SECOND ANNUAL NATHAN SHOCK CENTER SYMPOSIUM ON THE BASIC BIOLOGY OF AGING

University of Alabama at Birmingham
Alumni House
April 18, 2018
AGENDA

8:30-9:00 am  Registration, Coffee and Poster Set Up

9:00-9:10 am  Welcome

Steven Austad, PhD
Distinguished Professor & Chair
Director, Nathan Shock Center of Excellence in the Basic Biology of Aging
Department of Biology
University of Alabama at Birmingham

SESSION I: Chair, Steven Austad, PhD – ENERGETICS

9:10-9:40 am  Keynote Speaker, Charlotte Peterson, PhD
Professor and Director, Center for Muscle Biology
University of Kentucky College of Health Sciences
“The Evolving Roles of Satellite Cells in Skeletal Muscle Aging and Adaptation”

9:40-9:55 am  Kaleen Lavin, PhD
Postdoctoral Trainee, Laboratory of Marcos Bamman, PhD.
Department of Cell, Developmental, & Integrative Biology
University of Alabama at Birmingham
“Human Neuromuscular Aging: Sex Differences Revealed at the Myocellular Level”

9:55-10:10 am  Christy Carter, PhD
Assistant Professor
Department of Aging and Geriatric Research,
College of Medicine, University of Florida, Gainesville
“Multimodal exercise-, and pharmaceutical-based strategies for altering the gut microbiome during aging”

10:10-10:25 am  Khandaker Ahmed, PhD
Laboratory of Rakesh Patel, PhD
Department of Pathology and Center for Free Radical Biology
University of Alabama at Birmingham
“Oral nitrate reductase activity declines with age: implications for age-associated decrease in vascular nitric oxide bioavailability”

10:25-10:35 am  Coffee Break

10:35-11:05 am  Keynote Speaker, Peter Rabinovitch, MD, PhD
Professor of Pathology
University of Washington
“Restoring mitochondrial energetics improves function in aging mice”

11:05-11:20 am  Brian Spurlock
Graduate Student Trainee, Laboratory of Kasturi Mitra, PhD
Department of Genetics
University of Alabama at Birmingham
“Novel Mito-SinCe2 Method Identifies Quantitative Relationships between Mitochondrial Dynamics and Energetics”
11:20-11:35 am. **Nicole Riddle, PhD**  
Assistant Professor  
Department of Biology  
University of Alabama at Birmingham  
“Measuring respiration rates in Drosophila melanogaster using the Loligo system”

11:35-11:50 am **Michelle Johnson**  
Researcher, *Laboratory of Victor Darley-Usmar, PhD*  
Department of Pathology  
University of Alabama at Birmingham  
“Bridging the Gap between Metabolomics and Bioenergetics”

11:50-1:20 pm **Lunch with Poster Session**

**SESSION II: Chair, Scott Ballinger, PhD - LONGEVITY**

1:20-1:50 pm **Keynote Speaker, Richard Miller, MD, PhD**  
Professor of Pathology  
University of Michigan, Medical School  
“Drugs that Slow Aging in Mice”

1:50-2:05 pm **Sara Sims**  
Graduate Student Trainee, *Laboratory of Kristina Visscher, PhD*  
Department of Neurobiology  
University of Alabama at Birmingham  
“Functional Connectivity Networks in the Healthy Oldest Old”

2:05-2:20 pm **Robert Williams, PhD**  
Professor and Chair, Department of Genetics, Genomics and Informatics, University of Tennessee Health Science Center  
“Genetic Analysis of Longevity in Diverse Cohorts of Mice: Influence of Diets and Drugs”

2:20-2:35 pm **Michael Paul Fitch**  
Researcher, *Laboratory of Liou Sun, MD, PhD*  
Department of Biology  
University of Alabama at Birmingham  
“The Generation of a Novel GH mutant Mouse Model Using the CRISPR/cas9 System”

2:35-3:05 pm **Keynote Speaker, Arlan Richardson, PhD**  
Professor of Geriatric Medicine  
University of Oklahoma Health Sciences Center  
“New Insight into the Life Extending Action of Dietary Restriction”

3:05-3:20 pm **Rui-Ming Liu, MD, PhD, DABT**  
Professor  
Department of Medicine, Division of Pulmonary, Allergy, Critical Care Medicine, University of Alabama at Birmingham  
“Ozone exposure accelerates memory decline in old male apoE3, but not apoE4, targeted replacement mice”
3:20-3:35 pm  **Joseph Palmer**  
Graduate Student Trainee, *Laboratory of Melissa Harris, PhD*  
Department of Biology  
University of Alabama at Birmingham  
“*Transcriptomic analysis reveals depth of quiescence is altered in melanocyte stem cells with age*”

3:35-3:50 pm  **Daniel Smith, PhD**  
Assistant Professor  
Department of Nutrition Sciences  
University of Alabama at Birmingham  
“*Yeast phenomic analysis of associations between hydrogen sulfide production and chronological lifespan*”

3:50-4:05 pm  **Hai Vo**  
Graduate Student Trainee, *Laboratory of Gwendalyn King, PhD*  
Department of Neurobiology  
University of Alabama at Birmingham  
“*Evidence for the role of age-regulating protein Klotho in homeostatic plasticity*”

4:05-4:15  **Discussion**

4:15-4:30  **Closing Remarks and Awards**
Dr. Richard A. Miller, is a professor of Pathology and associate director for research of the Geriatrics Center at the University of Michigan. He received a B.A. degree in 1971 from Haverford College, and M.D. and Ph.D. degrees from Yale University in 1976-1977. After postdoctoral studies at Harvard and Sloan-Kettering, he moved to Boston University in 1982 and then to his current position at Michigan in 1990. Dr. Miller has served in a variety of editorial and advisory positions on behalf of the American Federation for Aging Research and the National Institute on Aging, and as an editor-in-chief of Aging Cell. He is the recipient of the Nathan Shock Award, the AlliedSignal Award, the Irving Wright Award and the Kleemeier Award for aging research. His main research interests all relate to the control of aging rate in mice, and include ongoing studies of mutations that slow aging, the relation of cellular stress resistance to longevity, mapping of genes that influence lifespan and age-sensitive traits, screens for drugs that extend lifespan in mice, and methods to improve function of T lymphocytes from old donors.

Drugs that Slow Aging in Mice

Drugs that slow aging, and thus also delay a very wide range of age-related maladies, have the potential to revolutionize preventive medicine. Although once consigned to science fiction, research in the last 10 years has shown that drugs can extend mouse lifespan by as much as 25%, by postponement of cancer and other lethal diseases, and that such drugs typically also retard age-related changes in many tissues and organs. This talk will focus on the discoveries of the NIA-funded Intervention Testing Program (ITP), whose consortium of three laboratories has demonstrated strong effects of rapamycin, acarbose, and 17-α-estradiol in one or both sexes of mice, consistent evidence on male-specific benefits from nordihydroguaiaretic acid, and new evidence for significant, though small, benefits of glycine on both sexes. The ITP results support aging science in four ways: (a) by dispelling the still too common belief that the aging process cannot be made to run slower; (b) by calling attention to specific physiological pathways that might have effects on aging and, therefore, many age-dependent diseases; (c) by providing research platforms to test hypotheses about shared molecular mechanisms of aging rate control; and (d) as an important first step towards the development of drugs that could slow aging in people.
**KEYNOTE SPEAKERS**

Dr. Charlotte A. Peterson is the Joseph Hamburg Endowed Professor and Director of the Center for Muscle Biology. She recently stepped down as Associate Dean for Research in the College, a position she served from 2006-2016, to pursue a new opportunity through an Intergovernmental Personnel Appointment at the NIH. She will provide scientific oversite to a new NIH initiative through the Office of the Director’s Common Fund entitled “Molecular Transducers of Physical Activity Consortium” (MoTrPAC). MoTrPAC is a multi-institutional consortium that includes pre-clinical animal studies, clinical trials, and analyses and bioinformatics centers with the goal of discovering the molecules and pathways responsible for physical activity’s benefits for human health. She will also continue to oversee her extramurally-funded research program in the College. Dr. Peterson’s research focuses on elucidation of cellular and molecular mechanisms controlling skeletal muscle structure and function. Dr. Peterson is a Fellow of the Gerontological Society of America and recently completed her term on the Board of Scientific Counselors for the National Institute on Aging at NIH. She serves on external advisory committees for academic institutions nationally and internationally.

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Charlotte Peterson, PhD  
Professor and Director  
University of Kentucky  
College of Health Sciences

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**Evolving Roles of Satellite Cells in Skeletal Muscle Aging and Adaptation**

Satellite cells are the primary stem cell in skeletal muscle, required for postnatal muscle growth and adult muscle regeneration. Satellite cells are activated to proliferate and normally contribute nuclei to growing myofibers in response to hypertrophic stimuli, although considerable growth can occur in the absence of myonuclear accretion through the expansion of the myonuclear domain. We utilized the discrete expression of Pax7 in satellite cells to develop the Pax7-DTA mouse, whereby the use of Cre-lox technology allows for the specific and inducible depletion of satellite cells following tamoxifen-induced expression of diphtheria toxin. Depleting satellite cells in adult sedentary mice neither accelerates nor exacerbates sarcopenia. Synergist ablation surgery, where removal of synergist muscles places mechanical overload on the plantaris, was used to stimulate robust hypertrophy. Depletion of satellite cells in the adult mouse prior to mechanical overload of muscle results in extracellular matrix (ECM) dysregulation and muscle fibrosis. In aged mice, growth of the plantaris in response to mechanical overload is severely blunted, regardless of satellite cell content. We characterized interactions of activated satellite cells and their daughter cells, myogenic progenitor cells (MPCs), with muscle fibroblasts, which are responsible for the majority of ECM accumulation in muscle. MPC-derived exosomes are capable of down-regulating fibroblast collagen expression. These findings provide evidence for a new role for satellite cells in the regulation of fibroblast ECM production and suggest MPCs are actively involved in the remodeling of the skeletal muscle extracellular environment during muscle hypertrophy.
Dr. Peter Rabinovitch is the founding director (1985) of the University of Washington Nathan Shock Center for Excellence in the Basic Biology of Aging, one of 6 such NIA funded Centers in the country. He has been the Director of a T32 Biology of Aging Training Grant at the University of Washington since 1997. He is current Chairman of the NIA-B Study Section (NIH Biological Aging Review Committee). He has been funded continuously by the NIA since 1981, including as the PI of a Program Project in aging since 2004. He is a current recipient of an Ellison Medical Foundation Senior Scholar in Aging grant award, and a Breakthroughs in Gerontology grant award from the American Federation for Aging Research. The focus of Dr. Rabinovitch’s laboratory research is on the use of genetically altered mouse models to examine the effects of cell signaling and reactive oxygen species (ROS) on lifespan and healthspan.

Peter Rabinovitch, MD, PhD
Professor of Pathology
University of Washington

Restoring mitochondrial energetics improves function in aging mice

Aging is associated with significant declines in the function of organs that have high energy demands that are supplied by mitochondrial activity. This includes skeletal and cardiac muscle, CNS and kidney. In muscle, even in healthy individuals, aging results in increased prevalence of sarcopenia, left ventricular hypertrophy, impaired diastolic function and reduced myocardial performance. These same changes are seen in aging mice, making them a useful model for these studies of healthspan. Early studies showed that transgenic mice expressing mitochondrial catalase (mCAT), displayed numerous phenotypes of delayed aging, including cardiac and skeletal muscle function. Subsequently, we have been interested in whether shorter term pharmacologic treatments might be able to reverse functional deficits of aging in old mice. The mitochondrial protective SS-31 peptide (elamipretide) offers similar benefits as mCAT in models of cardiac hypertrophy and failure; this agent has recently been shown to bind to cardiolipin and improve the electron carrying function of cytochrome c, while reducing its peroxidase activity. Brief treatment with elamipretide improves skeletal muscle ATP generation and contractile force. Reduced proton leak is seen in both cardiomyocyte and skeletal muscle. 24-26 month old mice that receive SS-31 for 8 weeks have improved skeletal muscle energetics, function and endurance, enhanced cardiac diastolic function, improved myocardial performance, reduced cardiac hypertrophy and increased exercise endurance. Our collaborators have also demonstrated improved vision in old mice treated with SS-31, as well as significantly reduced renal cell senescence and glomerulosclerosis. Thus, short-term treatments can enhance mitochondrial function and reverse muscle aging phenotypes in old mice and these benefits can be acute in onset. Based on this and other preclinical data, Elamipretide is presently in multiple Stage II and III human clinical trials. It is hoped that interventions that target mitochondrial function can have a high translational potential, with late-life treatments offering appreciable healthspan improvements.
KEYNOTE SPEAKERS

Dr. Arlan Richardson has devoted his career to aging research for the past 40 years. Dr. Richardson’s research has focused on various aspects of aging, among which: (i) the effects of aging and dietary restriction on gene expression in rats and mice, (ii) testing the oxidative stress theory of aging by measuring the effect of alterations in the antioxidant defense system on the lifespan and pathology of transgenic and knockout mice, and (iii) studying the effect of rapamycin on aging and age-related diseases. Dr. Richardson has a long history of studying the mechanism of action of dietary and was the first group to show that dietary restriction altered gene expression at the level of transcription. Dr. Richardson has received numerous awards for his research in aging, e.g., the Nathan Shock Award from the Gerontology Research Center at the NIA, the Robert W. Kleemeier Award from the Gerontological Society of America, the Harman Research Award from the American Aging Association, the Irving Wright Award of Distinction in Aging Research from the American Federation for Aging Research, and the Lord Cohen Medal for Services to Gerontology from the British Society for Research on Aging.

Arlan Richardson, PhD
Professor of Geriatric Medicine
University of Oklahoma
Health Sciences Center

New Insight into the Life Extending Action of Dietary Restriction

Over the past 50 years, dietary restriction (DR) has been shown to extend the lifespan of a wide variety of organisms, ranging from invertebrates to mammals, such as rodents, dogs, and non-human primates. Because DR has also been shown to delay the onset of most age-related diseases and improve most physiological processes that decline with age, DR is believed to increase lifespan by delaying/retarding aging. This presentation will focus on three novel questions related to DR:

1. Does DR reduce the lifespan of some recombinant inbred lines of mice as was reported by Laio et al. (2010)?
2. Do rapamycin and DR increase lifespan through similar mechanisms, i.e., is rapamycin a DR mimetic?
3. Does DR have early effects that create a cellular memory that persists even when DR is discontinued?
SHORT TALK ABSTRACTS

ORAL NITRATE REDUCTASE ACTIVITY DECLINES WITH AGE: IMPLICATIONS FOR AGE-ASSOCIATED DECREASE IN VASCULAR NITRIC OXIDE BIOAVAILABILITY

Kiyoung Kim1, Khandaker A. Ahmed1, Marcas M. Bamman2, William Van Der Pol3, Elliot J. Lefkowitz4, Casey Morrow2, Pamela V. O’Neal5 and Rakesh P. Patel1

1Department of Pathology and Center for Free Radical Biology, 2Department of Cell, Developmental and Integrative Biology, 3Center for Clinical and Translational Science, 4Department of Microbiology, 5College of Nursing, University of Alabama in Huntsville

Ingestion of nitrate-rich foods can improve cardiovascular function by increasing nitric oxide (NO) bioavailability. The proposed mechanism involves salivary nitrate reduction to nitrite by lingual nitrate-reductase (NR) expressing bacteria; nitrite then mediates systemic NO-signaling. However, little is known about how oral NR activity is regulated, and whether this is a modifiable factor for cardiovascular disease risk. In this study, we developed methods to screen oral NR activity on human tongue swabs and tested how this activity varied as a function of age. Volunteers were recruited into three age groups (I: 25~45 years, n=7; II: 51-73 years, n=13; and III: 67-95 years, n=14). Group I and II were recruited from Birmingham, AL and Group III from Huntsville, AL and comprised active, productive seniors. Tongue swabs were collected from the posterior tongue and NR activity measured *ex vivo* by following nitrate-dependent nitrite formation. Colony-forming units (CFU) were also determined to assess bacterial number. Oral NR activity was indexed by calculating the initial nitrate-dependent nitrite formation rate (INNFR) pre- and post-normalization to CFU. In addition, microbe composition was determined using Microbiome Analysis with 16S rRNA gene sequencing. INNFR was significantly lower in Group III relative to Group I or II (both p<0.0001). In addition, there was a negative correlation between age and INNFR (r(38)= -0.74, p<0.0001), but not between normalized INNFR and age (p=0.122) suggesting that lower bacterial number may play a role in lower oral NR activity in older subjects. Microbiome analysis revealed that the relative abundance of *Actinomyces sp.*, *Campylobacter sp.*, *Prevotella sp.*, *Veillonella sp.* and *Selenomonas sp.* correlated with nitrate reductase activity. The microbe composition of younger subjects had significantly higher abundance of *Actinomyces sp.*, *Campylobacter sp.*, *Prevotella sp.*, than older subjects (p<0.05). Oral NR activity decreases with age and may contribute to age associated decreases in NO-bioavailability.

MULTIMODAL EXERCISE-, AND PHARMACEUTICAL-BASED STRATEGIES FOR ALTERING THE GUT MICROBIOME DURING AGING

Christy Carter

*Department of Aging and Geriatric Research College of Medicine, University of Florida, Gainesville*

Dysbiosis may be major source of inflammation observed with age. We have previously shown that exercise and pharmaceutical interventions that influence function of the renin-angiotensin system decrease inflammation and improve physical performance in humans and rodent models of aging. Here we discuss the conditions under which each of these interventions alone or in combination impact function. We expand the discussion as to how each of these interventions may modulate the rat gut microbiota to influence inflammatory status. Finally, we introduce a novel probiotic methodology allowing for overexpression of specific, beneficial RAS components. Ultimately, combining these methods may be quite cost effective and translatable to humans while requiring fewer regulatory hurdles.

However, this dramatic elevation in H$_2$O$_2$ production is accompanied by a parallel increase in several antioxidant enzymes that all converts H$_2$O$_2$ into H$_2$O, consequently preventing oxidative damage. In stark contrast, the crosstalk between MuSC-MN is lost during aging and augmented myogenic activity following nerve stimulation is significantly reduced in the aged muscle. Furthermore, aged MuSCs fail to maintain redox balance, possibly due to increased oxidative stress. Taken together, our results show that in response to traumatic muscle injury, H$_2$O$_2$ function as a rheostat in MuSCs to coordinate signal transduction for neuromuscular synergy during muscle regeneration. Hence, a disruption in redox balance due to oxidative stress during aging cause a failure in redox-responsive myogenic signaling and ultimately, a deficit in muscle regeneration. These data reveal critical mechanisms in the MuSC-niche regulation and identify a promising therapeutic target to facilitate cell-based therapy.
The generation of a novel GH mutant mouse model using the CRISPR/Cas9 system

Michael Paul Fitch, Anil Kumar Challa, Liou Y. Sun

Department of Biology, University of Alabama at Birmingham

Growth hormone (GH) is naturally produced by somatotrophic cells in the anterior pituitary gland and plays an important role in growth and development. In the mouse, the hormone is produced as a 216 amino acid precursor protein with an N-terminal signal peptide (26 amino acids) essential for hormone secretion. The signal peptide is cleaved during the secretion process resulting in a 191 amino acid mature form of GH. Loss of function mutation in the growth hormone (GH) signaling in mouse has been attributed to an increased life span and increased insulin sensitivity. To further study the effects of the loss of GH gene on aging and health, we used the CRISPR/Cas9 system and identified a novel mutation of the mouse Gh gene. Here we report a small deletion (14 bp) in exon 3 that results in a frame shift allele, causing an early truncation of the predicted protein sequence and includes 79 mutant amino acids. In the homozygous state, this deletion allele causes embryonic lethality in a C57BL/6 and in a mixed C57BL/6 X BALBc background. Timed mating and analysis of harvested embryos show that homozygous mutant embryos are conceived and develop at least until E14; no live homozygous mutant pups were found in all the litters. This suggests that Gh is likely to be critical for embryonic development during the last week of gestation. Also, the mutant allele might produce a non-functional protein or be toxic to embryonic development.

Bridging the gap between metabolomics and bioenergetics

Michelle S. Johnson1,2, Gloria A. Benavides, Taylor Berryhill3, Stephen Barnes2,3 and Victor M. Darley-Usmar1,2

Department of Pathology, Mitochondrial Medicine Laboratory1, Center for Free Radical Biology2, Targeted Metabolomics and Proteomics Laboratory3, University of Alabama at Birmingham, Birmingham, AL 35294-0022

Metabolomics is the identification and quantification of small molecule intermediates within a cell or tissue. The field of metabolomics has developed methods in which cellular and tissue metabolites can be quantified using analytical techniques such as quantitative liquid chromatography-multiple reaction ion monitoring mass spectrometry (LC-MRM-MS). Two distinct methodologies exist to date: targeted metabolomics, which measures a defined set of analytes such as glycolytic, and TCA cycle intermediates and untargeted metabolomics. Techniques such as targeted metabolomics can be useful tools to investigate alternations in mitochondrial function and metabolism as a whole. Mitochondrial dysfunction and metabolic diseases such as type 2 diabetes, neurodegeneration and cardiovascular disease all are encompassed under the umbrella of aging-related diseases.

The purpose of this study was to determine how the flux in TCA cycle intermediates and glycolysis are coupled to changes in cellular oxygen consumption rates (OCR). Using 2-deoxy glucose (2-DG), a glucose analog that blocks glycolysis at hexokinase, and specific mitochondrial electron transport chain inhibitors, we hypothesized that blocking mitochondrial function and thereby decreasing ATP production should result in an increased flux through the glycolytic pathway corresponding with an increase in extracellular acidification rate (ECAR) as a measure of glycolysis. Moreover, using a mitochondrial un-coupler such as FCCP should result in increased TCA flux while an inhibitor of complex I such as rotenone would result in a decreased TCA flux most likely due to an impaired NAD+ pool.

These data can be used to validate metabolic changes that occur during metabolic aberrations that can occur during age-related pathologies. For example, using specific mitochondrial inhibitors showed that glutamate metabolism seems to only be associated with inhibition of complexes III and V, whereas glutamine metabolism, which relies heavily on the NAD+ pool, significant changes with complexes I, IV, V, and glycolysis inhibition can be seen.

Human neuromuscular aging: sex differences revealed at the myocellular level

Kaleen M Lavin; Brandon M Roberts; Gina M Many; Anna Thalacker-Mercer; Edward K Merritt; C Scott Bickel; David L Mayhew; S Craig Tuggle; James M Cross; David J Kosek; John K Petrella; Cynthia J Brown; Gary R Hunter; Samuel T Windham; Richard M Allman; Marcas M Bamman

University of Alabama at Birmingham

Age-related muscle loss (sarcopenia) is a major clinical problem affecting both men and women – accompanied by muscle weakness, dysfunction, disability, and impaired quality of life. Current definitions of sarcopenia do not fully encompass the age-related changes in skeletal muscle. We examined the influence of aging and sex on elements of skeletal muscle health using a thorough histopathological analysis of myocellular aging and assessments of neuromuscular performance. Two-hundred and twenty-one untrained males and females were separated into four age cohorts [mean age 25y (n=47), 37y (n=79), 61y (n=51), 82y (n=25)].

9
and 72y (n=44)]. Total (-12%), leg (-17%), and arm (-21%) lean mass were lower in both 61y and 72y than in 25y or 37y (P<0.05). Knee extensor strength (-34%) and power (-43%) were lower (P<0.05) in the older two groups, and explosive sit-to-stand power was lower by 37y (P<0.05). At the histological/myocellular level, type IIx atrophy was noted by 37y and type IIa atrophy by 61y (P<0.05). These effects were driven by females, noted by substantial and progressive type IIa and IIx atrophy across age. Aged female muscle displayed greater within-type myofiber size heterogeneity and marked type I myofiber grouping (~5-fold greater) compared to males. Differential mechanisms between aging males and females may contribute to whole muscle atrophy and functional decline: fiber atrophy in females vs. fiber loss in males. Future studies will be important to better understand the mechanisms underlying sex differences in myocellular aging and optimize exercise prescriptions and adjunctive treatments to mitigate or reverse age-related changes.

### Ozone Exposure Accelerates Memory Decline in Old Male APOE3, but Not APOE4, Targeted Replacement Mice

Rui-Ming Liu, Chunsun Jiang, Luke T. Stewart, William McGilberry, Hui-Chien Kuo, Stephanie Wall, Trent Tipple, Shannon Bailey, Dean Jones, Lori McMahon

*Dept of Medicine; Dept of Cell, Developmental, and Integrative Biology; Dept of Biostatistics; Dept of Pediatrics; Dept of Pathology, University of Alabama at Birmingham, Birmingham, AL; Dept of Medicine, Emory University, Atlanta, GA, USA*

Ozone exposure sensitizes memory decline in old male APOE3, but not APOE4, targeted replacement mice. APOE4 is a genetic risk factor. Which environment factor is responsible and how genetic and environmental risk factors as well as aging interact, leading to LOAD, however, is unknown. Ozone (O3), a highly reactive oxidant, is one of most abundant urban pollutants. In this study, we investigated whether O3 exposure synergizes with APOE E4 genotype and aging, leading to AD, using human APOE E4 and APOE E3 [represent the majority of human population who carries APOE E3 gene] targeted replacement (TR) male mice. Our results show, surprisingly, that exposure to a cyclic O3 exposure protocol, which mimics human exposure scenarios, impairs memory of old male apoE3, but not apoE4, TR mice nor the memory of young apoE3 TR mice. Astrocytes are activated in old male mice; cyclic O3 exposure further activates astrocytes and impairs neurogenesis in old male apoE3, but not apoE4, TR mice. The concentrations of glutathione (GSH) and cysteine, two important antioxidants, are lower in the hippocampus of male apoE4 TR mice compared to male apoE3 TR mice. However, male apoE3, not apoE4, TR mice experience age- and O3 exposure-related declines in the concentrations of these antioxidants. Together, the results suggest that a decrease in antioxidant defense at old age renders male apoE3 mice highly sensitive to O3-induced neuropathophysiology. Male apoE4 mice, which have decreased levels of GSH and cysteine at young age, likely have developed compensatory mechanisms to protect against O3-induced neuropathophysiology. As APOE E4 mainly affects females, our results also suggest that interaction between gene and environment as well as aging in AD pathogenesis may be sex-dependent.

### Transcriptomic Analysis Reveals Depth of Quiescence Is Altered in Melanocyte Stem Cells With Age

Joseph W. Palmer, Melissa L. Harris

*Department of Biology, University of Alabama, Birmingham*

The cellular state of quiescence is vital for concealing the long-term regenerative potential of somatic stem cells throughout the body. In recent years numerous studies have shown that this once perceived dormant state is in-fact highly adaptive and can influence stem cell fate upon subsequent reactivation. In this study we characterize how quiescence changes with age and how these changes contribute to the aging phenotype of hair graying. To answer this question we use the melanocyte stem cell model (McSCs). McSCs reside within the hair follicle niche and undergo well-defined cycles of activation during hair growth. While hair graying has traditionally been described as depletion of McSCs, an increasing number of clinical cases report near complete hair repigmentation in aged patients undergoing various treatments. This implies that a portion of McSCs are retained with age, and that fail to activate under normal physiological conditions. To investigate this curious phenomenon at a molecular level, purified McSCs were collected from birth, breeding age, and 2-year-old C57BL/6J mice and whole genome gene expression was performed. The resulting 913 differentially expressed genes in aged quiescent McSCs, were then subjected to a combination of gene ontology, network and transcription factor analysis. Interestingly, aged McSCs predominantly show changes in gene expression consistent with an overall reduction in translation, an increased regulation of homeostasis and adhesion, and no changes in genes associated with senescence. This signature suggests that aged McSCs exist in a deeper level of quiescence than young McSCs. We propose that aged McSCs become refractory to activation signaling due to the increased depth of quiescence, and this phenomenon can explain one aspect of age-related hair graying. Our future
direction is to further investigate the mechanisms governing the observed changes in stem cell quiescence and gain insight into how this population can be targeted for tissue regeneration in aged organisms.

**MEASURING RESPIRATION RATES IN DROSOPHILA MELANOGASTER USING THE LOLIGO SYSTEM**

R. Colton Ritchie¹, Louis P. Watanabe¹, Maria S. Johnson², Tim R. Nagy²,³, and Nicole C. Riddle¹,³

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² Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, AL
³ Nathan Shock Center of Excellence in the Basic Biology of Aging, UAB, Birmingham, AL

One important way to assess an organism’s metabolism is to measure its respiration. Changes in metabolism – precipitated for example by increased physical activity or exercise – lead to changes in respiration. Thus, respirometry can be used to assess an organism’s energy expenditures. Respiration is measured either as an increase in carbon dioxide (CO₂) or a decrease in oxygen (O₂). Measurement of CO₂ and/or O₂ concentration requires specialized sensors, and often, the equipment necessary is both expensive and designed for a particular study organism. At UAB, systems for respirometry studies are available for rodents and aquatic animals, but were lacking for our animal model, the fruit fly *Drosophila melanogaster*. In collaboration with the Comparative Organismal Energetics Core of the UAB Nathan Shock Center, we have developed protocols to adapt the Loligo system for respirometry studies in Drosophila. Our goal is to study how respiration changes in Drosophila in response to exercise and with increased age. To date, the Loligo system has been used mostly for aquatic organisms, and the protocol we present here is the first such protocol for Drosophila. As our approach demonstrates that respirometry analysis with the Loligo system is feasible for terrestrial organisms, this protocol will enable greater access to respirometry analysis for researchers working with a variety of organisms.

**FUNCTIONAL CONNECTIVITY NETWORKS IN THE HEALTHY OLDEST OLD**

Sara Sims¹, Paul Stewart², Diana Pizzaro³, Kristina Visscher²

¹University of Alabama at Birmingham, Department of Psychology, ²University of Alabama at Birmingham, Department of Neurobiology, ³University of Alabama at Birmingham, Department of Neurology

Functional networks are brain regions with temporally correlated activity. The fronto-parietal network is involved in attentional control, the cingular-opercular network is involved in maintenance of task demands, and the default mode network is active when there are no attentional or task goals. Since these networks can be impacted with age-related diseases, it is important to assess the functional activity in a healthy oldest old adult brain without the diseases related to aging. The Mcknight Brain Aging Registry (MBAR) is a multi-site study whose aim is to characterize the brains of the oldest old. In the MBAR study, participants are 85 years and older, do not have any major physical diseases, and are cognitively healthy (mean MOCA score of 24.44). As part of the MBAR protocol, participants have an 8-minute resting-state MRI scan with eyes open. To better understand connectivity networks in the oldest old, we performed a preliminary analysis with 28 subjects and we observed discrete functional connectivity networks including the default mode network, the fronto-parietal network, and the cingulo-opercular network. Future research with the entirety of the MBAR dataset will examine the relationship between these networks, and measures of behavior collected in our extensive MBAR cognitive battery.

**YEAST PHENOMIC ANALYSIS OF ASSOCIATIONS BETWEEN HYDROGEN SULFIDE PRODUCTION AND CHRONOLOGICAL LIFESPAN**

Daniel L. Smith, Jr.¹,², Sven Wang³, Rui R Chang³, Haley D. Albright⁴, Crystal M. MaHarrey⁴, John W. Rodgers⁴, Rick White⁵, Eric Schadt³, John L. Hartman, IV²,⁴

¹Department of Nutrition Sciences, ²Nathan Shock Center, ³Department of Genetics, University of Alabama at Birmingham, Birmingham AL, 35294 USA
⁴Department of Genetics and Genomic Science, Mount Sinai School of Medicine, New York NY, 10029 USA
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The use of yeast gene knockout mutants in genome-wide analysis of chronological lifespan (CLS) has revealed numerous genetic determinants of cellular aging; however, gene-nutrient and gene-gene interaction markedly influences the phenotype, leading to divergent results from various studies. To enable a more integrated understanding of aging networks we established a carefully controlled experimental design, using quantitative high throughput cell array phenotyping (Q-HTCP) for automated CLS assessment of the yeast gene deletion strain (YGDS) collection in parallel with estimation of H₂S production.
to investigate transsulfuration and hydrogen sulfide (H$_2$S) production, which has been implicated in longevity for multiple organisms. The YGDS collection (BY4741 parental strain) was systematically sampled for viability weekly over a two-month period. H$_2$S production was estimated separately after 7 days for each culture using a modified lead acetate media derived from the CLS media. Comparisons were made between our CLS results and those of previous screens, including H$_2$S production. Using conservative scoring and network construction criteria, mitochondria (structure and function), vacuolar processes and proton transport predominated for maintaining longevity. Of the individual YGDS found to increase H$_2$S production (enriched for processes related to sulfur amino acid metabolism), few were overlapping with previous publications, although more were found in common with the long-lived CLS deletion strains than the short-lived CLS from our screen. YGDS were longer lived in this new media than in previously reported studies, and reliant on mitochondrial function and organization (including the ubiquinone biosynthetic pathway), with perturbations of the F$_1$-F$_0$ ATP synthase extending CLS. These results suggest maintenance and utilization of mitochondrial membrane proton gradient, vacuole function, and previously uncharacterized genes play key roles in yeast CLS under conditions where cells are relatively long-lived. The limited overlap in gene deletions that increased H$_2$S production compared with previous studies suggests gene-nutrient interaction influences this phenotype too.

**NOVEL MITO-SINCE2 METHOD IDENTIFIES QUANTITATIVE RELATIONSHIPS BETWEEN MITOCHONDRIAL DYNAMICS AND ENERGETICS**

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Mitochondrial dynamics primarily refers to the opposing fission or fusion between individual mitochondria, which maintains steady-state mitochondrial morphology in cells. Modulation of mitochondrial dynamics impacts aging or its related physiology in multiple model organisms, but the mechanistic details are not clear. Mitochondrial dynamics and energetics impact each other to control cellular bioenergetics. The details of the bidirectional relationship between mitochondrial dynamics and energetics are unclear, and the methods to integrate these mitochondrial aspects are lacking. Here, we have developed, validated and tested an analytical method to study the quantitative relationship between mitochondrial dynamics (Fission / Fusion / matrix-continuity / Diameter) and energetics (ATP-synthesis / Redox) in single cells. We named the method Mito-SinCe2 (Mito-SinCe-SQuARED: Single Cell Simultaneous Quantification of ATP or Redox with Dynamics of Mitochondria). To test the proof of principal we applied the Mito-SinCe2 method on ovarian tumor initiating cells with stem cell properties. The findings from Mito-SinCe2 analyses led us to hypothesize that mitochondria dependent ovarian tumor initiating cells interconvert between 3 states with distinct mito-Dynamics-Energetics relationships, where mito-Dynamics-ATPsynthesis relationship is mutually exclusive with mito-Dynamics-Redox relationship. Moreover, mito-ATP synthesis relates to mitodynamics only beyond a certain level of mitochondrial fusion. Additionally, Mito-SinCe2 analyses can potentially predict new quantitative features of the relationship of opposing fission/fusion states, and classify cells with a distinct fission/fusion state into relevant functional classes. In summary, quantitative Mito-SinCe2 analyses can unravel complexities of the debated bidirectional mito-Dynamics-Energetics relationship, as well as that of energetic dependence of (normal or neoplastic) stem cells. Since, understanding stem cell regulation is critical in the field of regenerative medicine and aging, the novel quantitative Mito-SinCe2 method has significant potential to unravel the crosstalk of mitochondrial dynamics and energetics in physiological processes, including stem cell biology, that contributing to aging.

**EVIDENCE FOR THE ROLE OF AGE-REGULATING PROTEIN KLOTHO IN HOMEOSTATIC PLASTICITY**


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**Rationale:** Klotho (KL) is a protein that is expressed predominantly in the brain and kidney and is age-downregulated. Both increasing and decreasing KL expression profoundly impacts both lifespan and cognitive function. While the level of KL expression impacts cognition, the molecular mechanisms of KL action in brain are unknown. We recently reported that KL is localized to the synapse and impacts synaptic plasticity. Here, we investigate the role of KL in synaptic function with a focus on KL effects on neuronal morphology and homeostatic plasticity.

**Methods:** We examined the effect of KL on homeostatic plasticity through multi-electrode array recordings of cultured neurons. The morphology of neurons from these mice was assessed by performing Sholl and spine analysis on apical dendrites.
of CA1 pyramidal neurons. And interactions of KL with synaptic proteins were assessed by co-immunoprecipitation experiments.

**Results:** Previously we have shown that although KL-deficient mice are cognitively impaired, they exhibit enhanced synaptic plasticity. Conversely, the cognitively enhanced KL-overexpressing mice exhibited decreased synaptic plasticity. Our data now reveal that the KL-deficient synaptic plasticity change may result from neuronal hyperactivity as we observe a deficit in homeostatic downscaling and KL-dependent changes in spine morphology. As these results implicate KL as important for basic neuronal function, we probed for KL binding partners at the synapse and discovered KL binds PSD-95, a critical post-synaptic structural protein.

**Conclusions:** Electrophysiology results suggest that KL regulates both pre- and post-synaptic function. This along with the KL-dependent changes in neuronal morphology and the interaction of KL with PSD-95 suggest that KL regulates the activity of PSD-95, an important regulator of synaptic function and homeostatic plasticity. KL-deficiency occurs with age and this work is the first evidence of KL action directly at the synapse to impact cognitive function suggesting that KL is vital to maintain brain function with age.

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**GENETIC ANALYSIS OF LONGEVITY IN DIVERSE COHORTS OF MICE: INFLUENCE OF DIETS AND DRUGS**

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We are using two murine cohorts to evaluate the impact of DNA variants, diets, and drugs on longevity: (1) inbred BXD strains on high and low fat diets, and (2) the NIA UM-HET3 F2 intercross. Both segregate for millions of variants, making them ideal for the analysis of gene-by-treatment interactions that modulate lifespan.

**Methods:** (1) We measured longevity in 80 BXDs (females) on chow (CD) or high fat diet (HFD, 60% fat). Some cases were sacrificed between 6–24 months for omics analyses. All were sequenced at 40X. (2) The NIA cohort consists of >15,000 F2 progeny treated with compounds suspected to have effects on lifespan.

**Results:** (1) BXDs on HFD gain 5X more weight than those on CD by 500 days. Diet shortens life by 80 days (615 ± 13 SE days; CD 687 ± 13). Longevity under the two diets correlates poorly (r = 0.32). Remarkably, baseline weight and weight gain within diet do not correlate with longevity, demonstrating that diet itself, rather than weight gain per se modulates longevity in these female cohorts. Preliminary QTL mapping of CD and HFD BXD cohorts yielded suggestive loci (GeneNetwork Traits 18441 and 18435). Matched omics analyses of liver, fat, and eyes are in progress to help dissect molecular networks and predictors of metabolic aging. (2) Preliminary analyses based on a subset of UM-HET3 cases highlight longevity QTLs on ChrS 1, 4, and 15 in males and ChrS 1, 3, 9, and 15 in females. Haplotype hazard ratios range from 0.5 to 2 and LOD scores from 4.5 to 6.7. Some genetic determinants of longevity and weight gain in the UM-HET3 are sex- and site-specific. Additional samples from both populations will provide powerful and complementary resources to study the genetics of longevity in relation to sex, metabolic status, and drug interventions.
01. CONTEXT FEAR MEMORY FORMATION IS REGULATED BY NEAT1 LncRNA-MEDIATED HISTONE LYSINE METHYLATION CHANGES IN THE HIPPOCAMPUS

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Histone lysine methylation is critical for the formation and maintenance of long-term memories. While manipulation of histone methyltransferases and demethylases perturbs the transcriptional landscape, and is sufficient to either improve or impair the formation of long-term memories, the altered targeting of histone methylation mechanisms during long-term memory formation is poorly understood. Recently, long noncoding RNAs (lncRNAs) have been implicated in targeting of histone methyltransferases to numerous gene loci. Despite the importance of such mechanisms to long-term memory formation, few studies have examined these mechanisms in the neuronal context. Recent studies have identified expression of a large population of lncRNAs in the rodent hippocampus, a region critical for the conversion of short-term memories to long-term memories. We have examined the role of one aging-regulated lncRNA, Neat1, in the neuroepigenetic processes of hippocampus-dependent long-term memory formation. Using RNA immunoprecipitations, we found multiple chromatin modifying enzymes associated with Neat1 in cultured cells, including Ehtm2, which mediates H3K9me2, a repressive histone modification that our lab and others have observed to be dysregulated in the aging hippocampus. Using an informatics approach and publicly available RNAseq data, we identified several memory-related gene targets for Neat1, including the immediate early gene C-fos. siRNAs targeting of Neat1 in neuronal cells significantly reduced global levels of H3K9me2, and resulted in decreased H3K9me2 at the C-fos promoter corresponding with upregulation of the product mRNA. Moreover, reducing Neat1 expression in vivo via infusion of siRNAs into the dorsal area CA1 of the hippocampus enhanced long-term memory retention in a contextual fear conditioning (CFC) task, while in vivo overexpression of Neat1 in this brain region using CRISPRa impaired performance in the task. These results support our hypothesis that the aging-induced lncRNA Neat1 is a potent negative regulator of hippocampus-dependent long-term memory formation via regulation of histone lysine methylation.

02. DETERMINATION OF THE BIOENERGETIC AND METABOLIC SIGNATURES OF PLATELET SAMPLES FOR PRECISION MEDICINE

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Mitochondria integrate the cellular bioenergetic program, a unique set of bioenergetic relationships between oxidative phosphorylation and cellular metabolism. Due to the influence of age, genetic, lifestyle factors on mitochondrial activity, there exist a significant bioenergetic diversity between individuals. However, the mechanism of this bioenergetic diversity has not been described. Metabolism, which is also influenced by the innate factors, has been suggested to control the bioenergetic activity of cells. Hence, it is important to integrate metabolic and bioenergetic signatures develop a personalized approach for clinical management. Recent reports suggest that circulating platelets can act as surrogate markers of bioenergetic health in individuals. It is hypothesized that distinct correlations exist between the bioenergetic activity and the metabolomic signature of platelets which is influenced by pharmacological stress. In this study the correlation between metabolites and the bioenergetic activity of platelets from multiple individuals was tested with or without doxorubicin, a chemotherapeutic agent. Untargeted metabolomics analysis of 300 million platelets from 5 independent donors was performed using LC-Orbitrap-MS. Cellular bioenergetics of platelets samples was determined using the XF analyzer. The results show that platelets samples present distinct metabolic and bioenergetic signatures. Doxorubicin altered platelet bioenergetics, specifically increased basal respiration, proton leak and non-mitochondrial respiration and a decrease in ATP-linked and maximal respiration in platelets. These alterations also show typical profiles for different individuals suggesting the personalized nature of this parameter and are correlated with metabolites. Cellular metabolites levels and the bioenergetic activity show correlation in platelets. The bioenergetic and metabolomics sensitivity to pharmacological agents are not well correlated. This study suggests that distinct correlation exist between the bioenergetic activity and metabolite signature during normal and under systemic stress.

03. THE ROLE OF NUCLEAR FACTOR ERYTHROID 2-RELATED FACTOR 2 (NRF2)-REGULATED ANTIOXIDANT DEFENSES IN UVA-INDUCED MATRIX METALLOPROTEINASE-1 (MMP-1) IN HACAT KERATINOCYTE CULTURE AND MOUSE SKIN: THE PHOTOPROTECTIVE EFFECTS OF HISPIDULIN (HPD)

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UVA plays a role in premature aging of the skin through triggering oxidative stress-associated stimulation of MMP-1 responsible for collagen degradation, a hallmark of photoaged skin. Compounds which can activate Nrf2, a transcription factor regulating antioxidant gene expression, could thus be developed into anti-photoaging agents. We aimed to investigate whether Nrf2 silencing affected UVA (4 J/cm²)-induced MMP-1 through MAPK/AP-1 signaling in HaCaT cells. Then, we explored the anti-photoaging effects of HPD having abilities to activate Nrf2 on MMP-1 and collagen expressions in association with phosphorylation of MAPKs (ERK, JNK, and p38), c-Jun and c-Fos in the skin of BALB/c mice subjected to repetitive UVA irradiation; three times per week for 2 weeks and the total dose was 60 J/cm². Our findings revealed that Nrf2 knockdown promoted MMP-1 activity and mRNA levels in UVA-irradiated HaCaT cells. Nrf2-depleted HaCaT cells with MAPK inhibitors significantly suppressed MMP-1 and AP-1 transcriptional activity following UVA exposure. Our results also demonstrate the in vivo relevance of the in vitro findings as HPD capable of activating Nrf2 provided the protective effects on UVA-mediated MMP-1 induction and collagen depletion in correlation with decreased levels of phosphorylated MAPKs, c-Jun, and c-Fos in mouse skin.

In conclusion, Nrf2 plays a photoprotective role in UVA-induced MMP-1 through MAPK/AP-1 signaling. Additionally, HPD with Nrf2 activating properties could protect against UVA-induced MMP-1 expression through MAPK/AP-1 pathway and may represent promising anti-photoaging candidates.

04. RUNX2 DEFICIENCY IN MATURE OSTEOBLASTS LEADS TO OSTEOPENIA AND PREMATURE AGING

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Runx2 transcription factor is essential for commitment and differentiation of osteoblast. Runx2 global null mice fail to develop bone and die at birth. Surprisingly, Runx2 overexpression in osteoblasts leads to osteopenia indicating an inhibitory function of Runx2 in mature osteoblast and postnatal bone synthesis. However, role of endogenous Runx2 gene in mature osteoblast and postnatal skeletogenesis remains unknown. We recently generated Runx2-floxed mice. To study osteoblast function without affecting osteoblast differentiation, we deleted Runx2 gene in mature osteoblast using Osteocalcin-Cre mice. Interestingly, Runx2OB-/- mice were born alive and Alizarin red/Alcian blue staining at 1-week showed normal bone development. Thus, Runx2 function in mature osteoblast is not essential for embryonic bone development. Postnatally, Runx2OB-/- mice grow poorly and exhibit 35% and 55% less body weight than WT littermates at 1 and 5-months respectively. Growth retardation noted in both genders is unrelated to dental or any issues with feeding and suggest mature osteoblast regulate homeostasis of connective tissue beyond the skeleton. Failed postnatal growth in Runx2OB-/- mice was coupled with appearance of hunch back and signs of premature aging, including dull hair, reduced activity, priapism, and sarcopenia. Runx2OB-/- mice started to die from 6-weeks of age and none survived beyond 6 months, indicating that Runx2 activity in mature osteoblast is essential for completion of normal life span. Postnatal bones assessed at 5 months showed striking osteoporosis, with a 65% decrease in cortical BV/TV. Surprisingly, trabecular bone volume was increased significantly (42%) but trabecular thickness and length were decreased in Runx2OB-/- mice. Calcein double labeling and TRAP staining showed that bone loss was due to reduced bone formation, but not increased bone resorption, suggesting disrupted osteoblast function. Interestingly, mRNA levels of early marker ALP were similar between 5-month old littermates but expression of mature osteoblast/osteocyte markers (BSP, OC, DMP1, SOST) was decreased by 80%. Acid-etch SEM revealed poor mineral quality, abnormal morphology, and pattern of osteocytes distribution in the femur of Runx2OB-/- mice. Runx2 regulated signals from mature osteoblast control peripheral fat distribution, and marrow adipogenesis. In conclusion, Runx2 activity in mature osteoblast is essential for postnatal bone acquisition and to prevent premature aging.

05. ALTERATIONS IN BI-DIRECTIONAL CROSSTALK BETWEEN MUSCLE STEM CELL AND MOTOR NEURON COMPROMISE REGENERATIVE POTENTIAL OF AGED MUSCLE AND CONTRIBUTE TO SARCOPENIA

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Deterioration of neuromuscular junction (NMJ) integrity and concomitant loss of motor neuron (MN) innervation is known to play a causative role in sarcopenia and frailty. Emerging evidence also suggests that disruption in neuromuscular...
communication also contributes to age-acquired deficits in muscle stem cell (MuSC) function. However, the exact underlying mechanisms of MuSC-MN interactions during muscle regeneration and signal transduction pathways governing these processes remain elusive. Here, we demonstrate that intercellular redox signaling via H$_2$O$_2$ primes MuSC for myogenesis by enhancing protein synthesis and mitochondrial function, thereby promoting muscle repair. In the young animals, upon mild nerve injury (sciatic nerve pinch), MuSC number, myogenic activity, and mitochondrial bioenergetics are significantly increased compared to control. Conversely, when muscle fibers are chemically injured (barium chloride), Wallerian degeneration of motor axon follows, and neuromuscular synapses are remodeled in the regenerating muscle fibers. More intriguingly, following nerve injury, we found an approximately 20-fold increase in mitochondrial hydrogen peroxide (H$_2$O$_2$) in both muscle fibers and MuSC. However, this dramatic elevation in H$_2$O$_2$ production is accompanied by a parallel increase in several antioxidant enzymes that all convert H$_2$O$_2$ into H$_2$O, consequently preventing oxidative damage. In stark contrast, the crosstalk between MuSC-MN is lost during aging and augmented myogenic activity following nerve stimulation is significantly reduced in the aged muscle. Furthermore, aged MuSCs fail to maintain redox balance, possibly due to increased oxidative stress. Taken together, our results show that in response to traumatic muscle injury, H$_2$O$_2$ function as a rheostat in MuSCs to coordinate signal transduction for neuromuscular synergy during muscle regeneration. Hence, a disruption in redox balance due to oxidative stress during aging cause a failure in redox-responsive myogenic signaling and ultimately, a deficit in muscle regeneration. These data reveal critical mechanisms in the MuSC-niche regulation and identify a promising therapeutic target to facilitate cell-based therapy.

06. THE EFFECT OF DIETARY LITHIUM ON THE HEALTH AND LONGEVITY OF DROSOPHILA MELANOGASTER ARE GENOTYPE DEPENDENT

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Small doses of the metal lithium are commonly prescribed for treatment of bipolar disorder and suicide risk in humans. In addition, previous research suggests that dietary lithium supplementation has health and longevity benefits in the fruit fly, Drosophila melanogaster. However, this previous study was only conducted on two Drosophila genotypes in one laboratory, and the reproducibility of the effects of lithium across multiple genetic backgrounds has yet to be determined. Understanding genetic background effects of lifespan extending interventions is important as we attempt to translate findings in animal studies into humans. Here, we observe the effects of small doses of dietary lithium on the health and longevity of the fruit fly. We find that both the health and longevity effects of lithium are genotype and sex dependent, suggesting that one dose of lithium may not be ideal for every genetic background. Future research will look at more dose dependent effects of lithium on longevity, as well as feeding behavior of flies on lithium supplemented media.

07. BODY COMPOSITION AND AGE IN ZOO AFRICAN (LOXODONTA AFRICANA) AND ASIAN (ELEPHAS MAXIMUS) ELEPHANTS

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Aging is often associated with increased fat mass and decreased fat free mass (FFM), but if this relationship exists in the long-lived elephant is unclear. This study examined body composition in zoo female African (N=20; Loxodonta africana) and Asian (N=23; Elephas maximus) elephants across a range of ages (8 – 56 years). Maximum lifespan for female elephants is over 65 years. Elephants were weighed to the nearest pound, five pounds, or kilogram, depending on the institutions’ scale. Body composition was assessed by deuterium dilution. Each elephant ingested deuterated water (0.05mL/kg body weight) orally using bread as a vehicle. Venous blood was collected from an ear or leg vein prior to deuterated water administration, and then at regular intervals (~24, 120, 240, 360, and 480 h) post deuterated water administration. Age was determined based on zoo records. Body weight was positively associated with age for African elephants (p=0.001; age: 16 – 51 years) and Asian elephants (p=0.026; age: 8 - 56). Fat mass neither unadjusted nor adjusted for weight was not associated with age for either African or Asian elephants (p=0.236). Older African and Asian elephants had significantly greater unadjusted FFM compared to younger elephants (p=0.001, 0.009, respectively) but not adjusted for weight (p=0.809, 0.168, respectively). Older female African and Asian zoo elephants appear to have greater FFM but not fat mass compared to younger elephants. The greater FFM
may be attributed to indeterminate body length growth observed in elephants. Body composition changes are important to understand as unintentional weight loss is associated with morbidity and mortality in various mammalian species.

08. HIGH FAT DIET AND TIME-RESTRICTED FEEDING AT NIGHT REGULATE AUTOPHAGY IN THE MOUSE LIVER

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Autophagy is a highly regulated process responsible for recycling excessive or damaged lipids, proteins and organelles, and proposed to be sensitive to feeding/fasting cycles. Previous studies demonstrated that high fat diet (HFD) induces hepatic steatosis, increases mitochondrial reactive oxygen species, impairs nitric oxide availability and modifies the mitochondrial proteome in mice. We hypothesize that regulation of autophagy plays a role in cellular adaptation to HFD induced liver damage. Furthermore, since circadian clock plays an essential role in repair of cellular constituents due to oxidative stress that occurs during diurnal activities, environmental exposures and cellular energy production, we hypothesize that autophagy in the liver is also regulated by time-restricted feeding. To examine autophagy in a pre-clinical model of nonalcoholic fatty liver disease (NAFLD) in mice, we fed male C57BL/6J mice either a low fat control diet (10% fat) or a HFD (45% fat) for 20 weeks. During the last 2 weeks of the feeding study, mice were randomized into two feeding protocols: 1) ad lib feeding group and 2) time-restricted feeding at night group (food provided only during the active phase of the day between ZT 12 – 24, or the active/awake phase). After two weeks of either ad lib or time-restricted feeding, tissues were collected at ZT 5 (inactive period) and ZT 17 (active period) of the 24-h day. Western blot analyses of LC3, a marker of autophagosomes, demonstrated a significant effect of HFD compared to control diet, and a significant effect of ZT 17 versus ZT5. Mice subjected to time-restricted feeding at night showed a significant effect on autophagy in the HFD group. Ongoing studies are further dissecting the mechanisms and consequence of regulation of autophagy by diet, feeding schedule, and molecular clock in the liver.

09. DIETARY RESTRICTION, INTERMITTENT FASTING, AND LONGEVITY ACROSS GENOTYPES IN DROSOPHILA MELANOGASTER

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Various methods of dietary restriction have been used to significantly affect life span across different species from yeast to mammals, and the most well-studied form of dietary restriction, caloric restriction, involves feeding 20-40% fewer calories than ad libitum fed individuals. Recently, intermittent fasting as a form of caloric restriction has become a popular way to potentially improve lifespan and health. Intermittent fasting involves planned periods of fasting that can last for a couple hours a day up to days at a time. While health and longevity benefits of intermittent fasting have previously been discovered in rodent models, the longevity effects of fasting in the fruit fly, Drosophila melanogaster, are unknown. Here, we analyzed two forms of dietary restriction in the fruit fly across four genotypes and both sexes for their longevity effects compared to control, ad libitum fed flies. The first dietary restricted group were fed media ad libitum that consisted of 50% less yeast (i.e. protein) than the control diet. Our second dietary restriction method, an intermittent fasting diet, consists of flies being fed our control diet ad libitum every day of the week except Mondays and Thursdays upon which they were switched to a media that did not contain any substantial nutrients. Therefore, they were fasted two out of every seven days. Our preliminary results suggest the effects of the two dietary restriction protocols are genotype and sex specific. Pending results from this study will give insights into the mechanisms that promote longevity in Drosophila via caloric restriction, and we will determine the extent to which different modes of dietary restriction improve both longevity and health across different genetic backgrounds.

10. DEVELOP A FAMILY TREE ALGORITHM TO ESTIMATE YEAST REPLICATIVE LIFESPAN FROM TIME-LAPSED MICROFLUIDIC IMAGES

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**Background:** High-throughput microfluidics based assay of yeast replicative aging can significantly increase the speed and quality of yeast lifespan measurements. However, the major challenge is to efficiently convert large volumes of time-lapsed images into quantitative measurements of yeast cell lifespan.

**Results:** In this study, we address this challenge by prototyping an algorithm that can evaluate cell division events through a family tree of cells. We generated a null distribution using single cells inside microfluidic traps. Based on this null distribution, we developed a greedy algorithm for cell tracking between images at different time points. We inferred a cell family tree through a trace-back method. The branching patterns of the cell family tree are then used to infer replicative lifespan of the yeast's mother cells. The longest branch of a cell family tree represents a yeast mother cell movement at different time points. The replicative lifespan of this mother cell is the number of small branches bifurcating from the main branch of this family tree.

**Conclusion:** Overall, we prototyped a greedy algorithm using a Bayesian approach to infer yeast replicative lifespan measurements from time-lapsed images. This generic method has the potential to not only accelerate the efficiency but also expand the range of quantitative measurement of yeast replicative aging experiments.

11. YEAST BIOLOGICAL NETWORKS CHARACTERIZED BY REPLICA TIVE LIFESPANS OF SINGLE-GENE DELETION MUTANTS AND THE NETWORK ARCHITECTURES

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It is widely recognized that biological phenotypes and genotypes, including biomolecular structures, biological functions and pathways, have been shaped by complex interactions inside living cells. These interactions constitute different biological networks such as the protein-protein interaction networks (PPIs), genetic interaction networks (GINs), metabolic networks, among many others. Reconstructions of these networks largely advanced biological research in the past decade and they are serving as invaluable resources for understanding biology. In present study, a reverse procedure is applied to evaluate the global architectures of the biological networks. Two comprehensive networks, the BioGrid PPI network and the CellMap GIN network, of the budding yeast, *Saccharomyces cerevisiae*, have been quantified by the cell phenotypes and gene characteristics. The most important phenotype of a cell may be its lifespan. Here, the genes in the networks have been characterized by the replicative lifespans (RLS's) of the cells with single-deletion mutations of the two genes interacting to each other. Random permutations were constructed for comparisons with the original networks. We observed that in both BioGrid and CellMap networks interactions between essential gene and majority of nonessential genes are significantly reduced compared to the random networks. This trend has been ascertained via the study of a parallel PPI network, STRING. Multiple characteristics of the genes, including the protein length, protein intrinsic disorder contents, protein abundance, the detected mRNA abundance, as well as the gene connectivities (quantified by the other gene numbers that a gene connects) derived from the studied network, have also been used to evaluate the biological network architectures. The present inverse study of biological networks may shed light on understanding their architectures and stabilities, as well as providing potential directions for their maintainances and improvements.

12. PRELIMINARY ANALYSIS REVEALS LIFE HISTORY BENEFITS FOLLOWING UV-B IRRADIATION IN A MARINE COPEPOD

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Mitochondria are thought to have a biphasic response to the production of reactive oxygen species (ROS), where low levels of ROS benefit mitochondrial performance and high levels are damaging. As such, we hypothesized that moderate ROS production would lead to more advantageous life history characteristics, possibly caused by the signaling of antioxidants, biogenesis, or repair mechanisms. We used UV-B light as an oxidant to determine if moderate ROS production has any positive or negative influence on life history characteristics in a marine copepod (*Tigriopus californicus*). We measured longevity, clutch frequency, gestation length, and nauplii production between a control group (full-spectrum lighting without UV-B) and both short (1 hour) and long-term (3 hour) UV-B treatments. Through the use of zero-inflated negative binomial and linear regression, we find that longer UV-B exposure leads to shorter gestation lengths and an increased clutch frequency among females that reproduce. However, UV-B exposure has no effect on whether or not females produce clutches. In addition, females that do produce clutches (versus those that do not) tend to live longer; more specifically, higher clutch frequencies lead to greater longevity. UV-B exposure was found to have no effect on nauplii production. These findings indicate possible benefits to moderate UV-B exposure. Individuals with increased mitochondrial function may therefore be better adapted to survive and reproduce within a given population. Hence, exposure to such oxidants may explain certain light-dependent behaviors (e.g., diel vertical migration) in organisms such as copepods.
13. THE METABOLOMIC CONSEQUENCES OF SIZE AND AGE IN THE COMPANION DOG

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No mammalian species is as phenotypically diverse as the companion dog. In fact, dogs show an over 50-fold variation in body size that is negatively correlated with longevity; small dogs live up to twice as long as large dogs. While these phenotypic trade-offs in size and longevity in dogs have been recognized for decades, little is known about what genetic and metabolic factors contribute to the variation we witness. Studies of IGF-I, have shown that growth factors play an important physiological role in size and longevity across mammalian species, including dogs, but this pathway does not explain all of the variation seen in size and longevity in the dog. We hypothesized that metabolic regulation differences exist between large and small dogs which contribute directly to longevity and size. To study how underlying physiological differences contribute to size in the dog, we performed untargeted metabolomics on large and small dogs of various ages. We determined that size contributed more to overall variation in the metabolome than age, and the effects of size and age in the dog appear to be sex specific. Interestingly, we also found a significant effect of obesity on the metabolome, regardless of if a dog was large or small. Our metabolic pathway enrichment analyses suggest that amino acid metabolism is differentially regulated between large and small dogs, and future studies on these pathways may shed light on new targets to improve large dog health and longevity. Overall, these results further support the use of the companion dog as an ideal model to develop new hypotheses about how metabolic regulation impacts size, longevity, and obesity.

14. RE-EVALUATING LIFE HISTORY TRADE-OFFS WITHIN THE CONTEXT OF MITOCHONDRIAL HORMESIS

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The notion that reproduction has an effect on future performance and longevity is long-standing dogma in evolutionary biology, yet there is little understanding of the mechanisms that underlie this relationship. One variable that has emerged as a likely link between reproductive effort and longevity is oxidative stress. Specifically, it is has been proposed that reproduction increases oxidative stress and in turn, oxidative stress results in cumulating cellular damage that impacts an individual’s longevity. Support for this hypothesis has been limited. We propose that there is limited support because ROS (reactive oxygen species), the molecules implicated in oxidative damage, are not consistently harmful. Instead, cells display a hormetic response to ROS exposure. For this presentation, the results of multiple studies that characterize how the mitochondria respond to an induced oxidative event and to a reproduction event will be described. In addition, how ROS exposure prior to reproduction impacts reproductive performance and how prior reproduction impacts a female’s response to an induced oxidative event will also be presented. Cumulatively, these data suggest that, at least relatively early in an animal’s reproductive life, increased ROS exposure associated with reproduction is more likely to enhance than to hinder the performance of females. Based on this evidence, we will propose a new model for understanding the tradeoff between reproduction and longevity whereby the early benefits of reproduction act to maximize subsequent reproductive performance, but delayed consequences of prior oxidative damage could contribute to early senescence in animals with high reproductive output. We recommend that future studies be designed to test these interacting effects.

15. MELATONIN AND ITS METABOLITES ENHANCE THE DNA REPAIR IN HUMAN MELANOCYTES EXPOSED TO UVB

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Abstract.

Ultraviolet radiation (UVR) induces DNA damage in skin cells by producing cyclobutane pyrimidine dimers (CPD), pyrimidine photoproducts (6-4)PPs plus augmenting the production of reactive oxygen species (ROS) with deleterious effects on skin. It also induces tumor suppressor factor p53, as a part of response to DNA damage. Melatonin is hormone normally produced in pineal gland and is responsible for many functions, including circadian rhythm and aging. With age, melatonin production decreases, therefore the protection of body from noxious compounds is diminished. Melatonin and its metabolites: 6-
hydroxymelatonin (6-OHM), N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK), N-acetylsertotonin (NAS), and 5-methoxytryptamine (5-MT), reduce DNA damage caused by UVB irradiation in human melanocytes. We measured the DNA repair capacity of melatonin and its metabolites in melanocytes exposed to UVB. Treatment with melatonin or its metabolites caused significant reduction of CPD levels in cells exposed to UVB. DNA damage and repair were further assessed by comet assay. We exposed cells to UVB and treated them with the mentioned compounds. DNA damage and repair assessment showed that melatonin and its derivatives significantly reduced the tail moment of the comets (p<0.001). Melatonin is known to induce phosphorylation of p53 at Ser-15, thus activating p53. We tested the ability of melatonin and its metabolites to induce p53 phosphorylation at Ser-15 as a response to UVB damage. All molecules tested significantly enhanced the expression of Ser-15 phosphorylated p53. Further, melatonin and its metabolites actions directly affect nucleotide excision repair (NER). Using an Oligonucleotide retrieval immunoprecipitation (ORiP) technique we proved that melatonin or its metabolites significantly enhanced the XPC and XPA interactions with the DNA substrate. Thus, by documenting the melatonin’s capabilities to induce DNA repair mechanisms in melanocytes (shared also by its metabolites), we identify melatonin as the natural protector against UVR.

16. METABOLITE PROFILE SIGNATURES ASSOCIATED WITH ACCELERATED AGING AND FUNCTIONAL DEFICIENCY OF SUCCINATE DEHYDROGENASE IN S. CEREVISIAE

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S. cerevisiae chronological lifespan (CLS) is a genetic model for eukaryotic cellular aging, measured as stationary phase survival. Genes, nutrients and environmental factors influence yeast. Succinate dehydrogenase (SDH), encoded by SDH1-4, has been associated with aging, but biochemical mechanisms remain unclear. We applied isotope ratio outlier analysis (IROA) LC-MS metabolomics to compare the biochemical profiles of individual SDH subunit knockout strains in S. cerevisiae. 13C-labeled internal standards were used in conjunction with a mass spectrometry metabolite library of standards (MS-MLS) to perform IROA metabolomics and assess SDH knockouts vs. the parental reference strain, at 11 time points over 7 days, to identify age-associated profiles. Samples extracted with methanol/chloroform, lyophilized, and re-dissolved with an equal-mass solution of 95% 13C yeast extract standards (Cambridge Isotope Laboratories) were analyzed by reverse phase UHLC-HRMS, using a C18-PFP column and Thermo Q Exactive instrument with resolution of 35,000 at m/z 200. Resulting data was analyzed using ClusterFinder software.

ClusterFinder analysis detected over 400 distinct ions (300+ in positive mode and 100+ in negative mode). 60 unique adducts were identified with MS-MLS (52 positive mode and 18 in negative mode). 205 structures were predicted from public databases, 104 chemical formulae of unknown structure were predicted, and 43 ions of unknown identity were detected. Many 12C and 13C orphans were identified, suggesting a custom IROA library as a future direction. Temporal metabolite profiles revealed age- and strain-dependent differential expression, most apparent at time points corresponding to entry into cell cycle state G0 / quiescence (3-7 days). Age-associated differential metabolic expressions correlated with normal (wild type and sdh3 knockdown) vs. reduced (sdh1, sdh2, and sdh4 knockouts) CLS. Glutathione and glutamate were associated with longevity, while citrulline and inosine were associated with reduced longevity. In total, 72 metabolites exhibited profiles associated with sdh-dependent aging.

17. ORAL NITRATE REDUCTASE ACTIVITY DECLINES WITH AGE: IMPLICATIONS FOR AGE-ASSOCIATED DECREASE IN VASCULAR NITRIC OXIDE BIOAVAILABILITY

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Ingestion of nitrate-rich foods can improve cardiovascular function by increasing nitric oxide (NO) bioavailability. The proposed mechanism involves salivary nitrate reduction to nitrite by lingual nitrate-reductase (NR) expressing bacteria; nitrite then mediates systemic NO-signaling. However, little is known about how oral NR activity is regulated, and whether this is a modifiable factor for cardiovascular disease risk. In this study, we developed methods to screen oral NR activity on human tongue swabs and tested how this activity varied as a function of age. Volunteers were recruited into three age groups (I: 25~45 years, n=7; II: 51-73 years, n=13; and III: 67-95 years, n=14). Group I and II were recruited from Birmingham, AL and Group III from Huntsville, AL and comprised active, productive seniors. Tongue swabs were collected from the posterior tongue and NR...
activity measured ex vivo by following nitrate-dependent nitrite formation. Colony-forming units (CFU) were also determined to assess bacterial number. Oral NR activity was indexed by calculating the initial nitrate-dependent nitrite formation rate (INNFR) pre- and post-normalization to CFU. In addition, microbe composition was determined using Microbiome Analysis with 16S rRNA gene sequencing. INNFR was significantly lower in Group III relative to Group I or II (both p<0.0001). In addition, there was a negative correlation between age and INNFR (r(38) = -0.74, p=0.0001), but not between normalized INNFR and age (p=0.122) suggesting that lower bacterial number may play a role in lower oral NR activity in older subjects. Microbiome analysis revealed that the relative abundance of Actinomyces sp., Campylobacter sp., Prevotella sp., Veillonella sp. and Selenomona sp correlated with nitrate reductase activity. The microbe composition of younger subjects had significantly higher abundance of Actinomyces sp., Campylobacter sp., Prevotella sp., than older subjects (p<0.05).

Oral NR activity decreases with age and may contribute to age associated decreases in NO-bioavailability.

18. IMPACT OF AGE ON OUTCOMES OF TRANSVAGINAL NATIVE TISSUE REPAIRS FOR ATIPICAL VAGINAL PROLAPSE

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OBJECTIVES: There is a paucity of data regarding impact of aging on surgical outcomes in women undergoing vaginal prolapse repair. The primary aim was to compare subjective treatment success in older versus younger women at least 3 years post transvaginal native tissue repair for apical prolapse. Post-operative symptom severity, quality of life (QoL), overall symptomatic improvement, surgical complications, and retreatment were also examined.

METHODS: This was a retrospective cohort study of all women who underwent primary transvaginal native tissue apical prolapse repair between 2011 and 2013. Subjects were mailed validated questionnaires regarding symptom severity and life impact. Patients were categorized as “younger”(age<70) or “older”(age≥70). Primary outcome was subjective success defined as “no” to “do you usually have a bulge or something falling out that you can see or feel in your vaginal area.”

RESULTS: Of 641 eligible patients, overall response rate was 51%. Median follow-up time was 58 months for each group. Median age was 61 (IQR 11) for younger and 74 (IQR 5) for older subjects. Subjective success was similar between groups (table, p= 0.11). Older women had a greater improvement in PFDI score (table, p=0.01), but change in PFIQ and PGI-I were similar between groups (p=0.75, p=0.48). Composite success, defined as absence of bulge symptoms plus no retreatment, was higher in older subjects (p=0.04). Retreatment rate and surgical complications were similar between groups (both p>0.05).

CONCLUSION: Older and younger women had similar subjective success rates at least 3 years post transvaginal native tissue prolapse repair, but older women had significantly higher composite success as well as improvement in symptom severity. This information may be helpful in counseling regarding surgical expectations and decision-making.

19. METHIONINE RESTRICTION’S EFFECTS ON MIRNA COMPOSITION, LIPID DEPOSITION, AND GLUCOSE TOLERANCE IN THE SKELETAL MUSCLE OF RAINBOW TROUT

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Methionine Restriction (MR) has been shown to extend healthspan in rodents through alterations in metabolism including increased glucose tolerance and decreases in IGF1. In rainbow trout, Oncorhynchus mykiss, many of these hallmarks have also been observed including increased glucose tolerance and decreases in hepatic SREBP1. While a conservation of metabolic status was observed the mechanisms underlying MR were still poorly understood. This study aimed to understand if small RNA's (micro-RNA's) could be playing a role in the metabolic phenotype observed across species.

Myogenic Precursor Cells (MPCs) were cultured for three days in complete medium with 10% fetal bovine serum (FBS), before being switched to a differentiation medium (2% FBS) with or without methionine present. After 48h cells were either left in control medium, methionine deficient medium, or ‘rescued’ for an additional 24h. Changes in miRNA composition and gene expression were determined using miRNA microarray or RT-qPCR respectively. An in vivo eight week feeding trial was then conducted with animals receiving Methionine Sufficient or MR diets. Muscle samples were collected for miRNA composition analysis by RT-qPCR, a glucose challenge was performed, and samples were collected for fochl extraction to determine total lipid content.

In vitro muscle specific miRNAs miR-133a, miR-210, and miR-206 were down regulated during MR and partially ‘rescued’ after 24h. These changes correlated with changes in gene expression of MyoD1 and myogenin. Taken together these results indicated that MR was able to block differentiation of MPCs. In vivo animals receiving MR diets had no change in overall body
condition but exhibited increases in liver lipid accumulation with corresponding decreases in muscle lipid accumulation and increased glucose tolerance at 8 wks. miR-133a was also down regulated in MR animals at 4 wks. Overall this data suggests that changes in miRNA composition play a role in the metabolic phenotype observed during MR.

20. DYNAMIC MODELING OF TRANSCRIPTIONAL GENE REGULATORY NETWORK UNCOVERS NOVEL PATHWAYS DURING PLANT AGING

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**Introduction:** Age-dependent senescence is a multifaceted and highly coordinated developmental phase in the life of plants. Moreover, network sciences, in particular network construction and analyses, have emerged an important branch of biology in deciphering the transcriptional regulatory dynamicity. Thus, elucidating the dynamic network modeling and simulation of molecular events, in particular gene regulatory network during various stages of plant growth and developmental processes including the aging is essential.

**Methods:** Towards this, we analyzed a high-resolution time-series senescence dataset spanning from four-day-old seedlings to 30-day-old leaves marking the onset of senescence. We also performed a weighted gene co-expression network analysis (WGCNA) using differentially expressed genes. Subsequently, we built a computational pipeline that integrates co-expression network, transcription factors (TFs)-promoter associations and microRNA (miR)-target interactions. Our platform models transcriptional regulatory dynamics and simulates the kinetic relationships among TFs and their downstream targets by employing Boolean and ordinary differential equation (ODE) models. We use genetics-based mutant approach to highlight our findings.

**Results and conclusions:** The resulted co-expression network encompasses 9,014 nodes (genes) and 9,93,699 edges. Based on the cluster coefficient and connectivity of the genes, we extracted 34 modules encompassing a minimum of 100 highly correlated genes that are differentially expressed at onset of Arabidopsis aging. Subsequently, we inferred novel dynamic transcriptional regulatory models (2,423 genes, 214 TFs, 5,020 TF/target and 281 miRNA: target) in senescence using time-course gene expression datasets resulting 104 TFs and 14 miRNAs as significant regulators for 41 functional paths. Dynamic simulations and predictive network perturbation analyses followed by an experimental dataset illustrated the kinetic relationships among TFs and their downstream targets. In conclusions, our network science framework discovers cohorts of TFs and their paths with previously unrecognized roles in plant aging and provides a comprehensive landscape of dynamic transcriptional circuitry.

21. NEURAL CORRELATES OF SELF-GENERATION AND AGE DURING PAIRED-ASSOCIATE LEARNING


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**Objective:** To examine the neural correlates of memory performance improvement during self-generation of paired-associates.

**Background:** Self-generating verbally processed language-related information results in better retention over reading. The increase in memory performance may be related to recruitment of a frontotemporal encoding network.

**Design/Methods:** FMRI data from 173 healthy English-speaking participants (97F; 57LH; ages 19-76) were analyzed. In verbal paired-associate learning task, 60 related word pairs were presented with participants saying the second word aloud. In the "read" condition, both words were presented; in the "generate" condition the first word and first letter of the second word followed by asterisks were presented, and participants generated the second word. Post-FMRI forced-choice recognition test was performed. Data were preprocessed using AFNI. A one-sample t-test contrasted the generate-read conditions. Relationships between age and post-scan memory with encoding-related brain activity were examined using regression analyses.

**Results:** Group maps comparing generate-read conditions indicated increased activity for generate>read of bilateral posterior cingulate, right superior/middle temporal, left angular gyrus and right insula. Areas of increased activity for read>generate included bilateral middle/inferior frontal gyrus, bilateral superior/inferior parietal lobule, bilateral superior frontal gyrus, right insula, right cerebellum, left fusiform/middle temporal gyrus, and left caudate. Increased activation with age was observed for the generate>read contrast in left parahippocampal and inferior/middle temporal gyrus, right lingual gyrus, right cerebellum, left superior temporal gyrus, and left middle frontal gyrus (p=0.01, corrected).

**Conclusions:** These findings support differential cortical involvement for active vs. passive verbal encoding in both conditions results in increased right frontal activation, and differential cortical involvement relating to age of individual.
22. ROYAL JELLY RETARDS IMMUNOSENESENCE IN C. ELEGANS AGAINST S. AUREUS INFECTION THROUGH P38 MAPK, INSULIN SIGNALING AND WNT SIGNALING PATHWAY

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An aging organism encounters various age associated diseases including reduction in immunity called Immunosenescence. This renders them to be susceptible to an extended array of pathogens. Here, we study the molecular mechanisms involved in immunosenescence by providing dietary interventions that promote immunity in Caenorhabditis elegans and study their effects. In this study we provide royal jelly a nutraceutical to Caenorhabditis elegans along with pathogenic food sources. Royal jelly has been previously shown to prolong the lifespan and offer resistance to certain stresses in C. elegans. Here, we show that royal jelly supplementation can promote survival of C. elegans when infected with Staphylococcus aureus at different ages of the worm. Staphylococcus aureus is a nosocomial pathogen which has resistance to a variety of antibiotics. We show that royal jelly does not influence the growth or pathogenicity of S. aureus at the concentrations used in this study. Royal jelly improves the integrity of the C. elegans gut which contributes to improved immunity. Through epistasis genetic assays we identified the involvement of DAF-16/FOXO and the Insulin signaling pathway in royal jelly mediated improved immunity. We also found that royal jelly requires p38MAPK and WNT signaling to carry out this response.

23. DROPPING LIKE BUTTERFLIES: STUDIES ON AGING AT THE INTERSECTION OF ECOLOGY, EVOLUTION AND PHYSIOLOGY

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Studies on aging have long been biased towards a handful of laboratory organisms. While this approach has been fundamental for our understanding of many biological processes, a major limitation is that studies using established laboratory species often lack ecological depth. In the wild, organisms face varying conditions, and a key challenge is predicting how organisms respond to environmental change. Aging or senescence, defined as loss of function with age, can have negative effects on performance and fitness and therefore affect both individual and population level processes. We have studied effects of environmental variation on energetics and life-history in temperate butterflies. Specifically, we were interested in knowing whether butterflies show signs of reproductive and metabolic senescence, and whether the rate of senescence is affected by interventions such as dietary restriction and increased flight. Using two North American and one Eurasian species, we performed experiments where butterflies were reared under standard conditions, subjected to experimental treatments, and individually monitored until the end of the lifespan. We found that reproductive output, resting metabolic rate, and flight metabolic rate decreased with age. Dietary restriction reduced reproductive output and resting metabolic rate (investment in somatic maintenance), but did not affect flight capacity or lifespan. Increased flight improved early-life reproduction, and in the case of the Eurasian Glanville fritillary butterfly, increased total reproductive output. Increased flight elevated resting metabolic rate, and in the North American Mormon fritillary, increased the rate of senescence in flight metabolic rate. However, this was not reflected in lifespan, suggesting that metabolic senescence and longevity can be decoupled. Taken together, the findings show that while some traits are conserved, butterfly life-histories are flexible and respond to environmental change. Butterflies have adapted to live in a vast range of environmental conditions and serve as a promising model system for ageing studies.

24. AGING ALTERS RESPIRATORY BUT NOT GLYCOLYTIC CAPACITY IN HUMAN MONOCYTES

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Inflammaging is a condition of chronic low-grade inflammation due to the aging process and is associated with a variety of chronic diseases. Monocytes are innate immune cells which contribute to inflammation and are dysregulated during aging, demonstrated reduced phagocytosis, increased inflammation, and alterations in subset proportions. Metabolism is known to determine immune cell function, with quiescent and anti-inflammatory cells primarily relying on fatty acid oxidation, while activated and inflammatory cells primarily rely on glycolysis. We hypothesized that aging would result in a shift in the metabolic profile of monocytes, potentially contributing to the aged phenotype of these cells. Using Seahorse assays, we profiled mitochondrial respiration and glycolysis in classical monocytes isolated from older (60-80 yr, N=8) and younger (18-35 yr, N=8) adults. Monocytes isolated from older adults demonstrated impaired total mitochondrial respiratory capacity (p=0.017) and spare respiratory capacity (p=0.020). However, basal respiration was unchanged, and both basal glycolysis and glycolytic capacity were not altered by aging (p>0.05). Mitochondrial function has been recently demonstrated to be important in monocytes for cellular function during glucose starvation and in response to certain bacterial pathogens, and transcriptomics studies have identified cellular respiration as potentially differing across monocyte subsets. Therefore,
impaired mitochondrial function is a potential mechanism by which aging alters monocyte function, underlining the need for further investigation.

25. IMPACT OF VITAMIN D$_3$ ON RADIOSENSITIVITY OF MELANOMA AFTER PROTON BEAM THERAPY

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It is known that proton therapy have many advantages over conventional radiotherapy offering better conformality than other therapies. It results from its ability to confine of the precise treatment to tumor volume with high-dose and with minimizing radiation dose to surrounding normal tissue, simultaneously. On the other hand previous studies have demonstrated that vitamin D, which plays a major role in osteogenesis and regulation of mineral homeostasis, can change radiosensitivity of some cell lines. The goal of the present study was to evaluate whether radioprotective properties of some cell lines in response to proton radiation are affected by vitamin D. In our work the impact of two vitamin D$_3$ compounds (1,25(OH)$_2$D$_3$ and 25(OH)D$_3$) on radiosensitivity of three melanoma lines: SKMEL 188 (Human), BHM Ma (Bomirski Hamster Melanoma - pigmented), BHM Ab (Bomirski Hamster Melanoma - unpigmented) were investigated. The level of radiosensitivity as a function of proton absorbed dose (source of protons - AIC isochronous cyclotron operating at 60 MeV) was determined by analysis of surviving curves using two radiobiological models: multi target/single hit and linear-quadratic. The analysis of the shape of the survival curves shows that: (a) Vitamin D$_3$ strongly influences radiosensitivity towards proton irradiation of investigated melanoma cells) (b) 1,25(OH)$_2$D$_3$ and 25(OH)D$_3$ potentiate the radiosensitivity of BHM Ab and BHM Ma cells in a concentration-dependent manner.

26. A PROBABILISTIC GENE NETWORK MODEL OF CELLULAR AGING AND ITS APPLICATIONS

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Biological aging is a complex phenotype with many genes involved, and is characterized by an exponential increase of mortality rate. Dietary restriction is a lifespan extension method that is conserved among many species. We have developed a probabilistic gene network model for cellular aging that can capture the emergent aspect of cellular aging. We applied our network model to study the lifespan extension effect of calorie restriction, including lifespan data sets measured in yeast strains with deletion of SIR2 and TOR1, and in different glucose concentrations. Our results suggest that gene network robustness plays a major in the effect of dietary restriction. Our results show that network model for aging can offer new insights on molecular mechanism of cellular aging. Ongoing work and future plans to further study gene networks and aging will also be presented.

27. IMPROVED METABOLIC RATE BY TENEURIN C-TERMINAL ASSOCIATED PEPTIDE (TCAP)-3: A COMPARATIVE APPROACH ACROSS ZEBRAFISH LIFE-STAGES

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Exercise rewards its host with improved skeletal muscle health and increased metabolism, which improves overall energy regulation. Recent studies have shown teneurin C-terminal associated peptide (TCAP)-1, a bioactive peptide, increases glucose uptake in skeletal muscle both in vitro and in vivo in rodent models. Here we investigate the role of TCAP-3, a closely-related paralogue, as a novel activator of muscle metabolism in zebrafish. To assess metabolic rate, oxygen consumption was measured in larval and adult zebrafish using Loligo systems respirometry chambers and resazurin metabolic assay. TCAP-3 treatment increased maximum oxygen consumption rates and cumulative NADH production, seen up to 24 hours after treatment. These assays demonstrate the conserved effect of TCAP-3 across all life stages in zebrafish. Thus, this project will provide insights into the roles of TCAP-3 as a novel regulator of muscle metabolism and may have practical applications in the prevention of metabolism-associated muscle diseases, such as aging.
28. RIBOSOMAL BIOGENESIS DURING INFLAMMATORY AND HYPERTROPHIC STIMULUS IN PRIMARY HUMAN SATELLITE CELLS

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Background: Ribosome biogenesis and protein translation are finely coordinated and essential for cell growth, proliferation, differentiation, and muscle development. Furthermore, there is a significant positive correlation between the fold change in total muscle RNA content from pre- to post- resistance training and the increase in muscle fiber cross sectional area. Data suggest that inhibition of ribosomal biogenesis using a pharmacological agent can blunt hypertrophy in vitro. However, there have been no studies to determine if a physiological stimulus can impair ribosomal biogenesis and hypertrophy. The aim of the present study was to determine if inflammation inhibits myotube hypertrophy by interfering with ribosome biogenesis in human primary myogenic cells.

Methods: Skeletal muscle satellite cells were isolated from untrained older adults (n=6) after percutaneous needle biopsy of the vastus lateralis. Satellite cells were grown in DMEM containing 20% FBS, 5 ng/ml fibroblast growth factor, 100 μl/ml streptomycin, and 100 U/ml penicillin until they reached ~70% confluence. They were then placed in differentiation media (DMEM containing 2% horse serum, 100 μl/ml streptomycin, and 100 U/ml penicillin) for seven days to induce formation of multinucleated myotubes. Myotubes were treated for 48 hours with either 20% FBS, TNFα (5ng/mL), or 20% FBS + TNFα (5ng/mL). Cells were subsequently harvested for analysis of mRNA, muscle protein synthesis, ribosomal proteins, myotube size, and myofusion index.

Results: Myotubes treated with FBS increased myotube diameter by 20% compared to control. TNFα (5ng/mL) induced 16% atrophy, while a combination of both treatments caused 7% hypertrophy. Total RNA concentration (ng/ul) increased 32% in FBS treated cells but only 20% in response to the combination of FBS + TNFα. Phase II fusion was decreased in myotubes treated with TNFα or a combination of FBS + TNFα.

Conclusions: TNFα-mediated inflammation impairs human myotube hypertrophy, which may be driven by impairments in both ribosome biogenesis and phase II myoblast-myotube fusion.

29. INTERACTING EFFECTS OF AUXOTROPHY, NITROGEN AVAILABILITY, AND SPECIFIC AMINO ACIDS INFLUENCE CHRONOLOGICAL LIFESPAN IN SACCHAROMYCES CEREVISIAE

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Once yeast cultures reach stationary phase, chronological lifespan (CLS) is measured as maintenance of the percentage of cells capable of re-entering the cell cycle upon exposure to fresh media. We use quantitative high throughput cell array phenotyping (Q-HTCP) for growth curve analysis of the genomic collection of yeast gene knockout and knockdown (YKO/KD) strains to understand influences of, and interactions between, genetic background and diet composition on CLS. Target of rapamycin (TOR) is an evolutionarily conserved nutrient signaling pathway that modulates lifespan of all eukaryotes, rendering yeast informative for systems level genetic analysis of complex aging biology. Restriction of amino acids, such as methionine, which are sensed by TOR, also influences lifespan. We characterized the S. cerevisiae strain upon which the isogenic YKO/KD collection exists, regarding effects on CLS of TOR signaling, specific amino acid and total nitrogen availability, and auxotrophic mutations. Auxotrophic strains have variable CLS that is shortened relative to the prototrophic strain in most cases observed thus far. Amino acid supplementation can extend CLS of a corresponding auxotroph; e.g., leucine or uracil supplementation increased CLS of leu2 or ura3 auxotrophic strains respectively, but not for the prototrophic strain. Threonine supplementation had complex effects on CLS depending on met17 or lys2 auxotrophy. In contrast, methionine supplementation reduced CLS in the met17 auxotrophic strain, but only in the presence of ammonium sulfate, the standard nitrogen source in defined yeast media. Additionally, ammonium sulfate shortens CLS independent of auxotrophy. Interestingly, ammonium sulfate was required for CLS extension by reduced TOR1 expression, but CLS extension by rapamycin treatment appears to have greater dependence on met17 and lys2 auxotrophy than on ammonium sulfate. This work establishes the rationale for using systems level phenotyping of yeast CLS model to characterize complex effects of nutrient restriction and auxotrophy on eukaryotic longevity.
30. DROSOPHILA HP1 PROTEINS MAY IMPACT GENE REGULATION BY ALTERING RNA POLYMERASE II DYNAMICS

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Chromatin changes, particularly loss of heterochromatin, are a hallmark of aging. Elucidating the function of individual chromatin components is critical to understanding how these chromatin changes with age and contributes to the aging process. The Heterochromatin Protein 1 (HP1) family is central to a number of processes linked to aging such as DNA repair, DNA replication, chromatin structure maintenance, and gene regulation. However, the molecular mechanisms underlying many of these functions for individual HP1 family members are not well understood. In particular, HP1 has a complex role in mediating gene regulation. In Drosophila melanogaster, HP1a is associated with repression of some genes but is also necessary for activation of others. HP1B and HP1C null mutants also exhibit both up and down-regulated genes. Here, we investigate gene regulation by HP1 proteins by mining publicly available RNA polymerase II (RPII) ChIP-Seq, ChIP-chip, and GRO-Seq data focusing on how HP1 binding changes RPII dynamics. We find that transcripts bound by HP1 proteins have increased promoter proximal pausing compared to unbound transcripts. Further, RPII ChIP-chip profiles from HP1 null mutants show these patterns are altered compared to wild type individuals. These results support a model in which HP1 proteins mediate gene regulatory effects through changes in RPII dynamics. Additionally, we show HP1 bound transcripts overlap with R loops, three stranded DNA/RNA hybrids, at a frequency much higher than expected by random chance. These structures are associated with both increased promoter proximal pausing and genome instability. Together these findings will inform future investigations into a possible connection between HP1 gene regulatory effects and genome stability and how these processes change through aging.

31. NOVEL DROSOPHILA MODEL TO STUDY AGE-DEPENDENT ANTIVIRAL IMMUNITY

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Elderly individuals are particularly vulnerable to infectious diseases, including viral infections, due to the age-related functional decline of both the innate and adaptive immune systems. Although considerable progress has been made in understanding how aging affects the adaptive immune system, much less is known about the role of aging on innate immunity, which is the first line of defense against infections. With its short lifespan, lack of adaptive immunity and the presence of numerous genetic and genomic tools, the fruit fly Drosophila represents an excellent model system to investigate age-dependent innate immune reactions. Previous studies have shown that similar to humans older flies exhibit higher susceptibility to viral infections. Therefore, they can be used to study the impact of aging on antiviral immunity. We used the RNA-containing Flock House Virus (FHV), which is pathogenic to flies, to model age-dependent resistance to virus infection. Using a collection of 150 inbred, fully sequenced Drosophila lines we showed that this is a quantitative trait and that genotype plays a role in the age-dependent resistance to infection with FHV. We used the data from this study to perform a genome wide association (GWA) analysis and identified numerous SNPs located in 47 genes. Among them we identified genes involved in cellular processes including autophagy, glucose metabolism and transcription regulation. We are currently examining the role of some of these genes in the age-dependent immunity to infection and addressing the question whether resistance or tolerance mechanisms are involved in this process. Since innate immunity represents the first line of defense against pathogens, understanding the mechanisms by which aging alters immunity and organismal ability to fight infections and identifying new factors that mediate resistance to viral infection may lead to novel therapies that improve the health of the elderly.

32. REVERSING SKIN WRINKLES AND LOSS OF HAIR IN MICE BY RESTORING MITOCHONDRIAL FUNCTION

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Mitochondrial DNA (mtDNA) copy numbers decline with age, and such changes increase the risk for age-associated diseases. The causative association of the decline in mtDNA copy number in aging and aging-related diseases, however, has not been addressed. We created a mtDNA depleter and replerter mouse and demonstrate that aging-associated skin wrinkles and hair loss are regulated by mtDNA. To evaluate the consequences of depletion of mtDNA in the whole animal, we created an inducible mouse (mtDNA-depleter) expressing, in the polymerase domain of POLG1, a dominant-negative mutation to induce depletion of mtDNA in different tissues. These mice showed reduced mtDNA content, changes in mitochondrial protein expression and...
reduced stability of mitochondrial oxidative phosphorylation complexes. We demonstrate that ubiquitous depletion of mtDNA in mice has profound and predominant effects on the skin resulting in wrinkles and hair loss. Development of skin wrinkles was associated with the hyper proliferation of epidermis, increased expression of MMPs and decreased expression of TIMP1. We also found increased skin inflammation that may be an underlying contributing factor for skin phenotype. Histopathologic analyses revealed dysfunctional hair follicles. These mice also showed changes in expression of aging-associated markers including IGF1R, KLOTHO, VEGF, and MRPS5. Our rescue experiment revealed that, by turning off the mutant POLG1 transgene expression and repleting the mtDNA in the whole animal, the skin and hair phenotypes revert to wild-type phenotype upon. These studies present first in vivo evidence that the development of skin wrinkles and loss of hair associated with mitochondrial dysfunction can be restored by restoring mitochondrial function.

33. RESILIENCY IN MICE AS A PREDICTOR OF FUTURE HEALTH/LIFESPAN

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Aging is the number one risk factor for the majority of morbidities and mortalities that afflict developed countries. With the success of public health and medicine in the 20th century, life expectancy has increased by 30 years. Unfortunately, while individuals are living longer than ever on average, many older adults are spending decades of their life with frailty and its associated morbidities. This decline into frailty is preceded by a loss of resilience, the ability the recover from a significant stressor or challenge. However, the physiological changes that predict frailty and declines in resiliency are unknown. Here, we are attempting to develop a standardized set of resilience assays that singly or in combination predict the future healthspan in early-to-mid life mice. For the purposes of this study, resiliency is defined as a quantitative metric that describes the speed and completeness with which an animal recovers from an acute physical challenge. C57BL/6 mice of both sexes and ages 12, 18, and 24 months obtained from the NIA aging rodent colony. We will first optimize each resilience assay protocol each of which consists of an acute challenge and associated recovery. We will then determine which of the resilience assays optimized previously are predictive of increasing life-or health-span using interventions of known ability to extend life or health in one or both sexes: dietary restriction, rapamycin supplementation in food, and 17-α-estradiol. Once the most predictive resilience assay (or combination of assays) is discovered, we will be evaluated in other genetic backgrounds. Extending the generality of any positive results to other genotypes is important for the resilience assays to prove useful in humans. Results from this study will allow us to predict the impact on healthspan of mice from a few simple, quick, and inexpensive assays that have translatability to the clinic.

34. DYNAMIC REGULATION OF O-GlcNACYLATION OF SYNAPTIC AND TRAFFICKING PROTEINS AND IMPLICATION IN PARKINSON'S DISEASE

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O-linked attachment of β-N-acetyl-glucosamine (O-GlcNAc) to serine and threonine residues is an important pathway that senses nutrient availability and cellular stress and regulates diverse biological processes. Two enzymes, O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA), are responsible for addition to and removal of O-GlcNAc from proteins. Pharmacological increase of protein O-GlcNAcylation in the brain has been shown to decrease tau phosphorylation and neurotoxicity, and has led to clinical trials for Alzheimer’s Disease. Our recent studies however, have found that O-GlcNAcylation is increased in Parkinson’s disease brains. In neurons, inhibition of O-GlcNAc removal by a highly specific OGA inhibitor, thiamet G, increases MTOR phosphorylation, attenuates autophagy and increases α-synuclein protein accumulation. In rodents, protein O-GlcNAcylation is increased in the aging brain, and acute thiamet G exposure impairs learning and memory. To identify the target proteins that are most dynamically regulated and the most sensitive to thiamet G inhibition of OGA, we performed a proteomics analyses from hippocampal samples of male C57/BL6 mice at 3 months of age injected with saline or thiamet G i.p. at 10 mg/kg. Samples were harvested 3 h after injection. Using isobaric labelling with tandem mass tags, followed by chemoenzymatic photocleavage (CEPC) enrichment of O-GlcNAcylated peptides and a single liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) analysis, we have identified 635 unique peptides that are O-GlcNAcylated. From these, 91 peptides from 65 proteins are >1.5 fold in response to thiamet G compared to saline control. Pathway analyses revealed a thiamet G-dependent enrichment of synaptic proteins, trafficking, Notch/Wnt signaling, HDAC signaling and circadian clock. We found that DNAJC6, a protein involved in the endocytosis pathway and with mutations responsible for a subset of familial Parkinson’s disease, has 12-fold higher O-GlcNAc modification 3 h after thiamet G injection. Future studies will determine whether DNAJC6 O-GlcNAcylation levels in sporadic Parkinson’s disease.