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Nathan Shock Centers * Epigenetics * Chromatin * Lipids * Systems Biology * Longevity Factors

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Poster Abstracts

Epigenetic regulation of the aryl hydrocarbon receptor (AhR) in normal aging.

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Purpose: Older adults are more vulnerable to adverse drug reactions partly due to impaired drug metabolism in old age. Recent research has established that significant changes to epigenetics occur in normal aging and prior work by our lab has shown that these changes can affect drug metabolism. Specifically, we found that CYP2E1 intrinsic clearance was associated with age-related changes to DNA methylation (DNAm) and histone acetylation at the Cyp2e1 locus in mouse liver. Here we are investigating the aryl hydrocarbon receptor (AhR), a ligand-dependent transcription factor that controls a network of drug-metabolizing enzymes. We hypothesize that 1) DNAm at Ahr changes with age and that 2) AhR-regulated genes are differentially methylated with age

Methods: To assess DNAm at Ahr we used a C57Bl/6 mouse model to investigate a highly conserved Ahr regulatory region. We obtained liver tissues from the National Institute on Aging for 4, 18, 24, and 32 months, n=12 per group. Ahr DNAm levels were measured using high-resolution melt analysis. All reactions were performed in triplicate and results analyzed via ANOVA. To analyze AhR regulatory networks, we obtained publicly-available AhR chromatin immunoprecipitation sequencing (ChIP-seq) data and genome-wide age-associated differentially methylated regions (a-DMRs) for mouse liver. Enrichment of Ahr binding sites at genes with a-DMRs was tested via Fisher's exact test. **Results:** The average DNAm levels of 4, 18, 24 and 32 months were 100%, 83.9%, 92.2% and 92.9%, respectively. This change in DNAm at Ahr with age was significant (p=0.0024). Our data integration analysis of AhR ChIP-seq data and genome-wide liver a-DMRs showed a significant enrichment with a p-value < 2.2e-16 and an odds ratio of 4.8 suggesting that AhR target genes also exhibit DNAm changes with age. **Conclusions:** The variability in drug metabolism is a major issue in pharmacotherapy, especially in the elderly. Our results show that DNAm reduces with age at AhR and that AhR target genes are also affected by epigenetic aging. These initial studies suggest that a more detailed analysis of AhR regulation in aging is warranted and they open the door for future clinical studies regarding the role of AhR and the variability in drug metabolism.

Defining The Role of Cellular Resilience in Baboon Aging.

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Translational aging models are key in addressing the fundamental gaps in the mechanisms of human aging. Here, we tested the feasibility of primary fibroblasts isolated from nonhuman primates (baboons) as a model of cellular resilience in response to homeostatic challenge. We used a real-time live-cell imaging system to determine the influence of donor age and sex on cell proliferation responses to hydrogen peroxide (H₂O₂) and paraquat (PQ) in fibroblasts derived from baboons across the life-course (n=11/sex). Linear regression analysis indicated that donor age significantly reduced the ability of cells to proliferate following exposure to H₂O₂ (50 and 100 μM) and PQ (100 and 200 μM) challenges in cells from males (6.4-21.3 years; average lifespan 21 years) but not cells from females (4.3-15.9 years). These data demonstrate that with advancing age, fibroblasts derived from female baboons were less impacted by oxidative stress compared to males. In a follow-up study, we determined mitochondrial bioenergetics and proteasome activities in response to PQ, using an experimental design that include no recovery (NR) and 24h recovery (24h R) from PQ challenge. Seahorse bioanalyzer showed that paraquat dose-dependently inhibited maximal respiration in NR group and only at 200 μM PQ in 24h R group. Cells challenged with 100 μM PQ but allowed 24h recovery had similar maximal respiration rate as untreated cells, demonstrating metabolic recovery. In response to PQ, chymotrypsin-like activities of the 26S proteasome increased in NR group but reduced in 24h R group when compared with untreated cells. We are currently studying how donor age and sex modify mitochondrial bioenergetics and proteasome function in the presence of oxidative stress. Funding NIA1U19AG057758-01A1

Mitochondrial dysfunction contributes to fibrosis in aging-associated benign prostatic hyperplasia (BPH)

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Benign prostatic hyperplasia (BPH) is characterized by proliferation, smooth muscle changes, and fibrosis of the prostate. The single greatest risk factor for BPH is age, with 90% of men in their eighties impacted. Many men with BPH will develop lower urinary tract symptoms, which reduce their quality of life as disease severity progresses. Given the multifactorial nature of the disease, treatments have thus far been limited. While BPH has been clearly linked to aging, the molecular mechanisms have yet to be fully elucidated. In this study, we specifically examine how mitochondrial dysfunction caused by aging may contribute to fibrosis in BPH. **Methods:** To evaluate how mitochondrial dysfunction may contribute to fibrosis, we used both in vivo and in vitro models. We examined the complex I protein, NDUFS3 and a mitophagy associated protein, PINK1, via immunohistochemistry (IHC) in prostate tissue from young (2 months) and old (24 months) C57Bl/6J mice. Additionally, we quantified collagen using picrosirius red as an indicator of prostatic fibrosis. We assessed loss of complex I function in vitro using complex I inhibitor, rotenone, on prostate stromal cells (BHPs1) and determined collagen gene expression. Complex I rescue experiments using idebenone, a CoQ10 analog, were also tested. **Results:** IHC staining of mouse prostate tissue showed decreased levels of NDUFS3, suggesting reduced mitochondrial function, specifically associated with complex I of the electron transport chain (ETC). Furthermore, PINK1 was also decreased by IHC, indicating a reduction in Parkin dependent mitophagy. qPCR experiments on rotenone treated BHPs1 cells revealed increased gene expression for both Col1a1 and Col3a1, suggesting complex I dysfunction can contribute to increased collagen production, and therefore fibrosis. Finally, idebenone ameliorated overexpression, supporting the role of complex I in dysfunction. **Discussion:** Combined, this in vivo and in vitro data suggests that mitochondrial dysfunction, potentially originating from complex I of the ETC, is contributing to the production of collagen, potentially leading to a progression of fibrosis in BPH in aging men. MSN231296 (TL), MSN228588, MSN238245 (WAR)

The Mitochondrial Permeability Transition Pore Activates the Mitochondrial Unfolded Protein Response and Promotes Aging

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Mitochondrial activity determines aging rate and the onset of chronic diseases. The mitochondrial permeability transition pore (mPTP) is a pathological pore in the inner mitochondrial membrane thought to be composed of the F-ATP synthase (complex V). Oligomycin sensitivity-conferring protein (OSCP), a subunit of F-ATP synthase, helps protect against mPTP formation. How the destabilization of OSCP may contribute to aging, however, is unclear. We have found that loss OSCP in the nematode *Caenorhabditis elegans* initiates the mPTP, activates a maladaptive mitochondrial unfolded protein response (UPR_{mt}), and shortens lifespan, specifically during adulthood. Genetic or pharmacological inhibition of the mPTP inhibits the adult UPR_{mt} and restores normal lifespan. Loss of the c-ring proton rotor, the putative pore-forming component of the mPTP, during adulthood extends lifespan, suggesting that the mPTP normally promotes aging. Our findings reveal how the mPTP/UPR_{mt} nexus may contribute to aging and age-related diseases and how inhibition of the UPR_{mt} may be protective under certain conditions.

Cellular hallmarks of aging emerge in the ovary prior to primordial follicle depletion

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Ovarian aging and subsequent menopausal transition have been linked with a decline in lifespan and the emergence of co-morbidities such as osteoporosis and cardiovascular events. Despite these effects and the importance of ovarian aging to female reproduction, very little is known with regard to the basic mechanisms that promote ovarian aging. In the study, we determined the role cellular senescence, transcriptomics and epigenetics play in the decline of ovarian reserve with age using C57BL/6 mice (3M, 6M, 9M and 12M). Histological assessment following Sudan black staining of ovarian sections revealed an accumulation of lipofuscin aggregates with age suggesting an increase in senescent cells. Following RNA sequencing, principal component analyses of samples using all expressed genes revealed a separation of samples by age, with 3M and 6M samples segregated from 9M and 12M samples. Further transcriptomic and pathway analyses revealed a shift in transcriptomic pattern with increased pathways and processes linked with inflammation and cell-cycle inhibition with age. Additionally, we observed significant increases in senescent-related markers with advancing age following quantitative PCR analysis. Low-coverage Whole Genome Oxidative Bisulfite Sequencing revealed no DNA methylation or hydroxymethylation changes in the ovary with age. Taken together, our findings suggest that cellular senescence may contribute to the age-related decline in ovarian reserve. Cell-type specific analyses and higher depth sequencing are needed to fully elucidate the role of senescence and epigenetics in ovarian aging.

Elucidating the metabolic mechanisms that preserve the quiescent neural stem cell pool throughout aging

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Tight metabolic regulation is essential to maintain stem cell homeostasis and support healthy aging. With age, altered metabolic profiles are associated with neural stem cell (NSC) dysfunction and a decline in neurogenesis. However, the key mechanisms that contribute to these changes have yet to be elucidated. In this study, we are using a combination of genomics, metabolomics, and imaging approaches to investigate the metabolic mechanisms that support NSC function and how they are altered throughout aging. Through transcriptome profiling, we found that quiescent NSCs exhibit significant age-associated alterations in metabolic and mitophagic gene networks that were not deregulated in activated NSCs. To investigate the impact of these transcriptome-wide changes, we measured mitophagy using the mtKeima imaging system and first compared quiescent and activated NSCs. We observed a strong accumulation of mitochondria in acidic compartments in quiescent NSCs. In addition, preliminary analysis revealed that mitophagic activity becomes dysregulated throughout aging in the quiescent population. Together, these data suggest that quiescent NSCs may be sensitized to metabolic reprogramming throughout aging. In ongoing experiments, we are using targeted metabolomics to precisely define the metabolome changes affected by dysregulation of mitophagy with age. As dysfunctional mitophagy is associated with both healthy and pathological brain aging, elucidating the mechanisms underlying mitochondrial turnover may lead to important strategies to enhance neurogenesis in the context of aging and neurodegenerative disease. This work is supported by NIA/NIH R01 AG053268.

Beneficial effects of dietary protein restriction on metabolic health, cognition and AD pathology

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Age is by far one of the greatest risk factors for most chronic diseases including Alzheimer's disease (AD). Obesity and diabetes patients have an increased risk of AD, reiterating the fact that metabolic dysfunction is a key underlying risk factor for AD. Targeting therapeutics that can suppress aging or modify these risk factors have the potential to slow down AD in rapidly growing aging population. Here, we report our preliminary results of our investigation into the effects of dietary protein restriction (PR) on metabolic health, cognition and disease pathology in the 3xTg mouse model of AD. Six-month-old male and female 3xTg-AD mice were placed on either 21% protein (control) or 7% protein restricted (PR) diets starting from 6 months and continued until 11 months. Female and male mice on PR exhibited improvement in glucose tolerance, and blunted weight gain irrespective of their strain; however, females showed a stronger effect in glycemic control than males. Following metabolic phenotyping, we performed cognitive tests on these mice: Novel object recognition (NOR) and Barnes maze was used to assess the cognitive deficits in all the groups. The significant difference in NOR was seen in the 3xTg mice fed a low protein diet compared with the control fed animals in a long-term memory test. Furthermore, based on latency measurements in Barnes maze, we found significant differences between genotype and diet, suggesting improvement of memory. Following PR, female mice showed decreased tau phosphorylation, AMPK phosphorylation and p62 levels in the brain, as well as a trend towards reduced expression of mechanistic target of rapamycin complex 1 (mTORC1) signaling in the brain. These results are promising and will advance our understanding of dietary modifications as an intervention for the treatment of AD as well as key molecular mechanisms that may lead to the development of novel strategies to improve health related quality of life and delay or prevent AD.

Telomeric 8-Oxoguanine Drives Rapid Premature Senescence in the Absence of Telomere Shortening

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SUMMARY Oxidative stress is a primary cause of cellular senescence and contributes to the etiology of numerous human diseases. Oxidative damage to telomeric DNA has been proposed to cause premature senescence by accelerating telomere shortening. Here we tested this model directly using a precision tool to produce the common base lesion 8-oxoguanine (8oxoG) exclusively at telomeres in human fibroblasts and epithelial cells. A single induction of telomeric 8oxoG is sufficient to trigger multiple hallmarks of p53-dependent senescence. Telomeric 8oxoG activates ATM and ATR signaling, and enriches for markers of telomere dysfunction in replicating, but not quiescent cells. Acute 8oxoG production fails to shorten telomeres, but rather generates fragile sites and mitotic DNA synthesis at telomeres, indicative of impaired replication. Based on our results we propose that oxidative stress promotes rapid senescence by producing oxidative base lesions which drive replication-dependent telomere fragility and dysfunction in the absence of shortening and shelterin loss. Funding RPB: NIA F32AG067710-01; Hillman Cancer Center Postdoctoral Fellowship for Cancer Research Funding PLO: R35ES030396 and R01CA207342

TurnoverR: A Software Tool for the Analysis of Protein Turnover from Metabolic Labeling Studies in Aging and Disease

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Loss of protein homeostasis is a hallmark of aging and age-related diseases, including neurodegeneration, sarcopenia, and type 2 diabetes. While disruption of protein turnover machinery, such as autophagy and the ubiquitin-proteasome system, are often associated with aging and associated pathologies, the turnover rates of proteins do not necessarily reflect a reduction of these processes. Therefore, methods to measure the turnover rates of proteins directly, rather than surrogate measurements of translation and degradation machinery, are critically needed to accurately examine the stability of the proteome during aging and disease processes. Additionally, while the measurement of protein turnover is relevant in many biological settings, including aging, conducting a protein turnover study remains very computationally complex and difficult for most scientists. The development of versatile computational tools on widely accessible, open-source platforms is needed to make this approach more user-friendly. Here, we have developed a new computational tool – TurnoverR – for the accurate calculation of protein turnover rates from mass spectrometry analysis of metabolic labeling experiments in Skyline, a free and open-source proteomics software platform. Using data generated from metabolic labeling of mice with heavy leucine, we demonstrate how this tool enables the calculation of protein turnover rates entirely within a Skyline workspace using raw data acquired on multiple mass spectrometric platforms. We re-analyze data in calorie restricted and ad libitum-fed mice to show this approach re-capitulates turnover rates and differential changes in turnover rates between treatment groups calculated in previous studies. We hope that the addition of this external tool to the widely used Skyline proteomics software will facilitate wider utilization of metabolic labeling and protein turnover analysis in highly relevant biological models, including aging, neurodegeneration, and skeletal muscle atrophy. Acknowledgements: Nathan Shock Pilot Award (UW, Schilling), R01 AR071762 (NIH, Adams), and K99 AG065484 (NIH, Basisty).

Optogenetic elevation of mitochondrial membrane potential increases *C. elegans* lifespan.

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Mitochondria are organelles that make ATP to provide cellular energy, but they are also fundamentally linked to the process of biological aging. Mitochondria are necessary for life and for cellular function, however, as they dysfunction over time they may be a primary driver of cellular deterioration with age. Mitochondria use an electrochemical proton gradient across their inner membrane to power ATP production. This mitochondrial protonmotive force (PMF) also controls diverse metabolic signaling that mitochondria coordinate for cells and tissues, indicating the potential power of targeting PMF to affect broad cellular functions. Aim: PMF declines with age in organisms and tissues, indicating that targeting PMF specifically may illuminate novel mechanisms of mitochondrial control over aging. Our objective was to test if preserved PMF with age resulted in preserved health and longevity. Method: The recently developed optogenetic tool called "mitochondria-ON" (mtON) allows precise and reversible elevation of PMF using light. We used mtON in *C. elegans* to achieve our aim in vivo. Results: Light activation of mtON reversed age-decreased PMF and extended lifespan. In addition, mtON activation preserved healthy mobility as animals aged. These results indicated that decreased PMF is a fundamental driver of biological aging and mortality. Further, directly targeting PMF may be a powerful intervention to reverse dysfunction for diseases of aging and for preserving longevity. Funding acknowledgements: BJB: Biological Mechanisms for Healthy Aging Training Grant NIH T32AG066574. MK: Nathan Shock Center of Excellence in the Basic Biology of Aging NIH P30AG013280. APW: NIH R01NS115906.

Nematode FMO-2 rescues the negative effect of high glucose

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Changes in the quantity or composition of diet can influence systemic metabolism, health, and longevity. Reduced intake of nutrients, or dietary restriction (DR), improves health and longevity across multiple species. Variation in the concentration of a single metabolite can also influence longevity in different directions, for instance, restriction of glucose increases lifespan, however, excess glucose consumption exacerbates age-related diseases and shortens lifespan. Although extensive literature focuses on the mechanisms of DR-mediated longevity, there is less knowledge about how excess nutrients influence aging apart from metabolic diseases. We previously found that in *C. elegans*, a xenobiotic-metabolizing enzyme, flavin-containing monooxygenase-2 (FMO-2), is downstream of DR and is both necessary and sufficient to promote health and longevity. Additionally, mammalian FMOs can influence carbohydrate and lipid metabolism, and their expression is altered in human diabetic patients and rodent models of diabetes. We now find that while growing worms in high glucose (HG) media significantly shortens lifespan, overexpression of FMO-2 rescues this negative effect of HG on longevity. Untargeted and targeted metabolic profiling reveals that overexpression of FMO-2 alters one-carbon metabolism (OCM), leading to decreased methylation flux. Further genetic studies support the role of OCM and highlight the importance of lipid metabolism in regulating longevity during regular and high glucose diets. Taken together, our data suggest that FMO-2 modifies flux in the OCM network that then modifies lipid metabolism to constrain the negative effect of glucose on lifespan.

Acarbose Suppresses Symptoms of Mitochondrial Disease in a Mouse Model of Leigh Syndrome.

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Mitochondrial diseases are pathologies characterized by impairment in mitochondrial function. Mitochondrial dysfunction is also a hallmark of the aging process. Rapamycin, a drug that increases lifespan and reduces the incidence of age-related pathologies in multiple models, increases survival and reduces the impact of neurological symptoms in a mouse model lacking the complex I subunit Ndufs4. Here we show that acarbose, another drug that extends lifespan in mice, suppresses symptoms of disease and improves survival of Ndufs4^{-/-} mice. Unlike rapamycin, acarbose rescues disease phenotypes independently of mTOR inhibition. Furthermore, rapamycin and acarbose have additive effects on clasping and maximum lifespan in Ndufs4^{-/-} mice. Acarbose rescues mitochondrial disease independently of glycolytic flux and Sirt3 activity by potentially remodeling the microbiome. This study provides the first evidence that the microbiome may rescue severe mitochondrial disease and proof of principle that biological aging and mitochondrial disorders are driven by common mechanisms. Funding: NINDS R011NS098329

Worm-YOLO: A Machine Learning Approach to Analyzing Data from the WormBot Image Capture Platform.

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C. elegans has been a workhorse within the field of aging biology due to its short lifespan, easy culturing, and robust genetic tools. However, while some automated tools have been developed, the necessity of manual or semi-manual assays has limited the scope of what a single researcher can accomplish. Using our previously published robotic image capture platform for data collection, we present here a novel machine learning based approach to analyzing and extracting lifespan and measures of health from *C. elegans* within the context of drug discovery. Here we present the pipeline for analyzing results from several compound interventions and show how Worm-YOLO accurately recapitulates manually analyzed data while also allowing for new and deeper mining of behavioral and morphological metrics of health. The increased efficiency from automated analysis coupled with the ability to mine high-dimensional data allows for a holistic evaluation of each compound tested; linking metrics of health with end-of-life outcomes within the context of total lifespan. Furthermore, we also show how our pipeline allows for analysis of several of the *C. elegans* genetic models of aging associated diseases such as Alzheimer's Disease.

Individual protein dynamics in aged muscle and brain

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Proteins are the main drivers of many cellular processes. Maintenance of functional proteome requires protein turnover (synthesis and degradation). Loss of cellular proteostatic regulation is one hallmark of aging. The existing literature indicates that both aging and treatments that extend lifespan slow protein synthesis, thus creating a contradiction. Further, although it is thought that lifespan-extending treatments increase protein breakdown, many studies show increased half-lives with treatments that slow aging. We hypothesized that there is heterogeneity among individual proteins in that some increase and some decrease rates of synthesis with aging. To answer this, we used deuterium oxide (D₂O) labeling combined with proteomics to compare synthesis rates of individual proteins isolated from the brain cortex and gastrocnemius muscle of young (25 week) and aged (90 week) C57BL/6J female mice. Mice were labeled with D₂O for 4, 15, 45, and 60 days (n=3-5 per time-point per age). Total proteins were isolated from the homogenized cortex and gastrocnemius. Trypsin digested samples were analyzed using LC-MS/MS and data-dependent acquisition (DDA). Protein turnover rates were calculated using D₂Ome Software based on isotopomer distribution within peptides. Our analysis demonstrated that nearly an equal number of proteins increased synthesis as decreased synthesis within each tissue. Further, the data show which proteins have particularly slow synthesis rates, making them susceptible to age-related accumulation of protein damage. These data can be used to identify proteins of particular interest that impact the aging process and are amenable to treatment to maintain proteostasis.

Loxl2 is a mediator of cardiac aging in *Drosophila melanogaster*; genetically examining the role of aging clock genes.

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Transcriptomic, proteomic, and methylation aging clocks demonstrate that aging has a predictable preset program, while Transcriptome Trajectory Turning Points indicate that the end of human development is the likely stage at which the progressive loss of homeostatic control, and in turn aging, begins. Turning points in this developmental age range overlapping with human aging clock genes revealed 5 candidates that we hypothesized could play a role in aging or age-related physiological decline. To begin to examine these gene's possible effects on lifespan and health-span, we utilized whole body and heart specific gene knockdown of human orthologs in *Drosophila melanogaster*. Whole body Loxl2, fz3, and Glo1 RNAi positively affected lifespan as did heart-specific Loxl2 knockdown. Loxl2 inhibition also reduced cardiac arrhythmia and fibrosis. Loxl2 binds several transcription factors in humans. RT-qPCR confirmed that several transcriptional targets of this binding, including TNF α (egr), have expression levels that correlate with Loxl2 reduction in *Drosophila*. These results point to conserved pathways and a possible mechanism by which inhibition of Loxl2 can be beneficial to heart health and aging. NIH 2021 R01 AG

Gene correlation network alterations across the lifespan

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Despite large efforts towards identifying age-associated genes via absolute alterations in expression levels, a robust set of genes which are consistently changed with age has remained elusive. Recently, research has seen some promise in identifying more subtle alterations in the coordination of genes, showing robust co-expression changes. The framework of network science is particularly suitable for investigating questions about gene expression due to the coordinated nature of biology surrounding transcriptional mechanisms. Therefore, it follows to study transcriptional alterations in aging with methods that look beyond single gene expression level changes. Here, we investigate the transcriptional co-expression network and how it is altered over the lifespan, while focusing on the consistency of changes in the dyadic relations across two different datasets. For comparison, we find statistically significant gene simplices across these datasets with two methods: 1) differential of single gene expression via ordinal regression and 2) differential correlation of gene-gene expression. The overlap coefficient between gene simplices retrieved by these methods is used as a measure of consistency in order to test the hypothesis that the dynamic gene-gene correlation structure is a more robust feature of aging.

Right heart failure in aged mice and the sex-specific outcomes of metformin

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In age-related heart failure as well as other clinical contexts in which aging populations suffer significant morbidity and mortality, right ventricular (RV) function is the strongest predictor of survival. However, despite the clear link between RV dysfunction and mortality, little is known about the impact of age on the RV and no RV-directed therapies exist. We used a murine model of pulmonary hypertension (PH)-induced RV dysfunction by exposing 18 month old C57BL/6 male and female mice to hypobaric hypoxia (HH; 17,000 feet). By two weeks of HH exposure, pulmonary artery acceleration time (PAT) slowed, indicative of early PH. While early HH exposure resulted in RV hypertrophy, by week 3, RV systolic function declined and by week 4, the RV was dilated and was unable to maintain cardiac output. This model of PH and right heart failure is well-characterized by sex differences in young animals, however sex differences were not present in aged male or female mice which both demonstrated significant RV dysfunction. Our group and others have reported that the AMPK activator and longevity drug metformin protects against RV dysfunction in young mice, however, metformin has yet to be tested in the aging RV. Metformin (200mg/kg/day in drinking water) demonstrated clear sex differences with respect to metabolic and cardiopulmonary outcomes. Metformin lowered blood glucose in aged female but not male mice, attenuated RV mass in aged male mice but improved PAT in female mice. Metabolomics analyses also supported sex-specific effects of metformin on the heart, with females demonstrating changes in amino acid, nucleotide, and glutathione metabolism. Together, we suggest that the aged RV undergoes significant remodeling in response to HH and that metformin impacts RV function in a sex-specific manner. Ongoing efforts are aimed at elucidating the molecular basis for remodeling in the aged RV as well as understanding the mechanisms of metformin action in the failing right heart- a disease with significant clinical implications. This project was supported by NIH AG058810.

Heterochronic Plasma Transfer Alters Proteostatic Maintenance in Skeletal Muscle

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Heterochronic blood and plasma transfer (HPT) studies show that circulating factors modulate skeletal muscle to respond to damage. Yet, how the systemic environment influences skeletal muscle in the absence of muscle damage is unknown. Proteostatic maintenance (i.e., protein synthesis and breakdown) is necessary for cellular stability in skeletal muscle. One measure of proteostatic maintenance is how much protein is synthesized for cellular maintenance versus cellular proliferation (protein:DNA ratio). Here we determined if altering the systemic environment of old mice with young plasma and young mice with old plasma would alter skeletal muscle proteostatic maintenance. We hypothesized that compared to old mice that receive old plasma (OOp), old mice that underwent HPT from young mice (OYp) would improve proteostatic maintenance. Additionally, we hypothesized that compared to young mice that receive young plasma (YYp), young mice that underwent HPT from old mice (YOp) would decrease proteostatic maintenance. To test this, we implanted young (5-month, n=3 YYp and 5 YOp) and old (24-months, n=5 OOp, and 5 OYp) C57BL/6J mice with a jugular catheter. All mice underwent HPT, receiving 100 μ l plasma injections, every three days for 24 days. The day before HPT, mice began stable isotope labeling with deuterium oxide. After 4 weeks the gastrocnemius and tibialis anterior (TA) muscles were harvested and analyzed for DNA and protein synthesis to calculate the protein:DNA ratios. The protein:DNA ratio for the mitochondrial fraction trended toward a higher ratio in the TA of the OYp versus OOp (11.93 ± 1.161 and 8.928 ± 1.128 , respectively; $p=0.10$). The protein:DNA ratio for the myofibrillar fraction trended toward being lower in the gastrocnemius of the YOp compared to YYp (8.338 ± 0.429 and 6.281 ± 0.737 , respectively; $p=0.06$). These results suggest that manipulating the systemic environment alters skeletal muscle proteostatic maintenance to reflect the systemic environment. Thus, strategies to improve the systemic environment, like exercise, may be a feasible strategy to improve proteostatic maintenance in aged skeletal muscle. This work was funded by the NIH T32 Grant: 5T32AG052363-04.

Implications of bidirectional interactions between host and microbes on NAD metabolism

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Nicotinamide adenine nucleotide (NAD) is a redox cofactor and a co-substrate for signaling enzymes. NAD is essential to all living organisms, including microbes that reside in and on host. NAD metabolism has been studied for more than a century from the perspective of host cells. Nevertheless, microbial NAD metabolism and their impact on host cells is not well known. In this study we used isotopically labeled NAD precursors to negate the long-held notion that microbes primarily use dietary amino acids and vitamin B3 to synthesize NAD. We found that orally delivered vitamin B3, nicotinamide and nicotinic acid, are absorbed in the proximal parts of the small intestine. Intriguingly, we found that host-derived nicotinamide in circulation enter gut lumen and completely support NAD synthesis in the ileal microbiota. On the other hand, in the colon both host-derived nicotinamide and complex carbohydrates such as soluble fiber are essential to fuel NAD synthesis. We show that nicotinic acid produced by microbial deamidation of nicotinamide is required to by-pass salvage synthesis in host tissues. We found that microbial deamidation is accelerated in the ileal lumen of aged mice with implications on NAD metabolism in microbes and host. Thus, NAD precursors cycle between the host and gut microbiome in a mutually beneficial manner to maintain NAD homeostasis.

CHEMOTHERAPY-INDUCED ACCELERATED CEREBROVASCULAR AGING

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Studies on cancer survivors show that chemotherapy induces long-lasting adverse effects on cognitive function. Importantly, no strategies exist to prevent/reverse chemotherapy-induced cognitive impairment (CICI). Progress in this field is hampered by the lack of adequately controlled clinical studies, making it difficult to draw definitive conclusions regarding CICI mechanisms. Therefore, to address this question, we have established an innovative mouse model; cancer-free p16-3MR mice treated with the chemotherapeutic drug paclitaxel (PTX). Hypothesis: Our central hypothesis is that chemotherapy induced endothelial senescence is a major contributor in initiating several pathologies like neurovascular dysfunction, microvascular rarefaction and blood-brain barrier (BBB) disruption, which collectively contributes to decrease in cognitive functions in cancer survivors. Result: Behavioral assessment with radial arm water maze provided evidence for chronically impaired cognitive function (n=20, p<0.05). Although chemotherapeutics does not cross BBB; endothelial cell lining of cerebrovasculature (CVECs) is exposed to the highest concentrations of these drugs, making them uniquely vulnerable to drug-induced DNA damage. This DNA damage and activation of microglia (p=0.0001) was observed in PTX treated mice model with Immunohistochemistry. Flow cytometry-based analysis of cerebrovascular endothelial cells shows increase senescent endothelial cells in brain vasculature following PTX treatment. In addition, endothelial vasodilator function was also examined by assessing functional hyperemia responses & BBB integrity. Upon observation; endothelial-dependent cerebral blood flow responses were found to be markedly attenuated (p=0.07) and BBB integrity (p=0.0412) observed to be compromised in PTX treated mice. Treatment with PTX has also been observed to cause vascular rarefaction in the somatosensory cortex (p<0.05). Conclusion: Our findings concur with our hypothesis that PTX treatment induces cellular senescence in CVECs as a DNA damage response, which leads to decreased cerebral blood flow, NVC dysfunction and increased neuroinflammation contributing to cognitive impairment. Acknowledgment: This work was funded by the American Heart Association (AHA) Pre-Doctoral grant, Stephenson Cancer Center (SCC) Pilot/Trainee award.

ACE2/ACE imbalance in the aging-associated leaky gut in mice.

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Compromised barrier properties of intestinal epithelium (leaky gut) with aging leads to systemic inflammation. Recent studies point to the beneficial effects of the protective axis of Renin Angiotensin System (RAS), which constitutes Angiotensin converting enzyme-2 (ACE2)/ Angiotensin-(1-7) (Ang-(1-7))/Mas receptor (MasR). This is known to be counter-regulatory to the classical axis of RAS consisting of ACE/Ang II/AT1 receptor (AT1R). This study tested the hypothesis that leaky gut with aging is associated with ACE2/ACE imbalance and that activation of MasR with Ang-(1-7) would restore gut barrier integrity. Study was carried out in Young (3-4 months) or Old (20-24 months) mice. ACE and ACE2 enzyme activities were evaluated in the colon. Protein expressions of ACE, ACE2, AT1R, AT2R and MasR were determined by western blotting. Ang-(1-7) was administered by subcutaneous osmotic pump (1 µg/Kg/min) for four weeks and the gut permeability was evaluated by using FITC-dextran. Colon wall integrity was evaluated by immunohistochemistry of claudin 1 and occludin. Zonulin-1, IL-6, and TNF α were analyzed in the plasma. ACE2 protein and activity were decreased in Old group while that of ACE were increased (n=6). AT1R and MasR expressions in the gut wall were higher in the Old (P<0.007, n=4) while AT2R (P<0.005, n=4) expression was lower. Gut permeability was higher in Old mice (P<0.01, n=6) that was abolished by Ang-(1-7) treatment. Hematoxylin & Eosin staining revealed that aging was associated with atrophied villi in the gut wall that was normalized by Ang-(1-7). Expression of claudin 1 and occludin were lower in the Old gut wall that were restored by Ang-(1-7). Importantly, plasma levels of Zonulin-1, (P<0.01) IL-6 and TNF α (P<0.05) (n=8) were higher in the Old group compared to the Young, which were lowered by Ang-(1-7) (n=5). MasR-deficient mice have increased gut permeability (P<0.01) and higher plasma levels of zonulin (P<0.05) compared to the wild type mice (n=6) at younger age. Activation of MasR by Ang-(1-7) would be a promising approach for reversing leaky gut and reduce systemic inflammation with aging.

Caloric restriction improves lipid and lipoprotein profiles in rhesus monkeys

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Metabolic syndrome is correlated with both obesity and age. Caloric restriction (CR) without malnutrition is a dietary intervention that has been shown to extend lifespan and delay the onset of metabolic diseases. Using the highly translatable rhesus monkey (*Macaca mulatta*) model, we evaluated the changes in lipid and lipoprotein profiles for both male and female, median-age individuals on a control or CR diet. We performed biometric measures using dual-energy x-ray absorptiometry (DEXA). Plasma fatty acid composition was measured by gas chromatography for FFA, cholesteryl esters, diacylglycerol (DAG), phospholipid and triacylglycerol lipid classes, while lipoprotein profiles were generated by NMR. Circulating levels of metabolic hormones and inflammatory markers were determined by ELISA. We find that individuals on a CR diet have significantly lower levels of low and very low-density lipoprotein particles, as well as lower triglyceride and cholesterol levels. Additionally we identify shifts in phospholipid and triacylglycerol correlations, and cholesterol esters and diacylglycerols correlations between control and CR individuals. Together this data reveal changes to circulating lipid and lipoprotein profiles in monkeys on a long-term caloric restricted diet. Funding: AG040178

CD8+ T cell dysfunction regulates infection-induced mortality in aged mice

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Age and metabolic disease increase the risk of morbidity to numerous infectious diseases including SARs-COV2, the virus causing the COVID-19 pandemic. This increase in risk is, in part, due to age-related immune system dysfunctions including increased inflammation, a decrease in the number of naïve T cells, impaired humoral immunity, and accumulating cellular senescence. Exposing specific-pathogen-free (SPF) mice to a milieu of murine pathogens creates a “normal microbial experience” (NME), causing 100% mortality, elevated immune cell counts, and inflammation in aged, but not young mice. We hypothesized that the dysfunctional aged immune system could be targeted to prevent mortality to NME-related infection. To address the specific changes in immune cells, we used flow cytometry, RT-qPCR, and RNA-sequencing to analyze lymphoid and non-lymphoid tissues from young and old, SPF or NME-exposed mice. We found increased expression of inflammatory markers and multiple subsets of immune cells. RNA-seq showed distinct clustering of the individual groups, with strong enrichment for inflammatory pathways. Obesity, a condition that induces inflammation and senescence, was not sufficient to induce mortality to NME in middle-aged mice. There were no differences in the exhausted T cell response, inflammation, or antibody production when comparing the lean and obese NME-exposed mice. This data is the first to show protection against age-related mortality using checkpoint blockade therapy to improve T cell activity. This improves our understanding of how the aged immune system and T cell functionality affect aged individuals’ ability to defend against novel pathogens. This work is supported by the UMN Clinical and Translational Science Institute COVID-19 rapid response and the Fesler-Lampert Chair.

Dietary protein restriction attenuates high-fat induced inflammation in aging heart by activation of AMPK-ULK1 mediated mitophagy.

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Advanced age and obesity are risk factor for cardiovascular disease. Cardiac aging can result from defective mitochondrial functions such as energy production, protein quality control, chronic inflammation, and mitochondrial DNA (mtDNA) leak. mtDNA leak is a central mediator of inflammatory responses in human diseases. Protein restriction has emerged as a promising therapeutic intervention for age-related diseases. However, it is largely unknown if protein restriction alters mitochondria-mediated innate immune responses to prevent aged-related heart inflammation. Here, we examined the role of dietary protein restriction in cardiac inflammation of high-fat diet-fed mice. Methods: 12-month-old mice were randomly allocated to control (CTRL), protein restriction (LP), high-fat (HF), or high-fat combined with protein restriction (HF+LP) diet for 4 months. Heart tissue was analyzed by RNA-sequencing (RNA-seq), gene expression, immunoblotting, and electron microscopy (TEM). Results: KEGG enrichment demonstrated an up-regulation of inflammation associated with mitochondrial damage-associated molecular patterns in HF mice (vs. CTRL), whereas HF+LP downregulated this pathway (vs. HF). LP and HF+LP significantly decreased the presence of cytosolic mtDNA (vs. HF, $p < 0.05$) due to the clearance of dysfunctional mitochondria by mitophagy. DRP-1, PINK1, Parkin, Beclin, p62, and LC3II expression were also significantly increased in the mitochondrial fraction of HF+LP hearts (vs. all, $p < 0.05$). eiF2 Ser51 and AMPK Thr172 were significantly increased in LP and HF+LP (vs. CTRL, $p < 0.05$) while mTOR Ser2448 was increased only in HF animals (vs. CTRL, $p < 0.05$). AMPK mediated ULK1 Ser555 was significantly increased in LP and HF+LP (vs. CTRL, $p < 0.05$). TEM images confirmed the presence of autophagosome vacuoles within the mitochondria of HF+LP heart tissue (vs. HF, $p < 0.05$). Conclusions: Inflammation in the aging mouse heart was augmented by HFD due to impaired mitophagy, whereas dietary protein restriction reduces cardiac inflammation through activation of the AMPK-ULK1 mediated mitophagy.

Subcellular localization of PDE4D and HCN1 in rhesus macaque entorhinal cortex layer II: Signature of vulnerability in Alzheimer's Disease

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Tau pathology emerges in a distinct spatial and temporal pattern in Alzheimer's Disease (AD). Anatomical studies in AD subjects and rhesus macaques show earliest signs of tau pathology in the stellate cell islands in entorhinal cortex (ERC) layer II. However, the molecular mechanisms that confer vulnerability to ERC layer II cells early in the disease course is unknown. Our previous research in monkeys showed early calcium dysregulation in layer II ERC, where phosphorylated tau accumulated on the calcium-storing smooth endoplasmic reticulum (SER) under glutamatergic synapses, and PKA-phosphorylated ryanodine receptors on the SER showed evidence of calcium leak. cAMP-PKA magnification of calcium release has been seen in prefrontal cortex, associated with HCN channel opening to dynamically regulate synaptic strength. This process is regulated by phosphodiesterases (PDE), regulation that is lost with age. The current study examined whether this "signature of flexibility" could also be seen in layer II ERC, underlying vulnerability to tau pathology with advancing age. We used high-spatial resolution immunoEM to localize PDE4D and HCN1 in young rhesus macaque (7-10y) ERC layer II. Our results reveal that PDE4D and HCN1 were primarily observed in postsynaptic compartments in macaque ERC layer II. In dendritic spines, PDE4D was concentrated on the SER spine apparatus and in the postsynaptic density, and HCN1 expressed in the membrane near excitatory synapses. Within dendritic shafts, PDE4D labeling was observed along microtubules and near mitochondria, whereas HCN1 was organized in discrete clusters along the plasma membrane. PDE4D immunolabeling was also observed in astroglial leaflets ensheathing excitatory asymmetric synapses. In summary, PDE4D is optimally positioned to modulate cAMP microdomains and control calcium extrusion from the SER. HCN1 channels are localized in subcompartments to facilitate dynamic physiological representation of sensory experience and visual space governed by cAMP-PKA signaling. The anatomical patterns in ERC layer II corroborate our findings in vulnerable glutamatergic circuits in prefrontal cortex, suggesting conserved molecular features in association cortices most susceptible in AD. These data suggest that PDE4D and HCN1 are positioned to provide a signature of flexibility in postsynaptic compartments in ERC layer II stellate cells, which becomes a signature of vulnerability when abrogated by advancing age. Funding: AFAR

Assessing the Dynamic Mitochondrial Fission and Fusion Events in Skeletal Muscle in vivo

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Skeletal muscle plays an important role in systemic metabolism and overall health. Within skeletal muscle, there is a decline in mitochondrial function with age. Under normal healthy conditions, mitochondria remodel via two dynamic processes, mitochondrial protein turnover and mitochondrial dynamics (i.e., mitochondrial fission and fusion events). While capturing the dynamic nature of mitochondrial protein turnover in skeletal muscle is now possible, assessing mitochondrial fission and fusion relies on snapshots of protein markers from muscle homogenate at distinct timepoints. Recent technical advancements allow for measurement of mitochondrial dynamics in skeletal muscle in vitro; however, rates of mitochondrial dynamics in other tissues are significantly slower in vivo compared to in vitro conditions. Thus, the purpose of this study was to develop novel imaging methods to assess mitochondrial dynamics in vivo. Our hypothesis was that we could capture and assess in vivo mitochondrial fission/fusion events using a novel imaging approach. Using multiphoton microscopy, we activated a mitochondrial-targeted photo-activated GFP that was electroporated into the tibialis anterior (TA) muscle of 5 adult C57Bl6 mice to assess the rates of mitochondrial fission and fusion events. In vivo mitochondrial fission and fusion rates were ~ 50 times slower in comparison to previously reported rates in in vitro models. We used an additional imaging approach to explore changes in mitochondrial morphology over more prolonged periods of time. To do so, we imaged control and denervated TA muscles of 5 adult C57Bl6 mice using an electroporated mitochondrial-targeted GFP and TOM20_tdTomato. We found that two weeks of denervation decreased the cross-sectional area (CSA) that was co-stained in the denervated limb compared to a control limb, suggesting a decrease in cristae structure. In addition, two weeks of denervation decreased the CSA of the population of subsarcolemmal mitochondria in the denervated limb compared to a control limb. Our data emphasizes the importance of assessing mitochondrial dynamics in vivo. Additionally, changes to mitochondrial morphology are regional, which stresses the importance of assessing mitochondrial subpopulations. Funded by the NIH-T32 Grant: 5T32AG052363-04.

In silico identification of new potential senolytics.

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During aging, senescent cells (SC) accumulate in tissues and organ contributing to the progression of various chronic and degenerative diseases. Hence, the scientific community is searching for therapeutic agents, called senolytics, which are able to selectively induce SC cell death through the inhibition of SC anti-apoptotic pathways (SCAPs). Hence, our aim was to identify new potential senolytics in silico. We used a reference of 69 known molecules with senolytic activity. We assessed structural similarity, with 2D fingerprints, of molecules from databases of FDA-approved drugs, experimental drugs, natural products, and a database of molecules synthesized in our laboratory. The 100 molecules with the most remarkable similarity to the senolytic ones were selected. Then a consensus virtual screening based on the structure was performed, having as targets 4 SCAPs (SERPINE1, EFNB1, PDGFB, and PIK3CD), that were previously identified through co-expression network analysis of primary human prostate cell cultures induced to senescence with H₂O₂. Taking the former into consideration, the molecules that potentially inhibited at least 2 SCAPs were selected. After using network pharmacology and cheminformatic analysis, 11 promising molecules with potential senolytic activity were selected. Five FDA-approved drugs, 3 experimental drugs, and 3 molecules synthesized in our lab were identified as potential senolytics due to their high structural similarity (> 0.85) measured with the Tanimoto coefficient. Moreover, those molecules share an identical chemical space, so they might potentially inhibit more than 2 SCAPs and other known senolytic targets. The high chemical similarity with senolytics has allowed the identification of FDA-approved drugs that are potential inhibitors of various SCAPs; however, these molecules still need to be validated in biological tests. Drug repositioning is a powerful tool that will simplify the experimentation process to find more suitable senotherapeutic agents with fewer secondary effects. This work was supported by CONACyT "FORDECYT-PRONACES/263957/2020" KSODA is a CONACyT fellowship.

FGF21 prevents low protein diet-induced renal inflammation in aged mice.

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Low protein diets extend lifespan through a comprehensive improvement in metabolic health across multiple tissues and organs. Many of these metabolic responses to protein restriction are secondary to transcriptional activation and release of FGF21 from the liver. However, the effects of a low protein (LP) diet on kidney function in the context of aging has not been examined. Therefore, the goal of the current study was to investigate the impact of chronic consumption of a LP diet on renal function and histology in aging mice lacking FGF21. Wild type (WT, C57BL/6J) and FGF21 KO mice were fed a normal protein (NP, 20% casein) or a LP (5% casein) diet ad libitum from 3 to 22 months of age. The LP diet led to a decrease in kidney weight and urinary albumin/creatinine ratio in both WT and FGF21 KO mice. Although the LP diet produced only mild fibrosis and infiltration of leukocytes in WT kidneys, the effects were significantly exacerbated by the absence of FGF21. Accordingly, transcriptomic analysis showed that inflammation-related pathways were significantly enriched and upregulated in response to LP diet in FGF21 KO but not WT mice. Collectively, these data demonstrate that the LP diet negatively affected the kidney during aging, but in the absence of FGF21, the LP diet-induced renal damage and inflammation were significantly worse, indicating a protective role of FGF21 in the kidney. This project was supported in part by NIH R01 DK115749 (KS), HL148114 (DVI), DK105032 and DK121370 (CDM), DK096311 (TWG) as well as F32DK115137 (CMH). SG was partially supported by NIGMS U54 GM104940 which funds the Louisiana Clinical and Translational Science Center, and by funding from the National Medical Research Council, Ministry of Health Singapore (WBS R913200076263). This work utilized the following core facilities at Pennington Biomedical: Animal Behavior and Metabolism, Genomics and Cell Biology and Bioimaging Core -- that are all supported in part by COBRE (P20GM103528) and NORC (P30DK072476) center grants (NIH).

Epigenetic histone modifications alter the pSIRT1 pathway to promote Alzheimer's disease.

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Alzheimer's disease (AD) is a neurodegenerative disorder that affects a person's memory and other vital mental functions. It is the most common cause of dementia and is most common in adults that are 65 years or older. The buildup and presence of amyloid beta oligomers (A β O) have been associated with AD pathology, however, it remains unclear whether age-related metabolic changes and epigenetic markers increase the production of A β or if A β changes the histone marks to alter metabolism. To better understand the role that A β O has in AD, the effects of A β O on histones that control the PPAR γ coactivator 1 alpha (PGC-1 α)/sirtuin 1 (SIRT1) pathway will be examined this to determine how these alterations affect AD. Additionally, the nutraceutical treatment of (?)-epigallocatechin-3-gallate (EGCG) will be introduced and evaluated to determine if it is effective in preventing AD. The purpose of this study is to better understand how A β O affects AD as well as explore the potential that EGCG has as a treatment for AD. Gaining more insight into what role A β O plays in AD pathogenesis will pave the way for better and more specific AD treatments. This work was supported by the Brewer Lab at University of California, Irvine.

Machine learning-based one year mortality prediction among residents in senior living facilities

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Aim: Approximately 1/4 of deaths in the U.S. occur in long-term care facilities. Despite this, predictive tools for mortality of the residents within these facilities are suboptimal. **Objective:** We have developed a machine learning algorithm to predict mortality within one year among residents at Juniper communities serving older adults including skilled nursing facilities (SNFs), independent living facilities (ILFs), and assisted living facilities (ALFs) using data in the patient electronic health record taken from the first 90 days after admission. **Methods:** Our machine learning model uses gradient boosted trees to predict all-cause mortality in individuals at senior living facilities. The model utilized age and gender, and measurements of respiration rate, diastolic and systolic blood pressure, temperature, heart rate, SpO₂, glucose, CO₂, sodium, creatinine, potassium, calcium, chloride, blood urea nitrogen, albumin, and cholesterol. Summary statistics of vitals and laboratory measurements were calculated across the 90-day period after admission. The final dataset for the model contained 715 individuals, with 159 individuals in the positive class (22.2% prevalence). The model was optimized via a grid search across five standard hyperparameters and five-fold cross validation with an 80:20 train:test split. Feature importance was evaluated through Shapley values. Metrics for the area under the receiver operating characteristic curve (AUROC), sensitivity, and specificity were calculated on the 20% hold out test set. **Results:** The model achieved an AUROC of 0.75, a sensitivity of 0.71, and a specificity of 0.61 for predicting one-year mortality 90 days after admission. The most important features in the model were age and summary statistics for temperature, blood pressure, glucose levels, and oxygen saturation. **Conclusions:** Vital signs and age were identified as the major factors associated with machine-learning based one-year mortality prediction in this study. Machine learning algorithms may be beneficial for predicting one-year mortality in the older adults at SNFs, ALFs, and ILFs.

Endothelial metabolic shift from anaerobic glycolysis to oxidative phosphorylation with aging

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Endothelial dysfunction is a hallmark of vascular aging and is a key feature in the etiology of cardiovascular diseases that are associated with increased oxidative stress. Flux through the mitochondrial electron transport chain (ETC) is thought to be an important source of cellular reactive oxygen species that suppresses endothelial cell (EC) function in older humans but surprisingly little is understood about the influence of aging on EC metabolism. Younger ECs, typically utilize anaerobic glycolysis but with advancing age ECs shift toward a greater reliance on oxidative metabolism. This shift may explain enhanced superoxide production via the ETC. Here we aimed to determine the impact of aging on EC metabolism. In this present study, we utilized Seahorse technology to assess oxidative and glycolytic metabolism in primary lung ECs isolated from young and old C57BL/6 mice, a cell culture model of aging. We found that old ECs demonstrate a 37% higher rate of oxygen consumption ($p < 0.01$), 74% higher spare respiration ($p < 0.01$), and 92% higher proton leak ($p < 0.01$) compared with young ECs. In contrast, ATP production was 27% lower in old compared with young ECs ($p < 0.01$). The total extracellular acidification rate (glycolytic shift) was 47% ($p < 0.01$) lower and the maximal glycolytic rate was 22% lower ($p < 0.01$) in old compared with young ECs. Together, these data indicate reduced glycolysis and increased oxidative phosphorylation in old ECs. Next, we assessed mitochondrial reactive oxygen species (mtROS) production, as well as the expression of mitochondrial membrane complexes, and uncoupling protein 3 (UCP3). Interestingly, we observed 81% higher mtROS production ($p < 0.01$), and reduced expression of the mitochondrial membrane complex II (34%, $p < 0.05$), III (65%, $p < 0.01$), and IV (87%, $p < 0.01$) in old compared with young ECs. UCP3 protein expression was increased by 56% ($p < 0.01$) in old compared with young ECs. Taken together, these findings suggest that, despite less efficient mitochondrial coupling and lower protein expression of mitochondrial ETC proteins, aging results in a shift away from anaerobic glycolysis towards a greater reliance on oxidative phosphorylation. This age-related metabolic shift in ECs may contribute to increased mitochondrial superoxide production and subsequent endothelial dysfunction. Funding Information: National Institute of Health (R01 AG040297, R01 AG048366, K02 AG045339) and US Department of Veterans Affairs (1101BX002151).

Induction of multiple senescence markers by tumor inoculation in young C57BL/6 mice.

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Childhood cancer survivors often experience adverse long-term health complications related to pre-mature aging. Clinical studies have shown that senescence markers are increased in cancer survivors. It is assumed that cancer treatments such as doxorubicin and radiation therapy are the main drivers of pre-mature aging phenotype in cancer survivors. However, it is not known whether cancer itself may also induce pre-mature aging. In the present study, we evaluated the effect of inoculating EL4 lymphoma cells in immunocompetent mice on the expression of senescence markers and senescence-associated secretory phenotype (SASP). Four-week old male C57BL/6N mice were inoculated in the flank region with a single subcutaneous injection of EL4 lymphoma cells or sterile PBS and sacrificed 4 weeks following the tumor inoculation. Thereafter, we determined the effect of tumor on the gene expression of the senescence markers (p21, p16, p19, p53, Mmp13, and Pai-1) and SASP (Il-1a, Il-1b, Il-6, Tnf-alpha, Mcp-1, and Cxcl1) in the heart, kidney, and liver by real-time PCR. Serum cytokine levels were also measured by ELISA. EL4 tumor increased the gene expression of Mmp13, Pai-1, Il-1a, Il-1b, Il-6, Tnf-alpha, Mcp-1, and Cxcl1 in the heart. Tumor also increased the gene expression of p16, p19, p53, Il-1a, Il-1b, Tnf-alpha, Mcp-1, and Cxcl1 in the kidney. In the liver, p16, Pai-1, Il-1a, Il-1b, Il-6, and Tnf-alpha gene expression was induced in tumor-bearing mice as compared to tumor-free mice. The gene expression of p21 was not significantly altered in the heart of tumor-bearing mice but was reduced in the kidney and liver. A significant increase in TNF-alpha was observed in the serum; however, Il-6 and Mcp-1 were not significantly changed in tumor-bearing mice. Taken together, these results demonstrate that presence of tumor induces senescence markers in an organ-specific manner, suggesting that cancer itself may contribute to the pre-mature aging phenotype in cancer survivors. This work was supported by the National Heart, Lung, and Blood Institute (NHLBI) grant R01HL151740 and the National Institutes of Health's National Center for Advancing Translational Sciences grant UL1TR002494

Ablation of Poly-ADP-Ribose Polymerase 1 (PARP1) in muscle stem cells prevents full regeneration and alters the muscle gene program in mature muscle

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Poly-ADP-Ribose Polymerases (PARPs) attach NAD-derived ADP-ribose to proteins and regulate many cellular functions basally and under stress, including recruiting DNA repair enzymes. To combat DNA repair in BRCA-mutant cancers, PARP1 inhibitors are used clinically. Interestingly, PARP1 inhibition/ablation (KO) spares NAD-levels, activates SIRT1 and improves skeletal muscle metabolism in models of obesity. Notably, in mouse models of muscular dystrophy, Poly-ADP-Ribosylation levels parallel disease severity. PARP1 inhibitors are associated with clinical musculoskeletal-related adverse events. Furthermore, grip strength was reduced in whole body PARP1 KO mice. Subsequently, we generated 2 novel tamoxifen-inducible PARP1 KO mouse models: 1) a muscle stem-cell specific KO (PARP1-iMScKO) and 2) a mature muscle-specific KO (PARP1-iMKO). Following Tibialis anterior (TA) cardiotoxin injury and regeneration, PARP1-iMScKO muscles were significantly smaller than WT. Following deletion induction, PARP1-iMKO had decreased TA mass and decreased TA muscle fiber minimum feret diameter. Surprisingly, we observed decreased levels of the E3-ubiquitin ligases (i.e. Atrogin1 and MurF1) and autophagy genes (e.g. Map1lc3b and Sqstm1) suggesting the lower muscle mass was not due to increased protein degradation. We did observe a decrease in the muscle specific transcription factor MyoD1 and an increase Myh mRNA and protein levels. Additionally, we found that Olaparib (a PARP1 inhibitor)-treatment decreased muscle fiber minimum feret diameter within 7 days. In conclusion, these findings suggest that PARP1 preserves muscle mass and PARP inhibitors impair muscle mass maintenance implicating PARP1 as a positive regulator. Future research could yield new therapeutic avenues for treating sarcopenia and/or muscular dystrophies, as well as address musculoskeletal-related adverse events associated with PARP1-targeted chemotherapeutics.

Increased Tau Phosphorylation and Aggregation in Mitochondrial Disease Mice

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Most diseases of aging, such as Alzheimer's Disease, Parkinson's Disease, and cancer, are exacerbated by aberrant mitochondrial function. Its dysfunction is an early feature of most tauopathies and is associated with tau hyperphosphorylation that disrupts microtubules. However, the role of mitochondrial failure in tau pathogenesis is unclear. As age is the most critical risk factor in the onset of Alzheimer's Disease and Related Dementias, a better fundamental mechanistic understanding of the multivariate processes involved stands to illuminate innovative strategies to prevent these diseases. Housing Complex I deficient (NDUFS4-KO) mitochondrial disease mice in 11% oxygen extends median lifespan from 50 days to 500 days, however, the mechanism of this is unclear. Global phosphoproteomic analysis of brain extracts indicated NDUFS4-KO mice have 3-fold increases in tau phosphorylation and aberrant phosphorylation of cytoskeleton-regulating proteins. Follow-up studies suggest the presence of hippocampal pathologic tau aggregates in these mice. Housing mice at 11% O₂ prevents this abnormal tau phosphorylation. Furthermore, hypoxia decreases tau phosphorylation and doubles lifespan in transgenic *C. elegans* expressing human tau. Collectively, this work identifies a mechanistic framework connecting mitochondrial failure, oxygen toxicity, and tau pathogenesis. More broadly, these studies may provide fundamental insights in tau biology to discover innovative approaches to combat tauopathies. Anthony Grillo was supported by NIH Ruth L. Kirschstein NRSA Fellowship grant no. F32 NS110109. This work was also supported by NIH grant no. R01 NS098329.

Dietary amino acid balance for reproduction and storage in grasshoppers

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Diets ideally should match the species' needs to yield full reproduction and long lifespan. High protein diets increase reproduction, at the cost of longevity, while low protein diets extend lifespan, at the cost of reproduction. This importance of protein has motivated research on the amino acid composition of diets. One successful approach designed a diet with amino acids that matched those encoded for by the exome. This method produced full reproduction even on somewhat low protein diets in flies. The individual protein that best matched the amino acids of the exome-matched diet was yolk protein. Here we designed a diet that matched the amino acid composition of grasshopper vitellogenin (Vg), the precursor to egg yolk protein. Each day, each individual (n=90) was fed 1gm fresh lettuce (insufficient for reproduction on its own), fed ad libitum dry carbohydrate diet, and force-fed a solution for delivering amino acids. The four diets were: 1) amino acids matched to the composition of Vg (group ID: Vg-balanced & 1gm), 2) isonitrogenous but with amino acids out of proportion from Vg (Unbalanced & 1gm), 3) buffer and unlimited access to lettuce (Buffer & ad-lib), and 4) buffer only (Buffer & 1gm). Buffer & ad-lib had the highest reproductive output, because the delivery of liquid diets did not accomplish full feeding. Reproductive output was higher in the Vg-balanced & 1gm group than in the Unbalanced & 1gm group. Reproduction requires exactly the amino acids that were fed to the Vg-balanced & 1gm group. To initially test whether the Vg-balanced diet improved other developmental processes, we serially measured levels of storage proteins in these same females. Insects store high levels of hemolymph proteins that appear to provide amino acids for any developmental process. The major storage protein contains 170% more phenylalanine and 65% more tyrosine than Vg. Despite this, among groups fed 1gm daily, the Vg-balanced & 1gm group had 200% more cumulative storage protein. This suggests that the Vg-balanced diet may improve development generally, and not merely provide amino acids needed to make eggs. Future studies will test this diet in juvenile males. Funded by NIH 1R15AG050218-01A1 and UNF Terry Presidential Prof to JDH.

Improving mitochondrial function via drug treatments can reduce cellular senescence.

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Aging is a complex process culminating in loss of organ function and organism resilience. A great challenge in aging research is determining cause and effect: which insult(s) instigate aging and which are a consequence of it? For example, mitochondria dysfunction can be both upstream and downstream of cellular senescence. Our approach to addressing this challenge is to use a well-defined system in which, by definition, the primary insult is increased genotoxic stress in the nuclei of cells. We use *Erc1*^{-/-} mice and mouse embryonic fibroblasts (MEFs), in which *Erc1* is deleted and this destabilizes a critical DNA repair endonuclease ERCC1-XPF. As a consequence, the cells are missing several key DNA repair mechanisms required to protect the nuclear genome. Cells and tissue from mutant mice accumulate oxidative DNA damage faster than wild-type (WT) cells and mice and display accelerated onset of senescence. *Erc1*-deficient cells and mice also display mitochondrial dysfunction. Here, we seek to understand the mechanism by which mitochondrial dysfunction arises. We treated *Erc1*^{-/-} MEFs with mitochondrial-targeted drugs and synthetic molecules to inhibit signaling pathways, then measure the effect on mitochondrial ROS production, mitochondrial membrane potential, mitochondrial mass, mitochondrial bioenergetics, and markers of senescence. Preliminary results suggest that improving mitochondrial function reduces cellular senescence. Furthermore, targeting upstream signaling has more impact on mitochondrial health than trying to target mitochondrial per se. Future work is aimed at discovering better mitochondrial-targeted therapeutics that can improve mitochondrial homeostasis while reducing cellular senescence. Funding sources: NIH/NIA: U19 AG056278, R01 AG063543, P01 AG062413

Mitochondrial DNA deletion mutation frequency as a metric of biological age.

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With age, somatically-derived mitochondrial DNA (mtDNA) deletion mutations clonally accumulate within individual cells across multiple tissues. Attaining high intracellular abundance, these deletion mutation-containing mtDNA genomes disrupt the mitochondrial electron transport chain, oxidative phosphorylation and cellular function, and result in cell death. We hypothesize that the accurate quantitation of mtDNA deletion mutation frequency will provide an index of age-induced cell death and biological age. We have developed digital PCR assays that quantify mtDNA deletion frequency using total DNA samples from any tissue. This assay is quantitative, amenable to a 96-well format, and has a detection limit below one part per million. We found that muscle mtDNA deletion mutation frequency increased exponentially with age and that deletion frequency was lower in women (p=0.0093). On average, skeletal muscle mtDNA deletion mutation frequency increased 98-fold between the ages of 20 and 80. Deletion frequency predicted physical performance declines between 50 and 80. These data affirm the status of mitochondrial dysfunction and genome instability as hallmarks of aging and corroborate our hypothesis that deletion frequency is a metric of biological age. This work is supported by the National Institute on Aging (R56AG060880, R01AG055518, K02AG059847, and R21AR072950).

A computational solution for bolstering reliability of epigenetic clocks: Implications for clinical trials and longitudinal tracking

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Epigenetic clocks are widely used aging biomarkers calculated from DNA methylation data, but this data can be surprisingly unreliable. Technical noise produces deviations of 3 to 9 years between replicates for six prominent epigenetic clocks, substantially limiting their utility. We present a novel computational solution to bolster reliability, calculating principal components from CpG-level data as input for biological age prediction. Our novel principal-component versions of six clocks show agreement between most replicates within 0 to 1.5 years, improved ability to detect clock associations and intervention effects, and reliable trajectories in longitudinal studies and cell culture. This method entails only one additional step compared to traditional clocks, requires no prior knowledge of CpG reliabilities, and can be applied to any existing or future epigenetic biomarker. The high reliability of principal component-based clocks will be particularly useful for personalized medicine, longitudinal tracking, in vitro high-throughput screening, and clinical trials of aging interventions. <https://www.biorxiv.org/content/10.1101/2021.04.16.440205v1>

Role of the epigenetic writer SUV420H2 in Early Human Pluripotency and in quiescence

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Organismal health requires continuous regeneration. However, the mechanisms that govern the regenerative competence still remain unclear. The cells that are refractory for regenerative aging, pluripotent stem cells have the capacity to remain in pluripotent stage either by culture conditions in vitro or by diapause conditions in vivo. We have previously identified metabolic differences that regulate the ESC epigenetic state. Here we define a novel epigenetic regulator during implantation stage, SUV420H2. Our screen for early ESC regulators revealed SUV420H2 as a critical component in naïve to primed transition. Using the CRISPR/Cas9 system, we generated SUV420H2 mutant naïve hESC line to study its role in the pre- and post-implantation embryonic stages. We show that SUV420H2 mutants do not enter in vitro quiescence, but instead continue dividing. By immunoblotting, we also show mutant cells have higher levels of H4K16ac as well as increased rates of proliferation. This data suggests that mechanistically H4K20me3 repressive marks in key target genes are a pre-requisite for diapause, quiescent stage. Furthermore, our functional metabolic assays show that SUV420H2 mutant cells have an increased levels of fatty acid β -oxidation, mitochondrial respiration and glycolysis compared to wildtype, suggesting SUV420H2 as a potential metabolic inhibitor. We conclude that increased OXPHOS and glycolytic metabolism might be due to blocking the inhibitory effect of SUV420H2 on PPAR- β . The data reveal the mechanism for SUV20H2 requirement during naïve to primed embryonic transition. The epigenetic repression by H4K20me3 marks is a pre-requisite for the potential diapause and metabolic reprogramming that takes place during naïve to primed transition. Funding Acknowledgement: Biological Mechanisms of Healthy Aging Training Grant; NIH: 1T32AG066574-01

Activation of human small heat shock protein HSPB5 by zinc: Taking advantage of a multitude of possibilities.

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Small heat shock proteins (sHsp) are a cell's first stress response units. They function to delay protein aggregation, implying that sHsps are resistant to cellular stress conditions and may instead be activated by them. Indeed, stress factors known to lead to activation of sHsp chaperone activity include acidosis, and changes in metal ion concentration. Here we expand upon previous findings from our and other laboratories on the ubiquitously expressed human small heat shock protein HSPB5 (aka, β -crystallin) which showed that 1) a highly conserved histidine residue plays an important role in HSPB5 activation and 2) metal ions can activate and cause structural rearrangements in HSPB5. We sought to determine in detail the effects of zinc on HSPB5. Despite ~50% of HSPB5 sequence space being intrinsically disordered, it contains numerous conserved histidine residues: of the 9 histidines in its sequence, four are in the disordered region (NTR domain) and 5 are in the stably folded core domain (ACD domain). Our isothermal calorimetry data reveal 2 macroscopic zinc-binding constants. Paradoxically, although histidines in the structured ACD domain have previously been implicated in metal-ion binding, we observe a higher affinity binding that involves the disordered NTR. Furthermore, 9 single histidine-to-alanine mutations revealed that no single histidine is critical for either the strong or weak binding events. Such behavior implies remarkable plasticity in zinc binding and a role for disordered regions in zinc binding in HSPB5. We propose a model for the observed metal ion plasticity of HSPB5 in which binding sites are not pre-defined in the absence of metal ions and there exist multiple ways in which metal binding can be executed. Our findings shed light on the modes of action of HSPB5 in preventing protein aggregation by identifying plasticity as a key factor in zinc binding. Taking these findings into a bigger picture, metal imbalance can have calamitous effect on cells and induce protein aggregation. National Eye Institute: 2 R01 EY017370 Vision Training Grant: T32 EY07031 from the NEIBiological Mechanisms for Healthy Aging Training Grant: NIH/NIA T32 AG066574

Necroptosis contributes to chronic inflammation and fibrosis in aging liver

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Inflammaging, characterized by an increase in low-grade chronic inflammation with age, is a hallmark of aging and is strongly associated with various age-related diseases, including chronic liver disease (CLD) and hepatocellular carcinoma (HCC). Because necroptosis is a cell death pathway that induce inflammation through the release of DAMPs, we tested the hypothesis that age-associated increase in necroptosis contribute to chronic inflammation in aging liver. Phosphorylation of MLKL and MLKL-oligomers, markers of necroptosis, as well as phosphorylation of RIPK3 and RIPK1 were significantly upregulated in the livers of old mice relative to young mice and this increase occurred in the later half of life (i.e., after 18 months of age). Markers of M1 macrophages, expression of proinflammatory cytokines (TNF α , IL6 and IL-1 β), and markers of fibrosis were significantly upregulated in the liver with age and the change in necroptosis paralleled the changes in inflammation and fibrosis. Hepatocytes isolated from old mice showed elevated levels of necroptosis markers as well as increased expression of proinflammatory cytokines relative to young mice. Short term treatment with the necroptosis inhibitor, necrostatin-1s (Nec-1s), reduced necroptosis, markers of M1 macrophages, expression of proinflammatory cytokines, and markers of fibrosis in the livers of old mice. Thus, our data show for the first time that liver aging is associated with increased necroptosis and necroptosis contributes to chronic inflammation in the liver, which in turn appears to contribute to liver fibrosis and possibly CLD. Funding: This work has been supported by NIH grants R01AG059718 (DS), Merit grant I01BX004538

Senolytics reversibly improve metabolic health in nonhuman primates

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Senescent cells accumulate in adipose tissue (AT) with obesity, causing local inflammation and dysfunction. Senescent cells in aging nonhuman primate (NHPs) AT correlated with metabolic disease measures in our prior work. We evaluated the senolytic combination dasatinib plus quercetin (D+Q) on AT senescent cell burden and systemic metabolic health in older obese NHPs with naturally occurring type II diabetes (n=3) who had remained stable on insulin therapy for six months prior to study. NHPs were administered a single oral dose of D (5mg/kg) plus Q (50mg/kg), and monitored for effects over time. Blood samples were collected 1 week prior to, and 4 and 8 weeks post-treatment. Abdominal subcutaneous (SQ) AT biopsies were taken before and 1 week and 1 month after dosing. Senescence-associated beta galactosidase (SA- β -gal) staining showed 24% reductions in AT senescent cell burden which rebounded at 1 month. Concordantly, immunostaining for p21 showed large reductions in positive staining at 1 month, which returned to baseline at 3 months. Two animals had large reductions in senescence biomarker plasminogen activator inhibitor 1 (mean=55%). Following the pattern in senescence markers, we saw dramatic improvements in A1c (mean decrease 1.5%), fasting glucose (FBG), cholesterol (TPC) and triglycerides (TG) were reported in all subjects, in the absence of weight loss. A1c decreased by 1-2% and FBG by \pm 100mg/dL (Cohen's d = 3.81 and 2.78). Post-prandial glucose were unchanged. Similarly, TPC and TG reduced (Cohen's d = 0.73 and 1.55). A1c values rebounded in 3-4 months post-dose. Improvements in fasting and overall glycemic measures, and lipid profiles suggest effects on hepatic metabolism. Creatinine also lowered post-treatment without changes in blood urea nitrogen, which may indicate reduced muscle catabolism. Our results show single D+Q has potent and long-lasting effects for improved metabolism but are reversible, indicating that dosing at monthly or longer intervals is a viable approach. Funding: R01 HL142930, P40 OD010965, and UL1TR001420

Influence of iron dyshomeostasis in mitochondrial disease progression

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A commonality of aging amongst all species is mitochondrial dysfunction, however, mitochondrial dysfunction can also be caused by pediatric genetic neurodegenerative disorders that severely shorten lifespan. The Kaeberlein Lab advocates there is significant overlap between the etiology of mitochondrial dysfunction in aging and pediatric mitochondrial disease. To elucidate the biological mechanisms that can lead to mitochondrial dysfunction in humans, our lab uses NDUFS4-KO mice as a model of the human mitochondrial disease Leigh Syndrome. These mice have non-functioning Ndufs4 - an iron-sulfur cluster protein in Complex I of the oxidative phosphorylation system. We hypothesized a deficiency of Ndufs4 would lead to iron imbalances that could drive oxidative stress and neurodegeneration in these mice. Consistent with this, we observed an increase in total iron in the livers of NDUFS4-KO mice. We thus asked if a low-iron diet may alleviate the effects of mitochondrial disease progression. NDUFS4-KO mice fed a low-iron diet showed signs of microcytic, hypochromic anemia. Consistent with our hypothesis, this reduction in body iron delayed the onset of neurodegeneration, decreased lipid peroxidation, and extended lifespan. I next aimed to study the molecular consequences of a low-iron diet in NDUFS4-KO mice by quantifying mRNA transcript levels and protein expression of genes involved in iron uptake, storage, or efflux, and comparing these levels between wild-type (WT) and NDUFS4-KO mice fed a normal or low-iron diet. I observed an increase in protein expression and mRNA transcript levels of iron-uptake proteins such as TfR1 in both WT and NDUFS4-KO low-iron diet mice. When analyzing the expression of genes that respond to iron overload, such as ferritin, I observed a decrease in the low-iron diet NDUFS4-KO mice compared to control diet NDUFS4-KO mice. This observation shows that a low iron diet contributes to a reduction in excess iron. My data may support future research into the use of low-iron diets for those afflicted with mitochondrial dysfunction as a consequence of genetic disorders. Importantly, my data ultimately contributes to illuminating the role iron plays in energy production and can further our understanding of how mitochondria function influence the effects of aging.

Loss of DNA repair mechanisms in cardiac myocytes induces dilated cardiomyopathy.

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Cardiomyopathy is a progressive disease of the myocardium associated with cardiac dysfunction. Genotoxic stress can contribute to several degenerative cardiovascular diseases and cell-autonomous causes of cardiomyopathy are not well-understood. We generated Ckmm-Cre+/-; Ercc1-/- fl mice, which selectively delete the DNA repair gene Ercc1 in differentiated myocytes to test the hypothesis that the accumulation of DNA damage caused by the depletion of Ercc1 in cardiac myocytes would be sufficient to trigger a spontaneous cardiac phenotype in mice. The Ckmm-Cre+/-; Ercc1-/- fl mice expired suddenly at 6-7 months of age due to dilated cardiac myopathy, affecting both systolic and diastolic cardiac function. To elucidate the mechanism by which Ercc1 deletion caused heart disease, we studied cardiomyocytes isolated from the mutant mice in vitro. The cells were treated with doxorubicin, a genotoxic cancer chemotherapeutic known to cause heart disease. Doxorubicin rapidly induced gamma-H2AX foci, a marker of genotoxic stress, in cardiomyocytes isolated from Ckmm-Cre+/-; Ercc1-/- fl mice and wild-type littermate controls. Importantly, after 18 hours, the foci were resolved in WT cultures, but not cells in which Ercc1 was deleted, demonstrating that mutant cells are DNA repair-deficient. Cardiomyocytes from the Ckmm-Cre+/-; Ercc1-/- fl mice were susceptible to apoptosis (more round than rod-shaped cells). Genetic or pharmacological inhibition of p53 partially rescued apoptosis in cardiomyocytes or cardiac tissue from Ercc1-deficient mice and improved disease markers such as ANP and BNP. Taken together, these data support our hypothesis that endogenous DNA damage when not adequately repaired is sufficient to drive cardiac disease. Furthermore, it appears to do so through p53-dependent cardiomyocyte apoptosis rather than cellular senescence. Our data also demonstrated that Ckmm-Cre+/-; Ercc1-/- fl mice offer a novel and clinically accurate model of spontaneous cardiac dysfunction that could be used for rapidly testing therapeutic interventions.

The Interplay of peroxisome and mitochondrial dynamics during aging in Drosophila

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Damaged mitochondria are repaired and recycled through the mechanisms of mitochondrial dynamics in response to stress; this helps in restoring cellular homeostasis. Mitochondrial dynamics have emerged as a novel regulator of aging in recent years. During aging, alterations in mitochondrial morphology and structure have been observed. Researchers have performed genetic manipulations of genes involved in the fission and fusion of mitochondria, which extended the lifespan. However, the causes of the age-dependent alteration in mitochondrial dynamics remain unanswered. Our focus is to explore the involvement of the peroxisome in maintaining mitochondrial homeostasis during animal aging. Recent studies in our lab have shown mitochondrial morphology and function alteration due to impaired peroxisomal protein import in aging oenocytes (hepatocytes) of fruit flies. We found an increase in mitochondrial size in oenocytes during fly aging. Similarly, we have found that the knockdown of Pex5 alters mitochondrial morphology. Interestingly, knocking down the genes involved in peroxisomal plasmalogen synthesis resulted in enlarged mitochondria. Our future goal is to understand how peroxisomes contribute to age-related alterations of mitochondrial dynamics and functions.

Polarization of gamma delta T cells toward IL-17A production in natural aging and accelerated aging

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With aging, there is a gradual decline in immune surveillance along with an increase in chronic inflammation. Gamma delta T cells are less common than alpha beta T cells and may be triggered by different alarm signals including PAMPs and DAMPs. The presence of gamma delta T cells in peripheral organs makes them well positioned to contribute to immune-mediated aging. Here, we assessed how aging affects the accumulation of inflammatory gamma delta T cells in various organs including peripheral and central lymphoid organs. We find that aging increases the percentage of IL-17A-producing cells in gamma delta T cell population in spleen and liver in old mice. Notably, mature gamma delta T cells accumulate in aged thymus and among them, the percentage of IL-17A-producing cells dramatically increases in thymus, similar to the increase in the periphery. Similarly, in the Ercc1 hypomorphic mouse model of accelerated aging due to the accumulation of DNA damage, there is a preferential increase in IL-17A-producing cells compared to IFN-gamma-producing cells in gamma delta T cell population in spleen and liver. Similar to aged WT mice, there are less immature gamma delta T cells and a higher population of gamma delta 17 T cells in the thymus of Ercc1 hypomorphic mice, suggesting potential links between senescence and altered gamma delta T cell generation. Interestingly, the population of IL-17A and TNF-alpha co-expressing gamma delta T cells increases in both animal models. The TNF-alpha and IL-17A co-expression from the gamma delta T cells could be important to promote expression of other SASP factors such as IL-6 in senescent fibroblasts. Our future studies will examine the relationships between age-mediated senescence and our observed skewed composition of gamma delta T cells. Overall, these studies will provide a better understanding of the immunological changes with aging and potential therapeutic immune targets to slow aging. NIH grants T32 AG029796, P01 AG043376, U19 AG056278, R01 AG063543, P01 AG062413, R00 AG058800, the Fesler-Lampert Chair in Aging Studies, and AFAR Junior Faculty Award.

Pharmacogenomic and metabolomic predictors of healthspan interventions in natural populations.

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There is growing interest in developing pharmacological healthspan interventions. These interventions target known longevity-related pathways, like the mechanistic Target Of Rapamycin (mTOR) signaling pathway. However, such interventions are not well-studied in genetically diverse natural populations like humans, leaving a critical gap in our understanding of how genetic variation influences efficacy of these interventions. Other interventions that extend lifespan, like dietary restriction, show a genotype-dependent response where only some individuals within a population benefit. We seek to understand the broad utility of the mTOR inhibitor rapamycin within the context of natural genetic variation, and to identify genetic and metabolic biomarkers that predict rapamycin efficacy. To study natural genetic variation, we use the *Drosophila* Genetic Reference Panel (DGRP), a diverse collection of genetically variable fruit fly strains derived from a natural population. We assess rapamycin response among the DGRP by quantifying developmental timing, an easily measurable phenotype that is regulated, in part, through mTOR signaling. In a screen of 140 DGRP lines, we identified substantial variation in rapamycin response on developmental timing. In some strains, rapamycin treatment has no effect on developmental timing. In others, rapamycin nearly doubles development time in treated flies. Using GWAS combined with set-based statistical analyses, we are identifying genes and genetic pathways associated with rapamycin mediated developmental delay. In future work, we will characterize the metabolome of DGRP lines that are sensitive or resistant to rapamycin, raised with and without rapamycin treatment to 1) identify metabolic changes resulting from rapamycin treatment and 2) identify biomarkers that predict rapamycin sensitivity in untreated animals. Ultimately, these pharmacogenomic and metabolomic analyses advance a precision medicine approach where interventions can be tailored to genetic background and metabolomic profile to maximize individual healthspan. Funding: NIH T32 AG052354

FMO rewires metabolism to promote longevity through one carbon metabolism.

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An organism's ability to respond to stress is crucial for long-term survival. Stress responses are coordinated by distinct but overlapping pathways, many of which also influence longevity across species. Despite extensive effort, our understanding of these pathways and how they affect aging remains incomplete and thus is a key area of study in Geroscience. Our previous work identified flavin-containing monooxygenase-2 (*fmo-2*) as a key longevity-promoting gene downstream of at least three longevity promoting pathways, including the hypoxic response, the pentose phosphate pathway, and the dietary restriction pathway. Based on the commonalities of these pathways, we hypothesized that *fmo-2*, a classically annotated xenobiotic enzyme, might play a key endogenous role in responding to metabolic stress. Our resulting data, using metabolic profiling and further epistatic analysis, both support this hypothesis and link *fmo-2*'s mechanism to modifications to one-carbon metabolism (OCM), a key intermediate pathway between the nucleotide metabolism, methylation, and transsulfuration pathways. Using mathematical modeling and a novel metabolomics approach, we further identify the likely mechanism of *fmo-2*-mediated metabolic effects and connect them to both OCM and downstream components. We propose a model whereby Fmos represent a conserved class of enzymes that can substantially modify endogenous metabolism, similar to how transcription factors modify gene expression, and that *fmo-2* is a key member of a conserved metabolic stress response. Funding: NIH R21AG059117, AFAR Junior Faculty Award, Paul F. Glenn Center for Aging Research

Expression levels of GPLD1 regulated by Cap-Independent Translation in Long-Lived Mice

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Aging entails many physical, biological, chemical, and psychological changes, such as cognitive decline of the aged brain. Several longevity mouse models in which reduced GH and/or IGF-1 signaling leads to extended lifespan, such as Snell dwarf (DW) and growth hormone receptor knockout mice (GKO) were used to gain insight into the cellular and molecular drivers of the age-associated reduction of adult neurogenesis. In this study, we used DW, GHRKO and liver tissue-specific GHR^{fl/fl} mouse lines (LKO), to investigate the effects of altered GH signals on expression levels of GPLD1. Gpld1, a GPI degrading enzyme, hydrolyzes the inositol phosphate linkage in proteins anchored by phosphatidylinositol glycans (GPI-anchor) thus releasing these proteins from the cell membrane. Overexpressing Gpld1 in mice stimulates neurogenesis and improves learning and memory, mimicking known effects of exercise on the brain. Our work now shows that compared with wild-type (WT) mice, GKO, and DW mice have higher protein levels of GPLD1 in liver tissue. In contrast, higher protein levels of GPLD1 are not seen in liver-specific GKO mice, which are not long-lived. This is the first direct evidence that the GH signaling pathway functionally regulates protein expression of GPLD1 in liver tissue of mutant mice with extended lifespan (DW and GKO). Interestingly, mRNA levels of GPLD1 do not change in these mice model, suggesting the role of a post-transcriptional control pathway. Fibroblast cells were treated with 4EGI-1, which diminishes cap-dependent translation but increases translation of cap-independent mRNAs. 4EGI-1 led to higher protein levels of GPLD1, but had no effect on GPLD1 mRNA levels. This indicates that elevated levels of GPLD1 in liver of slow-aging mice is likely to reflect increased Cap-independent translation (CIT).

Protection against APOE4-associated aging phenotypes with a longevity-promoting intervention

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Two of the primary risk factors for late onset Alzheimer's Disease (AD) are aging and APOE genotype. While the causal relationship between aging and AD is not well defined, shared phenotypes, such as decreased metabolic function and increased inflammation, are strong leads. Thus, APOE genotype may drive AD phenotypes through the regulation of aging processes. The NIA Interventions Testing Program recently found that 17 β -estradiol (17 β E2) treatment increases male rodent healthspan and lifespan. Since 17 β E2 has been shown to act upon systemic and neural pathways associated with AD pathology, we propose that 17 β E2 may be a pleiotropic intervention strategy. Further, because APOE4 is associated with an accelerated aging phenotype, 17 β E2 may have APOE genotype-specific effects. Using 10-month-old APOE3 or APOE4 targeted replacement male mice maintained on normal chow in the absence or presence of 14.4 ppm 17 β E2 for 20 weeks, our initial results indicate genotype differences in the impact of 17 β E2 across multiple outcomes. As predicted, APOE4 mice on control chow exhibited an aged phenotype compared to APOE3, with APOE4 mice having a higher frailty index. Importantly, 17 β E2 treatment reduced the frailty index most strongly in APOE4 mice. In addition, APOE4 mice presented with impairments across multiple metabolic measures (e.g. body weight, plasma leptin, hepatic steatosis), which were significantly attenuated with 17 β E2 treatment. These data confirm and extend prior findings that APOE4 is linked to progeroid phenotypes both peripherally and neurally, outcomes associated with AD risk. Importantly, 17 β E2 significantly improved a range of measures across genotypes, but showed the strongest effects in the APOE4 genotype. This work may present a proof of principle for the use of pro-longevity interventions in the treatment of AD-related phenotypes. This research was funded by the Cure Alzheimer's Fund.

Neuron-specific mechanisms control the mitochondrial regulator PGC-1 α

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Mitochondrial dysfunction has been proposed as a hallmark of the aging process. Specifically, as a function of aging, mitochondria appear to have decreased enzyme activity and respiratory capacity and increase reactive oxygen species production. Brain aging is associated with morphological and homeostatic changes, including alterations in brain size, cognitive impairment, and white and grey matter integrity. However, the causes of these changes remain an open and actively pursued field of study. The ubiquitously expressed transcriptional coactivator peroxisome proliferator-activated receptor gamma-coactivator 1 (PGC-1 α) has been described as the master regulator of mitochondrial function. Despite the emerging connections between PGC-1 α and disease vulnerability, the regulation of PGC-1 α outside of the skeletal muscle, liver, and adipose tissue is not well defined. This is particularly true in the brain, where PGC-1 α is enriched in neurons, and alterations in expression levels have been linked to neurodegenerative disorders. Here we report that astrocytes and neurons differ substantially in mitochondrial status and the transcript variants of PGC-1 α expressed, including using a neuron-specific promoter. Taking advantage of the ability of the tau-kinase GSK-3 β to influence PGC-1 α expression, we investigate how transcript variants are differentially regulated in primary neurons and astrocytes. Neuronal PGC-1 α responds robustly to GSK3 β inhibition by lithium, switching the dominant promoter, leading to changes in isoform distribution and abundance, while astrocytes are refractory to lithium treatment. The data presented here highlight key mechanisms for neuron-specific metabolic regulation that are likely to be relevant to neurodegenerative diseases that have a link to mitochondrial dysfunction. This work was supported by funding from the NIH [AG057408(RMA) and AG067330(RMA)].

Endogenous variation in chaperone expression and variation in aging and cancer.

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Isogenic animals, even human monozygotic twins, will age at different rates and have differences in the incidence and severity of age-related disease. Differences in the expression of chaperones can cause differences in the rate of aging and cancer. Different low fidelity endogenous epigenetic silencing pathways cause differences in the expression of chaperones among isogenic individuals. It is our goal to understand the causes and consequences of endogenous variation in gene expression. Previously, we showed that GFP-expressing hsp-16.2 transcriptional reporters act as biomarkers of aging and cancer penetrance/expressivity by detecting differences in the dosage or activity of several distinctly regulated genes. Animals that express more of the biomarker will live longer. Yet, if animals bear a RAS G13E allele, they will develop more neoplasias. Animals that express more of the biomarker express more of just five chaperones, which we screened for effects on lifespan and cancer. Here we show that this lifespan/penetrance biomarker requires hsp-17/HSPB5 for lifespan prediction capability. We also detail mechanisms of cell to cell variation in gene expression and identify cis and trans elements controlling endogenous silencing of hsp-90. We thank the National Cancer Institute and the National Institute on Aging for Funding

Neurodegeneration in a murine model of accelerated brain aging.

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It is well known that senescent cells drive aging and age-related diseases including tauopathies that model Alzheimer's disease (AD), where selective clearing of senescent cells improves disease phenotypes, offering a new approach to treat AD. However, there remains controversy as to which central nervous system (CNS) cells senesce. Identifying those cell types would enable the development of senolytics that are optimally suited to age-related neurodegenerative diseases. To tackle this, we are deleting *Ercc1* in each cell type of the CNS. *Ercc1* encodes a subunit of the DNA repair endonuclease ERCC1-XPF that is critical for the stability of the holoenzyme. Lack of ERCC1-XPF causes accelerated accumulation of endogenous oxidative DNA damage and thereby accelerated senescence. Using the *CamKIIa* promoter to drive Cre recombinase expression, we first deleted *Ercc1* in forebrain neurons. Animals developed normally into adulthood. MRI studies revealed no differences in brain volume between mutant and littermate controls at 3 months of age. However, 6 and 10-month-old mutants exhibited progressive cerebral atrophy relative to controls. Histology indicated a neuron loss and an increase in inflammation (GFAP, IBA-1 immunostaining) in the hippocampus and cortex of 1-year-old *CamKIIaCre^{+/+};Ercc1^{-/-}* mice. While there were no significant changes in the expression of the senescence markers p16, p21 in the cortex of 10-month-old mutants, expression of inflammatory markers including *tnfa*, *il6* was increased. In contrast, in *Ercc1^{-/-}* mice, in which ERCC1-XPF is systemically deleted, p16, p21 expression and that of several senescence-associated secretory phenotype genes were increased in several brain regions including hippocampus and midbrain. Collectively, these data suggest that DNA damage drives senescence of non-neuronal cell types in the CNS, whereas neurons die in response to genotoxic stress. This holds promise that senolytics might be used in neurodegenerative diseases to selectively eliminate senescent cell types within the CNS that can be replaced. Funding: NIH/NIA R01 AG063543, U19 AG056278, P01 AG062413, Aligning Science Across Parkinson's ASAP-000592

Life-Shortening Effect of DMSO-Solubilized Rapamycin in y w Male *Drosophila melanogaster*

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This study was performed to screen a number of potential life-extending supplements, including inhibitors of the TOR signal transduction pathway, while confirming the effects of other, previously tested compounds. This initial screen was conducted in male flies of a comparatively long-lived y w strain of *D. melanogaster*. Unexpectedly, the main finding was a strong, dose-dependent decrease in life span using doses of rapamycin previously shown to extend life span in other strains. Several other supplements had no effect over dose ranges varying by up to five orders of magnitude, but the highest dose of wortmannin (10 μ M) also drastically decreased longevity and the highest dose of spermidine (10 mM) had a smaller adverse effect, while the highest doses of PI-103 HCl (10 μ M) and AZD8055 (10 μ M), an intermediate dose of AZD8055 (0.1 μ M) and lowest dose of WYE-132 (10 pM) slightly increased life span. Possible explanations of the rapamycin result include use of a DMSO solvent in contrast to ethanol used for past studies, or storage of DMSO-solubilized rapamycin in aliquots at -20 $^{\circ}$ C; however, DMSO alone had no effect on life span. No firm conclusion should be reached pending replication of the study in both sexes and other fly strains. This work was funded by anonymous donors.

Aggresome Mediated Neural Stem Cell Quiescence Exit

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Maintaining a healthy proteome throughout life is critical for proper somatic stem cell function, but the complexities of the stem cell response to increases in damaged or aggregated proteins remain unclear. Here we demonstrate that adult neural stem cells (NSCs) utilize aggresomes to recover from disrupted proteostasis and describe a novel function for the intermediate filament vimentin in proteostasis as a spatial coordinator of proteasomes to the aggresome. In the absence of vimentin, NSCs have a reduced capacity to exit quiescence, a time when NSCs are required to clear a wave of aggregated proteins, and demonstrate an early age-dependent decline in proliferation and neurogenesis. Taken together, these data reveal a significant role of vimentin and aggresomes in the regulation of proteostasis during quiescent NSC activation and in the age-dependent decline in adult neurogenesis. We thank our funding sources: NIH T32 T32GM008688 (to C.S.M.), Diana Jacobs Kalman Fellowship from AFAR (to C.S.M.), Wisconsin Graduate Fellowship (to C.S.M.), SciMed Graduate Research Fellowship (to T.J.P.), Sloan Foundation fellowship (to D.L.M.), DP2 NIH New Innovator Award (to D.L.M.), and Vallee Scholar Award (to D.L.M.).

Peripheral migration contributes to age-associated gamma delta(??)-T cell accumulation in visceral adipose tissue in mice.

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Age-associated adipose tissue dysfunction is a key contributor to chronic inflammation, which is directly linked to the development of frailty and cardio-metabolic disorders. We recently reported a significant, visceral adipose tissue (VAT) specific, increase of ??-T cells in aged mice and human. Aged TCR γ knock out mice (TCR γ ^{-/-}, lacking ??-T cells) showed a drastic reduction of IL-6 gene expression in VAT and significantly less circulating IL-6. As IL-6 is a prominent marker of age-related inflammation, these studies suggest that ??-T cells promote both local and systemic inflammation in aging. The goal of the present study is to unravel the mechanism of ??-T cell expansion in aged VAT. We hypothesize that the pro-inflammatory state of the aged VAT microenvironment promotes peripheral ??T cell migration. Using an isochronic parabiotic model, in which WT and TCR γ ^{-/-} mice were surgically joined to share the same circulatory system, we found ~10% ??-T cell chimerism in VAT of aged mice (22-24 months) and only ~2% chimerism in VAT of young mice (6 months). By PCR based lineage analysis of γ and δ chains, we evaluated relative abundance of ??-T cell subpopulations in VAT and blood in young (5 months) and aged (25months) mice. All γ chains were expressed in both blood and VAT with V γ 6 and V γ 7 being most abundant regardless of age, while V γ 2 and V γ 4 showed distinct age-associated increase in VAT suggesting that the age-associated increase of VAT ??-T cells may be subset specific. V δ 1 was expressed in both blood and VAT, but V δ 2 was uniquely expressed only in VAT suggesting that V δ 2 ??T cells may reside as a self-sustaining population, while V δ 1 ??T cells may be reconstituted from the circulation. Collectively these data suggest that peripheral migration of distinct ??-T cell subpopulations may contribute to age-associated accumulation. Funding: This study was supported by the National Institute on Aging (R56 AG061508) and the National Institute of General Medical Science (R01 GM129532) of the National Institutes of Health.

Dietary isoleucine restriction reverses diet-induced obesity in mice

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While calorie restriction (CR) is the gold standard for interventions that prolong mammalian lifespan and healthspan, adhering to reduced calorie diets is difficult for humans. Recent findings by our lab and others have shown that protein restriction (PR) promotes health and longevity in mice, and that lower consumption of dietary protein is associated with increased longevity and health in humans. Our lab has found that the key mediators of the metabolic health and longevity benefits seen on PR are the branched-chain amino acids (BCAAs). Restriction of all three BCAAs, or specific restriction of isoleucine (ile) or to a lesser extent valine (val), promotes metabolic health, fitness, and lifespan in mice. We hypothesized that an ile restricted diet would induce improvements in weight, fat mass, and metabolic health in diet-induced obese male and female mice. After a 4 month exposure to a high fat, obesogenic Western diet, C57B6/J mice were separated onto macronutrient and calorie matched diets: AA-defined control (no AAs restricted), AA-defined low BCAA, or an AA-defined low ile diet. The ile restricted diet group displayed significant improvements in body composition and glucose tolerance. While further research is needed to describe the mechanisms of ile restriction and its effects in humans, this study demonstrates a promising dietary intervention to improve health and potentially lifespan. The Lamming Lab is supported in part by the NIH/National Institute on Aging (AG056771, AG061635 and AG062328 to D.W.L.) and by funding from the University of Wisconsin-Madison School of Medicine and Public Health and Department of Medicine to D.W.L. M.M. was supported in part by a Research Supplement to Promote Diversity in Health Related Research (R01 AG062328 -01S1). The Lamming laboratory is supported in part by the U.S. Department of Veterans Affairs (I01-BX004031), and this work was supported using facilities and resources from the William S. Middleton Memorial Veterans Hospital.

Hyperglycemia-associated retinal changes in a murine model of Type II diabetes

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Retinal pigment epithelium (RPE) is a single layer of pigmented cells that act as a selective barrier and nourishes the retinal cells. Diabetes-induced complications on outer retinal layers are poorly understood. Though some studies describe the morphological changes of the RPE layer, the molecular changes are not clearly known. Understanding cellular senescence in diabetic retinopathy (DR) may provide novel therapeutic targets for DR. So far, there is no animal model which can mimic the development of DR as in human. Mice in which Ercc1 is knocked-out in β 2-cells were used in this study. Ercc1 encodes one subunit of a DNA repair enzyme (ERCC1-XPF), which is required for nucleotide excision repair, interstrand cross-link repair, and repair of some double-strand breaks. Knock-out of Ercc1 in pancreatic β 2-cells resulted in hyperglycemia, reduced β 2-cell mass, impaired glucose tolerance, and impaired insulin production and secretion. Eyes from hyperglycemic Ercc1 KO animals were collected to determine the morphologic and molecular changes in the RPE and retinal layers. The mice had high expression of senescence markers (p16 & p21) and senescence-associated secretory phenotype (SASP) genes in the RPE layer. But the senescence markers were not altered in neural retina. Interestingly, the gene expression of pro-apoptotic genes was significantly increased in neural retina. The retinal thickness was unaltered; however, the cell count was significantly decreased in the photoreceptor layer of the diabetic animals. Isolectin-B4 staining of blood vessels revealed retinal vascular degeneration. Together, these data suggest that hyperglycemia induces senescence of the RPE layer, which then promotes photoreceptor cell death and abnormal retinal vasculature, likely through their SASP. The data also suggest that Ins2-Cre^{+/-};Ercc1^{-/-} mice might be an accurate and rapid model of DR, which could be used to test therapeutics. Funding Acknowledgement: Dr. Narasimhan is generously supported by a post-doctoral fellowship sponsored by Dr. Cecilee A. Fester.

Cysteine-Specific Effects of Sulfur Amino Acid Restriction on Adipose Metabolism

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Dietary restriction of the sulfur amino acid (SAA) Met (MR) profoundly affects rodent adipose metabolism but exerts a modest effect in humans. However, the SAA makeup of animal and human MR diets is different in that rodent diet lacks Cys; although both have low Met. Despite their ability to synthesize Cys from Met, rodents cannot meet metabolic demand as Met in MR diet is low. Thus, they undergo both MR and Cys restriction (CR), i.e., SAA restriction (SAAR=MR+CR). Human MR diet results only in MR, as Cys level is typically unaltered. Epidemiological studies associate adiposity with plasma Cys but not with Met. Here, we report CR-specific effects of SAAR on adipose metabolism and molecular mechanisms. Through depletion-repletion studies, we found discrete effects of MR and CR on SAAR-induced phenotypes. A cohort of male F344 rats was fed four diets, all replete with Cys (0.5% w/w) but each with progressively depleted Met (0.17, 0.10, 0.07, and 0.05%). Another cohort was fed five diets, all with the same low Met (0.07%) but each with progressively depleted Cys (0.5, 0.25, 0.12, 0.06, and 0.03%). Dose-response data show that body weight, food intake, plasma IGF1, FGF21, and leptin are MR-dependent, but plasma adiponectin is CR-dependent. Among plasma amino acids that responded only to CR, Ser increased robustly. Molecular data confirm that CR increases hepatic Ser biosynthesis by depleting 3-phosphoglycerate from glyceroneogenesis. Studies are in progress to confirm if this results in the decreased availability of glycerol-3-phosphate (G3P), required for triglyceride synthesis. Preliminary data from knockout mice suggest that the NRF2 pathway mediates Ser biosynthesis. Data from other mouse models show that Ser biosynthesis and adipose metabolism changes are more pronounced in males than in females, in young- than in adult-onset, and on 60% fat diet than on 10% fat diet. We found a novel and CR-specific mechanism, increase in Ser biosynthesis, by which SAAR ameliorates adipose metabolism. We also provide compelling evidence for considering Cys levels in formulating human and animal SAAR diets. Funding: Orentreich Foundation

miR-146a-5p Modulates Cellular Senescence and Apoptosis in Visceral Adipose Tissue of Long-Lived Ames Dwarf Mice and in Cultured Pre-Adipocytes.

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Previous studies have demonstrated that the miR-146a-5p increases in mice during aging, while long-living Ames dwarf (df/df) mice maintain youthful levels of this miRNA over an extended period. Aim: The aim of this study was to elucidate the involvement of miR-146a-5p in modulating cellular senescence and apoptosis in visceral adipose tissue of df/df mice and in cultured pre-adipocytes. Methods: Wild-type and df/df female mice were divided randomly into groups of wild-type and df/df controls treated with miRNA-negative control-base, and df/df transfected with 4 or 8 µg/g of a miR-146a-5p mimetic. Effects of the miR-146a-5p mimetic were also evaluated in 3T3-L1 cells cultured under high (HG) or normal glucose (NG) conditions. Results: Treatment with 8 µg/g miR-146a-5p mimetic increased SA-β-galactosidase activity, p16Ink4a and IL-6 expression in visceral adipose tissue of df/df. RNAseq analysis showed that apoptosis signaling pathway was downregulated in visceral adipose tissue of df/df transfected with 8 µg/g miR-146a-5p mimetic. In 3T3-L1 cells the miR-146a-5p mimetic induced similar effects at NG but not HG. Importantly, 3T3-L1 HG cells expressed significantly more miR-146a-5p than 3T3-L1 grown in NG conditions. Conclusions: These results indicate that miR-146a-5p represents a marker for cellular senescence. This miRNA represents one of the significant SASP factors that if not precisely regulated, can accentuate inflammatory responses and stimulate senescence in non-senescent cells. The role of miR-146a-5p might be different in healthy vs. stressed cells, suggesting potential effects of this miRNA depend on overall organismal health, aging, and metabolic state. Funding: NIH grants R56 AG061414, R15 AG059190, and R03 AG059846.

Effects of whey protein on blood glucose concentrations with oral glucose intake in younger and older men

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The addition of protein to a carbohydrate meal represents a strategy to reduce the postprandial glycaemic response in nonelderly people with type 2 diabetes (T2D); it is not known if this is effective in people with or without T2D >65 yrs. We aim to determine the impact of age and T2D on the acute effects of oral whey protein and glucose intake, alone or in combination, on postprandial glycaemia in younger (18-50 yrs) and older (>65 yrs) men, with and without T2D. Results for healthy younger (HY) and healthy older (HO) men without T2D are presented. Methods: In randomised, double-blind order, 10 HO (age and BMI: 78±2y; 26.3±0.4kg/m²) and 10 HY men (28.6±2.1y; 27.3±1.4kg/m²) ingested 250ml drinks comprising (i) control (~2kcal) (C), (ii) 30g glucose (120kcal) (G), (iii) 30g whey-protein (120kcal) (P) and (iv) 30g whey-protein plus 30g glucose (240kcal) (GP) following an overnight fast on separate study days. Blood glucose concentrations were assessed using the glucose oxidase method (t=0-180min). Repeated-measures ANOVA was used. Results: Baseline blood glucose concentrations did not differ between ages or study days (5.1±0.04 mmol/L). The addition of whey-protein to glucose intake significantly attenuated the BGL response; blood glucose concentrations (AUC???? and peak;) were lower (P<0.05 for all) following GP (drink-condition effect: 5.7±0.1 mmol/L*180min; 7.2±0.1mmol/L) when compared to G (6.1±0.2 mmol/L*180min; 8.6±0.3mmol/L) and both higher than C (5.1±0.1 mmol/L*180min; 5.4±0.1mmol/L) and P (5.0±0.1 mmol/L*180min; 5.2±0.1mmol/L). Older, when compared to younger, men had higher blood glucose concentrations (age effect: younger: 5.2±0.1 mmol/L*180min, 6.5±0.3mmol/L; older: 5.8±0.1mmol/L*180min, 6.8±0.3mmol/L). There were no interaction effects of age by drink condition (P=0.26). Conclusion: Co-ingestion of whey protein with glucose in younger and older men, without T2D, markedly reduces postprandial blood glucose (30-50%) compared with glucose alone indicating that the addition of protein has beneficial effects on postprandial glycaemia. Funding: This research was supported by Diabetes Australia Research Grant.

Sexually divergent induction of microglial inflammatory pathways with hippocampal aging.

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Age and sex are the primary risk factors for developing Alzheimer's disease (AD), with a higher incidence in women at all ages from 60-100 years old. Sexual divergence with brain aging is responsible for emergent physiological and pathological differences, including disparate susceptibility to neuroinflammation and neurodegeneration. Extensive preliminary data shows activation of sexually divergent neuroinflammatory pathways with aging, pointing to a role of microglia in age-related hippocampal sex differences. However, isolation of microglia from brain tissue can lead to ex vivo activation and confound studies. In this study we compare two methods developed to minimize ex vivo activation confounds to study the sexually divergent response to aging in hippocampal microglia. To this end, we collected hippocampal tissue from young (5-6 mo) and old (22-25 mo) mice and isolated microglial transcripts by: 1) traditional cell isolation using Cd11b magnetic-activated cell sorting (MACS) supplemented with transcription and translation inhibitors from C57/BL6 mice and 2) Translating Ribosome Affinity Purification (TRAP) from Cx3cr1-NuTRAP mice without the need for cell isolation. Our data shows induction of sexually-divergent microglial inflammatory pathways with hippocampal aging that are not apparent from whole-tissue data. Future studies will examine the mechanisms regulating for these sex divergences (e.g., hormonal and chromosomal) and the functional impacts on AD pathogenesis. Funding: This work was supported by NIH NRSA Fellowship (F31AG064861), NIH Grant (R01 AG059430), VA Merit Award (I01BX003906), Geroscience Training Grant (T32 AG052363), and Presbyterian Health Foundation.

Adiponectin receptor activation impacts skeletal muscle aging in mice.

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The loss of skeletal muscle mass and function with age, known as sarcopenia, is accompanied by reduced muscle strength and physical performance. Currently there is no effective pharmacological intervention for sarcopenia. Adiponectin, an adipose-tissue derived hormone, stimulates mitochondrial metabolism in target tissues and been linked to delayed aging with caloric restriction. AdipoRon, an adiponectin agonist, has been shown to stimulate metabolism in skeletal muscle in young mice; however, its effects on skeletal muscle in older mice and its functional consequence is still largely unknown. The purpose of this study was to investigate if AdipoRon could be used as a novel agent to treat or reverse the effects of sarcopenia by preserving muscle metabolism, mass, and function. Male and female mice presenting with early (18 months) or late (24 months) stage sarcopenia were treated with AdipoRon in the diet (50mg/kg) for 4 months. Physical performance, body composition, and glucose regulation were assessed at baseline, 12 and 18 months, and after treatment. Mitochondrial respiration and muscle contractile force were performed ex vivo in perfused muscle fibers. At advanced age, AdipoRon improved fasting glucose in both males and female. In males, age-related declines (24-28 months) in functional performance were attenuated with AdipoRon treatment. In females, functional measures were equivalent between 24 and 28 months, although age-related loss in body weight and body fat was prevented by AdipoRon treatment. These data show sex dimorphism in skeletal muscle aging, with loss of function progressing in aged males and loss of body fat in aged females. AdipoRon has potential therapeutic clinical applications for functional and metabolic declines linked to sarcopenia. Funding: Department for Veterans Affairs Merit Award (BX003846) and NIH Training Fellowship (DK007665)

Metformin and tBHQ treatment combined with an exercise regime prevent osteosarcopenic-obesity in middle-aged female Wistar rats.

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Osteosarcopenic obesity (OSO) is a pathologic event characterized by the loss of bone density, muscle mass and strength, in conjunction with an increase in adipose tissue, which concur with a decrease in mobility and physical activity. The main factors that induce this deterioration are the inflammatory environment induced by adipose tissue, and oxidative stress. Exercise enhances muscle fibers and decreases adiposity preventing OSO. We have recently found that combining exercise and metformin (MTF) slows the onset of sarcopenia in a mechanism involving the decline of adipose tissue and oxidative stress. Also, tert-butylhydroquinone (tBHQ), a Nrf2 inducer is known to decrease oxidative damage. Hence, our aim was to use the combined triple treatment (exercise-MTF-tBHQ) to reverse OSO in a model of middle-aged rats subjected to high fat diet (HFD). Female Wistar rats were fed with a HFD from 21 days after birth until their euthanasia at 15 months of age. Different interventions, including combining a Fartlek-type exercise routine with MTF and tBHQ administration were performed from 10 to 15 months. Body composition, fat, bone mineral density, and total lean mass without bone were determined using the DXA scanner. The grip force and the redox state (GSH/GSSG), as well as protein and lipids oxidation were also evaluated. Our results showed that the combined exercise-MTF-tBHQ treatment increased muscle mass and strength, and decreased body weight and mass index, fat percentage, and improved redox status, thus increasing animal survival. We thank Dr. Guerrero-Aguilera from UAM-I for animal supply. This work was supported by FOSSIS-CONACYT-262302 and 272256; and INGER SIREs-DI-003-2015. RTP, SPLC, DHA, BMN and GPV are CONACYT fellows.

Role of hepatic mTORC1 Signaling in Mediating the Calorie Restriction Response

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Calorie restriction (CR) extends both lifespan and healthspan in diverse species. However, the exact mechanism of how CR works still remains elusive. CR may work in part by regulating the activity of the mechanistic Target of Rapamycin Complex 1 (mTORC1), a serine/threonine protein kinase serving as a central integrator of numerous nutrient and hormonal cues including amino acids and insulin. Genetic or pharmaceutical inhibition of mTORC1 signaling extends the lifespan of yeast, worms, flies, and mice, and it has generally been assumed that CR promotes health and longevity at least in part via inhibition of mTORC1. However, this has never been formally tested in a mammal, and it is not clear that a CR diet reduces mTORC1 signaling *in vivo*, as investigation of the effects of a CR diet on mTORC1 signaling is substantially complicated by time-of-feeding effects of CR. A classic CR regimen, where mice are fed once per day, imposes a prolonged fasting period - animals consume their entire daily portion within ~2 hours, in contrast to the normal food consumption pattern of a mouse, in which eating occurs mostly during the dark cycle. This makes the assessed mTORC1 activity at any single time point a poor proxy for the overall effect of CR on mTORC1 activity. Here, we comprehensively determined the requirement of hepatic mTORC1 signaling in mediating the CR response over the course of a 24-hour cycle. In contrast to our expectations, we observed that mTORC1 signaling was higher in CR-fed mice immediately after feeding. To test the role of mTORC1 signaling in the metabolic response to CR we utilized male and female mice lacking hepatic TSC1 (TSC1-LKO), inhibitor of mTORC1. TSC1-LKO mice on CR had similar improvement in glucose homeostasis as the wild-type, suggesting suppression of hepatic mTOR does not mediate the CR response. Taken together, our results suggest that hepatic mTORC1 activity is dependent on fed-fasted conditions and may not be required to mediate the CR response. Funding: NIA F31-AG061635, AG056771, AG062328, AG066311, and I01-BX004031

Effect of riboflavin deficiency on Alzheimer's Disease (AD) in the 5XFAD mouse model

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Riboflavin (vitamin B₂) is an essential nutrient for organismal health. Riboflavin deficiency is strongly correlated with loss of body weight, vision impairment and skin disorders in human populations and animal studies. The mechanism by which riboflavin elicits its biological effects is attributed to serving as a co-factor for the formation of flavin adenine dinucleotide (FAD) via flavin mononucleotide (FMN). Flavoproteins including NADP(H): quinone oxidoreductase (NQO1), rely on FAD for protein stability and optimal function. *In vitro* studies have shown that riboflavin plays an essential role not only for NQO1 stability but also that mutant NQO1 isoforms co-aggregate with amyloid β^2 in cell-free experiments (Martinez-Limon, PNAS 2016). The exact relationship between riboflavin, NQO1 and amyloid β^2 -driven diseases, including Alzheimer's Disease, has not been examined in an *in vivo* model. Therefore, in the present study, we examined the effect of riboflavin deficiency on outcomes related to AD in wild-type and 5XFAD mice of both sexes. Mice were fed either control or riboflavin-deficient diets from weaning to 4 months of age. Data related to brain and serum levels of amyloid β^2 as well as metabolic and other biological alterations associated with riboflavin deficiency will be presented. Additionally, the role of NQO1 in riboflavin-mediated AD pathogenesis in the 5XFAD mouse model will also be explored. This research was funded by the Intramural Research Program of the National Institute on Aging.

Acute Induction of Senescence Triggers eIF2a Phosphorylation Without ATF4 Expression

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Cellular senescence is a state of indefinite cell cycle arrest that can result from sublethal damage incurred to the genome. Senescence primarily serves as a protective mechanism to prevent potentially tumorigenic cells from replicating, however senescent cells also accumulate during aging and are associated with aging-related disease. The characteristic of senescent cells most associated with disease is the Senescence-Associated Secretory Phenotype (SASP), which describes a robust secretory program with effects ranging from paracrine activation of senescence to induction of tumorigenesis. The SASP also represents a unique phenotype of senescence, since it requires substantial protein synthesis even though overall translation is reduced, suggesting that senescent cells may employ a unique translational program to upregulate the SASP. To explore the uniqueness of translation in senescent cells, we performed mass spectrometry to compare senescent cells to both cycling cells and contact-inhibited quiescent cells. We found that, while the proteome of senescent cells was more similar to that of quiescent than cycling cells, there were several translation regulators with expression levels unique to senescent cells. Notable among these proteins was EIF2AK4 (GCN2), which phosphorylates eIF2a to inhibit translation initiation as part of the integrated stress response (ISR). By performing western blot analysis, we confirmed that EIF2AK4 expression was high in senescent cells and further found that eIF2a phosphorylation was increased in senescence relative to cycling cells. Interestingly, we also found that eIF2a phosphorylation was increased in quiescent cells, and thus examined the expression levels of ATF4 (GCN4), a downstream transcription factor expressed when eIF2a is phosphorylated. We found that ATF4 expression was highly elevated in quiescent cells but not in senescent cells, and RT-PCR analysis of mRNA levels indicated that this was not due to differential transcription of ATF4. These results indicate that senescent cells may utilize eIF2a phosphorylation in a way that is unique from the traditional ISR and may further identify a characteristic that effectively separates the cell cycle arrest in senescence from that in quiescence.

Metformin suppresses SARS-CoV-2 induced inflammation in monocytes independent of AMPK activation.

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Severe acute respiratory distress syndrome coronavirus 2 (SARS-CoV-2) causes disproportionate severe coronavirus disease 2019 (COVID-19) in older adults which is associated with hyperinflammatory activation of myeloid cells. We aimed to define mechanisms underlying monocyte inflammatory responses to SARS-CoV-2 and to characterize therapeutics useful for targeting these responses. Stimulating human monocytes with spike protein subunit 1 (S1) from SARS-CoV-2 caused a dose-dependent activation of glycolysis and inflammatory cytokine gene and protein expression. This response was dependent on hypoxia inducible factor 1- α , as both glycolytic activation and cytokine gene expression were blocked by chetomin. Metformin pre-treatment also blocked glycolytic activation and suppressed mitochondrial metabolism in S1-treated monocytes and abrogated cytokine production. Metformin was also efficacious in blocking glycolytic activation of monocytes by the SARS-CoV-2 envelope protein, and it additionally suppressed interleukin-6 production in monocytes infected with 0.5 MOI SARS-CoV-2 strain WA1/2020. The effect of metformin is likely not dependent on activation of AMP-activated protein kinase (AMPK), as co-treatment with metformin and the AMPK inhibitor compound C failed to rescue immunometabolic activation or cytokine production of S1-treated monocytes. However, the metformin effect is also not likely to be solely due to mitochondrial complex I (CI) inhibition, as the CI inhibitor rotenone failed to suppress metabolic activation and cytokine production in S1-treated cells. Metformin represents a promising therapeutic for ameliorating COVID-19 associated hyperinflammation, which is linked to excessive morbidity and mortality in older adults infected with SARS-CoV-2. However, the mechanism by which metformin acts to suppress SARS-CoV-2 mediated inflammation appears to be complex. Principal funding was provided by a UofM/UTHSC CORNET award for COVID-19 research to TC and BP.

Many chronological aging clocks can be found throughout the epigenome: implications for quantifying biological aging

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Epigenetic alterations are a hallmark of aging and age-related diseases. Computational models using DNA methylation data can create “epigenetic clocks” which are proposed to reflect “biological” aging. Thus, it is important to understand the relationship between predictive clock sites and aging biology. To do this, we examined over 450,000 methylation sites from 9,699 samples. We found ~20% of the measured genomic cytosines can be used to make many different epigenetic clocks whose age prediction performance surpasses that of telomere length. Of these predictive sites, the average methylation change over a lifetime was small (~1.5%) and these sites were under-represented in canonical regions of epigenetic regulation. There was only a weak association between “accelerated” epigenetic aging and disease. Furthermore, pan-tissue clocks did not detect key tissue-specific methylation differences. Despite the reproducible and accurate age predictions from DNA methylation data, these findings suggest they may have limited utility as currently designed in understanding the molecular biology of aging and may not be suitable as surrogate endpoints in studies of anti-aging interventions. Purpose-built clocks for specific tissues, age ranges, or for examining aging acceleration/deceleration may have better performance for their specific utility. FundingThe presenters would like to thank NIH grants #5P30AG050911 (to J.D.W. and W.M.F.), #2P20GM103636 (to J.D.W.), #1T32AG052363-01 (To William Sonntag), #1F31AG063493 (to H.L.P.), R01AG059430 (to W.M.F.) and VA grant #101BX003906 (to W.M.F.)

An evolving role for the long non-coding RNA H19 in aging and senescence.

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Long noncoding RNAs (lncRNAs) regulate diverse cellular processes and are associated with many age-associated diseases. However, the function of lncRNAs in cellular senescence remains largely unknown. Here we characterize the role of lncRNA H19 in senescence. H19 is a highly conserved maternally imprinted gene that is critical in controlling embryonic growth in conjunction with the neighboring Igf2 gene. Loss of H19 results in fetal overgrowth associated with Beckwith Weidemann syndrome, while elevated H19 occurs in human cancers. Most adult tissues experience strong repression of H19 levels with age, with the exception of skeletal muscle cells and tumors. Here, we show that the loss of CTCF mediates the repression of H19 associated with cellular senescence as part of the stress response pathway. Consistent with a role for lncRNA H19 in somatic cell growth and proliferation, targeting H19 induces senescence. Mechanistically, the loss of H19 as a result of cellular stress induces premature senescence, at least in part, through its ability to regulate miRNAs of the Let-7 family. It appears that Let-7 mediated reduction of EZH2 occurs during senescence to regulate several genes orchestrating the senescence program. Thus, gradual loss of H19 is crucial for the establishment of the senescence program in somatic cells. Funding Acknowledgement: Drexel Aging Initiative, NIA R56AG071815and Pennsylvania Commonwealth Universal Research Enhancement Program (CURE)

Metabolic Rewiring of Aged Myoblasts and Restores Regenerative Potential of Progeric Skeletal muscle

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Skeletal muscle (SkM) comprises 45-55% of body mass and plays essential physiological roles in the body such as enabling skeletal movements and regulating metabolism. With age adult SkM is known to decrease in muscle mass, strength and functional capacity, a state known as sarcopenia. Sarcopenia correlates with loss of metabolic function, disabilities, and mortality. Here we investigate the age-related metabolic rewiring that occurs in myoblasts using in vitro and in vivo models of aging and rejuvenation. Bioenergetics and the source of energy production play crucial roles in cell function and skeletal muscle regeneration. In this study, we hypothesize that metabolic rewiring of ATP production is critical for the rejuvenation of aged myoblasts. We provide evidence that aged myoblasts cells experience a disruption to constitutive bioenergetics, ultimately utilized a different energy source than young cells, leading to DNA damage and loss of cellular function, as well as compromised regenerative capacity and myotube formation. Interestingly, we found that expressing the transcription factor NANOG reversed the metabolic rewiring leading to marked improvements in skeletal muscle physiology including increased insulin sensitivity, upregulation of glycolysis and restored the ability of aged myoblasts to differentiate into striated skeletal muscle capable of spontaneous contraction. Our investigation links metabolic reprogramming to skeletal muscle aging and rejuvenation and provides possible means for addressing sarcopenia, one of the most important causes of functional decline in older adults.

Shifts in epigenetic context associated with development and aging influence regulatory variation and disease risk

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A hallmark of aging is the accumulation of alterations to the epigenome (the organization and activity of genomic DNA) that impact transcriptional networks at the regulatory level. These shifts may modify the effects of genetic regulatory variants during aging, and may contribute to dysregulation of biological pathways involved in aging-associated diseases. However, these shifts occur in the existing backdrop of epigenetic changes experienced through an individual's development into adulthood; thus, the phenotypic, and ultimately fitness, impacts of regulatory variants subject to developmental- versus age-associated epigenetic shifts may differ considerably. The forces of natural selection may therefore act differently on variants depending on their changing epigenetic context, which we propose as a novel lens through which to consider regulatory sequence evolution and concomitant phenotypic effects. In our studies, we define genomic regions undergoing changes to chromatin accessibility, a key component of epigenetic regulation, as tissues transition from their fetal to adult forms, and subsequently from early to late adulthood. Using these epigenomic datasets, we examine patterns of evolutionary sequence constraint and potential functional impacts of genetic variation (e.g. heritable disease risk associations). We find that while the patterns observed with developmental epigenetic changes are consistent with stronger fitness consequences (i.e. negative selection pressures), they tend to have weaker effects on risk associations for aging-related diseases. Conversely, we see stronger effects in terms of risk association when considering variants with increased local accessibility in adult tissues, particularly for young-adult samples when compared to old. Based on our findings, we propose a model for how the epigenetic context of a region may influence the effects of sequence variation subject to evolutionary forces, and suggest that such a perspective on gene regulatory networks may elucidate our understanding of aging biology and associated disease mechanisms. Funding: Research was supported by The American School of Prehistoric Research, Harvard University.

How to stall neuron aging.

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Neurons live as long as the animal in which they reside. Just as animals show characteristic signs of aging, neuron aging is likewise accompanied by characteristic cellular features, including morphological alterations, synapse loss, and metabolic dysfunction. How these features come about remains unclear. Understanding the mechanisms underlying neuron aging is important because aging is the primary risk factor for neurodegenerative disease: loss of cellular homeostasis in neurons likely contributes to this increased risk. We investigated how aging affects neuron cell biology by exploiting the ability of the nematode *Caenorhabditis elegans* to de-couple the symptoms of aging from chronological age. Under standard laboratory growth conditions, *C. elegans* live in a proliferative state, in which they develop, age and die in two weeks. In the alternate organismal state called dauer, however, animals survive for months. Importantly, their neurons continue to function. We asked, how is neuron aging stalled in the dauer state? This initial work focused on aspects of neuron morphological aging. We first determined that morphological aging is regulated cell-intrinsically. Through a neuron-specific genetic manipulation, we induced a dauer-like suspension of neuron morphological aging within aging (non-dauer) animals. We term this manipulation "dauerization." Next, we used dauerization to ask, what are the cell-intrinsic drivers of neuron morphological aging? Surprisingly, we found that dauerization intensely suppresses the constitutive endocytic pathway. Synaptic vesicle cycling was uniquely preserved. We propose that this physiological suppression of the constitutive endocytic pathway simultaneously turns down multiple pro-aging processes supported by the endosomal system. A flip-side to our results is the well-established connection between abnormal endocytic pathway function and neurodegenerative diseases, suggestive that there is a conserved interplay between the endocytic pathway and aging. Funding: HHMI, NIH R01-NS103037 and R01-NS091144 to KS, Human Frontier Science Program LT000127/2016-L to CY.

Sulfur amino acid metabolism in the regulation and function of *C. elegans* flavin-containing monooxygenase-2.

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C. elegans flavin-containing monooxygenase-2 (*fmo-2*) is activated by and necessary for dietary restriction- and hypoxic response-mediated lifespan extension, and FMO-2 overexpression is sufficient to extend lifespan. Despite this, the mechanisms of *fmo-2* regulation and function in longevity are not defined. FMOs are highly conserved, cytosolic enzymes that utilize NADPH and O₂ to oxidize nitrogen- and sulfur-containing molecules, including endogenous thiols. Here, I use the genetic power of worms to test the hypothesis that endogenous sulfur amino acid metabolism is necessary for both the regulation and function of *fmo-2*. I find that S-adenosylmethionine (SAM) synthesis and subsequent phosphatidylcholine (PC) synthesis are necessary for *fmo-2* activation. I also find that FMO-2 overexpression-mediated longevity is eliminated or even reversed by glutathione reductase (*gsr-1*) RNAi, and that *fmo-2(ok2147)* loss-of-function mutant worms have approximately half the total glutathione of wildtype worms. These results are consistent with a model in which excess methionine cycle flux activates *fmo-2*, which then hormetically oxidizes glutathione to stimulate counterbalancing transsulfuration flux and subsequent glutathione synthesis. These results are also consistent with a model in which FMOs are important regulators of NADPH- and thiol-dependent redox homeostasis as a counterbalancing force to glutathione reductase and thioredoxin reductase.

Gpnmb is Chondroprotective in a Mouse Model of Age-related Osteoarthritis

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Musculoskeletal Research Group, NEOMED, Ohio Degenerative joint diseases, such as Osteoarthritis (OA), affect half of the global population over the age of 65. Affected individuals experience chronic pain and inflammation, loss of mobility and a decreased quality of life. OA is characterized by physical and proteolytic degradation of the articular cartilage, synovitis (synovial inflammation), abnormal bone formation (osteophytes) and subchondral bone remodeling. The risk of cartilage damage increases with age and currently there are no therapeutic options for altering or reversing OA. Instead, treatment consists of pharmacological interventions to minimize the pain associated with chronic inflammation until the end stages of disease development where arthroplasty is required. Chondroprotective or chondro-inductive compounds are currently being evaluated to reduce inflammation and delay or prevent these irreversible surgical procedures. Osteoactivin/Gpnmb is a naturally occurring anti-inflammatory glycoprotein that has been shown to reduce inflammation in lymphocytes, macrophages and microglia. Here, we evaluated the potential therapeutic effect of Gpnmb in primary chondrocytes in vitro and in primary OA (age-related) in vivo. Primary murine chondrocytes and HTB94 cells were treated with IL-1 β alone or a combination of IL-1 β and recombinant GPNMB (rGpnmb). Gpnmb treatment significantly reduced inflammatory cytokine expression induced by IL-1 β . In vivo, we assessed the chondro-protective efficacy of Gpnmb in male DBA/2J male mice and age-matched DBA/2J.Gpnmb+ male controls. DBA/2J mice have an inactivating point mutation in the Gpnmb gene and exhibit high levels of pro-inflammatory cytokines when challenged with IL-1 β . DBA/2J.Gpnmb+ have the wild-type Gpnmb allele knocked-in to the DBA/2J strain and the return of this gene results in reduced inflammation. Using aged mice (~75 weeks), we examine joint morphology in these two strains and results showed elderly DBA/2J mice develop significant cartilage degradation and loss were compared to age-matched control animals. Together, our data suggest Gpnmb is chondroprotective, reduces inflammatory cytokine expression and cartilage degradation and may serve as a candidate therapeutic for OA.

Effect of obesity in the brain inflammatory state and its relationship with cognitive decline during the aging process in female Wistar rats.

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Aging is a multifactorial progressive process characterized by the loss of cellular, molecular and physiological functionality. During aging the brain is characterized by a shift from the homeostatic balance of anti-inflammatory mediators to a pro-inflammatory state called neuroinflammation. In addition, when aging is accompanied with obesity, this inflammatory response can be exacerbated. Studies have shown that rodents fed with a high-fat diet have an increased blood-brain barrier permeability in the hippocampus, a brain region that participates in learning and memory. Thus an obese phenotype during aging might increase the inflammatory state and might contribute to cognitive deterioration. Hence, our aim was to determine the effect of obesity in the establishment of inflammation and cognitive deterioration in the rat brains during the aging process. In this study we used female Wistar rats that were fed with a hypercaloric diet from 21 days after birth until their euthanasia at 13 months of age. We evaluated the pro-inflammatory and anti-inflammatory cytokines levels (IL-6, IL-10, TNF α , MCP1 and IL-10) in serum, as well as in cerebral cortex and hippocampus by ELISA assay. Memory and learning processes were evaluated using the novel object recognition test. An increase in weight was observed in the rats subjected to the hypercaloric diet from the third month and on. We observed changes in cytokines levels in serum, cortex and hippocampus. Concurring with a decrease in time and interactions number with the novel object was observed in the rats subjected to the hypercaloric diet, supporting the idea that obesity increases inflammation and might be playing an important role in cognitive deterioration. We thank Dr. Guerrero-Aguilera from UAM-I for animal supply. This work was supported by CONACyT grant FORDECYT-PRONACES/263957/2020T. Santín-Márquez, Salas-Venegas & Cortés-Rodríguez are CONACyT scholarship holders.

Molecular interaction analysis of heat shock cognate protein A8 and TLQP-21 by surface plasmon resonance assay

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A TLQP-21 receptor, one of the VGF derived peptides, nomenclated Heat Shock Cognate Protein A8 (HSPA8) was "fished out" in human neuroblastoma SH-SY5Y cell line using biotinylated TLQP-21 as a probe through avidin agarose affinity chromatography and mass spectrometry (MS)-based protein identification. In addition, molecular docking and protein modeling studies showed the docking compatibility of TLQP-21 into the HSPA8 peptide binding pocket. In this study, binding of TLQP-21 to its receptor HSPA8 was detected by Surface Plasmon Resonance (SPR) assay. SPR assay has become a recognized method for quantifying molecular interactions. The experiment involves one immobilized reactant on a surface and observing its interaction with a second component in solution. Different parameter conditions of temperature, buffer and flow rate were tested to see the tendency of the interactions between HSPA8 (receptor) and TLQP-21 (ligand). Overall study here shows that the binding was electrostatic and weak, indicating that the SPR Assay technique is not a preferable model to show the interaction and binding between HSPA8-TLQP-21. Though the binding was improved comparatively at low temperature rather than higher temperature. Further studies are recommended to optimize the conditions of SPR Assay to get better interaction and binding between HSPA8-TLQP-21, the receptor-ligand.

Effect of sulforaphane-treatment in preventing age-associated cognitive decline in female and male Wistar rats, and its relationship with the cortical and hippocampal redox state

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The nervous system is morphologically and functionally affected during aging. Oxidative stress is involved in age-related damage, leading to cognitive decline and neurodegenerative processes. Sulforaphane (SFN) is an antioxidant-response inducer that improves the antioxidant and anti-inflammatory pathways. Hence, our objective was to evaluate if SFN was able to prevent the age-associated cognitive decline. Middle-aged and old female and male Wistar rats were treated with 0.5 mg/Kg for 5 days per week for 3 months. Then, memory and learning were evaluated at 15 (adult) and 21 (old) months of age, using the novel object recognition test and the Barnes maze test. Young (4 months old) rats were used as an age-control. The antioxidant response induction and the redox state (GSH/GSSG) were also determined in the brain cortex (Cx) and the hippocampus (Hc). Age-dependent memory and learning decayed in both, female and male aged rats, and SFN-treatment improved this cognitive decline in both sex adult groups, but not in the old groups. SFN-treatment increased Nrf2 levels and antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) in Hc and Cx, which in consequence, increased redox state in both evaluated regions in females and males adult rats, but these were not observed in the old animals. SFN treatment prevented memory deterioration in adult rats, but not in the older groups. Our results suggest that SFN might prevent rather than revert neural damage. We thank Dr. Guerrero-Aguilera from UAMI for animal supply. This work was supported by CONACyT grant FORDECYT-PRONACES/263957/2020. RSM, VSV, SPLC and RTP are CONACyT scholarship holders. UHA was a PRODEP postdoctoral fellow.

Senolytic Treatment Reduces Cell Senescence and Necroptosis in Sod

1 Knockout Mice that is Associated with Reduced Chronic Liver Disease, and Hepatocellular Carcinoma. Ramasamy Selvarani1, Sabira Jazir1, Evan H Nicklas1, Jacob L. Brown2, Benjamin F. Miller2,3, Holly Van Remmen2,3, Arlan Richardson1,3, and Sathyaseelan S. Deepa1. 1Department of Biochemistry & Molecular Biology, OUHSC, OKC, Oklahoma; 2Aging & Metabolism Program, OMRF, OKC; and 3Oklahoma City VA Medical Center, OKC, Oklahoma.

Sod1 (Cu/Zn Superoxide dismutase) is the major superoxide dismutase isozyme that catalyzes the conversion of superoxide anions to hydrogen peroxide. It is found in all cells and is localized in the cytosol and the intermembrane space of the mitochondria. Knocking out Sod1 enzyme results in accelerated aging as shown by reduced lifespan, a decline in physiological functions, including cognition, and increased pathology. The Sod1KO mice are unique as they exhibit chronic liver disease, e.g., fatty liver, fibrosis and spontaneously develop hepatocellular carcinoma, the major end-of-life pathology disease observed in the Sod1KO mice. Our laboratory has shown that cell senescence is increased in kidney of the Sod1KO mice, suggesting that cell senescence could play a role in the accelerated aging phenotype observed in Sod1KO mice. Therefore, we treated Sod1KO mice with the senolytics, dasatinib and quercetin (D+Q), which have been shown to target senescent cells. Liver tissue was obtained from 4 groups of 6-month-old female mice, which were treated with vehicle or D+Q for 7 months: wild type mice treated with vehicle or D+Q and Sod1KO mice treated with vehicle or D+Q. D+Q treatment reduced the increase in cell senescent (i.e., p16 transcript levels) observed in the liver of the Sod1KO mice to levels observed in the WT mice. In addition, D+Q reduced pro-inflammatory cytokines (TNF- α , IL-6, IL-1 β) in the livers of Sod1KO mice compared to vehicle treated Sod1KO mice. In studying the role of inflammation in aging, surprisingly, we found that necroptosis (phosphorylated Mlkl) was also reduced in the Sod1KO mice treated with D+Q. Importantly, D+Q treatment reduced fibrosis (desmin, collagen1) and reduced tumor incidence in Sod1KO mice by nearly 80%. Thus, our findings show that senolytics, D+Q, dramatically reduced HCC in the Sod1KO mice and this appears to be due to the reduction in chronic inflammation, which appears to arise from cell senescence and possibly necroptosis.

Epidemiological Models of Aging Identifies Biomarkers Associated with Aging Rates

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Physiological processes that control the aging rate of our species are poorly understood. Large scale biobank datasets with deeply phenotyped participants hold promise for discovery of these processes, however most current epidemiological frameworks are incapable of capturing full complexity of interactions between physiology and survival. Here we describe a first of its kind epidemiological model that bridges the physiological network with a Gompertz survival layer in a single framework. This fully parametric Bayesian model allows us to distinguish between features that affect mortality hazards at the baseline from features that interact with time and contribute to differences in aging rates. We carry out a number of network simulations, and show that variables that significantly affect aging rate can be captured in cohorts as small as 5,000 participants. We apply our model to two longitudinal cohorts (MrOS and HABC) with ~ 16 year follow up and describe the most significant variables that influence aging rates and survival times. We find that most of the lifespan variation is captured by existing risk factors such as elevated IL6, increased calcification of abdominal artery (AAC), and increased red blood cell distribution width (RDW). Consistent with the Gompertz framework, the age of participants at recruitment does not change the rate at which they are aging. More importantly, we identified three variables associated with increased aging rate in MrOS cohort - decreased kidney function, increased phosphate level, and reduced RBC counts. To our knowledge this is the first time these variables have been linked to the aging rate of our species. We further show the utility of the model to infer impact of potential aging therapeutics by simulating potential interventions and by propagation of information throughout the network structure. In the future, full power of bridged physiology/survival models can also be extended and applied to larger sized biobanks for modeling human diseases and lifespan.

ELOVL2: Not just a biomarker of aging.

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DNA methylation of the ELOVL2 (Elongation Of Very Long Chain Fatty Acids-Like 2) promoter is one of the most robust molecular biomarkers for chronological age, but whether ELOVL2 plays a functional role in aging has barely been explored. ELOVL2 encodes a transmembrane protein involved in the synthesis of very long polyunsaturated fatty acids (VLC-PUFAs). These fatty acids play important roles in retinal biology and photoreceptor renewal, key processes implicated in age-related eye diseases such as age-related macular degeneration (AMD). Here, we report that Elov12 regulates age-associated functional and anatomical aging in vivo, focusing on mouse retina, with direct relevance to age-related eye diseases. We show that an age-related decrease in Elov12 expression is associated with increased DNA methylation of its promoter. Mice carrying a point mutation C234W that disrupts Elov12-specific enzymatic activity show electrophysiological characteristics of premature visual decline, as well as early appearance of autofluorescent deposits, well-established markers of aging in the mouse retina. Finally, based on our findings, we provide new ideas of potential treatment to slow down aging of the retina. Our findings indicate that ELOVL2 activity regulates aging in mouse retina, provide a molecular link between polyunsaturated fatty acids elongation and visual functions, and suggest novel therapeutic strategies for treatment of age-related eye diseases. Funding: Bright Focus Foundation, Edward N. and Della L. Thome Memorial Foundation, NIH R01EY027011

Effect of acarbose on metabolic health and cognitive function in a mouse model of Alzheimer's disease.

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Alzheimer's disease (AD), a neurodegenerative disease in which patients exhibit impaired memory, motor function, and language due to neuronal damage, is rapidly growing in prevalence as the population grays. As AD is a disease of aging, and other diseases of aging including diabetes and obesity are risk factors for AD, geroprotective interventions may be of use in the prevention and treatment of this disease. Here, we report our investigation into the effects of the geroprotector acarbose, a common anti-diabetic drug which extends the lifespan of UM-HET3 mice, on cognition and disease pathology in the 3xTg-AD mouse model of AD. We treated 6-month-old male and female 3xTg-AD mice with acarbose for 3-6 months to determine effects on AD pathology, metabolic health and cognition. We found that acarbose-treated male and female 3xTg mice showed a trend in decreased tau phosphorylation (which is a marker for AD progression) and reduced expression of mechanistic target of rapamycin complex 1 (mTORC1) signaling in the brain. Surprisingly, acarbose did not promote metabolic health, suggesting the effects of acarbose may be independent from its effects on glucose homeostasis. We will report on ongoing studies using acarbose at later time points, but these early results suggest that the use of geroprotectors as therapies for AD has potential, and may also provide additional insights into the mechanisms of action by which geroprotectors function.

An Lsd1 histone demethylase inhibitor as a novel senotherapeutic.

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Senescent cells are characterized by an irreversible cell-cycle arrest and production of a senescence-associated secretory phenotype (SASP), which includes pro-inflammatory cytokines and chemokines. Removal of senescent cells has been shown to ameliorate age-related pathologies. One initial strategy for the development of senotherapeutic drugs has been to make use of current cancer therapeutic agents that target shared pathways between cancer cells and senescent cells. LSD1 is a histone mono- and di-demethylase, targets K4me1/me2 and K9me1/me2 on H3 and on non-histone targets such as p53 and p65, which are key regulators of senescence. Inhibitors of LSD1 are currently in clinical trials for treating several cancers. In this study, we demonstrate that LSD1 inhibition constitutes a novel serotherapeutic target. First, LSD1 inhibition reduces SASP factor expression through downregulation of the p65 NF- κ B pathway in senescent cells. Next, LSD1 inhibition induces senolysis by triggering apoptosis through activation of the p53 pathway.

Healthy lifespan extension through 3-hydroxyanthranilic acid.

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The metabolism of tryptophan by the kynurenine pathway is increasingly linked to aging and age-associated disease. Kynurenine pathway enzymes and metabolites influence a range of molecular processes critical to healthy aging, including regulation of inflammatory and immune responses, cellular redox homeostasis, and energy production. Aberrant kynurenine metabolism is observed during normal aging and has been implicated in a range of age-associated pathologies, including chronic inflammation, atherosclerosis, neurodegeneration, and cancer. In previous work, we and others identified three genes—*kynu-1*, *tdo-2*, and *acsd-1*—encoding kynurenine pathway enzymes for which decreasing expression extends lifespan in invertebrate models. We now find that knockdown of *haao-1*, a fourth kynurenine pathway gene encoding the enzyme 3-hydroxyanthranilic acid dioxygenase (HAAO), extends lifespan by ~30% and delays age-associated decline in health in *Caenorhabditis elegans*. This lifespan extension is mediated by increased physiological levels of the HAAO substrate 3-hydroxyanthranilic acid (3HAA). 3HAA increases resistance to oxidative stress during aging by directly degrading hydrogen peroxide and activating the Nrf2/SKN-1 oxidative stress response. Beyond oxidative stress, 3HAA induces activation of a broad spectrum stress response. Finally, knockdown of *haao-1* further increases resistance of aging *C. elegans* to the pathogenic bacteria *Pseudomonas aeruginosa*. Aging mice fed a diet supplemented with 3HAA are similarly long-lived. Our results identify HAAO and 3HAA as novel therapeutic targets for age-associated disease. This work provides a foundation for more detailed examination of the molecular mechanisms underlying the benefits of 3HAA, and how these mechanisms interact with other interventions both within and beyond the kynurenine pathway. We anticipate that these findings will bolster growing interest in developing pharmacological strategies to target tryptophan metabolism to improve health aging. This work was supported by NIH P30AG038070 and NIH R35GM133588.

The role of necroptosis in age-associated neuroinflammation

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Inflammaging (chronic sterile inflammation) is a characteristic feature of aging, and is termed neuroinflammation when it occurs in the central nervous system (CNS). Necroptosis, an inflammatory cell death pathway, is shown to play a role in neuroinflammation in various neurodegenerative diseases. We found that necroptosis is increased in the accelerated aging (*Sod1*^{-/-}) mouse model. Based on the findings, we hypothesized that an age-associated increase in necroptosis might contribute to increased neuroinflammation with age. To test our hypothesis, changes in the expression of necroptosis marker, phospho-MLKL (P-MLKL), was assessed in young (7-month) and old (24-month) wild type mice brain using immunofluorescence staining. Old *Mkl1*^{-/-} mice (24-month) were used as a control. Expression of P-MLKL was significantly upregulated in CA3 region of the hippocampus (4-fold) and cerebral cortex layer V of old mice (5-fold) relative to young mice, whereas P-MLKL staining was absent in old *Mkl1*^{-/-} mice. Nearly 80% of P-MLKL immunoreactivity is localized to neurons and 10% is localized to microglia. Changes in neuroinflammation was assessed using neuroinflammatory markers, Iba-1 and GFAP. Immunofluorescence staining showed that expression of Iba1 and GFAP is increased in the brains of old WT mice relative to young mice and blocking necroptosis genetically using *Mkl1*^{-/-} mice reduced expression of Iba-1 and GFAP in old mice. Similarly, treating old mice with a necroptosis inhibitor, necrostatin-1s, significantly reduced the levels of TNF- α , IL-6 and IL-1 β in the brain. In summary, expression of necroptosis markers increased with age in the brain in a region-specific manner, primarily in neurons and blocking necroptosis reduced levels of pro-inflammatory cytokines in the hippocampus and cortex. Thus, our data support a role of necroptosis in age-associated neuroinflammation and suggest that necroptosis inhibition could be a potential strategy to reduce neuroinflammation and improve age-associated cognitive decline. The work has been supported by R01AG059718 to DS and I01BX004538 to AR

Gene network analysis of senescence genes present in human adipose tissue

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In the Human digital ageing atlas, a total of 187 genes were identified in the adipose tissue of female. Expression of 88 genes was increasing with age, and 99 genes were decreasing with age. In both the expressions, Cytohubba showed the top 10 nodes ranked by Maximum Clique Centrality (MCC). The top 10 hub genes showing increased expression with age are VCAM1, VEGFC, FLNB, NOTCH3, TPD52, ADM, SHROOM5, PHACTR3, PDE4B, ARL4A. Increased expression of Vascular cell adhesion molecule 1 (VCAM1) with age indicates the migration of leukocytes to inflammatory sites. Functional enrichment using STRING in Cytoscape for the top 10 hub genes was performed. Gene Ontology (GO) process displayed are cellular response to interferon-gamma, B-cell differentiation, cAMP metabolic process, cell differentiation, morphogenesis of embryonic epithelium. KEGG pathways shown are focal adhesion, AGE-RAGE signalling pathway in diabetic complications. The top 10 hub genes showing decreased expression with age are COL5A1, COL1A1, COL1A2, COL4A2, THY1, PLOD1, SMAD7, ECM1, IRS1, RET. Reduced expression of Collagen type V, I, IV indicate the collapse of connective tissue during aging. Gene Ontology (GO) process displayed are heart morphogenesis, positive regulation of transmembrane transport, regulation of cell-substrate adhesion, biomineral tissue development, regulation of epithelial to mesenchymal transition, TGF-beta receptor signalling pathway, ureteric bud development, eye development, cellular response to retinoic acid, angiogenesis. GO functions are extracellular matrix structural constituent and signalling receptor binding. GO component are basement membrane, collagen type 1 trimer. The nearest neighbour networks identified are RET signalling, neurotrophin signalling pathway, platelet-derived growth factor binding, and hydroxylysine metabolic process.

Role of Methionine sulfoxide reductase A (MsrA) in the longevity effects of Methionine Restriction.

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Restriction of the essential amino acid methionine in the diet has been shown to confer metabolic and lifespan benefits, however many questions remain about its mechanisms and effects. Methionine can also become oxidized to form methionine sulfoxide which can alter protein function and may have roles in redox signaling. This damage can be repaired by Methionine sulfoxide reductase A (MsrA). It is not well understood how methionine repair may be involved during restriction. In this work we addressed these questions with a lifespan and health span longitudinal study utilizing a MsrA knock-out mouse line. We observed that commencing methionine restriction in 9-month-old adult mice resulted in some benefit to glucose metabolism over time mainly in wild type males as measured with HbA1c and fasting blood glucose. We also observed in wild type mice that methionine restriction decreased body weight for the first few months on diet, but then drove an increase in body weight which surpassed control animal weights. Health span studies involving frailty, grip strength, rota-rod, rearing assay, and voluntary wheel running showed that methionine restriction improved some of these metrics for the MsrA knock-out, but generally decreased performance for wild type mice. Lifespan of all groups was also similar, showing no significant effect of either methionine restriction or the MsrA knock-out. While the presence of MsrA is important for resistance to oxidative stress, its presence does not appear to be required for the beneficial effects of methionine restriction. This study also demonstrates that the age at which methionine restriction is started can have significant impacts on its long-term benefits. Funding: T32 Training Grant â€” 5T32AG021890-15.

Profiling epigenetic age in single cells.

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DNA methylation dynamics emerged as a promising biomarker of the mammalian aging process, with the advent of multivariate machine learning models (epigenetic clocks) that enable robust measurement of biological age in bulk tissue samples. While bulk sequencing or microarray approaches reveal average changes in methylation with age across many cells, they inherently obscure the epigenetic heterogeneity that exists at the single-cell level. However, intrinsically sparse and binarized methylation profiles of individual cells have so far precluded the assessment of aging in single-cell data. To address this fundamental limitation, we developed scAge, a statistical framework for epigenetic age profiling at single-cell resolution, and validated our method in mice. This approach leverages CpG-specific aging patterns in bulk methylation data and a statistical profiling algorithm that together enable accurate estimation of epigenetic age from partial single-cell profiles. Our novel method recapitulates the chronological age of tissues while simultaneously uncovering some heterogeneity among cells. We report accurate tracking of the aging process in hepatocytes and refine previous observations of attenuated epigenetic aging in muscle stem cells. We also apply scAge to reveal, at the single-cell level, a natural rejuvenation event occurring during early embryogenesis. We introduce our framework as a resource to enable exploration of epigenetic aging trajectories at single-cell resolution. This work was supported by NIA grants to Vadim N. Gladyshev and is covered under a provisional patent application filed by Brigham and Women's Hospital naming Alexandre Trapp, Csaba Kerepesi, and Vadim N. Gladyshev as inventors.

Amyloid-Beta 42 levels in companion dog brains increase with age and correlate with cognitive scores

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The companion dog is an increasingly important model in aging research because it shares the human environment, is exposed to similar environmental risk factors, receives comparable medical care, and develops similar age-related pathologies. This includes Canine Cognitive Dysfunction (CCD), which shares many of the clinical features of Alzheimer's Disease (AD), including progressive loss of cognitive function, disrupted sleep, anxiety, and aimless wandering. Amyloid-beta 42 (A β 42) plaques similar to these found in humans with AD are known to naturally occur in the brains of aged dogs, making them an intriguing potential model for AD in humans. As part of the Dog Aging Project, we studied frozen samples taken from the frontal cortex, medial temporal cortex, entorhinal cortex, and hippocampus of n=24 companion dogs that were euthanized for unrelated health reasons and donated by their owners. Samples were frozen within 4 hours post mortem. Canine Cognitive Dysfunction Scores (CCDS) were obtained by owner questionnaires. We used a novel quantitative Luminex assay to measure A β 42 in these samples and correlated them with the dogs' ages and CCDS. We found a statistically significant positive correlation between age and A β 42 levels (P=3E-5), as well as a statistically significant positive correlation between CCDS scores and A β 42 levels (P=0.01), in all three brain regions considered. Our results show that A β 42 levels in companion dog brains increase with age and correlate with CCDS. We will now investigate histopathology in formalin fixed samples from the same dogs and brain regions, investigate whether we can also measure Tau in these samples, as well as correlate our findings with the known clinical CCD phenotypes as measured by owner and/or veterinarian questionnaires in these dogs. Funding: NIH grant 3U19AG057377-02S3, European Research Council (ERC) Grant Agreement No. 680040

Interplay between iron and pH homeostasis and aging

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During aging, there is a loss of acidity in the lysosome/vacuole across diverse species. The vacuolar ATPase is the conserved proton pump that acidifies the yeast vacuole and metazoan lysosome. In yeast, disruption of the V-ATPase complex results in loss of vacuolar acidity and pleiotropic phenotypes including shortened lifespan. We previously found that multiple phenotypes resulting from loss of the V-ATPase are related to defects in iron homeostasis. We also observed that following loss of vacuolar acidity, wildtype cells display indications of disrupted iron homeostasis during aging. Interestingly, at the single cell level, genetically and environmentally identical aging cells displayed divergent phenotypes. One subpopulation of cells strongly activated the yeast transcriptional response to low iron (the iron regulon) and displayed limited iron-sulfur cluster defects, while another subpopulation of cells failed to mount a strong activation of the iron regulon and displayed elevated indicators of iron sulfur cluster defects. We tested the hypothesis that constitutive activation of the iron regulon would promote a single aging trajectory and be beneficial for lifespan. However, we found no beneficial effect on the lifespan of wildtype cells. Constitutive activation of the iron regulon increased the lifespan of V-ATPase mutants, but other phenotypes tested in these mutants were negatively impacted. BMW was funded by a University of Houston-Clear Lake Faculty Research Support Fund (A03S21).

Quinolate Phosphoribosyltransferase Knock Out (QPRT KO) Mice Develop Frail-like Characteristics at Middle Age

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Chronic inflammatory pathway activation increases with age and is epidemiologically linked to multiple aging-related pathophysiological processes, phenotypes such as physical frailty and sarcopenia, and early healthspan declines in aging organisms. Despite this, molecular mechanisms that directly connect chronic inflammation to these conditions remain poorly characterized. Based on our recently published findings, preliminary data, and numerous lines of consilient evidence, we hypothesize that chronic inflammation contributes to important aging-related phenotypes by increasing the degradation of dietary tryptophan into multiple kynurenines with unique physiological properties, via the 'kynurenine pathway' (KP). To understand the impact of elevated KP metabolites on mammalian healthspan we utilized quinolate phosphoribosyltransferase knock out (QPRT ko) mouse which lacks the terminal enzyme of the KP and thus develops increased levels of potentially toxic, downstream kynurenines. We tested the effects of this mutation on metabolism (using indirect calorimetry), glucose handling, spontaneous motor activity and body composition in male and female mice young, middle aged, and older mice. We observed that this mouse has decreased activity, lean mass and VO₂, and impaired glucose clearance as early as 12 months compared to age and sex matched mice. This data indicates that the KP may play a role in the development of frailty in mammals and thus is a potential target for strategies to intervene on frailty and functional decline.

The synergistic effects of elamipretide and NMN on restoring function in aged mouse hearts.

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We sought to determine what effects SS-31 (AKA elamipretide) and NMN, individually and in combination, have on aged (24 months old) mouse hearts. Our integrative approach assessed heart function, metabolism, and proteome state. Echocardiography revealed that SS-31 treatment restored diastolic function (Ea/Aa) halfway to that of young (5-6 months old) mice. NMN restored high work systolic function (fractional shortening) fully to that of young mice. Correlation of these data with abundance proteomics revealed a subset of proteins that play opposing roles in maintaining systolic and diastolic function with age. NMN and the combined treatment increased nicotinamide and 1-methylnicotinamide levels in aged hearts, indicating greater NAD⁺ utilization. However, only the combined treatment resulted in significantly greater steady state NAD(H) levels. PCr/ATP decreased in response to increased workload in aged control, but not young, hearts and both drugs were able to normalize PCr/ATP dynamics. Both drugs also increased mitochondrial NAD(P)H production under higher workload but only NMN increased NAD⁺. These measures did not shift in hearts given the combined treatment, which may be owed to the enhanced NAD(H) levels in the resting state. Both drugs were effective in restoring acetylation of cytosolic proteins that is lost with age but only NMN significantly reduced acetylation that accumulated with age in mitochondrial pathways. SS-31 greatly reduced the age-related glutathionylation of cysteine residues. SS-31 also restored the phosphorylation state of key contractile proteins, including Myot and cMyBP-C. These results demonstrate that the different modes of action used by each drug allow them to act synergistically to restore function in aged mitochondria and hearts greater than can be accomplished by either individually. Funding: GAATG T32AG000057-40, P01 AG001751, UW Nathan Shock Center P30 AG013280

Reducing methylglyoxal rescues obesity related phenotypes and lifespan in a leptin receptor deficient mouse model.

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The occurrence of obesity has been closely tied to an increase in sugar consumption with chronic hyperglycemia enhancing glycolysis. One of the byproducts of glycolysis is methylglyoxal (MGO), a reactive precursor for advanced glycation end-products (AGEs). It is hypothesized that AGEs formulate a positive feedback loop for increased food intake, but the mechanism remains uncertain. Here, we demonstrate that treatment with glycation byproduct lowering compounds (GLY-LOW), a customized chemical cocktail which blocks the production of MGO, leads to the regulation of food consumption and metabolism parallel to the leptin pathway in mice fed a high carbohydrate chow diet. Conversely, exogenous supplementation of MG-H1, an MGO-derived AGE, selectively bound ependymal cells of the third ventricle and increased food consumption rates and weight gain. Furthermore, GLY-LOW treatment in a leptin receptor deficient mouse model rescued weight gain, diabetic phenotypes, and lifespan, suggesting a satiation mechanism independent of leptin sensitivity. RNA sequencing of the hypothalamus of leptin receptor deficient mice treated with GLY-LOW showed significant downregulation of Rax, a gene responsible for tanycyte differentiation, and several genes involved in feeding and aging. Thus, we propose that AGEs, a macromolecule formed by reactive byproducts during glycolysis, upregulates hypothalamic tanycyte expression and drives food consumption rates independent of the leptin system. We will also discuss evidence for the potential of GLY-LOW as a new class of therapeutics that reduce the effects of glycation. Funding sources: Larry L. Hillblom Foundation, NIH

Female sarcomeric relaxation and acetylation are uniquely altered in the aging heart

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As women age, they have an increased propensity to develop a specific type of heart failure called heart failure with preserved ejection fraction (HFpEF) which is characterized by prolonged relaxation. It is possible that sex-specific differences in the heart of aging females lead to functional changes that enable this type of heart failure. The objective of this study is to determine sex-specific differences that occur in cardiac myofilament relaxation during aging. It has previously been shown that increased acetylation of myofilament proteins shortens the duration of relaxation. We hypothesize that changes in acetylation of sarcomeric proteins as women age leads to prolonged relaxation. Myofibril-enriched proteins were isolated from human explanted non-failing donors and assessed using mass-spectrometry and sarcomeric relaxation was measured. Myofibril-enriched proteins from the hearts of females over 60 years of age were less acetylated than proteins isolated from the hearts of females between 20-40 years of age. Importantly, myofibrils isolated from the hearts of females over 60 years of age relaxed more slowly than the myofibrils isolated from the hearts of females between 20-40 years of age. These differences were not observed in myofibrils isolated from male hearts of either age. These data demonstrate for the first time that there are key sex differences at the level of the sarcomere that occur during normal cardiac aging that may contribute to the propensity of females to develop HFpEF. Funding Acknowledgment: K01 AG066845-01

Age-dependent response to the soluble fiber inulin supplementation.

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Accumulating evidence suggest that leaky gut and dysbiotic microbiota/metabolites are factors contributing to age-associated inflammation. In addition to pro-inflammatory shifts in gut microbiota with aging, lack of dietary soluble fiber has been suggested to increase the risk of developing chronic inflammatory diseases, while supplementation of diets with soluble fiber might offer an array of health promoting benefits. Inulin, a soluble, fermentable dietary fiber, is thought to exert anti-inflammatory effects through promoting growth of bacteria that metabolize the fiber to short-chain fatty acids (SCFA). This study aims to systematically evaluate the capacity of SCFA production in aging mice when given dietary inulin supplement, and its impact on the structure of gut microbiome. Male C57BL/6J mice across young (5 months), middle (11 months) and old (26 months) age were subjected to a control diet for two weeks, followed by 6 weeks of inulin-containing diet. We performed targeted metabolome analysis of fecal samples collected before and 4 weeks after the diet-switch. We showed that inulin supplementation lead to significant increase in butyric acid and propionic acid levels across all age groups. Interestingly, the increase in butyric acid levels was most prominent in the middle age group, while the increase in propionate acids showed age-dependent reduction. These data suggest that the older mice still retain the capability to ferment soluble fiber and produce SCFA, albeit to a lesser extent. Consistently, fecal microbiome analysis showed that the SCFA producing bacteria populations, such as Roseburia of the family Lachnospiraceae, was most abundantly increased in the middle age group. Together, our data suggest an age-dependent effect of inulin. Acknowledgement: This work was supported by The Jackson Aging Center Pilot Project Award, the National Institute on Aging R21AG061726 and US Army Medical Research and Development Command (grant W81XWH-20-1-0127) to C.-S. Wu.

Geriatric Mice are Leaner and have Improved Glycemic Control after Dietary Isoleucine Restriction

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Dietary intervention remains one of the most efficacious ways to improve healthspan and extend longevity. A low protein diet recapitulates many of the metabolic benefits of caloric restriction, including improved metabolic health and increased lifespan. We recently demonstrated that restricting the dietary branch chained amino acid isoleucine (1/3 ile) alone is sufficient to induce tremendous physiological benefits, especially when fed lifelong in young male mice. Here, I investigate whether this intervention remains beneficial as a late-life intervention. Using naturally aged 20 month+ C57BL/6J mice, we observed that the 1/3 ile diet remains efficacious in both male and female mice. Immediately following the start of the diet, the animals lose 20-30% of their bodyweights over a period of 4 weeks with significant escalation of compensatory food consumption. Body composition MRI revealed that the animals fed the 1/3 ile diet were leaner, but loss in both lean and fat mass were observed. Metabolic chamber experiments confirmed increased energy expenditure and altered respiratory exchange ratio in animals fed the 1/3 ile diet. Importantly, the 1/3 ile diet enhanced glycemic control as evaluated by an intraperitoneally injected glucose tolerance challenge and a refeed post-prandial glucose test. Male mice fed the 1/3 ile diet exhibited improved rotarod staying time compared to the control group but both male and female mice underperformed in the inverted cling assay. These current results suggest that dietary isoleucine restriction may be a potent intervention for inducing rapid weight loss and improving the metabolic health of specific geriatric patients. Funding Acknowledgement: NIH R01-AG056771-04 (DWL), T32-AG000213-27 (CY)

An aged immune system drives senescence and aging of solid organs.

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Aging of the immune system, or immunosenescence, contributes to morbidity and mortality of the elderly. To define the contribution of immune aging to organism aging, Ercc1, encoding a critical DNA repair protein, was selectively deleted in murine hematopoietic cells to increase the burden of endogenous DNA damage and thereby senescence in the immune system only. Vav-iCre^{+/-};Ercc1^{-/fl} mice were healthy into adulthood then displayed premature onset of immunosenescence characterized by attrition and senescence of specific immune cell populations and impaired immune function, similar to changes that occur with aging in wild-type mice. Remarkably, non-lymphoid organs also showed increased senescence and damage, suggesting that senescent, aged immune cells can promote systemic aging. Indeed, transplantation of splenocytes from Vav-iCre^{+/-};Ercc1^{-/fl} or aged WT mice into young animals induced senescence in trans, whereas, transplantation of young immune cells attenuated senescence. Rapamycin treatment of Vav-iCre^{+/-};Ercc1^{-/fl} mice improved immune function and reduced markers of senescence in immune cells. These data demonstrate that an aged, senescent immune system plays a causal role in driving systemic aging and therefore represents a key therapeutic target to extend healthy aging. Funding: This work was supported by the NIH grants P01 AG043376, R56 AG059676, and R01 AG044376, and Irene Diamond Fund/AFAR Postdoctoral Transition Award.

Metformin treatment in young mice alters innate immunity

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To understand the effects of early treatment of metformin on the innate immunity, female and male heterogeneous mice (UM-HET3) were treated with metformin or saline between the ages of 15 and 56 days (i.p. 200mg/kg). In peripheral blood, metformin treatment did not alter concentration of leukocytes. Flowcytometry assay revealed that there is no significant change in CD14, a marker of monocyte. However, TLR4, a mediator of lipopolysaccharide (LPS) induced reaction, was significantly reduced in the circulating leukocytes of the metformin treated mice (P<0.05). In an ex-vivo study, blood samples were treated with LPS (100ng/ml) for four hours. In the metformin treated mice, LPS treatment significantly increased the expression of CD14 but reduced the expression of TLR4. In the LPS treated group, leukocytes in peripheral blood of metformin treated mice have significantly higher CD14 but lower TLR4 than that of saline treated mice (P<0.05). In both LPS untreated and treated samples, females have significantly lower TLR4 levels than the males (P<0.05). Furthermore, using ELISA, we tested the production of IL-6, IL-1 β and TNF- α by leukocytes, with or without LPS stimulation. Without LPS stimulation, there is no significant difference in these cytokines between metformin and saline treated mice. With LPS stimulation, leukocytes collected from the metformin treated mice produced significantly higher levels of IL-6 and IL-1 β (P<0.05). Interestingly, in the LPS treated samples, metformin treatment associated with significant higher levels of IL-6 and IL-1 β in males than that in females. These results indicate that the metformin treatment may regulate the immune reaction to infections and the effects might be different between sexes.Funding: William E. McElroy Charitable Foundation, NIA R21AG062985, ADA 1-19-IBS-126, to AB. Research seed grant of Southern Illinois University School of Medicine to RY.

Targeting cellular senescence with novel senotherapeutics by design.

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Senolytics have emerged as an effective therapeutic approach to eliminate senescent cells to improve aging phenotypes and associated co-morbidities. Despite their promising potential, only a handful of senolytics have been reported, including a natural flavonoid fisetin discovered by our group. Fisetin has been shown to reduce senescence and extend healthspan in aged mice. However, its moderate potency and poor bioavailability likely limit its clinical effectiveness. Therefore, there is a clear need to identify more effective fisetin analogues (FAs) with lower toxicity that can be translated into clinical applications for treatment of age-related diseases. This project aims to develop novel FAs with more potent senolytic activity, safer toxicity profiles and to extensively evaluate their senotherapeutic potential in vitro, ex vivo and in vivo. By leveraging drug design, medicinal chemistry and high-content imaging analysis, we have successfully optimized the senolytic activity of fisetin, leading to the identification of several improved FAs with better physicochemical properties. In both senescent murine and human fibroblasts, the new FAs showed more potent senolytic activity and less cytotoxicity than fisetin. To assess their in vivo therapeutic potential, a cohort of old wild-type C57BL/6J mice were treated acutely and evaluated for tissue senescence. The FAs were found more effective than fisetin at reducing cellular senescence and SASP factors in multiple tissues including kidney, brain and lung. To evaluate the long-term therapeutic potential on healthspan, the FAs are being tested in the Ercc1- Δ mouse model of accelerated aging. Finally, to determine the translational potential, FAs are currently being tested ex vivo using surgically excised explants from human adipose tissues, which have increased senescent cell burden of not only pre-adipocytes, but immune infiltrating cells and endothelial cells. The results of the senolytic activity of FAs compared to fisetin in these model systems will be presented. This work was supported by NIH grants P01 AG043376, U19 AG056278, RO1AG063543, P01AG062413, and Glenn Foundation for Medical Research Postdoctoral Fellowships in Aging Research.

Metformin treatment of juvenile mice alters aging-related developmental and metabolic phenotypes

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Metformin is a widely used drug for the treatment of type 2 diabetes with strong potential of long-term effects that may extend healthspan and longevity. However, the effects of early life metformin treatment in healthy animals, as well as the effects on development, have not been investigated. In the current study, heterogeneous mice (UM-HET3) were treated with metformin between the ages of 15 and 56 days. The results show that early life treatment with metformin has profound effects on developmental and metabolic traits. Body weight and food consumption were increased in both sexes. Age of sexual maturation was significantly delayed in females, but not affected in males. Interestingly, tail length and circulating insulin-like growth factor 1 (IGF1) levels were significantly increased in both sexes. Glucose tolerance was improved in both sexes, but no significant difference in insulin tolerance was found. Circulating adiponectin and insulin levels were altered by metformin treatment in a sex-specific manner. Analysis of quantitative insulin sensitivity check index (QUICKI) suggests that metformin treatment significantly increased insulin sensitivity in female pups, but, unexpectedly, had opposite effect in male pups. This study revealed that early life metformin treatment alters development and metabolism of mice in both sex-specific and non-specific manners. These effects of metformin may have long-term impacts on aging-related traits.Funding:This study is supported by William E. McElroy Charitable Foundation, NIA R21AG062985, ADA 1-19-IBS-126, to AB.

Metformin modulates doxorubicin-induced senescence phenotype in endothelial cells.

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Doxorubicin (DOX) is a cardiotoxic chemotherapeutic agent. In addition to cardiotoxicity, DOX treatment is associated with endothelial dysfunction and increased markers of vascular aging in cancer survivors, contributing to DOX-induced cardiovascular complications. Metformin, an anti-diabetic drug, has been shown to have anti-aging effects in different models of aging. Therefore, in our current work, we first established an in vitro model of DOX-induced senescence in EA.hy926 human endothelial-derived cells and Human Umbilical Vein Endothelial Cells (HUVECs). Then, we identified the effects of metformin on DOX-induced senescence phenotype. Both HUVECs and EA.hy926 human endothelial-derived cells were treated with increasing DOX concentrations for 24 hours. Thereafter, DOX was removed and cells were incubated in DOX free media for another 72-120 hours. Protein expression of senescence markers were measured using Western blot analysis. Cell cycle analysis was assessed using flow cytometry. Senescence-associated beta-galactosidase (SA-beta GAL) activity was measured by SA-beta GAL assay. Next, cells were incubated with DOX +/- increasing metformin concentrations. Effects of metformin on DOX-induced senescence markers and cell cycle were assessed. To delineate the molecular mechanisms, the effects of metformin on major signaling pathways were determined. DOX significantly induced expression of senescence markers in endothelial cells in a concentration-dependent manner. Moreover, DOX caused cell cycle arrest in the G2/M phase and increased SA-beta GAL staining. Metformin corrected DOX-induced upregulation of senescence markers, which was associated with a significant inhibition of the JNK pathway. Intriguingly, metformin had no effect on DOX-induced cell cycle arrest and slightly corrected the increased expression of SA-beta GAL. Our current work shows that metformin modulates DOX-induced senescence phenotype in endothelial cells, suggesting it may protect against DOX-induced vascular aging and endothelial dysfunction. Funding Acknowledgment: Research was supported by the National Heart, Lung, and Blood Institute, grant R01HL151740, the National Institutes of Health's National Center for Advancing Translational Sciences, grant UL1TR002494.

Transcriptomic and epigenetic profiling of neurodegenerative disease models in Drosophila.

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Aging in *Drosophila melanogaster* is characterized by loss of repressive heterochromatin structure and loss of silencing of reporter genes in constitutive heterochromatin regions. In addition, aging results in desilencing and activation of transposable elements (TEs) normally resident in heterochromatin. Similarly, studies in various animal models of neurodegenerative disease have demonstrated alterations in chromatin structure and epigenetic marks in disease states, as well as loss of heterochromatin and activation of deleterious TEs. Using genetic models of neurodegenerative disease in *Drosophila* (*elav* > human A β 42, *elav* > human tau), we profiled the transcriptome of fly brains at single cell resolution. We also profiled the genomic localization of a number of histone marks including H3K9ac, H4K16ac, H3K9me2, and H3K27ac in these models using the CUT&RUN technique. We find that Kenyon cells, the neurons in the fly mushroom body that are responsible for learning and memory, exhibit transcriptional profiles indicating acute ER stress/unfolded protein response as well as decreased synaptic function in disease model animals compared with controls. In addition, these cells are underrepresented in the brains of affected animals compared with controls, indicating likely cell death. This work was funded by grants from NIGMS (GM109035) and NIA (AG070529).

Transplantation of brown adipose tissue from the long-lived Ames dwarf mice improves high-fat diet-induced metabolic abnormalities.

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Obesity is a major risk factor for many age-related diseases, including diabetes and dementia. Increasing the amount of adipose tissue is the key characteristic of obesity. There are two functionally different types of adipose tissue: white adipose tissue, the primary site of triglyceride storage, and brown and the related beige adipose tissue (BAT), which specializes in thermogenic energy expenditure. BAT also exerts its metabolic benefits via the secretion of signaling molecules that mediate inter-organ cross-talks. Ames dwarf homozygous (Dwarf) mice have a marked extension of longevity due to a loss of function mutation (*Prop1*) and display enhanced BAT activity. Using integrated metabolomics analyses, we identified several lipid species that are increased in BAT and circulation of the Ames dwarf mice. Many of these lipid species have been linked to key features of healthy aging, such as mitochondrial activity and insulin sensitivity. Based on these findings, we hypothesized that the BAT-derived lipid mediators could deliver metabolic benefits. To test this hypothesis, we investigate the metabolic impacts of transplanting BAT from Dwarf or their heterozygous littermates (Het) mice into recipient mice fed with a high-fat diet. Compared with mice receiving Het-BAT transplants, the mice carrying the Dwarf-BAT transplant had greater oxygen consumption and higher energy expenditure without changes in food intake, leading to reduced weight gain. Furthermore, mice receiving Dwarf-BAT transplant mice exhibited improvements in glucose metabolism and insulin sensitivity. Interestingly, the metabolic benefits of Dwarf-BAT transplantation appeared to be specific to male mice as the phenotypes were not observed in female cohorts. Taken together, these findings highlight the metabolic impact of Dwarf-BAT and underscore its role in lifespan and healthspan. This work was supported by NIH grants (R01DK122808 and R01DK122898).