ T Cells in Aging and Disease

Aging is the strongest clinical risk factor for atherosclerosis. T cell populations and functions are significantly changed with age as well. Yet the role of aging on T cells on development of atherosclerosis is unclear. We found that depletion of $CD8^+$ T cells attenuated atherogenesis in aged, but not young, mice. Furthermore, adoptive transfer of CD8+ T cells from aged mice but not young mice into recipients lacking CD8+ T cells significantly enhances atherosclerosis in mice. Finally, we characterized T cells in healthy and atherosclerotic young and aged mice by single-cell RNA-sequencing and T cell receptor sequencing. We found that with aging effector and memory central memory $CD8^+$ T cells preferentially accumulate within atherosclerotic plaques and almost no naïve CD8+ T cells were present in atherosclerotic plaques in both young and old mice. We also uncovered significant aging-related transcriptomic differences related senescence, cytotoxicity, and migration along with CD8+ T cell clonal expansion with aging. Overall, these data suggest that memory $CD8^+$ T cells contribute to atherosclerosis in aging and represent a potential therapeutic target for atherosclerosis.

# SESSION II: UAB SPEAKERS

**Anna Thalacker-Mercer, Ph.D.**
Associate Professor
Department of Cell, Developmental
and Integrative Biology
University of Alabama at Birmingham

**Dr. Thalacker-Mercer** received her doctorate degree through the Interdepartmental Nutrition Program in the Department of Nutrition Science at Purdue University. She continued her research training as a Postdoctoral Fellow at the University of Alabama at Birmingham (UAB) in the NIH funded Obesity Training Program, the Center for Aging Translational Research Program, and the Center for Exercise Medicine. Throughout her research career she has studied age-, inflammatory-, metabolic-, and nutrition-related alterations in human skeletal muscle using cellular, molecular, and -omics approaches in whole muscle tissue and primary cell culture. The overarching objective of her research program is to identify and understand nutrient and metabolic requirements (i) for maintenance of skeletal muscle homeostasis with advancing age and disease and (ii) for skeletal muscle regeneration following traumatic injury. Findings from her research have far-reaching impact for several populations that face skeletal muscle deterioration and declining quality-of-life

**Nutrient and Metabolic Determinants of Skeletal Muscle Regeneration in Older Adults**

Globally, the population over the age of 65 y is expanding parallel with increased human life-expectancy. Despite living longer, older adults are ultimately faced with decreased quality of life and loss of autonomy with advancing age. Among adults, 65 y and older, mobility is the most commonly reported disability (census.gov). Skeletal muscle deterioration is a common and debilitating consequence of aging. When exposed to the same muscle injury, older adults experience impaired muscle regeneration, which potentiates pathological muscle remodeling (e.g., loss of muscle mass and adipose and fibrotic tissue infiltration). Muscle regeneration is an obligatory process for repairing damaged tissue and returning muscle to a homeostatic state following injury. Regeneration is dependent on a well-choreographed myogenic program that includes the activation of muscle-specific stem cells (MuSCs) and expansion of MuSCs and their committed progeny, progenitor cells (MPCs), followed by terminal differentiation of MPCs into mature multinucleated muscle cells. MuSCs are generally in a quiescent state until activated by injury (e.g., mechanical overload, contusion, laceration) associated cues. Loss of or dysfunctional MuSCs/MPCs leads to defective muscle regeneration and remodeling. Heterochronic parabiosis studies demonstrated that factors present or absent in aged blood and local extracellular, muscle environment impair the regenerative process and enhance tissue remodeling. To date, much research has focused primarily on the accumulation of proteins that occur with age and adversely affect regeneration. Due to more powerful and accurate methodologies, the influence of nutrient availability and cell metabolism as determinants of the muscle regenerative process is being recognized. I will discuss recent advances in the identification and understanding of nutrients that are essential for the regenerative process but reduced with advancing age.

# POSTER ABSTRACTS

## 1. Angiotensin (1-7)-Expressing Probiotic and Aerobic Exercise Training Alters Gut Microbiome Diversity and Abundance in Aged Male Rats

Liliana C. Baptista[1,2,3]; Emily L. Zumbro[1]; Zacchary A. Graham[4,5,6]; Abigail L. Hernandez[1]; Taylor Buchannan[1]; Yi Sun[1]; YouFeng Yang[1]; Anisha Barnejee[1]; Amrisha Verma[7]; Qiuhong Li[7]; Christy S. Carter[1]* & Thomas W. Buford[1,4]*

*[1] Department of Medicine, Division of Gerontology, Geriatrics and Palliative Care; University of Alabama at Birmingham; Birmingham; AL; USA*
*[2] Research Center for Physical Activity, Health and Leisure; Faculty of Sport; University of Porto, Porto; Portugal*
*[3] Laboratory for Integrative and Translational Research in Population Health; University of Porto; Porto; Portugal*
*[4] Birmingham/ Atlanta Geriatric Research Education and Clinical Center, Birmingham Veterans Affair Medical Center; Birmingham; AL; USA*
*[5] Florida Institute for Human & Machine Cognition; Pensacola; FL; USA*
*[6] Department of Cell, Developmental, and Integrative Biology; University of Alabama at Birmingham; Birmingham; AL; USA*
*[7] Department of Ophthalmology, College of Medicine, University of Florida; Gainesville; FL; USA*

Pre-clinical therapeutic interventions with Ang (1-7) have been shown to improve cognition along with mitigating tau deposition and amyloid-β induced changes in biochemical and molecular outcomes on cognitive impairment and Alzheimer's disease. Ang (1-7) treatment also prevents skeletal muscle atrophy by inhibiting the myostatin signaling pathway in sarcopenic mice. However, Ang (1-7) involvement and interactions in humans remains elusive due to its short half-life and low oral bioavailability. To overcome these treatment limitations and explore the therapeutic potential of Ang (1-7) on physical and cognitive decline, we developed a gut microbiota-targeted therapeutic method- a genetically modified probiotic (GMP) designed to express Ang (1-7). Using an older rat model, we analyzed gut microbiome changes via 16S RNA sequencing on fecal samples collected following our multimodal intervention. Rats were subjected to moderate-intensity exercise or sedentary and one of three probiotic groups: control, probiotic, or probiotic + Ang (1-7). After 12-weeks of intervention, the GMP and exercise training distinctly altered fecal microbiome composition. The Inverse Simpson ($F[2,56] = 4.44$; $p = 0.02$) and Shannon-Wiener ($F[2,56] = 4.27$; $p = 0.02$) indices showed significantly higher alpha diversity amongst male rats receiving our experimental GMP. Exercise significantly altered beta diversity across groups ($F[1,56] = 2.39$; $p = 0.01$). ANCOM analyses were repeated at the genus level, and showed that 3 genera were significantly altered by probiotic supplementation: Faecalitalea, Entororhabdus and unclassified Muribaculaceae. Our findings suggests that our GMP enhanced gut microbial diversity. However, further research is needed to determine the exact mechanisms by which the gut microbiota exerts its modulatory effects, particularly on tryptophan and kynurenine local metabolites (i.e., skeletal muscle, liver and central nervous system) along with genes that were upregulated by our pathway analysis to confirm these findings.

## 2. Sex Differences in Socioemotional Behavior and Changes in Ventral Hippocampal Transcription Across Aging

Nina E. Baumgartner[1,2], Mandy C. Biraud[2], Elizabeth K. Lucas[1,2]

*[1]Department of Psychiatry and Behavioral Neurobiology, Heersink School of Medicine, University of Alabama at Birmingham. Birmingham, AL, 35233. [2]Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University. Raleigh, NC, 27607*

Socioemotional health is positively correlated with improved cognitive and physical aging. Despite known sex differences in socioemotional behaviors and the trajectory of aging, the interactive effects between sex and aging on socioemotional outcomes are poorly understood. We performed a comprehensive assessment of sex differences in socioemotional behaviors in C57Bl/6J mice across aging. We found that females exhibited decreased anxiety-like behavior and social preference, but increased social recognition, as compared to males. Additionally, we report that anxiety-like behavior and social preference increase, whereas cued threat memory recall decreases, with age. To investigate potential neural mechanisms underlying these sex- and age-related behavioral changes, we analyzed a panel of 780 transcriptions related to neuropathology in ventral hippocampal transcripts from the same mice and found age-related changes in genes related to autophagy, activated microglia, cytokines, and other functions. Interestingly, we observed sex differences in the timing, direction, and magnitude of changes. Strikingly, the autophagy pathway, as well as the individual differentially expressed autophagy genes *Cd68*, *Gusb*, *Man2b1*, and *Hexb*, were correlated with age-related behavioral changes in females but not males. Together, findings suggest critical sex differences in the trajectory of ventral hippocampal aging that may contribute to sex- and age-related differences in socioemotional behaviors.

## 3. Transcriptomic Analysis of Mice Exposed to Acute Modulation of the O-GlcNAcylation Pathway

Margaret Bell[1], Xiaosen Ouyang[1], Mariame Selma Kane[1], John C. Chatham[1], Martin E Young[2], Victor Darley-Usmar[1], Jianhua Zhang[1,2]

*[1]Department of Pathology, [2]Department of Medicine, University of Alabama at Birmingham, Birmingham, AL*

Enhancing protein O-GlcNAcylation by pharmacological inhibition of the enzyme OGA is explored as a strategy to decrease tau and amyloid-beta phosphorylation, aggregation, and pathology in Alzheimer's disease (AD). There is still more to be learned about the acute impact of enhancing global protein O-GlcNAcylation, which is important for understanding the mechanistic path of using OGA inhibition to treat AD. In this study, we investigated the acute effect of pharmacologically increasing O-GlcNAc, using Thiamet G (TG), on normal mouse brains. We sacrificed the mice and dissected the brain after 3 hours of saline or 50 mg/kg TG treatment. We next performed mRNA sequencing using NovaSeq PE 150 (n=5 each group). mRNA sequencing analysis discovered 27,941 differentially expressed genes (DEG) with TG treatment, with 1,234 DEG being statistically significant ($p < 0.05$; 593 up, 641 down). Gene Ontology (GO) analysis discovered significant downregulated GO terms related to the Biological Process: angiogenesis (count = 39; adj p = 5.52E-04), Cellular Component: receptor complex (count = 30; adj p = 2.90E-06), and Molecular Function: actin filament binding (count = 19; adj p = 1.12E-05) were revealed. Additional KEGG pathway analysis showed no significant upregulated pathways; however, the top significant downregulated pathway was related to PI3K-AKT signaling (count = 27; adj p = 5.66E-03). The mRNA sequencing analysis in this study uncovers a unique transcriptomic profile in the brain in as soon as 3 hours after TG treatment. Given that Pi3K-AKT signaling plays important roles in metabolism, ongoing studies will measure mitochondrial activity biochemical assays and be correlated to gene expression data using Kendall's $\tau$ correlation. Data and future analysis in this study will help provide the network landscape important for evaluating the mechanistic approach behind using OGA inhibition to treat AD.

## 4. Evaluation of Mitochondrial Function in Human Brain Tissue in Conjunction with Oxidative Stress Markers

Gloria A. Benavides, Ran Tian, Jianhua Zhang and Victor Darley-Usmar

*Mitochondrial Medicine Laboratory, Department of Pathology. University of Alabama at Birmingham*

Mitochondria dysfunction and oxidative stress are implicated in common diseases such as neurodegenerative disorders, cardiomyopathies, cancer and aging. The study of mitochondrial respiration is an important tool to determine mitochondrial function in response to stress, but until recently the evaluation of complex

activities was limited to fresh mitochondrial isolation from cells and tissues. Here we present a new experimental approach that reconstitutes maximal mitochondrial respiration in previously frozen samples. Optimal conditions has been determined for complexes I to IV in post-mortem brain tissues from not Alzheimer's disease (ND) and Alzheimer's disease (AD) with a reliable and sensitive high throughput method. Post-mortem tissues from brain were pulverized in a liquid nitrogen mini mortar and then homogenized with MAS buffer. Optimal conditions has been determined for complexes I to IV in the homogenates with a reliable and sensitive high throughput method. The activities of citrate synthase, a mitochondrial matrix enzyme, and lactate dehydrogenase, a cytosolic enzyme were determined. To investigate the correlation between complex activity and oxidative stress total GSH was quantified in the homogenates. In summary, the optimization of a new method to determine mitochondrial respiratory chain enzymes in snap-frozen tissue that require micrograms of a homogenate can be used in tissue from biobanks that could significantly impact translational studies of human diseases. Acknowledgement: We thank the staff of the Alabama Brain Collection as well as the families and donors of the brain tissue used in this study. This work was supported in part by UAB Nathan Shock Center P30 AG050886 (VDU, JZ), R56AG060959 (JZ) and I01 BX-004251-01 (JZ) and by the National Center for Advancing Translational Research of the National Institutes of Health under award number UL1TR003096.

## 5. The Integration Institute: Sex, Aging, Genomics, and Evolution (IISAGE)

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In many animals, aging shows sex-specific patterns, often with one sex aging faster or having a shorter lifespan. What causes the diverse patterns of sex-specific aging across the animal kingdom is unknown. The IISAGE Biology Integration Institute will determine how diverse biological processes contribute to sex differences in aging and uncover their evolutionary history. IISAGE will bring together expertise from across biology to identify the molecular mechanisms and generalizable rules that govern differences in aging between females and males. We will test hypotheses focused on differences between females and males in genome architecture, organismal biology, and phenotypic plasticity to understand differences in aging. IISAGE will define how processes at the molecular, organismal, and population level interact to generate sex differences in aging. IISAGE will produce novel analysis tools and hundreds of matched datasets profiling gene expression and chromatin in dozens of species, including several Drosophila species, houseflies, and lepidoptera. By integrating across disciplines, approaches, and levels of biological organization, IISAGE will develop predictive models for how genome architecture, organismal biology, and phenotypic plasticity can interact and lead to differences in aging. Integrated with its scientific mission, IISAGE's training, education, and outreach program will increase diversity in STEM and prepare trainees to work in diverse careers and in multidisciplinary teams. The IISAGE summer program will engage > 50 undergraduates from groups underrepresented in STEM. A citizen science project will engage pet owners and K-12 students to collect data for IISAGE scientific goals.

## 6. Scalable Lifestyle Interventions for Older Cancer Survivors: Improving Diet, Physical Activity, Weight Status, Body Composition, Overall Health, and Physical Performance Solely via Remote Delivery and Assessment

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Problem: While >80% of individuals diagnosed with cancer experience >10-year survival, they are at greater risk for second malignancies, comorbidity, and functional decline. Such decline can threaten independence and quality-of-life, especially for the >67% of survivors age 65+. The RENEW and BEAT Cancer interventions effectively improved physical functioning in older survivors via clinic-based and telephone-counseling approaches. Online programs with remote assessments are needed to increase reach. Thus to scale-up, adaptation and further testing was warranted.

Methods: Ten focus groups (n=57 cancer survivors; mean age 65±8.27) informed adaptation of RENEW and BEAT Cancer to the web-based AMPLIFY (AiM, Plan, and act on LIFestYles) program. Telephone surveys were conducted among 74 older cancer survivors to determine feasibility of remote assessments. To evaluate whether valid and reliable anthropometric and physical performance data could be collected remotely, one in-person and two remote assessments were completed and compared for 110 individuals [mean age=58 years].

Results: Focus groups revealed that content quality, privacy, ease-of-use, attractiveness, and health care providers' input were key issues in online programs. Survey results showed that 83.8% of older cancer survivors were willing to participate in virtual videoconferencing assessments. Respondents age 70+ vs. <70 were significantly less likely to favor virtual visits (p=0.04), though 61% indicated willingness. No adverse events occurred during remote assessments; validity/reliability testing showed ICCs for remote vs in-person assessments ranging from moderate (8' walk=0.47), to strong (8' up-and-go=0.74), to very strong (30 s chair stand=0.80; sit-and-reach=0.86; 2-min step test=0.87; back scratch=0.90; weight=0.93; waist circumference=0.98)(p-values < 0.001). Perfect concordance (100%) was found for side-by-side and semi-tandem balance, and 87.5–90.3% for tandem balance tests conducted using both assessment modes.

Conclusions: These favorable results informed the adaptation and testing of the AMPLIFY intervention. The efficacy trial has recruited >300 cancer survivors with results pending in 2024.

## 7. Cellular Senescence in the Cystic Fibrosis Bronchial Epithelium is Inhibited by Fibroblast Growth Factor Receptor Blockade

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Cystic Fibrosis (CF) is characterized by impaired mucociliary dysfunction, recurrent airway infections and chronic inflammation in the lungs. Cellular senescence is a feature of aging associated chronic inflammatory pulmonary diseases and characterized by, cell cycle growth arrest (p16, p21), apoptotic resistance

(BCL2/BCL-xL), increased secretion of interleukin- 6 (IL-6), IL-8 and IL-1β known as the senescence associated secretory phenotype (SASP) and increases in senescence associated β- galactosidase staining (SA-βgal). We and others have observed increased expression of cellular senescence markers in CF airways. Our lab has shown an increase in fibroblast growth factor receptors (FGFR) 1-4 expression in the CF bronchial epithelium. We hypothesize that inhibition of FGFRs can attenuate cellular senescence in the CF airway epithelium. We used primary human bronchial epithelial cells from non-CF and CF (ΔF508) donors (HBEs). HBEs were treated with FGFR1-3 or FGFR4 inhibitor then collected for analysis. In vivo, we used CFTR knockout rats and wild-type littermates as controls. Expression levels of IL-6, IL-8 and IL-1β, p16, p21 and BCL2 were examined by RT-qPCR or western blot from the cells and rat lung. Immunohistochemistry was performed on rat lung sections for p16, p21 and BCL-xL. Cellular senescence markers were increased in both CF *in vitro* and *in vivo* models when compared to controls. HBEs treated with FGFR inhibitors revealed a significant decrease in p16, p21 and BCL-xL protein levels. Cellular senescence markers are increased in both *in vitro* and *in vivo* models of CF. Our data suggests that FGFR inhibition significantly decreases expression of cellular senescence markers in CF via FGFRs may be an amenable target for future "anti-aging" therapies in CF airway disease.

## 8. Neuroprotective Function of Fucoxanthin in Rodent Models of Aging *In Vitro* and *In Vivo*

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Problem

Aging is associated with overproduction of reactive oxygen species (ROS) altering mitochondrial respiration and membrane permeability, ultimately leading to neuronal loss. DJ1 is an oxidative stress sensing protein that protects neurons from oxidative damage by scavenging free radicals. Fucoxanthin is a carotenoid found in brown seaweeds with strong antioxidant properties. However, there are limited studies demonstrating the role of fucoxanthin in brain aging. In this study, we hypothesize that treatment with fucoxanthin protects the brain against aging-associated oxidative stress by increasing the expression of DJ1.

Methods

Rat primary hippocampal neurons were grown with or without fucoxanthin for 6 wk. Time course viability analysis was performed at 3, 4, 5, 6 wk and mitochondrial membrane potential was measured at 6 wk by applying PI and TMRM staining, respectively. We further challenged neurons with hydrogen peroxide to mimic aging-associated oxidative stress. Then mitochondrial superoxide and the mitochondrial membrane potential were measured using mitoSOX and TMRM, respectively. Middle-aged male Sprague Dawley rats were supplemented with or without fucoxanthin (1mg/kg, 5d/w for 4wk). After supplementation was completed, brain tissues were harvested, and DJ-1 protein levels were quantified using immunoblotting.

Results

Fucoxanthin protected primary hippocampal neurons from age-associated death and loss of mitochondrial membrane potential. Fucoxanthin also prevented mitochondrial superoxide accumulation and loss of mitochondrial membrane potential against oxidative stress. Oral supplementation of fucoxanthin increased DJ-1 protein levels in the hippocampal tissues isolated from middle-aged rats.

Conclusions

In conclusion, fucoxanthin treatment increases protein levels of DJ-1 in the hippocampus *in vivo* and protects mitochondria during ROS challenges in primary hippocampal neurons *in vitro*. Our findings suggest neuroprotective potentials of fucoxanthin against ROS-associated mitochondrial dysfunction during aging.

## 9. Ferroptosis Regulators in Alzheimer's Disease Brains

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Alzheimer's Disease (AD) is associated with the perturbation of a variety of cellular functions including mitochondrial function, redox signaling, oxidative stress, and protein O-GlcNAcylation. In advanced cases, these dysfunctions can lead to neuronal death. Contributors to this neuronal death are not well-understood, but an increasingly large body of evidence suggests that ferroptosis, an iron-mediated form of regulated cell death, may be involved. Ferroptosis is characterized by iron-dependent lipid peroxidation, which has been shown to be exacerbated by abnormal iron metabolism and buildup, uncontrolled reactive oxygen species generation, and decreased in glutathione peroxidase 4 (GPX4) activity—many of which are consistent with the hallmarks of AD pathology. Preliminary studies from our laboratory and others identified changes of protein O-GlcNAcylation, a post-translational modification involving the addition of a GlcNAc moiety to target proteins, as well as proteins involved in glucose metabolism and nitric oxide synthesis, in AD brains. It remains unclear, however, whether these metabolic parameters are correlated with levels of ferroptosis regulators in the AD brain. In this ongoing study, we use western blot analysis and immunohistochemistry to evaluate the levels and localization of ferroptosis-related proteins in human AD brains. Proteins of interest include transferrin receptor 1 (Tfr1), ferroportin 1 (Fpn1), ferritin heavy chain (FTH), GPX4, cathepsin B, Cystine/glutamate antiporter (xCT), and solute carrier family 3 member 2 **(**CD98**)**. How the levels of these proteins are correlated with levels of protein O-GlcNAcylation and OGA activities in AD brains will be determined using bi-variant analyses. This study will help uncover the role of ferroptosis in neuronal death and AD, as well as the associations between ferroptosis and known markers of AD pathology.

## 10. Impact of Regional Microbiome Specificity on Age- and Alzheimer's Disease-Related Metabolic Impairment

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As the number of individuals living beyond the age of 65 is rapidly increasing, so is the need to develop strategies to combat the age-related cognitive declines that threaten independent living. Furthermore, increasing lifespan has led to an increased number of individuals living with Alzheimer's disease (AD), which further impairs quality of life. Unfortunately, therapeutics strategies for the treatment of AD, which mostly target central nervous system pathology, have failed to translate into humans. Therefore, alternative strategies must be considered, including those utilizing the strong relationship between gut and brain health. Which remains a largely untapped resource for the targeting of age- and AD-related cognitive impairment. These systems have strong, reciprocal links with several parameters of metabolic health including glucose utilization, insulin function and resistance, lipid function, waist circumference and more. Altered gut microbiome composition and/or density, collectively known as 'dysbiosis', can lead to impaired metabolite production and nutrient sensing, insulin resistance and increased intestinal permeability. All of these avenues provide potential link between dysbiosis and brain functioning, as they all are prevalent in aging and AD. Moreover, studies that have sought to target gut dysbiosis for treatment of AD have utilized information gained from investigations of microbiome content of fecal matter. However, fecal samples do not represent gut microbiome composition from along the length of the intestine. In fact, fecal samples most closely resemble colonic microbiome, but many microbiome-related disease states are the result of altered microbiome composition in other regions of the intestine. Moreover, age- and AD-related cognitive impairments correlate with impaired carbohydrate consumption and digestion, but this process occurs through gut microbes within the small intestine. Therefore, microbiome samples from multiple regions of the

intestine were assessed to determine the degree to which regional niches differ across genotype in a rodent model of AD, the Tg344-AD rat.

## 11. Altered Temporal Discounting, Reduced Motivation, and Impaired Executive Functions in the TgF344AD Rat Model of Alzheimer's Disease During Young Adulthood may Associate with Inflammation in the Basolateral Amygdala

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The relative weight that individuals give to rewards and "costs" (such as delay to reward delivery) in making decisions varies significantly across the population and disease states. One aspect of decision making involves weighing the relative benefits and costs associated with immediate versus delayed outcomes. This aspect of decision making is often referred to as temporal discounting (or intertemporal choice) and can be assessed on tasks in which subjects are required to choose between small, immediate rewards and larger rewards delivered after varying delays. Furthermore, intact executive functioning and motivation are foundational to the decision-making process. Recent literature shows there is a great deal of variability in the temporal discounting phenotype between individuals with mild cognitive impairment, frontal temporal dementia, and Alzheimer's Disease (AD), with some showing no differences relative to healthy controls and others showing altered rates of temporal discounting. Even beyond these disorders, individual differences in choice behavior (either maladaptive or normative) in young adults predict a variety of life outcomes, including educational success and socioeconomic status. The current study used a behavioral and molecular approach to determine the effect of genotype on phenotypic differences in temporal discounting, motivation, and executive function and neuroinflammation in the TgF344AD (TgAD) rat model of AD.

Young adult (6-7 mo) wild-type (WT; n=6) and TgAD (n=6) were trained on a several operant tasks including temporal discounting (decision making), progressive ratio (motivation), set shifting (cognitive flexibility), and delayed response (working memory) tasks. Basolateral amygdala (BLA) tissue was then isolated and processed for use in a multiplex ELISA to assess markers of inflammation. Results suggest TgAD rats showed a greater preference for the large, delayed reward relative to WT, however, this preference did not translate to a greater number of total rewards earned. Additionally, TgAD rats were less motivated to obtain the larger reward, were less cognitively flexible, and showed impaired working memory relative to WT controls. Additionally, AD-associated markers of inflammation were higher in the BLA of TgAD rats relative to WT. Interestingly, young TgAD rats show a similar decision-making and cognitive phenotype as old rats, and these data suggest AD pathology may manifest as maladaptive decision making in addition to impaired executive functions early in life.

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## 12. Skin Aging Caused by UVA Induced ROS is Attenuated by the Treatment with Novel Vitamin D3 and Lumisterol Hydroxyderivatives

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Many genetic and environmental factors contribute towards the effects aging has on the skin. Studies have shown that of the various environmental causes of skin aging UV radiation from the sun (UVA, UVB and IRA) and tanning beds (primarily UVA) plays a large role in skin damage. UV rays can cause wrinkles in the dermal layer and atrophy and barrier dysfunction in the epidermal layer resulting from mitochondrial DNA damage and altered gene expression. Aging skin shows a variety of molecular mechanisms that can be targeted by small molecules. In vitro UVA exposure has been shown to elicit oxidative stress in both the dermis and

epidermis, senescence in the germinal layer of epidermal tissue and modification of the collagen network in the dermis.

Vitamin D hydroxyderivatives protect skin from aging by inducing antioxidant interactions, preventing DNA damage, and maintaining skin homeostasis. The use of topical vitamin D derivatives on skin as an advisable method to prevent aging is an area where continued research is needed. We used hydroxyderivatives of vitamin D3 and lumisterol for treatment and protection of human skin fibroblast against UVA induced stress.

Human fibroblast were isolated from neonatal skin and exposed to different intensities of UAV irradiation: 10 J/cm$^2$ or 20J/cm$^2$. Before or after UVA exposure, the cells were treated with vitamin D3 hydroxyderivatives (1,25(OH)$_2$D3, 20(OH)D3, 20,23(OH)$_2$D3, 1,20(OH)$_2$D3, 20,25(OH)$_2$D3) or lumisterol hydroxyderivatives (L3, 20(OH)L3, 22(OH)L3, 25(OH)L3). The protective effects of treatment were measured by production of reactive oxygen species (CM-H2DCFDA assay) and cell viability (MTS assay).

Preliminary data show that the tested novel vitamin D3 and lumisterol hydroxyderivatives decrease production of harmful radical oxygen species (ROS) in skin cells, thus protecting against oxidative damage caused by UVA with cutaneous antiaging activity.

## 13. Aging, Plasminogen Activator Inhibitor 1, Brain Cell Senescence, and Alzheimer's Disease

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The etiology for late-onset Alzheimer's disease (LOAD), which accounts for >95% of Alzheimer's disease (AD) cases, is unknown. Emerging evidence suggests that cellular senescence contributes importantly to AD pathophysiology, although the mechanisms underlying brain cell senescence and by which senescent cells promote neuro-pathophysiology remain unclear. In this study we show for the first time that the expression of plasminogen activator inhibitor 1 (PAI-1), a serine protease inhibitor, is increased, in correlation with the increased expression of cell cycle repressors p53 and p21, in the hippocampus/cortex of senescence accelerated mouse prone 8 (SAMP8) mice and LOAD patients. Double immunostaining results show that astrocytes in the brain of LOAD patients and SAMP8 mice express higher levels of senescent markers and PAI-1, compared to astrocytes in the corresponding controls. In vitro studies further show that overexpression of PAI- 1 alone, intracellularly or extracellularly, induced senescence, whereas inhibition or silencing PAI- 1 attenuated H2O2-induced senescence, in primary mouse and human astrocytes. Treatment with the conditional medium (CM) from senescent astrocytes induced neuron apoptosis. Importantly, the PAI-1 deficient CM from senescent astrocytes that overexpress a secretion deficient PAI-1 (sdPAI-1) has significantly reduced effect on neurons, compared to the PAI-1 containing CM from wild type PAI-1 (wtPAI-1) overexpressed senescent astrocytes, although sdPAI-1 and wtPAI-1 induce similar degree of astrocyte senescence. Together, our results suggest that increased PAI-1, intracellularly or extracellularly, may contribute to brain cell senescence in LOAD and that senescent astrocytes can induce neuron apoptosis through secreting pathologically active molecules, including PAI-1.

## 14. Heavy Metal Exposure Induces Senescence as a Mechanism of Dopaminergic Neurodegeneration

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Heavy metal exposure is implicated in the etiology of neurologic diseases of aging such as Parkinson's Disease (PD) and Alzheimer's disease (AD) and metals represent an ongoing environmental risk factor for many populations around the world. Despite this well-known connection, the mechanisms behind metal-induced neurodegeneration remain unclear. Work from our lab suggests that a common feature of toxicant exposure in neurons is the induction of cellular senescence, which accelerates cellular aging, promotes inflammation, and impairs protein degradation pathways. We hypothesized that exposure to heavy metals induces senescence within dopaminergic neurons as a mechanism of environmental influence on aging and age-related neurodegenerative diseases.

To assess this, we used an *in vitro* platform within the dopaminergic neural N27A cell line that express human alpha-synuclein (αSyn) and treated neurons with 5 μM manganese ($MnCl_2$), lead 2.5 μM ($PbNO_3$), or vehicle for 24 hours. Using immunocytochemistry, we detected significantly elevated levels of the senescence protein p21, a cell cycle regulating protein, in dopaminergic neurons following both metal treatments ($p<0.0001$). As senescent cells are reported to have autophagic impairment, we assessed lysosomal function via the Lysosomal Membrane Associated Protein (LAMP)-1, which was significantly decreased following manganese and lead exposure ($p<0.0001$). Furthermore, we observed an accumulation of αSyn within N27A cells, suggesting that heavy metal-induced senescence caused a reduction in protein degradation pathways that promoted αSyn aggregation.

Together, these data suggest that heavy metal exposures may induce senescence as a mechanism that accelerates neurologic aging and influences neurodegeneration. In addition, these data indicate that inhibition of senescent proteins such as p21 may be effective at protecting neuronal damage from exposure to heavy metals such as manganese and lead. Experiments to further assess autophagy and block senescent pathways in dopaminergic neurons to preserve autophagy and limit αSyn accumulation are currently ongoing in our lab.

## 15. Impact of Elevation of Protein O-GlcNAcylation on Mitophagic Flux in the Brain

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O-linked N-acetylglucosamine (O-GlcNAc) post-translational modification of nuclear and cytoplasmic proteins represents a key regulatory pathway in the brain. Previous work has shown that loss of O-GlcNAc glycosylation in forebrain excitatory neurons induce neurodegeneration. However, little is known about O-GlcNAc modifies mitochondria under those conditions. We acutely treated male and female MitoQC mice with 10mg/kg and 50mg/kg of the highly selective orally bioavailable inhibitor of the enzyme responsible for removing O-GlcNAc glycosylation (OGA), Thiamet G, to increase O-GlcNAc in the brain. We find that increased O-GlcNAc behave differently in neurons, astrocytes, and microglia in a gender dependent manner. Histological quantitation of the size and number of lysosomal mitochondria in 3 m/o animals colocalized to each cell type indicates that increased O-GlcNAc can influence mitochondrial flux and provide a therapeutic target to alter mitoptosis in the brain.

## 16. Role of Aging Associated Vascular Senescence on Ovarian Cancer Burden

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Ovarian cancer is the most lethal gynecological cancer, typically diagnosed at advanced stages post-menopausally. The ovaries, which are a primary site of tumor growth for all ovarian cancers, are subjected to rapid changes in the vasculature upon the onset of menopause. In a corollary manner, the vasculature is a primary therapeutic target for the management of advanced ovarian cancers indicating the significance of tumor angiogenesis in diseases progression and metastasis. Given the association between aging endothelial senescence and angiogenesis, we hypothesized that pre-existing senescent endothelial cells in aged and/or menopausal ovaries could contribute to ovarian cancer growth and invasion in the ovaries.

To first distinguish the effects of follicular changes associated with the onset of menopause versus the effect of age we used 4-vinylcyclohexene diepoxide (VCD) for inducing menopause in young mice and a physiologically aged model. Menopausal changes of the ovary in both models were confirmed by follicle count and hormonal profile. Intrabursal implantation and assessment of tumor growth kinetics of mouse ovarian cancer cells (MOVCs) (ID8p53-/-) into the bursa of the ovaries from both these models revealed that physiologically aged mice had high tumour growth kinetics as compared to young (6 week old) mice. However, tumour burden in VCD induced mice was not significantly altered as compared to age-matched control mice suggesting that age-associated changes in ovaries contribute to tumor growth. We next assessed whether ovarian endothelial cells contribute to tumour growth, invasion into the ovary, by implanting MOVCs mixed with primary mouse ovarian endothelial cells (MOECs) intrabursally, and comparing to MOVCs alone. We find that mice implanted with MOVCs and MOECs together had significantly higher tumour burden as compared to MOVCs alone. Examining MOECs from aged mice indicate strong senescence features as evident by β-galactosidase staining, γH2Ax immunofluorescences, p16, TRF-1 (Telomeric repeat factor-1) and PAI-1 (plasminogen activators inhibitor-1) levels. We find that condition media from senescent MOECs from older mice promoted invasion of cancer cells *in vitro*. These findings suggest a potential direct contribution of the aged and senescent vasculature to tumour growth and invasion into the ovary. These findings and ongoing studies will be presented.

## 17. Differential Response to the Fasting Hormone Glucagon in Long-Lived Growth Hormone-Releasing Hormone Deficient Female Mice

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Loss of function mutations in the growth hormone (GH) signaling pathway are among the most reproducible methods for lifespan extension in the laboratory rodent. Previous work from our group has demonstrated that genetic knockout of the gene coding for GH-releasing hormone (GHRH) in mice indeed confers longevity extension. In addition to the reduced body size and elevated insulin sensitivity that has been well documented in this model, these animals also display mild hypoglycemia and preferential metabolism of lipids relative to carbohydrates during periods of fasting. These suggest there is a fundamental response to fasting in this model of healthy aging. We hypothesized that differences in the response to glucagon, a canonical fasting hormone secreted from the α-cells within the pancreatic islets of Langerhans, contribute to these differences. To this end, we employed glucagon, pyruvate, and glycerol tolerance tests to gauge capacity for endogenous glucose production. We also carried out indirect calorimetry during a glucagon challenge to gauge the metabolic response in vivo. These tests revealed a reduced capacity for glucose production but greater energy expenditure in response to glucagon administration in GHRH-KO mice. To explore potential mechanisms for these changes, we employed immunofluorescent staining of pancreas sections and gene expression analyses for genes associated with glucose production and lipid metabolism. These revealed an altered islet composition in GHRH-KO mice with a significantly elevated ratio of α-cells to insulin-producing β-cells and dampened expression of genes related to hepatic glucose production. Taken together these suggest that the unique response to fasting is, at least partially, mediated by an alternative response to glucagon.

## 18. Investigating the Role of MicroRNAs (miRNAs) in *Drosophila* Aging Antiviral Immunity

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microRNAs (miRNAs) are non-coding RNAs which post-transcriptionally regulate gene expression. Possessing a plethora of targets, miRNAs play roles in many biological processes including aging and innate immunity. A gap in knowledge exists regarding how miRNAs orchestrate innate antiviral responses in aging hosts. As older individuals are more susceptible to viral infections, uncovering such mechanisms could identify novel targets to improve infectious outcomes among the elderly. Using a *Drosophila melanogaster*-Flock House virus (FHV) host-virus model, we have previously shown that aging is associated with a robust transcriptional response and results in decreased survival of FHV infection due to impaired tolerance. Here, we used small RNA-seq analysis to identify differential changes in miRNA expression in young and aged cohorts 48h following FHV infection, finding younger flies differentially expressed more miRNAs than older flies. Subsequent Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analysis on targets of differentially expressed miRNAs showed neurogenesis enrichment for both young and aged-infected flies and reproduction and metabolic process enrichment for aged-infected flies. We then generated 17 lines with knockdown (KD) of candidate miRNAs and examined survival outcomes after FHV infection as a function of age and sex. We found that aged flies generally displayed increased susceptibility compared to younger counterparts and that in every line examined, females outlived age-matched males. miRNA KD also had a greater age-dependent effect on survival in males compared to females. For miR-311, which was chosen for further study, we found KD and overexpression of this miRNA led to significantly decreased survival of FHV for males, associated with comparable virus titers across samples. Taken altogether, our work provides insights into the role of miRNAs during FHV infection and identifies miRNAs with potential roles in innate immunity in the context of aging, highlighting miR-311 as one miRNA responsible for regulating FHV tolerance.

## 19. Studying Innate Immune Activation in the Drosophila Brain Following Bacterial Infection

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Background: Significant neuroinflammatory responses associated with neurodegenerative diseases including Alzheimer's disease (AD) could result from microorganisms invading the brain. When excessive, such responses could lead to the death of healthy cells in the tissue. However, the exact mechanisms leading to disease manifestation and progression in infectious contexts are not fully understood. The model organism Drosophila melanogaster represents an ideal genetically tractable system to investigate the processes associated with infectionmediated neurodegeneration because both the mechanisms of neural development and function and innate immunity are highly conserved from flies to humans.

Prior work in Drosophila demonstrated that brain bacterial infection with a mixture of non-pathogenic Escherichia coli (Gram-negative) and Micrococcus luteus (Gram-positive) activates the evolutionarily conserved NF-kB immunity, leads to age-dependent neurodegeneration and impeded mobility. These phenotypes depend on NF-κB activation in both neurons and glia in the fly brain. Nevertheless, there is a shortcoming in understanding how NF-kB immunity in the Drosophila brain is activated following bacterial infection. Our goal is to dissect the activation of the fly's NF-kB inflammatory pathways following a non-lethal brain bacterial infection over time.

Methods: Infection in the Drosophila brain of NF-kB pathway mutants or controls is performed by poking one side of the head with a fine, metal needle, dipped into concentrated pellets of overnight cultured E. coli or M. luteus. A poke with a sterile needle is used as a control for the injury alone, and thorax injections as a control for the effect of peripheral activation of immunity. Gene expression of Drosomycin (Toll pathway) or Diptericin (IMD pathway) is carried out to assay the reliance of canonical NF-κB pathways.

Results: Our preliminary data show that brain bacterial infection leads to increased expression of Drosomycin and Diptericin. Currently, using quantitative reversetranscription PCR we are testing whether the expression of these genes is affected in fly mutants for multiple components of the NF-kB pathways Toll and IMD.

Conclusions: Our fly-based model could be used to determine how inflammatory responses evolve over time, furthering our understanding of the pathophysiological mechanisms of acute and chronic brain infections and their link to neurodegeneration.

## 20. Effects of a Long-Term Chronic Methionine Restriction (MR) Diet Feeding in the Gut Microbiota Composition and Physiology in Male Mice

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Methionine restriction (MR) diet feeding has been consistently found to delay aging and extend lifespan in various model organisms including mice and rats. In our study, we have examined the long-term effects of MR diet feeding in genetically heterozygous male mice with the diet starting at 6 months of age. MR diet feeding has been known to produce improved metabolic homeostasis in the animals apart from delayed aging. We have observed similar results, MR mice have reduced body weight with increasing lean mass and reduced fat mass along with improved glucose tolerance and increased insulin sensitivity. Indirect calorimetry was performed on these animals, and they showed reduced expiratory exchange ratio (RER) with an increased oxygen consumption and increased CO2 production after controlling for their body weight. They also exhibited increased energy expenditure (EE) compared to the control diet (CD) fed mice. Longitudinal 16S rRNA amplicon sequencing was performed on fecal samples of mice collected after 1 week, 12 weeks and 57 weeks of the experimental diets feeding. For alpha diversity metrics, faith's phylogenetic diversity and observed features was analyzed and we found no differences overall between diet groups, but we found differences between the different timepoints. For beta diversity metrics, Unweighted Unifrac and Jaccard distance metrics were analyzed and similarly we found no differences between the diet groups but we found differences between the different timepoints. LefSe was utilized to determine the bacterial taxa differentially represented among the experimental groups. We observed dietary changes at 1 and 12 weeks of diet for various bacterial taxa but none at 57 weeks. Overall, we found that age had a bigger impact on the microbiota compared to experimental diet feeding.

## 21. Chronic ACE Inhibition with Lisinopril Mitigates Motor Deficits and Neurodegeneration in a Sex-Specific Manner in a Drosophila Model of Alzheimer's Disease

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Evidence arguing for a protective role of angiotensin-converting-enzyme inhibitors (ACEis) against Alzheimer's disease (AD) is inconsistent. Like vertebrates, orthologues of human ACE are present in *Drosophila melanogaster* and the activity of the fly ACE, AnCE, is inhibited by the ACEi lisinopril. We have recently shown that treatment with 1mM lisinopril ameliorates physical and cognitive deficits in a young Drosophila model of AD. Whether the same therapeutic benefits extend to later in life remains to be determined. Here, we tested the effect of chronic lisinopril treatment on the locomotion and cognitive deficits in an old fly model of AD. We used a Drosophila line expressing the human amyloid precursor protein and the human ß-site APP-cleaving enzyme in neurons as AD model. Newly eclosed flies were supplemented or not with 1mM lisinopril for 30 days after which locomotion and memory were tested via a negative geotaxis assay and an aversive phototaxic suppression assay, respectively. Fly heads were dissected and underwent standard histological procedures to quantify brain neurodegeneration (i.e., appearance of vacuolar lesions). Additional fly heads were collected to determine levels of tryptophan-derived metabolites and neuroinflammatory gene expression by LC/MS/MS and RT-qPCR, respectively. In this study, 1mM lisinopril significantly ameliorated locomotion deficits ($p$=0.0320) and decreased neurodegeneration in 30-days-old female flies ($p$=0.002), independent of genotype, compared to their untreated counterparts. This effect, however, was not detected in males. Similarly, lisinopril mitigated memory deficits in 30-days old AD females but not males ($p$=0.0214). Our findings provide strong evidence that long-term ACE inhibition with lisinopril mitigates the age-associated behavioral deficits in our wild-type and fly model of AD, but it does so in a sex-specific manner. Differential activation of the kynurenine pathway of tryptophan metabolism and differential expression of the NF-kB neuroinflammatory genes in our fly model of AD highlight potential new therapeutic targets of intervention.

## 22. O-GlcNAcylation and Neuropathology in APP $^{NL-G-F}$ Knock-in AD Mouse Model

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Alzheimer's disease is the main cause of dementia and is quickly becoming one of the most expensive and devastating diseases. Although there are pharmacological treatments include anti-amyloid β, anti-tau, and anti-inflammatory strategies at clinical trials stage, so far, no effective treatment to attenuate disease progression. O-GlcNAcylation (addition of O-linked-N-acetylglucosamine) is a ubiquitous post-translational modification that occurs in nucleus, cytosol, and mitochondria. There are 3000-5000 target proteins for O-GlcNAc in the brain. Changes of O-GlcNAcylation has been noted in human AD. In the current study we aim to determine in $APP^{NL-G-F}$ knock-in ($App^{ki}$) AD mouse model on C57BL/6J background whether O-GlcNAcylation pathway proteins are altered. We found $App^{ki}$ mouse brain has increased O-GlcNAcylation in Hippocampus (HP) and cortex area compared to normal C57BL/6J (B6) control brain by immunofluorescence. O-GlcNAcase (OGA), the key enzymes for removal of O-GlcNAc modifications was not changed. However, O-GlcNAc transferase (OGT), the key enzyme for addition of O-GlcNAc modifications was reduced in cortex and HP area ($p < 0.05$). OGT expression pattern was dramatically changed. We also found that lysosome-associated membrane protein 1 (LAMP1) was accumulated in amyloid plaque in $App^{ki}$ mouse brain by double labelling with an amyloid antibody, while its level not surrounding the plaques was reduced in cortex and HP area by image density/area quantification ($p < 0.05$). Interestingly, OGT and Lamp1 has similar pattern change by double labelling OGT and Lamp1 in $App^{ki}$ mouse brain compared to B6 control. Thus, OGT is also concentrated in amyloid plaque. Ongoing study aims to clarify if increasing O-GlcNAcylation is beneficial or detrimental.

## 23. Natural Genetic Variation and Sex Regulate Diet-Dependent Lifespan Extension Under Methionine-Restricted Conditions in *Drosophila melanogaster*

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Caloric restriction (CR) has long been extensively studied as an intervention in the aging process for its ability to extend lifespan and improve several measures of health in a broad range of animal models. Increasingly, research suggests that other factors such as amino acid content play a significant role in longevity and other age-related phenotypes and that restriction of a single amino acid, such as the essential amino acid methionine, is sufficient to robustly increase life and 27hosphor27n. However, prior studies have generally used only one or a few genetic backgrounds to examine the effects of methionine restriction and the degree to which the pro-longevity and geroprotective benefits of this intervention are genotype and sex-dependent is unclear. To address this, we have characterized the impact of a methionine restricted diet on lifespan and age-dependent changes in physical activity in *D. melanogaster* utilizing the natural genetic variation of the Drosophila Genetic Reference Panel. Interestingly, we find that a MetR diet can uncouple longevity extension from a common measurement of Drosophila 27hosphor27n, climbing ability. Additionally, we show that sex and genotype regulate diet-dependent changes in lifespan under MetR conditions. Altogether, our study highlights the complex role that genetic variation and sex have in determining the response of age-related traits to amino acid restriction.

## 24. Disparities and Barriers to Oral Healthcare Among Older Adults in Alabama

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Problem: Limited data exists on the oral health status of the older adult population of Alabama; improved surveillance programs are needed for estimations and planning.

Aim: This study compares gender, racial, and economic differences in 27hosphor to oral healthcare in the older adult population in Alabama, where limited data exists.

Methods: Self-reported, voluntary surveys including sociodemographic, oral care behaviors, and care utilization were completed at senior centers and senior living communities. Univariate analyses were conducted using Chi-Square or Fisher's Exact test, $p<0.05$ considered meaningful.

Results: 263 respondents were assessed: mean age 72.5 (SD 8.2), 68.4% female, 65.0% Black/African American (AA), 35.0% Caucasian (Ca), 86.6% sole source of income Social Security/Disability. AA were more likely than Ca to have dental insurance (50.9 vs 35.3%, p=0.02), and to have used dental insurance (32.7 vs 21.2%, p=0.02), but less likely to have had a cleaning/check-up (39.2 vs 76.2%, p=0.009). Those visiting a senior center were more likely to have had a cleaning/check-up compared to those residing in a living facility (76.0 vs 36.2%, p=0.001). Regardless of race, those receiving less than $1500/month were more likely to report cost as a barrier to dental care compared to higher income levels (34.2 vs 17.7%, p=0.03). Females were more likely than males to report transportation as a barrier to care (15.3 vs 4.6%, p=0.03)

Conclusion: The preliminary analysis points to the need to further explore access to oral health services for older Alabamians living independently. Further data is needed for planning and policy making. Barriers to care were not explored for those who are homebound.

## 25. Infrequent and Incomplete Data and Code Sharing in Nathan Shock Center Funded Research in the Last Five Years

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Background**:**

Data and code sharing facilitate reproducibility and trust when results can be verified and support efficient reuse of research resources. Growing recognition of the importance of data and code sharing among researchers, journals, and funding agencies led the Nathan Shock Centers (NSC) Coordinating Center to propose documenting and supporting data and code sharing in aging research. As a baseline for these efforts, we characterized recent data and code sharing practices in NSC-funded research.

Methods:

We surveyed articles citing NSC grants published 2017-2022 indexed in PubMed. We screened full texts in two phases. In phase 1, we excluded articles that did not generate or analyze data. For included articles, we classified statements about data and code availability as available via repository, included in supplemental file, included in paper, available on request, explicitly not available, no statement was included, or other. In phase 2, we checked articles indicating open data and code to see if we could find raw data or statistical code according to each paper's statement.

Results:

Of 507 articles screened, 400 were included. Of included articles, 43% had no data availability statement, and 90% no code statement. Data availability statements indicated that 30% were available in repositories, 26% in supplementary files, 10% included in the papers, and 14% available on request. Of code statements, 6% indicated code was available in repositories, and

≤1% for each of the other categories. Of articles indicating open data or code, open data and code were accessible for 68% and 50%, respectively.

Conclusions:

Data and code sharing statements and availability in aging research funded by NSCs occur with low to moderate frequency. Future work will evaluate how these practices improve over time in NSC-funded research with the NSC Coordinating Center support and evolution of journal and funder guidelines.

## 26. Mimicking Permanent Phosphorylation of HP1a Causes Sterility

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The Heterochromatin protein 1 (HP1) family are non-histone chromosomal proteins that are key factors in the formation of heterochromatin and in transcriptional regulation. HP1 proteins are found in many eukaryotic organisms including plants, animals, and fungi. HP1a from *D. melanogaster* was the first HP1 protein discovered, and it has been intensively studies for more than three decades. HP1a can act both as a repressor and an activator of transcription. Like many other proteins, HP1a undergoes post-translational modifications such as phosphorylation. However, little is known about the functions of HP1 post-translational modification, including phosphorylation. To advance our understanding of HP1a's post-translational modifications, we produced two HP1a mutants that either mimic or block phosphorylation. Specifically, we replaced serines (S) 88/89/91 (S88/89/91) either with glutamic acid € to mimic permanent phosphorylation

or with alanine (A) block phosphorylation. Using these mutant strains, we investigated how phosphorylation of HP1a impacts its known functions. Western blot analysis demonstrated that the 29hosphor-mimic HP1a protein is stable and accumulates to similar levels as wildtype HP1a. Polytene chromosome analysis indicated that the 29hosphor-mimic HP1a protein continues to localize to heterochromatic regions of the genome, including the centromeres and telomeres. On the organismal level, we found that homozygous 29hosphor-mimic HP1a mutants have a significant reduction in fertility for both males and females compared to heterozygous and wildtype animals. We observed a significant decrease in ovary size for the homozygous 29hosphor-mimic HP1a mutant females when compared to heterozygous and wildtype animals. Parallel experiments with the HP1a 29hosphor-block mutant strain are ongoing. Overall, our results show a significant fertility decrease, possibly due to the decreased ovary size in homozygous mutants, but no disturbance of HP1a localization on polytene chromosomes. These results suggest that phosphorylation of HP1a proteins at the site we modified (S88/89/91) might have specific functions in the Drosophila germline.

## 27. Glutamine Mediated Metabolically Derived Factors Boosts Muscle Progenitor Cells Proliferation in Aging

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Skeletal muscle (SkM) possesses a high capacity for regeneration that is reliant on a myogenic program that includes proliferation of SkM progenitor cells (MPCs) and is determined in part, by the availability of nutritionally non-essential amino acid (NEAA) glutamine. Cellular dysfunctions alter the entire SkM metabolism with advancing age. Metabolomic analysis of blood from older adults demonstrated that glutamine is reduced with advancing age. Muscle glutamine, taken up from the extracellular space, is important for MPC proliferation. Further, it is recognized that coordination of the myogenic program is impacted by dynamic changes in intermediary metabolism, however, the current understanding of the crosstalk between glutamine, intermediary metabolism and the myogenic process is limited. Therefore, the objective of the present study was to investigate the effect of extracellular glutamine availability on metabolic signaling and MPC proliferation.

Murine MPCs (C2C12 cells) were cultured in high glucose DMEM without glutamine (0 mM) or with various doses of glutamine (2, 6, or 12 mM) to determine the effect of glutamine dose on MPC proliferation. MPC counting and cell cycle were assessed using imaging cytometry and FACS, respectively. Next, using immunoblot we measured the dose-dependent change in protein expression of glutaminase (GLS), which catabolizes glutamine to glutamate. Glutamate, through α-ketoglutarate, is further metabolized to succinyl-CoA and succinate by the enzymatic proteins DLST and SUCLA2, respectively, which we measured via immunoblot. Importantly, this metabolic pathway generates the succinyl donor for succinylation and potentially the epigenetics of cells, ultimately leading to changes in the cell state.

We observed that MPC proliferation was retarded in the absence of extracellular glutamine, as demonstrated with imaging cytometry and FACS. There was no difference in cell death count with doses of glutamine. We demonstrated through succinyl-proteomics that DLST is a target of succinylation, to our surprise, DLST succinylation was increased with 0mM vs 6mM glutamine. DLST can localize to the nuclear fraction. Indeed, in 0mM glutamine, the DLST protein expression was increased in the nuclear fraction. We expect that this due to histone succinylation, which we also identified in the 0mM glutamine, with proteomics and will verify with subsequent studies. Our ongoing research demonstrates that glutamine availability, which is reduced in advanced age, leads to changes in protein modifications that alter the cell state and suggest a potential therapeutic mechanism to improve the myogenic program in older adults.


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