Identification of age-related transcriptional programs associated with cognitive resilience in Alzheimer's Disease.

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The onset, rate and severity of age-related cognitive decline varies across the elderly population, with a subset of individuals showing little to no decline and are therefore considered cognitively resilient. Approximately a third of the population will present cognitive resilience even in the presence of neuropathological signs of Alzheimerâ€™s disease (AD) suggesting that these individuals carry protective factors that prevent or delay age and AD related cognitive impairment. Given the inherent difficulties in identifying cognitively resilient individuals and the lack of phenotypic range associated with conventional model organisms of aging and AD, the genetic and molecular mechanisms that promote resilience are not understood. To address these challenges, we assessed short-term memory using contextual fear memory paradigm in the AD-BXD mouse reference panel, a genetically diverse mouse population that models AD and better mimics the phenotypic variation observed in humans. Resilience was defined based on the change in memory acquisition relative to that of the entire AD-BXD population, where strains showing lower than average decline were considered resilient. To investigate the transcriptional programs associated with resilience we selected 7 resilient and 7 susceptible strains and profiled ~220K nuclei from the hippocampal formation using single nucleus RNA seq. We identified 32 clusters representing the major cell types in the hippocampus including glutamatergic and GABAergic neurons, astrocytes, oligodendrocytes and microglia. Cognitive resilience was associated with lower age-related transcriptional change mainly in excitatory neurons, oligodendrocytes and astrocytes. In neurons, aged resilient strains showed lower expression of ribosomal and mitochondrial genes, higher expression of genes involved in chromatin organization, and synaptic signaling and architecture required for synaptic plasticity and cognition. Our findings suggest that resilient strains can maintain a youthful transcriptional profile in excitatory neurons by preserving neuronal function and maintaining lower energetic demand that often increases with aging and AD. Funding: NIA R01AG057914, BrightFocus Foundation.

Effects of Hyperbaric Oxygen on Brain Function and Markers of Neural Health

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Aging is a major risk factor for Alzheimer's disease (AD), a condition affecting approximately 6.2 million people in the USA. Currently, approved treatments of AD only manage symptoms, therefore it is important to seek out new therapeutics that can reverse and/or slow down the progression of this devastating disease. Hyperbaric Oxygen Therapy (HBOT), an intervention that has been used safely for conditions such as wound healing and decompression sickness, has now been associated with improved brain function in neurological conditions like stroke. HBOT benefits on brain function may be related to reduction in oxidative stress and inflammation, which play a large role in AD pathogenesis. Furthermore, HBOT altered the regulation of 8101 genes in human microvascular endothelial cell. The goal of our study was to determine the viability of HBOT as an intervention in a severe model of AD and identify the underlying mechanisms associated with the expected benefits. Male and female 5xFAD and wildtype (WT) mice were randomly assigned to one of four experimental groups consisting of WT-HBOT, WT+HBOT, 5xFAD-HBOT and 5xFAD+HBOT. HBOT daily (5 days/week) was started at 9-10 months and continued until the mice were euthanized at 12-13 months. Behavioral tests were carried out at 1 month into the treatment with HBOT. Brain regions, namely hippocampus and cortex, were isolated upon euthanization, and saved for future biochemical/molecular evaluations. Differential sex outcomes were found in the cognitive task, in which HBOT improved spatial learning and memory in males only and improved cognitive flexibility in females only. Furthermore, our preliminary data suggest that HBOT moderately affected global DNA methylation in the hippocampus. This work demonstrates that HBOT reverses cognitive impairment associated with an AD phenotype and affected epigenetic changes. Future work will continue to evaluate the underlying mechanisms associated with the beneficial outcomes of HBOT on brain function.IACUC-2021-0026 BvB Foundation Dallas; Office of Vice President for Research and Innovation, the Institute for Healthy Aging, and National Institutes of Health/National Institute on Aging (T32 AG020494); GSBS seed grant

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

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Oxidative stress is a primary cause of cellular senescence and contributes to the etiology of numerous human diseases including cancer. Oxidative damage to telomeric DNA has been proposed to cause premature senescence by accelerating telomere shortening, however this model was based on indirect, correlative studies using oxidants which damage the whole genome or organism. Here we directly tested whether oxidative telomere damage causes cellular aging by using a precision chemoptogenetic tool to produce the common lesion 8-oxo-guanine (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 including Î²-gal staining, cGAS positive micronuclei, and the senescence associated secretory phenotype. 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 or increase the number of critically short 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. Our study shows that telomeres are acutely sensitive to oxidative damage and reveals a novel mechanism of telomere driven senescence linked to oxidative stress that is independent of telomere shortening.

High-resolution cognitive testing paradigm identifies transcriptional signatures of cognitive heterogeneity in aging mice.

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Background: Age is, by far, the greatest risk factor for cognitive decline in the elderly and in Alzheimerâ€™s disease. Importantly, many individuals have impaired cognition, while some retain functionality despite their age. This heterogeneity in cognitive function is routinely observed in aged individuals but has been problematic to address in pre-clinical models with current testing methodology. The primary focus of this study was to assess cognitive heterogeneity in mice and the associated transcriptional signatures in the brain. Methods: Aged (24-27 mo) C57Bl/6J male mice were stratified into cognitively â€˜intactâ€™ and â€˜impairedâ€™ subgroups based on a 90% confidence interval of young (6 mo) performance (<1,000 entries in reversal; intact group) using a high-resolution automated home-Cage testing paradigm (Noldus PhenoTyper). Spontaneous activity, acquisition, and reversal-learning were assessed using a food-reward-based spatial discrimination task over a 90-hour period. Following behavioral testing, the hippocampus was dissected and analyzed by RNA sequencing and quantitative PCR. Pro-inflammatory cytokines were measured in serum. Results: We show that greater than 90% of young mice and approximately 41% of aged mice classified as cognitively intact, which is not the result of differences in movement or foraging behavior. Cognitive flexibility (reversal efficiency) was significantly reduced in the aged-impaired (>1000 entries) mice compared to controls. RNAseq analysis of hippocampus revealed transcriptional downregulation of mitochondrial oxidative phosphorylation and Sirtuins (Sirt1 and Sirt3) in the aged-impaired subgroup along with increases in markers of astrogliosis. Aged-impaired animals also showed increased levels of pro-inflammatory cytokines, including IL-6, TNF-alpha, etc., in serum. These data suggest a metabolic defect that is associated with cognitive impairment and inflammatory status specific to cognitive impairment. Funding: NIH R00AG056662, P20GM125528, and AG057424

Hyperactive mTORC1/4eBP1 Signaling Accelerates Cardiac Aging

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The mechanistic target of rapamycin complex 1 (mTORC1) has a major impact on aging. Studies have shown that partial inhibition of mTOR signaling by rapamycin reverses the age-related declines in cardiac function and structure in old mice. However, the downstream signaling pathways involved in reversing cardiac aging have not been established. TORC1 phosphorylates 4E-binding protein 1 (4EBP1) and suppresses its binding to eukaryotic initiation factor 4E (eIF4E), releasing eIF4E for the initiation of cap-dependent translation. The aim of this project is to examine the role of the mTORC1/4EBP1/eIF4E axis in age-related cardiac dysfunction. We utilized a whole-body 4EBP1 KO mouse model, which mimics a hyperactive 4EBP1/eIF4E axis, to investigate the role of mTORC1/4EBP1 signaling in cardiac aging. Echocardiographic measurements showed that young 4EBP1 KO mice exhibit no difference in cardiac function at baseline compared to WT mice. Interestingly, middle-aged (14-15 month-old) 4EBP1 KO mice display impaired diastolic function and myocardial performance at similar levels as a 24 month-old WT mice, indicating 4EBP1 KO mice exhibit accelerated cardiac aging. Old 4EBP1 KO mice show further declines in systolic and diastolic function compared to middle-aged 4EBP1 KO mice, and display worse systolic and diastolic function than age-matched old WT animals. Although we observed an exacerbated decline in cardiac function with age in 4EBP1 KO mice, age-related cardiac hypertrophy and transcript levels of hypertrophic markers (Nppa, Nppb and Myh7/Myh6) are not different between 4EBP1 KO and WT mice. Similarly, expression levels of cellular senescence marker p16 and senescence-associated secretory phenotype (SASP) component IL6 are not different between 4EBP1 KO and WT at any age. Overall, our results support an important role of TORC1/4EBP1/eIF4E axis in mediating age-related cardiac dysfunction. Further investigation of mTORC1-dependent signaling pathways and mechanisms involved in cardiac aging will facilitate the development of novel therapies with minimal off-target effects.We acknowledge support from NIH R00 AG051735 (YAC).

Demonstration of age-related increases in blood-brain barrier permeability and microvascular rarefaction mouse cerebral cortex

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Uncovering the Role of Cyclin D1 in Aging and Senescence

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Hepatocellular carcinoma (HCC) is an emerging health crisis due to rapidly increasing incidence rates and a 5-year survival rate of 18%. Age is one of the biggest risk factors for HCC, and to uncover how aging drives the increased risk for HCC, we profiled hepatocytes from young and old mice to look at transcriptomic and epigenetic changes with age. RNA seq revealed an elevation of oncogenic pathways of Myc, Ctnnb1 and Ccnd1 among others as well as elevations in the p53 tumor-suppressive pathway. Considering these observations, we hypothesize that the tumor suppressor and oncogenic pathways exist in a precarious balance within old hepatocytes, making them more prone to oncogenesis. Among these oncogenic drivers of HCC, Ccnd1 is significantly upregulated at both the mRNA and protein level with age. Additionally, epigenetic analysis with H3K27Ac Hi-ChIP along with H3K27Ac and H3K4me1 ChIP seq shows that Ccnd1 gains enhancer contacts in the old liver, which could be potentially driving the increased expression of Ccnd1 with age. We will validate this using a dcas9-KRAB mediated inactivation of these enhancer regions. To determine the molecular pathways being regulated by Ccnd1 and how this aging event promotes HCC, we are taking a two-pronged approach- knocking out Ccnd1 using CRISPR and alternatively, ectopically expressing Ccnd1- in young and old wildtype mouse hepatocytes using AAV infection. A well-known feature of aging is the accumulation of senescent cells. Chronic presence of senescent cells is known to promote tumor progression, and thus we also explored senescent cells to better understand the link between senescence, aging, and cancer. Interestingly, independent analyses of senescent human fibroblasts reveal that CCND1 is also upregulated with senescence. Thus, the role of CCND1 in senescence might parallel the role of Ccnd1 in aging. In conclusion, we seek to uncover how aging promotes HCC by profiling changes in young and old livers using a variety of large descriptive datasets, AAV-directed genetic alterations in mice and a parallel model of senescence.Funding by NIH- P01 AG073084-01, P01- AG031862.

DNA damage alters metabolic-epigenetic axis to drive aging.

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Although DNA damage is intricately linked to cellular metabolism, the metabolic alterations that occur in response to DNA damage are not well understood. We use a DNA repair-deficient model of ERCC1-XPF in C. elegans to gain insights on how genotoxic stress drives aging. Using an integrated multi-omic approach, we discover that nuclear DNA damage promotes mitochondrial Î²-oxidation and drives a global loss of fat depots. This metabolic shift to Î²-oxidation generates acetyl-CoA to promote histone hyperacetylation and an associated change in expression of immune-effector and cytochrome genes. We identify the histone acetyltransferase MYS-1, (mammalian TIP60), as a critical regulator of this metabolic-epigenetic axis. We show that in response to persistent DNA damage, PUFAs, especially arachidonic acid (AA) and AA-related lipid mediators are elevated and this is dependent on mys-1. Together, these findings reveal that DNA damage alters the metabolic-epigenetic axis to drive an immune-like response that can promote age-associated decline. Funding Acknowledgement: This work was funded by NIH grants R00AG049126 (AUG), R01GM132261 (NWS), NIH ZIA AG000679 (PS), AI145406, CA165065,CA243142,AI068021, GM113908, HL114453, AI156924, NS076511, AI156923, NS061817 (H.B./ V.K.). Metabolomics assistance was provided by the Metabolomic Core at Pitt S10OD023402. Strains were provided by the Caenorhabditis Genetics Center, which is funded by the NIH Office of Research Infrastructure Programs (P40 OD010440).

Myonuclei can replicate DNA

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Skeletal muscle is a heterogeneous tissue composed of multinucleated myofibers and supporting cells like satellite cells (SCs). Myonuclei are believed to be post-mitotic and unable to proliferate. Consequently, it is hypothesized that growth of muscle is dependent on the donation of nuclei from SCs. Although some studies suggest that under certain conditions myonuclei could replicate, it has been difficult to address whether this replicative potential exists in vivo. We hypothesized that skeletal muscle myonuclei possess the ability to replicate in vivo. To test this hypothesis, we used the HSArtTA;Tet-O-H2B-GFP (HSA-H2B) mouse model, which uses doxycycline (DOX) treatment for temporal labeling (Tet-ON) of residential myonuclei. After removing DOX, and thus turning off GFP-labeling, we provided the stable isotope deuterium oxide (D2O) for 8 weeks to measure DNA synthesis. At the end of D2O labeling, we harvested muscles and sorted GFP+ myonuclei using an antibody-independent approach and a restrictive gating strategy. Both GFP+ (myonuclei) and GFP- (non-myonuclei) fractions were then analyzed for D2O incorporation into DNA. By our approach, deuterium-enrichment in DNA from GFP-positive myonuclei indicates myonuclei replication. We observed deuterium enrichment into DNA in 6 out of 7 plantaris (PLA), 4 out of 7 extensor digitorum longus (EDL), 4 out of 7 tibialis anterior (TA), 6 out of 7 gastrocnemius (GAS), and 7 out of 7 quadriceps (QUAD). The average fractional synthesis rates (FSR, %/day) of replicating myonuclei were: 0.0351 Â± 0.017 in PLA, 0.0156 Â± 0.015 in EDL, 0.0220 Â± 0.018 in TA, 0.0223 Â± 0.018 in GAS, and 0.02692 Â± 0.043 in QUAD. These data are the first to unambiguously demonstrate that myonuclei replicate DNA in vivo. The proposed project challenges current dogma and might significantly impact skeletal muscle treatment to maintain muscle mass with age and disease. Funding: NIH R21 AR077387-01A1

Nutritional Stress and Aging: The Role of Flavin Monooxygenase

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Health, longevity, and systemic metabolism can be influenced by diet. Reduced nutrient intake, or dietary restriction (DR), enhances health and longevity across multiple species. In addition, there is evidence that variations in the concentration of a single metabolite can influence longevity both positively and negatively. For instance, restricting glucose intake can increase lifespan, while excessive glucose intake can increase the risk of age-related diseases and shorten lifespan. Despite significant literature on the mechanisms of DR-mediated longevity, little is known about how excess nutrients impact aging. 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 play an important role in carbohydrate and lipid metabolism, and their expression is modified in diabetic patients and rodent models of the disease. We now find that while worms grown on high glucose (HG) media have a significantly shorter lifespan, overexpression of FMO-2 reverses this negative effect. Metabolic profiling by untargeted and targeted methods reveals that overexpression of FMO-2 alters the metabolites in the One Carbon Metabolism (OCM) pathway and decreases flux through methylation processes. Genetic studies confirm the role of OCM during regular and high glucose diets and emphasize the role of lipid metabolism plays in regulating longevity. In summary, our findings suggest FMO-2 modulates flux in the OCM network to reduce glucose's negative impact on lifespan.

Universal DNA methylation age across mammalian tissues

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Aging is often perceived as a degenerative process resulting from random accrual of cellular damage over time. In spite of this, age can be accurately estimated by epigenetic clocks based on DNA methylation profiles from almost any tissue of the body. Since such pan-tissue epigenetic clocks have been successfully developed for several different species, it is difficult to ignore the likelihood that a defined and shared mechanism underlies the aging process. To address this, we generated data using 11,754 methylation arrays, each profiling up to 36 thousand cytosines in highly-conserved stretches of DNA, from 59 tissue-types derived from 185 mammalian species, representing 19 taxonomic orders. Our samples ranged in age from prenatal to 139 years (bowhead whale). The considered species had maximum life span ranging from 1.9 (cinereus shrew) to 211 years (bowhead whale). From these, we identified and characterized specific cytosines, whose methylation levels change with age across mammalian species. These cytosines are proximal to the genes that are greatly enriched in polycomb repressive complex 2-binding sites, involved in mammalian developmental process, and associated with age-related traits, including birth length, age at menarche, human longevity, Alzheimerâ€™s disease, etc. From these methylation profiles, we constructed two sets of age predictions, each with a single mathematical formula, termed universal pan-mammalian clocks that are equally accurate in estimating ages (r>0.96) of any mammalian species and tissue. Collectively, these new observations support the notion that aging is indeed evolutionarily conserved and coupled to developmental processes across all mammalian species - a notion that was long debated without the benefit of this new compelling evidence.

Impaired DNA repair mechanism induces retinal aging in mice

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MNERCC1 is a non-catalytic subunit of the endonuclease ERCC1-XPF complex. It is required for nucleotide excision repair, interstrand cross-link repair, and repair of some double-strand breaks. Humans with mutations or reduced expression of this enzyme complex develop progeroid symptoms and multi-organ failure at a young age. Systemic knockdown of Ercc1 in mice (Ercc1 -/Î”) models the human progeroid syndrome and causes the mice to age ~6 times faster than normal. These mice display increased DNA damage signaling and senescent cell accumulation in various organs including liver, kidney, adipose tissue, brain, pancreas, and lungs. The present study was designed to study the morphological and molecular changes in the retina of Ercc1 -/Î” mice. The retinal thickness and the photoreceptor cell count were measured in H & E-stained retinal cross-sections and were found to be decreased in Ercc1 -/Î” mice. Microvascular tufts were observed in the retinal whole-mounts stained with Isolectin-B4. Along with this, RPE flat-mount staining revealed morphological heterogeneity in cell shape and size. These changes are similar to those observed in old mice. Gene expression of senescence marker (p16 and p21) and SASP factors (Il6, Tnf, and Mcp) were increased in RPE from the mutant mice. RPE cells from the Ercc1 -/Î” mice were isolated and cultured to measure glycolysis. The cells from Ercc1 -/Î” mice displayed an abnormal metabolism evidenced by an increased glycolytic rate compared to cells isolated from age-matched wild-type control mice. From these results, it is evident that endogenous DNA damage promotes senescence of RPE. The metabolic shift in senescent RPE could contribute to photoreceptor cell death and abnormal retinal vasculature. This demonstrates that Ercc1 -/Î” mice could be a suitable model to study age-associated retinal changes and to test senotherapeutics for aging-related eye diseases. Funding acknowledgement: This work was generously supported by a donation to the University of Minnesota Foundation.

Leveraging the Ndufs4-/- mouse as a platform for testing longevity interventions.

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Mitochondrial dysfunction is one of the hallmarks of biological aging, as well as the driving factor for mitochondrial diseases. Up to 30% of mitochondrial disorders are due to mutations affecting the activity of Complex I in the electron transport chain. Loss of the Complex I subunit Ndufs4 recapitulates symptoms of Leigh Syndrome, a pediatric mitochondrial disease, in mouse. Ndufs4-/- mice suffer developmental delays, early onset of neurological symptoms and extremely reduced lifespan. Several studies have now shown that Ndufs4-/- mice are exquisitely responsive to treatments and interventions of interest in the biology of aging, such as rapamycin, NAD+ precursors, reduced oxygen tension, alpha-keto-glutarate precursors, and the antidiabetic drug acarbose. These results point to common mechanisms underlying both aging and mitochondrial disorders. To put this hypothesis to the test, we show that Ndufs4-/- mice are responsive to a wide range of longevity interventions previously tested in worms, mice, and by the National Institute on Agingâ€™s Intervention Testing Program. These observations support the hypothesis that mitochondrial and metabolic dysfunction induced by Complex I deficiency may be a key component of biological aging as well as mitochondrial disease. Furthermore, we propose that the Ndufs4-/- mice provide an affordable testing ground for candidate longevity interventions.Funding: NINDS 1R01NS98329; NIA 3P30AG013280

Myocardial fibrosis and remodeling due to aging are accelerated in Intrauterine Growth Restriction (IUGR) baboons.

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Introduction: IUGR offspring (F1) experience insulin resistance (IR) similar to diabetics. IR causes a shift in cardiometabolic substrate from glucose to fatty acids (FA). Acidic FA metabolic products promote myocardial scarification, increasing extra-cellular volume (ECV). Additionally, the heart shrinks along the central axis, becoming more spherical. We sought to determine if age-related ventricular remodeling (VR) increases in IUGR. Methods: Mothers of IUGR F1 ate 70% food eaten by control (CTL) mothers in pregnancy and lactation. After weaning, all F1 ate normal colony diet. Thus changes are due to developmental programming by poor maternal nutrition. A MOLLI MRI sequence was used to measure myocardial T1 in 5 image planes across the left ventricle (LV). ECV measurements were calculated according to differences in T1 and averaged across all image views. Sphericity Index (SI) is the ratio of volume of a sphere calculated from LV central axis radii at the end-diastolic volume measured with 3D cine MRI. A paired t-test was used to evaluate changes. Results: CTL F1 (n=7) averaged 5.7 yrs. while IUGR F1 (n=9) averaged 5.2 yrs. Subjects ranged from 6-10 years at the age of the younger scan and 11-15 yrs. at the older scan. IUGR F1 showed a greater increase in SI and ECV (0.36 Â± 0.07 to 0.57 Â± 0.20, p = 0.02 and 25.1 Â± 2.7% to 28.1 Â± 2.5%, p=0.004, respectively) vs. CTL (0.50 Â± 0.08 to 0.49 Â± 0.08, p=NS and 26.3 Â± 2.0% to 27.9 Â± 1.4%, p=NS). Changes in CTL measurements were too small to discern a difference. Conclusions: ECV and SI increases are normal signs of aging in baboons and humans. This longitudinal analysis shows a greater rate of increase for IUGR subjects compared to CTL subjects. These data demonstrate that LV imaging biomarkers reveal accelerated aging in IUGR F1 baboons an indication of IUGR programmed aging effects. The findings indicate that people born IUGR may be predisposed to cardiometabolic dysfunction later in life. NIA U19AG057758, NIH P51OD011133.

Impaired myocardial contractility in intrauterine growth restricted (IUGR) baboon offspring (F1) is improved with aging.

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Introduction. Maternal caloric deficits during development program impaired later life effect myocardial contractility and elasticity (cardiac strain). Young IUGR F1 show impaired left ventricular (LV) strain but strain changes with aging are not known. We aimed to determine if LV function improves longitudinally as F1 age. Methods. Mothers of IUGR F1 ate 30% calorie reduced diet vs. control mothers in pregnancy and lactation. After weaning, all F1 ate the same normal diet. MRI covered the entire heart in long axis (LAX) and short axis (SAX). MRI sessions were conducted in young and old F1. Images were analyzed with feature tracking in cvi42Â® cardiac analysis software. Endo- and epicardial contours were drawn on all slices containing the LV cavum in end-systole and end-diastole. LAX and SAX slices were analyzed, producing global measurements of peak radial, longitudinal and circumferential strains. Strain values are listed as a percent of change in length between end-diastole and end-systole for each of the 3 strain dimensions. A paired t-test was used to evaluate the changes from young to old. Results. IUGR F1 (n=9) age difference ~7.2 yrs. and control F1 (n=10) age difference averaged 6.7 yrs. between the two MRI acquisition timepoints. In IUGR F1, in SAX views, both radial and circumferential strain improved (10.1Â±3.4% to 17.6Â±5.2%, p=0.002 and -8.0Â±2.1% to -12.5Â±2.7%, p<0.001, respectively). In LAX image views, IUGR F1 improved in both the radial and longitudinal directions (11.6Â±4.1% to 21.6Â±5.9%, p<0.001 and -9.0Â±2.7% to -14.2Â±2.7%, p<0.001, respectively). Aging changes to strain in control F1 were not significant for any parameter. Conclusions. Global strain values across all IUGR F1 increased in magnitude with age indicating that gestational insults to cardiac metabolism, present during youth, may improve if a healthy diet and lifestyle are adopted. In all measurements, strain in older IUGR F1 reached similar levels to control F1. Proper nutrition throughout life is important for cardiac health but may not be as important as good nutrition in development. NIA U19AG057758, NIH P51OD011133.

Left-ventricular function and output in Intrauterine Growth Restricted (IUGR) baboons are impaired in early life but normalize in later life: Need for life course studies.

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Introduction. Maternal undernutrition in pregnancy and lactation programs offspring (F1) cardiac function. Myocardial fibrosis reduces ejection fraction (EF) and cardiac index (CI). We used a longitudinal study design to determine if LV function is improved or impaired as IUGR F1 baboons reach adulthood. Methods. Mothers of IUGR F1 ate 30% calorie reduced diet vs. control (C) mothers in pregnancy and lactation. After weaning, all F1 ate the same normal diet. MR cine images were taken of 4-chamber and 2-chamber long axis views and one short axis view covering the entire heart. MRI scanning was performed at young and old age. Endo- and epicardial contours were drawn on all slices containing the LV cavum with cvi42Â® cardiac analysis software combining imaging planes and computing cardiac function parameters in a 3D analysis. A paired t-test was used to evaluate the changes from youth to old age. Results. IUGR F1 (n=9) aged ~7.2 yrs. and control subjects (n=10) aged an average 6.7 yrs. between the two MRI acquisition timepoints. In all F1, EF and CI, improved with age (41.6Â±13.2% to 53.3Â±9.7%, p=0.006 and 2.42Â±1.09 L/min/m2 to 3.11Â±0.82 L/min/m2, p=0.04, respectively). Additionally, F1 changes in CI correlated linearly with changes in age (p=0.047, R2=0.21). EF and CI both improved in IUGR F1 (31.9Â±10.7% to 52.7Â±8.6%, p=0.004 and 1.66Â±0.62 L/min/m2 to 2.99Â±1.00 L/min/m2, p=0.02, respectively), but EF and CI changes were minimal in controls (50.7Â± .0% to 53.9Â±11.0%, p=NS and 3.10Â±0.97 L/min/m2 to 3.22Â±0.64 L/min/m2, p=NS, respectively). Conclusions. Both EF and CI improved in IUGR F1 indicating that developmental programming may only impair LV function during youth. Control subjects maintained similar values from youth to old age. Old IUGR subjects had CI and EF values similar to controls in youth. This study suggests that IUGR effects on cardiac function and output are reversible later in life assuming poor diet and lifestyle are ameliorated and demonstrates the need for life course studies. NIA U19AG057758, NIH P51OD011133.

Role of Porphyromonas gingivalis-derived virulence factors in the promotion of age-dependent periodontitis

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Aging is associated with increased prevalence and severity of pathogenic outcomes of periodontal disease, including soft tissue degeneration and bone loss around the teeth. Although lipopolysaccharide (LPS) derived from the key periodontal pathogen Porphyromonas gingivalis (Pg) plays an important role in the promotion of inflammation and osteoclastogenesis via toll-like receptor (TLR)4 signaling, its pathophysiological role in age-associated periodontitis remains unclear. This study investigated the possible effects of Pg-LPS on RANKL-primed osteoclastogenesis and ligature-induced periodontitis in relation to aging using young (2 months old) and aged (24 months old) mice. To the best of our knowledge, our results indicated that expression of TLR4 was significantly diminished on the surface of osteoclast precursors isolated from aged mice compared with that of young mice. Furthermore, our data demonstrated that the TLR4 antagonist (TAK242) dramatically decreased the numbers of tartrate-resistant acid phosphatase positive (TRAP+) osteoclasts differentiated from RANKL-primed young osteoclast precursors (OCPs) compared with those isolated from aged mice in response to Pg-LPS. In addition, using a ligature-induced periodontitis mouse model, we demonstrated that Pg-LPS elevated (1) secretion of senescence-associated secretory phenotype (SASP) markers, including the pro-inflammatory cytokines TNF-Î±, IL-6, and IL-1Î², as well as osteoclastogenic RANKL, and (2) the number of OCPs and TRAP+ osteoclasts in the periodontal lesion induced in young mice. In contrast, Pg-LPS had little, or no, effect on the promotion of periodontitis inflammation induced in aged mice. Altogether, these results indicated that periodontal disease in older mice occurs in a manner independent of canonical signaling elicited by the Pg-LPS/TLR4 axis.This work was supported by a Nova Southeastern University President Faculty Research Development Grant and NIH Grants R03 AG-053615, R01 AG-064003, R03 DE-027153, R15 DE-028699.

Characterizing mitochondrial dysfunction of the aging prostate via mass spectrometry methods.

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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; BPH occurs in 60-80% of men in their sixties, which increases to 90% of men in their eighties. BPH can cause bothersome lower urinary tract symptoms (LUTS), which significantly reduce the quality of life of aging men. Current approved treatments fail to consider the role of the aging prostate in the development and progression of BPH/LUTS, largely due to the lack of mechanistic understanding of the aging prostate. While more work is necessary to elucidate the mechanisms of aging in the prostate, preliminary data generated by our lab has identified mitochondrial dysfunction, a hallmark of aging, as a critical player in disease development. In this study, LC-MS/MS was performed on human prostate stromal cells treated with a complex I inhibitor (rotenone) alone, or in combination with complex I bypass agents. To assess the impact of age in a mouse model of BPH/LUTS, matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) was used to gather both spatial and quantitative protein profiles. In combination, these techniques and models provide both mechanistic insight and physiological relevance regarding mitochondrial dysfunction associated with BPH/LUTS. Additionally, this method can be used with various models investigating other hallmarks of aging going forward. U54DK104310, R01DK131175, K01AG059899, T32GM141013

Impact of the Aged Bone Marrow on the Anti-tumor Response to Melanoma.

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Melanoma is responsible for the most skin cancer-related deaths in the United States. Melanoma occurrence and severity increase with age while the anti-tumor responses decrease. There have been robust responses to immunotherapy treatments, however, there remains a subset of patients who do not benefit from these modalities. Understanding more about immune cell development and age-related changes in the bone marrow can improve patient outcomes. We hypothesize that during aging, changes in Wnt signaling reshape the aged bone marrow niche, driving precursor cells to become suppressive. This ultimately decreases the anti-tumor response to melanoma due to the skewing of hematopoiesis to yield decreased immune function. In a young and aged setting, we assess the effect of a Wnt inhibitor, sFRP1, on the development of immune cell subtypes by using flow cytometry. We have preliminarily observed in situ that sFRP1 is increased in the aged bone marrow through immunohistochemical staining. We also saw a decrease in CD4 and CD8 T-cells and an increase in CD11b positive myeloid cells in the tumors of young mice treated with recombinant sFRP1 compared to age-matched mice treated with PBS by using immunohistochemical staining. We hypothesize that the increase in sFRP1 in the bone marrow niche will impact immune cell development in the niche and the immune response at the tumor site. As a future direction, we plan to investigate combination treatments of anti-sFRP1 with immunotherapies, such as anti-PD-1. These insights could lead to improve outcomes for elderly patients with melanoma.

Morphological and microstructural brain alterations in the rhesus macaque model of normative aging

Alison Weiss
Rhesus macaques are known to naturally undergo age-related changes in brain structure and organization, as well as experience cognitive decline in multiple domains (executive function, declarative memory), but do not develop overt AD-like pathology accompanied by robust neuronal lossâ€”making them an excellent model of normative aging. Here, we report a large cross-sectional study identifying morphological and microstructural biomarkers of aging in this model. In a cohort of 40 animals (20F/20m, ages 5.3-28.2y) we collected high-resolution (0.5 mm isotropic) T2w SPACE images, and diffusion weighted (DW) scans in 1.0 mm isotropic resolution. Anesthetized NHPs allow longer scanning times compared to humans, thus enabling the acquisition of very high-quality MR data. Using Tensor Based Morphometry (TBM), we characterized a region-specific pattern of significant (p<0.01) age-associated atrophy in areas of the dorsolateral and ventrolateral prefrontal cortex, caudate, putamen, and medial thalamus, as well as age-associated ventricular enlargement. We next characterized microstructural alterations in cerebral white matter with voxel-based analyses of diffusion tensor imaging (DTI) data. Using this approach, we identified a number of white matter tracts with areas of significant (p<0.01) age-associated reductions in fractional anisotropy (FA) including the superior longitudinal fasciculus, corona radiata, superior temporal gyrus, stria terminalis, superior fronto-occipital fasciculus, internal capsule, and uncinate fasciculus. Additional analyses are underway to assess age-sex interactions in the structural and DTI data. Taken together, this work broadly suggests that aged macaques undergo morphological and microstructural changes in cortical, striatal, and thalamic regions. as well as in the white matter fiber pathways that interconnect these areas, that are detectable using high-resolution MRI and DTI. Funding Sources: NIH NIA T32AG055378-04, U24 AA025473, OD-011092, and a grant from the M.J. Murdock Charitable Trust.

miR-146a-5p Modulates Cellular Senescence in Visceral Adipose Tissue and Liver of Long-Lived Ames Dwarf Mice and in Human Endothelial Cells.

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Abstract: Changes in several microRNAs (miRNAs) expression profiles are strongly associated with downstream effects on aging and longevity. Previous studies have demonstrated that circulating miR-146a-5p increases 5-fold in normal mice during aging, while long-living Ames dwarf (df/df) mice maintain youthful levels of this miRNA. Aim: The aim of this study was to elucidate the involvement of miR-146a-5p in modulating cellular senescence in visceral adipose tissue and liver of df/df mice and Human Endothelial Cells (HUVEC). Methods: To test the effects of miR-146a-5p overexpression on visceral adipose tissue and liver, wild-type and df/df mice, were treated with miRNA-negative control-base, and df/df were treated with 8 Î¼g/g of a miR-146a-5p mimetic. Effects of miR-146a-5p overexpression were also evaluated in HUVECs early passage and late passage (P7 and P13, respectively), and cells cultured under high glucose conditions (HUVEC-HG). Results: Treatment of df/df mice with the miR-146a-5p mimetic increased cellular senescence and inflammation in visceral adipose tissue and liver. In addition, the miR-146a-5p mimetic induced similar effects in HUVEC P7, but not in HUVEC P13 and HUVEC-HG. Importantly, HUVEC P13 and HUVEC-HG showed significantly higher expression of miR-146a-5p than HUVEC P7 grown in normal conditions. Conclusions: These results indicate that miR-146a-5p represents one of the significant SASP factors that if not precisely regulated, can accentuate inflammatory responses, and enhanced cellular senescence in healthy cells, especially cells surrounded by senescent cells that over-express miR-146a-5p, possibly to target distant tissues through extracellular vesicle. The role of miR-146a-5p is different in healthy vs. stressed cells, suggesting potential effects of this miRNA depend on overall organismal health, aging, and metabolic state. However, analysis of the effects of other miRNAs identified in long-lived mouse models and in human centenarians on senescence and aging is currently underway. Funding: NIH grants R56 AG061414, R15 AG059190, and R03 AG059846.

Dietary intervention as a modulator of chemotherapy response

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Chemotherapy is the therapy for many types of cancer. It is also frequently administered together with other treatments such as radiotherapy and surgery to ensure maximal removal of tumor cells and prevent relapse. Lack of specificity for cancer cells and side effects are two of the main limitations of chemotherapy. Modulating external factors to augment chemotherapy efficiency could represent an alternative to significantly improve cancer treatment. Caloric restriction has been shown to limit tumor growth and metastasis in different cancer models, and a fasting mimicking diet potentiates tumor remission in response to chemotherapy. However, how dietary interventions can improve the response to chemotherapy in terms of both tumor elimination and overall health status has not been systematically addressed. In this project we aim to decipher whether mild caloric restriction and fasting cycles can improve chemotherapy response across cancer mouse models of different strains, sex and ages. We will assess the histopathological status of primary tumors and metastasis together with well-being parameters such as bodyweight, blood cell count, specific set of functions in various tissues (liver, intestine, kidney), and signs of pain and distress. The outcomes of this project could have great clinical implications and it could be easily translatable to the clinics and implemented. A simple dietary intervention in a safe, medically supervised manner could bring significant benefits in the treatment and quality of life for cancer patients.
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A novel mitochondrial-derived peptide regulates pathways critical to aging, obesity, and inflammation.

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The term ‘inflammaging’ defines an age-gradient increase in pro-inflammatory cytokines, and demonstrates a dynamic crosstalk between aging immunity and metabolism. However, the underlying mechanisms behind this phenomenon remain poorly understood. Here, we propose a novel regulator of inflammaging in the form of a mitochondrial-derived peptide (MDP). MDPs are a subclass of microproteins deriving from small open reading frames (smORFs) in the mitochondrial genome that have rapidly reshaped our understanding of human systems biology. By applying mitochondrial-targeted gene expression analyses in peripheral blood mononuclear cell samples across two representative cohorts, we detected significant shifts in the expression of a previously unannotated MDP. Further, through mitochondrial-genome wide association analyses, we have identified two mutations within the MDP smORF which associate with both immune and metabolic phenotypes. Additional in vitro characterization in the immune cell line THP-1 demonstrates protective effects against pro-inflammatory stimulation by LPS, showing decreased pro-inflammatory cytokine production and increased cell viability. Further assessment of adipose-specific effects also yields promising results, wherein peptide treatment in the mouse-derived adipose cell line 3T3-L1 significantly shifts metabolic activity. These observations correspond to in vivo results, showing trending weight loss in peptide-treated C57BL/6 mice fed high fat diet. Thus, this newly characterized MDP sits at the interface between metabolic tissues and immunity, revealing a novel regulator of inflammation as it relates to common phenotypes of age, including obesity and immune system dysfunction. Based on these results, we anticipate this novel MDP to continue to demonstrate unique and protective effects against the progression of inflammation, and associated disorders, that are commonly observed with age. Funding Acknowledgements: T32AG000037

Longitudinal tracking of accelerated brain aging after mild traumatic brain injury.

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Longitudinal tracking of accelerated brain aging after mild traumatic brain injury. Amgalan, Anar 1, Maher, Alexander 1, Ghosh, Satyaki 1,2, Chui, Helena 3, Irimia, Andrei 1,4. 1 Ethel Percy Andrus Gerontology Center, Leonard Davis School of Gerontology, University of Southern California, Los Angeles, CA, USA, 2 Department of Electronics and Electrical Engineering, Indian Institute of Technology, Guwahati, Assam, India, 3 Department of Neurology, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA, 4 Corwin D. Denney Research Center, Department of Biomedical Engineering, Viterbi School of Engineering, University of Southern California, Los Angeles, CA, USA. After mild traumatic brain injury (mTBI), the risk of complications and mortality increases with chronological age (CA) at injury. Gaussian process regression was used to estimate the brain’s biological age (BA) from T1-weighted magnetic resonance images (MRIs) of 133 participants (51 females) aged 20-83 (CA ?±? = 42.6±17 years) who had experienced their first mTBI. MRIs were acquired acutely (~7 days) and chronically (~6 months) after injury. Using rigorous statistical model selection, brain BA acceleration was estimated, modelled, and studied as a function of CA at injury and sex. Adults older than ~40 exhibited brain BAs 15.3±6.9 years older than expected in healthy controls (HCs) of the same CA and sex. Adults younger than ~40 had brain ages 1.8±5.6 years older than HCs, a significant difference (Welch’s t32=-9.17, p?9.47 × 10-11). In adults under 60, each additional decade of life translated in ~3 additional years of excessive brain aging after mTBI; in adults over 60, this figure was ~7 years. No significant sex difference was found (Welch’s t108 = 0.78, p > 0.78). Within-subject comparison found no significant increase in brain age between baseline and follow-up (t264 = 0.41, p > 0.66, power = 80%), suggesting that most mTBI-related brain aging had occured within ~7 days post-injury. Our results highlight the gravity of mTBI effects on brain aging in older adults and single out the acute stage of injury as critical for therapeutic interventions. This study was supported by the National Institutes of Health grant R01 NS 100973 to A.I., by the US Department of Defense contract W81XWH-18-1-0413 to A.I., by a Hanson-Thorell Family Research Scholarship, and by the James J. and Sue Femino Foundation.

Dj1 expression and dopamine deregulation in hiv-1 infected and antiretroviral therapy associated neurodegeneration.

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According to the world health organization, 37.7 million people worldwide in 2020 are living with the human immunodeficiency virus (HIV-1). In the US, combination antiretroviral therapy (cART) drugs have prolonged the lifespan of people with HIV aiding these patients to age with the virus. Consequently, the long-term effects of cART in the aging population are causing neuropsychiatric disorders such as depression. Among the aging population, depression is prevailing. Clinical evidence has established the prevalence of depression among cART treated patients. However, the underline molecular mechanism that cART induces depression-related neurodegeneration has not been established. This study is focused on pursuing a dopamine deregulation (DA) marker in cART treatment in HIV-1 infection through in-vitro conditions. Results suggest differentiated neuronal cell viability did significantly change with an increase in the concentration of cART for a long exposure of time. The morphology of neuronal cells treated at different concentrations indicates toxicity after long exposure at a high concentration compared to the control. Additionally, this study will establish the role of human protein DJ-1 as a neuronal marker. Characterizing the correlation between HIV-1 and cART mediated neurodegeneration will contribute to the early detection of depression. NIH Funded Grant Number, 1R01AI147731-01A1, 1R15NS108815-01.

Limiting polyamine production in Down syndrome fibroblast cells lead to reduction in cellular senescence.

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Down syndrome (DS) is a genetic condition characterized by a full or partial copy of chromosome 21 (T21) resulting in premature aging and a variety of neurodevelopmental and physical disabilities. Recent published data by others indicate that senescence-associated phenotypes in DS are linked to a global transcriptional dysregulation. The number of pathways leading to increased senescence in DS is broad and the many components involved are not well defined in their relationship with aging. Polyamines (PA), which are polycationic small molecules involved with cell proliferation, gene regulation, autophagy, and apoptosis, have been reported to be beneficial for aging. While a larger percentage of DS cells in a population becomes senescent faster than normosomic cells, we have found that they also carry a significantly increased presence of PAs. Thus, there is a lack of consensus regarding the role of PA in aging, and in this study, we investigate how PA impacts cellular senescence in DS using flow cytometry to reliably and rapidly measure levels of senescence and death. We prevented endogenous PA production through the addition of Î±-difluoromethylornithine (DFMO), an inhibitor for the enzyme ornithine decarboxylase, which is the rate limiting enzyme in the PA synthesis. Next, we added in exogenous PA (putrescine, spermidine, spermine) individually to investigate how each PA impacts senescence in human DS and control fibroblasts. Early experimentation showed neither PA nor DFMO alone was sufficient in altering senescence. Interestingly, when we combined DFMO with exogenous introduction of individual PA (putrescine and spermine), senescence in trisomic cells was significantly reduced. This data describes how the altered production of PAs relates to aging and related diseases. Further dissection of the role of PAs in DS-induced cellular senescence could reveal novel therapeutic targets for altering senescence rates, a common mechanism in many age-related diseases.Funding Agency: National Institute of Aging (AG070297)

A Complex I deficiency induces tau aggregation in mitochondrial disease mice.

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Mitochondrial dysfunction through reduced Complex I activity of the Electron Transport Chain is one of the earliest features of age-progressive tauopathies such as frontotemporal lobar degeneration and Alzheimer's Disease. However, it is widely debated whether mitochondrial dysfunction plays a causal role in tau proteotoxicity or if it is simply a consequence. Eliminating this ambiguity to understand the molecular pathways that initiate age-progressive neurodegeneration stands to illuminate innovative strategies to prevent these diseases. Mice missing the Complex I subunit NDUFS4 are a leading model of the pediatric neurometabolic mitochondrial disease Leigh Syndrome. These mice exhibit minimal Electron Transport Chain activity, reduced growth, neuroinflammation, and premature death within two months of birth. I observed age-progressive tau pathology in Complex I-deficient mice such as tau hyperphosphorylation and tau positive inclusion bodies in hippocampus. Reduced Complex I activity increases brain O2 in NDUFS4-KO mice to promote neuroinflammation and cause premature death. Consistent with this, housing NDUFS4-KO mice in low O2 chambers corrects hyperoxia to increase lifespan 10-fold. I used hypoxia as a probe to ask whether aberrant O2 status resulting from a Complex I deficiency underlies the observed tau pathobiology. I found hypoxia reduces oxidative stress and prevents tau aggregation in NDUFS4-KO mice and in transgenic nematodes expressing human tau. Collectively these data establish that mitochondrial dysfunction is implicated in initiating tau pathology and suggest that the abnormal tau speciation is dependent on O2 status. This presentation will discuss the O2-dependent tau pathology in mitochondrial disease mice, and will explore the mechanistic underpinnings that drive tau hyperphosphorylation and aggregation in these mice.
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Intrinsic mitochondrial function impacts the outcomes of metformin treatment on skeletal muscle mitochondrial morphology in aged rats.

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Aging is a risk factor for many chronic diseases. Metformin (Met) appears to slow aging, but evidence for this is not uniform. Our previous data suggest that Met benefits adults who are relatively insulin resistant and may have negative outcomes in those who are insulin sensitive. We hypothesized that the effects of Met on mito morphology and remodeling in skeletal muscle differ based on intrinsic mito function. Here we tested the effects of Met on mito morphology and remodeling in skeletal muscle based on intrinsic mito function. We used High- and Low-Capacity Runner (HCR/LCR) rat models, which are two selected lines of genetically heterogeneous rats that diverge for intrinsic exercise capacity, mito respiratory function, and longevity. We treated 16-month-old HCR and LCR male rats with Met in the drinking water for 28 days and compared to untreated controls (4-6 per group). To assess protein turnover, we labeled animals with deuterium oxide for seven days and analyzed hindlimb muscles. To determine differences in mito morphology, we electroporated DNA constructs that encode for mito targeted fluorophores into the tibialis anterior (TA) muscle. Normalized mass of the gastrocnemius (GA) and plantaris muscles were lower in Met treated, compared to untreated, HCR rats. Normalized mass of the TA muscle was lower in both Met treated rat lines compared to control. Bulk protein synthesis of the myofibrillar and mito fractions of the TA, GA, and soleus muscles were unaffected. The number of mito branches observed in a cross section of TA fibers was lower in Met-treated, compared to untreated, LCR rats. Mean CSA of each mito branch increased in Met-treated, compared to control, LCR rats. These data suggests that LCR rats with low intrinsic mito maintain mito CSA per fiber following Met treatment with the subsarcolemmal mito population more impacted than intermyofibrillar mito population. Met treatment had no effect on mito morphology in HCR rats. Our data support our hypothesis that Met differential effects in skeletal muscle based on intrinsic mito function. Funding: NIH Gerontology T32 Grant 5T32AG052363-04

A novel genetically heterogenous rat model (OKC-HETB/W) for aging research

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We have developed a new and unique genetically heterogeneous laboratory rat model that can be used to evaluate putative life- and health-extending interventions. The rat has numerous advantages over the mouse for interventional aging research including more human-like physiology and pathophysiology, more cognitive sophistication, and greater genetic diversity compared with standard mouse strains used in research. Inspired by the UM-HET3 mouse model, which is used by the Interventions Testing Program, our rat model (OKC-HETB/W) is also populations of genetically heterogeneous F2 descendants of 4 divergent, inbred strains. Based on the phylogenetic diversity as well as the commercial availability of inbred strains of rats, we chose the following 4 strains of rats: Brown Norway (BN), Fischer 344 (F344), Lewis (LEW), and Wistar Kyoto (WKY). The OKC-HETB/W rats are generated in two steps. First, two F1 lines are generated (BN/F344F1 and WKY/LEWF1). In the second step, these two F1 lines are used to generate two F2 lines. A significant difference from the UM-HET3 mice, our breeding scheme takes advantage of the ratâ€™s substantial mitochondrial genomic diversity compared with the mouse to create a population half of which carries the BN strain mitochondria (OKC-HETB), the other half carries the WKY strain mitochondria (OKC-HETW). These mitochondrial genomes mimic the human mitochondrial diversity in that they differ at 95 nucleotides involving 11 of 13 mitochondrial protein-coding genes, 5 tRNAâ€™s, and both ribosomal subunits. Our preliminary data show that OKC-HETB and OKC-HETW rats respond differently to exercise endurance, grip strength, and responsiveness to 17Î±-estradiol and show differences in the function of mitochondria isolated from skeletal muscle. A limited number of OKC-HETB and OKC-HETW rats will be made available to the research community to study the impact of nuclear and mitochondrial genetic diversity on pathways important in aging. Funding: NIH grant R21AG072137.

Transcriptomic changes in epigenetic regulators of oligodendrocyte differentiation in a monkey model of aging.

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Normal aging cognitive decline is free of neuronal death, but strongly associated with myelin loss in the primate brain. While myelin damage has likely a multifactorial cause, previous studies have shown that aging compromises the differentiation potency of old oligodendrocyte precursor cells (OPCs), and this is a bottleneck to remyelination in the aging brain. In addition, recent findings have revealed that epigenetic factors are key players to the fine tuning of gene expression during remyelination. Here we screened an array of epigenetic regulators that are known to affect OPC differentiation, to examine if they show age related changes in the Rhesus monkey brain. For this, we used fresh frozen tissue from 3 young (<10 years) and 5 old (>20 years) cognitively characterized male rhesus monkeys. We isolated total RNA and performed quantitative PCR analysis using primers against epigenetic regulators. To verify whether changes were specific to oligodendroglia, we applied RNA scope on a similar cohort of monkeys, using fixed frozen tissue cut in 30um slices. Results were analyzed relative to age and our Cognitive Impairment Index (CII). We found that the expression of histone deacetylase and DNA methyltransferase genes is reduced in aging, possibly allowing for the aberrant activation of genes in oligodendroglia. Together with our previous results showing decreased OPC differentiation potency in aging, we hypothesize that increased epigenetic gene activation in aging might promotes OPCs progenitor state, preventing their differentiation. These results collectively support the hypothesis that with aging, OPCs fail to differentiate into new mature oligodendrocytes, likely impairing remyelination in the aging brain. Interventions to stimulate OPC differentiation and promote remyelination could potentially reduce or reverse the accumulation of age-related myelin and damage associated cognitive decline. This study was supported by NIH/NIA grants 1RF1AG062831-01 and 2RF1AG043640-06.

The effect of reproduction on cellular senescence markers in the brain, liver, and skeletal muscle of wild-derived female house mice.

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Tradeoffs between reproduction and longevity observed across taxa are assumed to be reinforced by physiological constraints. According to the disposable soma theory (DST), females reallocate resources reserved for maternal somatic maintenance to offspring. This reduction in maternal somatic stores is hypothesized to incur a longevity cost through reduced efficacy in somatic maintenance, however, little evidence indicates reproduction affects the health, aging or longevity of soma. Cellular senescence (CS) commonly associated with cellular aging and age-related pathologies, is the result of mitotic cells reaching their Hayflick limit and can further be generalized as poor functioning cells lacking the ability to respond to external stimuli to accommodate post-mitotic senescent cell types. Senescent cells may affect the function of neighboring cells, can secrete senescence associated secretory phenotypes (SASPs) like interleukin 6 (IL-6), and can be mediated by nicotinamide adenine dinucleotide (NAD+) dependent histone deacetylase longevity marker, sirtuin-1 (SIRT1). Given resource redistributions occurring to maternal physiology during reproduction under the DST, we investigated the effect reproductive effort has on the senescence markers IL-6, NAD+, and SIRT1, within the brain, liver, and skeletal muscle of wild-derived house mice maintained in semi-natural enclosures. Hematoxylin and eosin (H&E) stains of the organs and mass measurements were also acquired to detect somatic abnormalities and changes in relative organ mass due to age, reproduction, and environmental factors. Supported by NSF-CAREER Award 1453784.

Soluble tau aggregates impair neurovascular coupling and cognitive outcomes.

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The brain consumes large amounts of energy but lacks energy reservoirs. The coupling between neuronal activity and increased blood flow (neurovascular coupling, NVC) that ensures the delivery of energy substrates ‘on demand’ to active neurons, is essential to brain function. Microvascular dysfunction occurs early in Alzheimer’s disease (AD), leading to chronic brain injury and neurodegeneration. Pathogenic forms of tau protein are causally implicated in AD and other dementias. We recently reported accumulation of pathogenic soluble tau aggregates (tau oligomers) in AD microvasculature. The functional consequences of brain microvascular tau accumulation in AD, however, are not yet understood. To define the impact of microvascular soluble tau aggregate accumulation on NVC and define mediation by this tau species, we used tau oligomer-specific monoclonal antibody (TOMA) immunotherapy to remove soluble tau aggregates, but not other forms of tau, from mice expressing non-mutant human tau (hTau mice) that specifically model tauopathy in AD. We found that profound NVC dysfunction in hTau mice was driven by malfunction of the neuronal isoform of nitric oxide synthase (nNOS), critical for NVC. Decreased nNOS activation may be due to a pathogenic tau-induced defect in Ca2+ signaling. A single course of TOMA immunotherapy in hTau mice at midlife prevented nNOS dysfunction at later stages of AD-like disease, restoring NVC as well as spatial and recognition memory. Taken together, our data indicate that soluble tau aggregates impair nNOS activation and thus drive NVC and cognitive impairment in a model of AD tauopathy. Tau immunotherapy may thus have potential to treat AD and potentially other tauopathies. NIA RF1AG057964-01, Merit Review Award 5I0 1BX002211, NCATS CTSA UL1TR002645.

How to Age With Elegans: Do environmental stressors affect aging differently in combination vs. alone?

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How to Age With Elegans: Do environmental stressors affect aging differently in combination vs. alone? Bradford Hull 1, Allison Michael 1, George Sutphin 1. 1 University of Arizona MCBCells encounter various forms of stress over time–oxidative stress, protein misfolding, DNA damage–and respond by activating specific, well-defined stress response pathways. As we age, the burden of stress increases while our cells' ability to deal with the resulting damage becomes diminished due to dysregulation of cellular stress response pathways. The majority of past work has focused on understanding responses to individual stressors. In contrast, how pathology and stress responses differ in the presence of multiple concurrent stressors is relatively unknown. We are working to understand how the presence of multiple distinct stressors alter the cellular stress response and impact downstream pathology. As part of a broader screen designed to identify stress combinations that produce non-additive effects on survival in Caenorhabditis elegans, we observed antagonistic toxicity (i.e. longer than expected survival) when copper sulfate (a heavy metal/oxidative stress inducer) was combined with dithiothreitol (DTT, an endoplasmic reticulum/misfolded protein stress inducer), golgicide A (a Golgi stress inducer), or sodium chloride (NaCl, a hyperosmotic stressor), suggesting an interaction either between the mechanisms of toxicity or the cellular response to copper sulfate and DTT/golgicide A/NaCl. We are now evaluating the impact of copper sulfate, DTT, golgicide A, and NaCl individually and in combination on oxidative, unfolded protein, Golgi, and osmotic stress response pathways to understand the underlying mechanism of the interactions above. We aim to identify the specific molecular mechanisms that mediate the interaction observed in the context of survival. Ultimately, this project will aid in building a comprehensive stress network around copper sulfate that can be used to identify key intervention points and developing targeted treatments for age-associated disease related to copper sulfate/oxidative stress. More broadly, by building a more comprehensive understanding of how cells respond to the complex combinations of stress encountered during normal physiology we aim to slow age-associated deterioration and develop treatment targets for age-associated disease.

Mitochondrial membrane potential is required for the lifespan-extending effects of dietary restriction in C. elegans.

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Overview: Mitochondria are conceptually central in many theories of biological aging; mitochondrial dysfunction is one of the well-investigated hallmarks of aging. Because mitochondria are such multifunctional organelles, longstanding hypotheses on their role in physiologic decline with age remain untested. Specifically, the electrochemical gradient that powers ATP production in mitochondria has been difficult to control and study. The gradient consists of a mitochondrial membrane potential (MMP), which regulates ATP production and various mitochondrial signaling mechanisms. Aim: MMP decreases with age in cells and tissues. We hypothesized that targeting MMP experimentally may reveal mechanisms of mitochondrial control over aging. Dietary restriction is a potent longevity intervention that is thought to function through incompletely understood metabolic pathways. Our objective was to test if DR affected, or could be affected by, changes in MMP. Method: We directly controlled MMP using pharmacology and genetics in C. elegans to test whether longevity following DR required MMP, specifically. We also tested if MMP affected autophagy in the context of DR. Finally, we used optogenetics to artificially control MMP using light to test if MMP in isolation was sufficient to affect lifespan. Results: We confirmed MMP decreases with age in worms. Longevity following DR was sensitive to decreased MMP, indicating that proper MMP regulation is required for the benefits of DR. Further, increasing MMP in isolation from other metabolic changes increased lifespan. These results showed that MMP is a fundamental driver of biological aging, and that targeting MMP therapeutically may be possible. 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.

Mapping the ketogenic system across ages, sexes, and diets.

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Understanding how our cells maintain energy homeostasis has long been a focus of aging biology. A decline in energy metabolism is central to many age-related diseases such as Alzheimerâ€™s disease, heart failure, frailty, and delirium. Intervening on pathways involved in energy homeostasis can extend healthy lifespan. When glucose is scarce, ketone bodies (i.e. beta-hydroxybutyrate (BHB), acetoacetate, acetone) are used. Aging is associated with glucose intolerance and insulin insensitivity, yet what role ketone body metabolism might play in compensating for impaired glucose utilization in age-related diseases is understudied. Here, we investigated how the bodyâ€™s endogenous ketone body production and utilization pathways are modulated by age across the lifespan of female and male C57BL/6N mice (3 mo. old, 12 mo. old, 22 mo. old). We show that metabolic changes between fasting and diet-induced ketogenesis are sex dependent. In fasting, females had a higher ketogenic capacity, resulting in higher plasma BHB levels. However, in fed-conditions, plasma BHB levels were higher in males but not females. Furthermore, we show that hepatic ketogenesis declines in normal aging, but could be increased with a ketogenic diet. We further demonstrate that tissues that undergo ketolysis could also produce ketone bodies to compensate for the age-related decline in hepatic ketogenesis we observed. Overall, we show that sexes, ages, and diets all influence ketone body metabolism and that older animals use ketone bodies to meet energetic demands. Additionally, the differences in ketogenic capacity in females and males also raise important questions about the efficacy of ketogenic therapies in aging. Financial Disclosure: NIA T32 AG052374, NIA R01 AG067333.

White matter fractional anisotropy in a nonhuman primate model of aging: relationships with social status and physiologic measures of stress.

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Psychosocial stress is associated with increased risk of pathological brain aging, and white matter (WM) may be particularly vulnerable to the effects of chronic stress. Cynomolgus macaques have complex central nervous systems and naturally develop neuropathologies with age. Female macaques form social status hierarchies in which subordinates exhibit behavioral and physiological signs of stress, and increased stress-related pathologies similar to humans. Thus, these primates are useful translational models for studying the effects of stress on WM integrity. We used diffusion tensor imaging to examine the relationship between psychosocial stress and fractional anisotropy (index of WM microstructural integrity (FA)) in 38 middle-aged females living in small social groups. We classified subjects as either dominant or subordinate based upon the outcomes of agonistic interactions. We assessed FA in relation to social status and stress physiology (blood pressure, resting cortisol, and heart rate variability (HRV)) in several regions of interest (ROIs) including the splenium, body and genu of the corpus callosum (CC), frontal, temporal, occipital, and total cerebral, and cerebellar WM. Repeated measures ANOVA showed that dominants had significantly higher FA than subordinates in the genu and splenium of the CC, and the occipital and frontal cortices (all p<0.05). Dominants also had higher FA, on average, in the body of CC (p=0.07) and cerebrum (p=0.10). Dominants and subordinates did not differ in temporal or cerebellar FA (both p>0.7). FA was inversely correlated with blood pressure, directly correlated with HRV (pâ€™s<0.05), but not correlated with serum cortisol. The psychosocial stress of subordination and physiologic indices of stress were associated with diminished WM integrity in several brain regions. These studies lay the groundwork for investigations of interventions on psychosocial stress to reduce risk of pathological brain aging. Funding: NIH R01HL087103, RF1AG058829, R01 HL122393, U24 DK097748, T32AG033534, WFADRC P30AG049638, WF Claude D. Pepper OAIC P30AG012332, and the Department of Pathology, WFSM.

Identifying FDA approved drugs that inhibit mTOR signaling.

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Toxicity is one of the major reasons that drug development projects fail. Repurposing existing clinically approved drugs has the potential to circumvent many of the safety and regulatory challenges and cut the massive cost of drug development. Some of the interventions found to extend the lifespan of mice via the National Institute of Aging Interventions Testing Program (ITP) are drugs that were clinically approved for other uses. One of these drugs is rapamycin, a known inhibitor of the mTOR protein kinase, an important conserved regulator of cell growth implicated in aging across species from yeast to mice. Rapamycin is now a promising drug with the potential to increase human healthspan and lifespan. However, rapamycin has some risk of adverse side-effects that warrant the development of additional inhibitors of mTOR. TOR inhibitors, including rapamycin-like inhibitors, can be identified by comparing growth sensitivities of wildtype yeast, tor1, and fpr1 mutant strains. Yeast mutants lacking TOR1 are sensitive to rapamycin and other mTOR complex I inhibitors, while mutants without FPR1 are resistant to rapamycin due to rapamycin forming a complex with Fpr1 for its specific mechanism of Tor1 inhibition. We are using yeast growth assays to screen through a commercially available library of Federal Drug Administration (FDA) approved drugs to help seek out clinically safe drugs that might have an off-target effect on the mTOR pathway and potential impacts on longevity. Funding: BMW was funded by an Impetus Longevity Grant (Norn Group)

Hypoxia-inducible factor-2a increases with age in hippocampal astrocytes

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Our lab reported basal levels of hypoxia-inducible factor-alpha isoforms 1 & 2 (HIF1a & HIF2a) increase with age in mouse hippocampus. HIF1a & HIF2a are preferentially expressed by neurons or astrocytes, respectively, and astrocytic HIF2a is integral to memory recall. Depending on isoform presence, genes transcribed by HIF are cell specific with HIF1a initiating glycolytic genes (Pdk1, Pgk1, Ldha, Cox4i1, Cox4i2) and either isoform transcribing transporter genes (e.g. Glut1) and trophic genes (e.g. Epo and Vegf). Changes in astrocytic HIF2a may diminish neural support and precede aging-related cognitive deficits, but exact roles for each isoform within aging hippocampal cell types have not been fully studied. To investigate the hypothesis that HIF2a expression in hippocampal astrocytes changes with age and affects HIF-associated gene expression, we combined 2-3 hippocampi per sample followed by magnetic cell sorting to isolate primary hippocampal astrocytes (HA) from 3-month, 9-month, and 18-month C57bl6/J mice. Samples (n = 3-4 per age) were probed for protein expression of HIF1a and HIF2a using ELISA and mRNA expression of Hif1a, Epas1, Pdk1, Pgk1, Ldha, Cox4i1, Cox4i2, Glut1, Vegfa, Epo, and EpoR using PCR. In 3-month HA, HIF1a and HIF2a protein concentration was comparable, but HIF2a increased 4- and 5-fold in 9- and 18-month HA. Further, Hif2a, Cox4i2, and Vegfa mRNA exhibited inverted U-shaped curves with significant elevation of these genes in 9-month HA. Pdk1 increased in a stepwise fashion with age whereas Cox4i1 was 50% elevated at both 9- and 18-months. No significant differences were observed in Hif1a, Pgk1, Glut1, Ldha, Epo or EpoR between age groups. These results suggest HIF2a becomes the predominate isoform in aged HA, and expression of genes transcribed by HIFa is altered with age. Because the genes are in glycolytic and trophic pathways, these results may reflect mechanisms by which astrocytes become more reactive during aging and less capable of providing neuronal support within the hippocampus. Future studies will be focused on determining which mechanisms serve to elevate HIF2a in aged astrocytes and how HIFa accumulation in the hippocampus affects synaptic transmission. Contributed equally. Funding provided by the Margaret Milam McDermott Foundation

Unacylated ghrelin ameliorates muscle wasting and contractile dysfunction in age-associated loss of muscle mass and function

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Sarcopenia, the progressive loss of muscle mass and dysfunction, universally affects the elderly and is closely associated with frailty and reduced quality of life. Despite the inevitable consequences of sarcopenia and its relevance to healthspan, no pharmacological therapies are currently available. Ghrelin is a gut-released hormone that increases appetite and body weight through acylation, which binds to and activates its receptor, GHSR1a, in the brain. Growing studies demonstrate that both acyl and unacylated ghrelin have direct impacts on muscle proliferation, fusion, and growth, independent of receptor activation. We hypothesized that unacylated ghrelin ameliorates muscle atrophy and contractile dysfunction in aged skeletal muscle. Young and middle-aged female mice were treated with saline or unacylated ghrelin for 10 months beginning at 4 and 18 months respectively. Unacylated ghrelin did not affect food consumption or body weight as expected. Muscle weights of gastrocnemius and quadriceps were ~20-30% decreased in old mice (28 month) compared to adult mice (14 month), but unacylated ghrelin ameliorated the loss by 15-30% for both muscle groups. Specific force (force per cross-sectional are) of isolated extensor digitorum longus muscle was diminished by ~30% in old mice, but unacylated ghrelin prevented the force deficit by ~80%. RNA-seq analysis further revealed that unacylated ghrelin shifted mRNA expression of 366 genes towards younger states that are differentially regulated in old mice. We further identified that unacylated ghrelin led to upregulation of protein kinase A and hyper-phosphorylation of FoxO3a signaling pathways. Unacylated ghrelin also improved mitochondrial respiration specifically from complex I, while reducing generation rate of mitochondrial reactive oxygen species. Our data demonstrate a direct role of unacylated ghrelin in sarcopenia independent of changes of food consumption or body weight, implicating unacylated ghrelin as a potential therapeutic strategy that can enhance quality of life and healthspan of growing elderly population.

Sialylation of Microglia and Alzheimer's Disease Substrates

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Patients with age-related neurodegenerative diseases such as Alzheimer's Disease (AD) have reported acute adverse neuropsychiatric side effects when treated with the antiviral agent Tamiflu. We have observed a specific form of sialylation, alpha 2,6 sialic acid (SA) bonds, near amyloid Beta (Abeta) plaques in both 5XFAD mouse model and human AD cases. We hypothesize that Tamiflu's mechanism as a sialidase inhibitor blocks the removal of SA residues and leads to an increase in sialylation levels near Abeta plaques leading to decreased plaque clearance. We investigated the effects of Tamiflu (or vehicle) treatment on 5XFAD mice and wild-type (WT) littermates. After initial oral treatment, mice performed Open Field (OF) behavioral task for anxiety-like behaviors. Following drug administration, Novel Object Recognition (NOR) and a second OF test were conducted. Mice were euthanized and brain tissue was immunostained for Abeta plaques, microglia, and SA. A 3-way ANOVA (treatment x genotype x timepoint) revealed no effect of Tamiflu treatment but 5XFAD mice exhibited greater velocity compared to WT. 5XFAD mice lacked habituation behavior in OF with no decrease in velocity over time compared to WT. Investigation of Abeta plaque pathology with IHC indicated no Tamiflu effect on plaque area nor total plaque count. A 2-way ANOVA (genotype x treatment) demonstrated 5XFAD mice have significantly increased alpha 2,6 SA levels compared to WT mice across brain regions, yet there was no Tamiflu treatment effects. Our findings confirm 5XFAD mice are hyperactive and lack normal habituation. The significant increase in alpha 2,6 SA in 5XFAD mice compared to WT suggests an interesting disease associated increase in sialylation and that SA distribution is localized within the plaque microenvironment. Planned analyses will closely examine the distribution of SA and compare SA levels in plaque-associated microglia to better understand the role of sialylation in microglial interactions with disease pathology. Supported by Biology of Aging T32 AG021890; Reed Precision Medicine Fund, Biggs Institute; NIA R21AG072423 and NIA K01AG066747 to SCH.

An LSD1 histone demethylase inhibitor as a potential 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. LSD1 is an H3K4me1/me2 and H3K9me1/me2 demethylase. LSD1 inhibitors are currently being investigated to treat certain cancers. LSD1 also targets non-histone proteins such as p53 and RelA/p65, which contribute to senescence and the secretory phenotype of senescent cells. Our results suggest first that LSD1 inhibition in senescent cells reduce their pro-infammatory SASP via downregulation of NF-kB activation and induces senolysis by p53 activation. In addition, H3K9 methylation has been shown to play a key role in aging and cellular senescence. Therefore, we hypothesized that removal of LSD1 by using an inhibitor rescues H3K9me1/2 levels and abrogates SASP expression. This hypothesis is currently being tested by performing a ChIP experiment. Finally, our novel Lsd1 inhibitor will be tested in a progeria mouse model to corroborate its senotherapeutics effects in vivo. Keywords: Aging, Epigenetics, senolytic, histone lysine methylation. Acknowledgements: This work was supported by NIH grant U19 AG056278.

Sex-specific reduction of aging kidney dysfunction with long-term mitochondrial protection in the context of Western diet.

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NCIn humans, aging compounded with a Western diet is associated with higher rates of kidney disease and it has been proposed that preventing mitochondrial dysfunction is key to reducing renal decline. Previously, we showed that an 8-week systemic treatment of aged 24-month-old (mo) male mice with a mitochondrial targeted protective tetrapeptide, Elamipretide (ELAM) significantly decreased kidney glomerulosclerosis typical by 26-mo. As these studies did not extend to female mice, we sought to determine the effect of longer ELAM intervention in both sexes. To exacerbate kidney injury 18-mo male and female NIA C57bl/6 mice were fed regular chow (RC) or a high fat, high sucrose diet (HFHS) for 10 months and treated with ELAM or saline vehicle. Urines were collected monthly to assess kidney function with kidneys harvested at 28-mo end point. Our results show that damage in the kidney due to age and diet is ameliorated by ELAM intervention but manifests differently in males and females. Both sexes gained significant weight on HFHS diet, with maximum male weight gain at a 40%, and females at an 80%, increase of starting weight. In both sexes, ELAM significantly reduced HFHS weight gain. In males, kidney injury as urinary albumin to creatinine ratio (ACR Âµg/mg) increased in HFHS fed mice relative to RC fed mice (HFHS = 519.9 vs. RC = 125.9; p<0.0001) and was significantly reduced by ELAM (312.7; p = 0.036 vs HFHS saline). ACR did not correlate to weight, thus protection by ELAM was not secondary to lower weight. While ACR in HFHS female mice did not significantly increase, kidney glomeruli from HFHS+ELAM female mice had significantly lower expression of kidney podocyte injury marker, Desmin, than in HFHS saline mice. Desmin was not reduced by ELAM in male mice. Finally, kidneys of HFHS+ELAM mice had reduced macrophage marker F4/80 relative to HFHS saline mice in both sexes. These data show the need to assess mito-driven kidney aging and therapeutics in males and females, as indicators of dysfunction differ between sexes. NIA P01 AG001751, NIA K01 AG062757

The longevity-promoting intervention 17Î±-estradiol protects against aging phenotypes in APOE4 mice

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The two largest 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. APOE genotype may be linked to AD phenotype through the regulation of aging processes. The NIA Interventions Testing Program found that 17?-estradiol (17?E2) treatment can increase male mouse lifespan. Since 17?E2 has been shown to act upon systemic and neural pathways that have also been associated with AD pathology, we propose that 17?E2 may constitute a pleiotropic intervention strategy. Further, because APOE4 is associated with age-related phenotypes, 17?E2 may preferentially improve outcomes in the context of APOE4 genotype. We maintained 10-month-old APOE3 or APOE4 targeted replacement male mice on normal chow in the absence or presence of 14.4 ppm 17?E2 for 20 weeks. Our results indicate genotype differences in the impact of 17?E2 across multiple outcomes. APOE4 mice exhibited an aged phenotype compared to APOE3, with higher frailty and impairments in multiple metabolic measures. Treatment with 17?E2 treatment yielded improvements in both APOE genotypes but with greater effects in APOE4 mice on several measures including body weight, plasma leptin, and hepatic steatosis. Plasma shotgun lipidomics showed 17?E2 diminishes genotype-specific differences between the treated groups. Interestingly, transcriptomic analysis of isolated microglia from both APOE3 and APOE4 mice revealed that 17?E2 treatment downregulated genes relating to inflammation and upregulated those in metabolic pathways. These data confirm and extend prior findings that APOE4 is linked to progeroid effects both peripherally and neurally, outcomes associated with AD risk. Importantly, although 17?E2 significantly improved a range of measures across genotypes, it showed the strongest effects in the APOE4 genotype. This research was funded by the Cure Alzheimer's Fund.

Oolonghomobisflavans from Camellia sinensis increase Caenorhabditis elegans lifespan and healthspan

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Tea polyphenols are widely considered as excellent antioxidant agents which can contribute to human health and longevity. However, the identification of the active biomolecules in complex tea extracts that promote health and longevity are not fully known. Here we used the nematode Caenorhabditis elegans to analyze the health benefits and longevity effects of Camellia sinensis oolong tea extracts (QFT, NFT and CFT) and oolonghomobisflavan A (OFA) and oolonghomobisflavan B (OFB), which are present in oolong tea extracts. Our results showed that oolong tea extracts and oolonghomobisflavans prolong lifespan and improved healthspan by curtailing the age-related decline in muscle activity and the accumulation of age pigment (lipofuscin). We found that the lifespan and healthspan promoting effects of oolong tea extracts and oolonghomobisflavans were positively correlated with the stress resistance via DAF-16/FOXO transcription factor. Furthermore, oolong tea extracts and oolonghomobisflavans displayed protective effects against AÎ²- and polyQ-induced neuro/proteotoxicity. Overall, our study provides new evidence to support the health benefits of oolong tea and importantly identify oolonghomobisflavans as potent bioactive molecules that promote health when supplemented with a normal diet. As such, oolonghomobisflavans represent a valuable new class of compounds that promote healthy aging.Funding Acknowledgements: This work was funded by the NIH R01AG058610 to S.P.C.

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 conclusions regarding CICI pathomechanism. Objective: To address this issue, our goal was to address the pathomechanism of chemobrain by establishing an innovative mice model, i.e., cancer-free p16-3MR mice treated with the chemotherapeutic drug paclitaxel (PTX). Result: In vivo P16-3MR senescence-reporter mice model was subjected to a clinically relevant protocol of PTX treatment. To establish a timeline of PTX-induced chronic cognitive impairment, we assessed the effect of senolytic treatments on six months post-PTX treated mice using a radial-arm water maze test—the output from this test help to encode the function of learning and memory. Our findings confirm that both learning plasticity and memory are impaired after PTX treatment. (n=20 each group, p<0.05). Further, microscopic examination of the brain was done to assess functional hyperemia responses in vivo. Endothelial-dependent cerebral blood flow (CBF) responses (p<0.05) and BBB integrity (p<0.05) were subsequently observed to be compromised in PTX treated mice. Moreover, treatment with PTX has also been observed to cause vascular rarefaction in the somatosensory cortex (p<0.05). Although the majority of chemotherapeutic drugs do not cross BBB, the endothelial cell lining of cerebromicrovasculature (CMVECs) is directly exposed to the highest concentrations of these drugs, making them uniquely vulnerable to drug-induced DNA damage leading to senescence. Therefore, to identify cell types undergoing PTX induced senescence in the brain, we sorted the p16-RFP+ cell fraction. We found a significantly increased percentage of CD31+/p16-RFP+ cells (40%), indicating that PTX induces significant endothelial senescence. Conclusion: Our findings suggest that senescent cells induce vascular changes in response to chemotherapy drugs leading to cognitive dysfunction.

Evaluation of changes in oral health during aging in a novel non-human primate model

Wang, H1, Abdul-Azees, PM1, Pizzini, J1, Dean, DD1, Reveles, KR1, Chun, Y-H1, Chen, X-D1, Salmon, AB1,2, Yeh, C-K1,2. 1 University of Texas Health Science Center at San Antonio & 2South Texas Veterans Health Care System, San Antonio

Evaluation of changes in oral health during aging in a novel non-human primate modelWang, H1, Abdul-Azees, PM1, Pizzini, J1, Dean, DD1, Reveles, KR1, Chun, Y-H1, Chen, X-D1, Salmon, AB1,2, Yeh, C-K1,2. 1 University of Texas Health Science Center at San Antonio & 2South Texas Veterans Health Care System, San AntonioObjective: Oral health is a major contributor to nutritional intake and frailty in older adults. Thus, poor oral health is considered a geriatric syndrome. However, the effect of aging on the biology of oral tissues is still unclear due to a lack of appropriate animal models. Here, we evaluated the oral health of a short-lived non-human primate (i.e., marmoset) as a potential aging model for human oral health. Methods: Oral health was assessed in live young marmosets (<7 years; n=7) and young (<7 years; n=6) and old (>9 years; n=6) marmoset cadavers. Oral diseases and other criteria for oral health were documented in both live and cadaveric animals. 16S rRNA analysis of the microbiome was performed using gingival tissues from cadaveric animals. Results: In live young animals, average salivary secretion was 17.3 ± 8.5 µL/min, gingival crevicular fluid was 1.42 ± 0.62 µL/min, and dental plaque collected was sufficient for microbial assays. Older animals had higher levels of tooth loss (5.2 ± 2.5 vs. 2.6 ± 1.4, p=0.16), dental caries (1.3 ± 0.4 vs. 0.38 ±0.38, p<0.02) and dental attrition/erosion (3.2 ± 1.3 vs. 0.92 ± 0.54, p<0.001) than younger animals. Microbiome analysis of gingival tissues from the marmoset revealed the presence of bacteria from 10 phyla, 24 classes, 44 orders, 84 families, 139 genera, and 291 species. Older marmosets had a higher, but statistically insignificant, average Chao1 (40.32 vs. 28.27, p=0.918) and Shannon (3.89 vs. 3.62, p=0.757) diversity index than young marmosets. Older marmoset tissues had a lower average abundance of Moraxallaceae (0.89 vs. 9.86, p=0.046), Acinetobacter (0.78 vs. 9.63, p=0.077) and Acinetobacter bouvetii-johnsonii-schindleri (0 vs 5.49, p=0.043) than those from young marmosets. Conclusion: We have established methods for collecting oral samples from live and cadaveric marmosets to assess changes in oral physiology with aging. Like humans, marmosets develop more caries and wear/attrition with aging. These results support the notion that marmosets may be a good pre-clinical model for better understanding the impact of aging on oral health. (Supported by NIA P30AG044271 and NIDCR DE028271A)

Beta-guanidinopropionic acid, a creatine analog, induces mitochondrial genotoxicity and myopathy in skeletal muscles of aged rats

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Beta-guanidinopropionic acid (GPA) is a creatine analog suggested as a treatment for hypertension, diabetes, and obesity, which manifest primarily in older adults. A notable side effect of GPA is the induction of mitochondrial DNA deletion mutations. We hypothesized that mtDNA deletions contribute to muscle aging and used the mutation promoting effect of GPA to compare the impact of mtDNA deletions on muscles with differential vulnerability to aging. Rats were treated with GPA for four months starting at 14- or 30-months of age. We examined quadriceps and adductor longus muscles as quadriceps exhibit profound age-induced deterioration, while adductor longus is maintained. GPA decreased body and muscle mass and mtDNA copy number while increasing mtDNA deletion frequency with the greatest effects on the quadriceps. The interactions between age and GPA treatment observed in the quadriceps were not observed in the adductor longus. GPA increased the number of mtDNA deletions and muscle aging phenotypes in the quadriceps, an age-sensitive muscle, while the adductor longus was spared. GPA has been proposed for use in age-associated diseases, yet the pharmacodynamics of GPA differ with age and include the detrimental induction of mtDNA deletions, a mitochondrial genotoxic stress that is pronounced in muscles that are most vulnerable to aging. Further research is needed to determine if the proposed benefits of GPA treatment for hypertension, diabetes, and obesity outweigh the detrimental mitochondrial and myopathic effects.FUNDINGThe National Institutes of Health R56AG060880, R01AG055518, K02AG059847, R21AR072950 and UCLA CFAR 5P30 AI028697.

Skeletal muscle mitochondrial ADP sensitivity correlates to indices of metabolic health in humans

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The dogma that mitochondria dysfunction is implicated in the progression of age and age-associated metabolic dysfunction is challenged by equivocal measurements of maximal mitochondrial respiratory capacity. Recent evidence in insulin resistant mice suggests mitochondrial respiration at sub-saturating ADP concentrations and the apparent sensitivity of ADP is impaired but this relationship has not been explored in humans. We tested the hypothesis that skeletal muscle mitochondrial ADP sensitivity is related to several indices of metabolic health in middle-aged to older men and women without diabetes (N=33, Age 43-70, BMI 19.4-40.2 kg/m2). We performed a fasting blood sample, skeletal muscle biopsy, and 3-hr hyperinsulinemic-euglycemic clamp. We used high-resolution respirometry with titration of sub-saturating to saturating ADP concentration (0.125 to 12mM) to evaluate the apparent Km of ADP (ADP sensitivity), submaximal and maximal coupled oxidative phosphorylation (CIP, CI+IIP). Mitochondrial ADP sensitivity was correlated (P<0.05) to fasting plasma insulin (r=0.51), HOMA-IR (r=0.53), and HbA1c (r=0.38). Similarly, mitochondrial respiration at sub-saturating doses of ADP (0.125 and 0.25 mM) and maximal oxidative phosphorylation correlated (r=0.62, 0.60, 0.53, P<0.01) to the glucose infusion rate required to maintain euglycemia during the insulin clamp. Additionally, we found no correlation with sub-maximal respiration or maximal CIP and CI+IIP to insulin, HOMA-IR, or HbA1c. Here, we identified a correlation of mitochondrial ADP responsiveness to several indices of insulin sensitivity within a group of relatively healthy middle-aged to older adults. Future work should 1) include a larger sample size to confirm these initial relationships between mitochondrial ADP sensitivity and metabolic health and 2) focus on mechanistic basis of whether changes to mitochondrial ADP sensitivity contribute to aging and age-related chronic conditions.Funding: NIH R01AG064951

Investigating the effects of pathogenic tau on nuclear tension

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Investigating the effects of pathogenic tau on nuclear tension Claira Sohn, Bess Frost Ph.D.University of Texas Health San Antonio Background: Nuclear biophysics is an emerging field focused on the nucleus as a mechanosensor. Nuclear mechanotransduction is a process in which the nucleus transforms mechanical signals into biochemical responses. Within the aging neuron, the nucleus has been observed to have a less stiff structure. While the nuclei of human Alzheimer's disease brain, brains of Drosophila models of tauopathy, and iPSC-derived neurons from patients with tau mutations harbor nuclear invaginations and blebs, it is currently unknown if age or tau-associated morphological changes to the nucleus affect nuclear biophysics, tension, and subsequent mechanotransduction. Methods: I have developed an in vivo FRET-based nuclear tension sensor in Drosophila melanogaster. I am using this tool to evaluate nuclear tension as a function of age in the tau transgenic Drosophila. In addition, I am utilizing a cell culture-based nuclear tension sensor to determine if nuclear tension is dysregulated as a consequence of pathological forms of tau and if changes to nuclear tension can be restored by pharmaceutical and genetic interventions. I also plan to over-express Progerin in the cell culture-based nuclear tension sensor to observe if that rescues the nuclear morphology and structure. Results: I find an age-dependent decrease in nuclear tension in neurons of the Drosophila brain. Ongoing experiments are designed to determine the effects of pathogenic tau on nuclear tension using in vivo and cell-culture based approaches. I speculate that the rigidity of the culture environment alters nuclear tension and that nuclear tension will be altered by pathogenic forms of tau.Conclusion: Natural aging and pathological forms of tau are known to cause structural changes to the nucleus, and these changes to the nuclear morphology are causal factors driving further neurodegeneration. A FRET-based nuclear tension sensor will allow for a greater understanding of the role of pathological tau on mechanotransduction and nuclear stability.

Investigating P19INK4D Associated Neuronal Senescence in ALS

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Amyotrophic Lateral Sclerosis (ALS) is an age-associated neurodegenerative disease mainly characterized by the denervation of musculature. We discuss evidence for overlap between age and ALS-associated hallmarks, potentially implicating cell type-specific ageing as a key contributor to this multifactorial and complex disease. ALS is always fatal, with its progression including the loss of both upper and lower motor neurons. Motor neurons are vulnerable to age-related phenotypes, with ageing likely a prerequisite to ALS, leaving aged neurons susceptible to disease-specific mechanisms. The SOD1G93A mouse model of ALS has revealed that motor neurons lose contact with their target muscles, have decreased mitochondrial function, altered RNA and impaired protein homeostasis, but without apoptosis until well after denervation. This observation led to the investigation of senescence as the underlying process driving these dysfunctions with the evasion of cell death. Cellular senescence is a complex cellular aging stress response to genotoxic damage or cellular stress where cells become apoptosis resistant and exhibit a senescence associated secretory phenotype (SASP) that is detrimental to surrounding cells. Previously, our lab had found that the most profoundly upregulated marker in senescent brain tissue from older adults was P19INK4D, a CDK inhibitor/ tumor suppressor, that regulates p53. It functions by responding to oncogenic stresses by interfering with the inhibitory effects of Mdm2 on p53, thereby enhancing p53 activity and its antiproliferative functions. The SOD1 mouse model showed an age-associated increase in p19 expression whereby 38.37% of motor neurons were p19 positive at 60 days old and 60.07% of SOD1 motor neurons at 80 days old, with 0% in the WT controls. Similarly, evaluating postmortem human tissue from Veterans with ALS to has revealed p19 expression. High resolution digital spatial profiling has revealed that p19-expressing cortical neurons in ALS display canonical features in ALS to provide evidence for p19 and neuronal senescence as a conserved stress response across age-associated neurodegenerative diseases.

Ambulatory and cognitive function in aging marmosets.

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The common marmoset (Callithrix jacchus) has recently been developed as a model to study and explore the biology of aging. While a number of new tools have been developed to study age-related change in marmosets, few tools existed to evaluate impairment in marmoset ambulatory and cognitive function. The Noldus Catwalk was originally designed for use in small rodents, such as mice, but it can be modified to fit adult marmosets. In order to determine if aged marmosets exhibit impaired mobility we have evaluated the gait of 17 marmosets ranging in age from 4-17 years (young adult to geriatric). Many gait measurements differ significantly between young and old individuals, for example swing pace (seconds) (young = .27+.13, old = .18 + .04; $F(1,18)=4.587$, $p=0.046$); and base of stance (cm) (young =2.07+.23, old = 3.65+.72; $F(1,18)=22.35$, $p=0.000$) suggesting evidence of impaired ambulation in aged marmosets. To evaluate if executive function (working memory and impulse control) varies with age in marmosets, we have employed two tasks, the detoured reach task, and the conveyor belt. Geriatric animals are slower to reach criteria of learning for both tasks and have increased prevalence of persistence errors. The ability to assess both ambulatory function and cognitive decline in geriatric marmosets enhances their use as a model for aging sciences. This work was supported by NIH U34AG068482, NIH P30AG044271, and NIH P51OD011133.

Skeletal Muscle Circulating Factors as Novel Regulators of CNS Aging

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Exercise is a powerful behavioral intervention against Central Nervous System (CNS) aging and plays an essential role in maintaining healthy neurocognitive function and immune metabolism in the aging brain. We have developed a new transgenic mouse model that moderately over-expresses transcription factor master regulator of proteostasis, Transcription Factor E-B (TFEB), in skeletal muscle (cTFEB;HSACre mice). Despite living sedentary lifestyles, our model shows significantly ameliorated proteotoxicity, increased BDNF levels and neurogenesis while improving neurocognitive function and decreasing neuroinflammation in the aging CNS. These neuroprotective effects are markedly reminiscent of those observed in the aging CNS post-exercise, suggesting enhancing muscle proteostasis may be sufficient to replicate the local and systemic benefits of exercise. Skeletal muscle is a powerful endocrine organ, secreting bioactive molecules, cytokines, known as â€˜myokinesâ€™, such as irisin/FNDC5 and cathepsin B that can modify CNS metabolism and function, which very likely contribute to the exercise-associated benefits on cognition. Exercise activates the production and secretion of these myokines from skeletal muscle into circulation, directly implicating skeletal muscle metabolism in the CNSâ€™s response to exercise. However, to date, the precise origin and function of these exercise-responsive, pro-neurogenic circulating factors remains largely unexplored. Characterizing a list of circulatory factors, influenced by the over-expression of TFEB in skeletal muscle, we will be able to cultivate a list of possible therapeutic targets for the stresses of aging on the CNS. Utilizing an array composed of highly-spoken about inflammatory and growth markers, I will determine the expression of 96 specific proteins in circulation, via serum, and in the muscle of young and aged mice. Upon completing the analysis of this data, I will then perform a correlation analysis of the most heavily increased myokines in circulation and muscle, to that of RNA data gathered from the brain. Completion of this project will lead to a curated list of targets to pursue as potential therapeutics for inflammation throughout the CNS during aging. Funding: NIH/NIA 3RF1AG057264-03S1

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

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Targeting and Transgenic Shared Resource, Roswell Park Cancer Institute. 4 Department of Rehabilitation Science.Skeletal muscle (SkM) comprises approximately 40% of total body mass and plays essential physiological roles in the body such as enabling skeletal movements and regulating metabolism. Age-related muscle loss, sarcopenia is a major medical problem facing the elderly and correlates with loss of metabolic function, falls leading to cranial fractures, type II diabetes, and cardiac insufficiency. Here we investigate the age-related metabolic rewiring that occurs in myoblasts using in vitro and in vivo models of aging and rejuvenation. RNA sequencing and pathway analysis data revealed that several metabolic pathways changed significantly upon senescence. We also provided evidence aged myoblasts have less glycolysis and insulin sensitivity, which leads to utilize a different source of energy to generate ATP. Our results suggested that aged myoblasts preferred to catabolize amino acids mostly methionine for ATP production and this came at the expense of accumulation of ammonium that leads to DNA damage and impaired cellular function, compromising regenerative capacity and myotube formation. Interestingly, we found that expression of the embryonic transcription factor, NANOG, in senescent cells restored insulin sensitivity, Akt2 signaling, glucose uptake and utilization of glucose for ATP production. In addition, NANOG decreased expression of methionine adenosyl-transferase (MAT) 2A and ammonium levels. Interestingly, inhibiting MAT2A using shRNA showed similar results as NANOG, including restoration of insulin sensitivity, Akt2 signaling and increased glycolysis. Most notably, decreasing methionine catabolism by NANOG expression or MAT2A inhibition led to dramatic improvements in skeletal muscle strength in a mouse model of premature aging.

Ultrastructural localization of PDE4D and HCN1 in rhesus macaque entorhinal cortex layer II: Molecular mechanisms mediating susceptibility to tau pathology 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 AD 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 aging. We used high-spatial resolution immunoEM to localize PDE4D and HCN1 in young rhesus macaque (8-10y) ERC layer II. 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 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. These data suggest that 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.

Molecular mechanism of age-related stress response -- lesson from retina.

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Glaucoma is a group of diseases with diverse molecular mechanisms of pathogenesis, all of which converge on a common pathway leading to typical optic nerve damage and consequent characteristic patterns of visual field loss. Of all the glaucoma-associated risk factors, age is by far the strongest and consistently reported. We, therefore, hypothesized that the intraocular pressure elevation (IOP) will have a significantly different impact on retinal ganglion cells (RGC) and their function depending on the age of the subject. We evaluated the effects of mild IOP elevation on RGC survival with respect to animal age and observed that the extent of RGC death correlated directly with the age of mice. Loss of RGC in aged animals was associated with significant loss of axons in the optic nerve head and with loss of visual response. The analysis of transcriptional response to IOP elevation in young and old animals revealed significant upregulation of genes associated with response to stress including Inflammaging and senescence specifically in aged animals. Surprisingly, chromatin accessibility analysis has not detected accompanying age-specific differences upon IOP elevation. Our further detailed chromatin analysis revealed key transcriptional differences in molecular mechanism of response to stress between young and old animals and suggested new approaches in preventing RGC death upon IOP elevation in aged individuals. Funding - R01 EY027011

Metformin rescues disease-related phenotypes in a C. elegans model of Alzheimer’s Disease

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Proteostasis dysregulation is a major hallmark of aging and age-related diseases. With age, proteins have an increased tendency to misfold as the cellular processes that are meant to repair these errors gradually lose function. Over time, the accumulation of misfolded proteins leads to the formation of clumps and protein aggregates that are involved in the pathogenesis of many human diseases, including Alzheimer’s Disease (AD) and Type 2 diabetes mellitus (T2DM). AD is an age-related, progressive neurological disease associated with the irreversible loss of cognitive and intellectual abilities, including reasoning, memory, and social functioning. Biochemically, AD is characterized by aggregates of hyperphosphorylated tau protein that make up neurofibrillary tangles, and beta-amyloid aggregates within senile plaques. These features have been directly linked to neurotoxicity and the successive degeneration of neurons which is demonstrated by the onset of cognitive impairment. Studying this process in humans is challenging due to a longer lifespan and greater complications monitoring protein aggregation and regulation. We used a model organism, C. elegans, a nematode that has functional counterparts in humans, a fast life cycle, and transparent body plan, to study molecular and behavioral aspects associated with Alzheimer’s disease. Previous studies have demonstrated that metformin, a first line monotherapy for the treatment of Type 2 Diabetes (T2D) in people, reduces mortality in T2D patients and increases lifespan across multiple model organisms. Metformin’s effects on human aging and age-related diseases, including AD, is an active area of investigation. In this study, we asked whether metformin impacts lifespan and healthspan metrics in a C. elegans neurodegeneration model that mimics aspects of human AD. Our results show that animals receiving metformin have improved behavioral and molecular markers associated with aging and proteostasis dysfunction. Ongoing work focuses on characterizing the mechanisms of action responsible for these effects.

Pathology in the Study of Longitudinal Aging in Mice (SLAM): what did we learn so far?

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Research: The Study of Longitudinal Aging in Mice (SLAM) is a large mouse population study instituted within the Intramural Research Program at the National Institute on Aging to comprehensively characterize normal aging in two commonly used strains of mice - the inbred C57BL/6J and outbred HET3 - of both sexes, and to validate their usefulness as preclinical models for human aging and age-related diseases. To date, over 2400 mice, logistically separated into cohorts of 200 mice, have been enrolled providing an actively expanding database of mouse physiological measurements and corresponding biorepository of tissues for each mouse. The tissues processed for histology are harvested either during planned terminal collections at different ages, between 6 and 36 months, or at necropsy and all the slides are systematically inventoried and then digitalized. This endeavor is establishing the first comprehensive murine geropathology whole-slide digital library matched to longitudinal, phenotypic, and molecular measurements for each individual animal. The histopathological characterization of each individual pathological conditions (e.g., neoplastic and degenerative diseases) is underway for all tissues with preliminary data showing significantly different sex and strain pattern of pathologies and severity of disease burden across ages. As the collection and in-depth analysis of data generated by SLAM move forward, this information will be used to identify associations between phenotypic characteristics, molecular biomarkers, and histopathological correlates, providing a strong foundation for translational research.Funding Acknowledgement: This research was supported by the Intramural Research Program of the National Institute on Aging of the National Institutes of Health.

Astaxanthin Lowers Global Thiol Oxidation in Older Adults.

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Previous work from our laboratory has demonstrated that older individuals have an impaired redox signaling response to acute exercise compared to young. Furthermore, we have shown that this is due to elevated basal levels of nuclear Nrf2, presumably driven by increased production of reactive oxygen species (ROS). The aim of the present pilot study is to test whether basal global thiol oxidation can be reduced in older individuals, using a phytochemical antioxidant intervention. Astaxanthin (AX) is a carotenoid that has been shown to have significant antioxidant effects. Importantly, AX acts directly on the mitochondria where the majority of basal production of ROS occurs. We hypothesized that a 2-week AX supplementation would lower global protein thiol oxidation in older individuals and improve the response to an ex vivo oxidative stimulus. The study cohort consisted of men and women over the age of 55y, (target n=11). The participants completed a screening visit that included health history, lifetime physical activity questionnaire, anthropometric measures, and resting blood pressure. Baseline blood draw after an overnight fast was taken and again after 14-days of AX supplementation. Peripheral blood mononuclear cells (PBMCs) were isolated and processed for measurement of global protein thiol oxidation. Briefly, free thiols were blocked with NEM, and then the sample was reduced with TCEP and labeled with maleimide infrared dye. A Bradford assay was used to determine protein concentration and a western blot was run to detect global protein thiol oxidation. To date we have 5 participants (mean age: 66 ± 7y) that have completed the study with recruitment still in process. Compliance to the supplementation is 99%. Western blot data show decreased non-stimulated global thiol oxidation by 54% after the intervention (p<0.005) while the response to the H2O2 stimulation is more variable. These early results indicate that astaxanthin supplementation is improving basal redox balance in older adults and enhancing cell signaling response, however, additional data are needed and are in progress. Funded by NAU-HURA and LSAMP to E.V. Astaxanthin supplement provided by AstaReal.

Limiting mitochondrial STAT3 improves immune cell mitochondrial function and inflammation.

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Age is a non-modifiable risk factor for the onset of many inflammatory and metabolic disorders. Altered immune cell mitochondrial function is implicated in the general decline of cellular health during aging. We observed significantly higher basal and maximal mitochondrial respiration in CD4+ T cells from lean older adults (BMI<24; Age >60 yrs) compared to lean younger adults (BMI<24; Age<35 yrs). Higher levels of mitochondrial superoxide, lower activation-induced switch to glycolysis, higher mitochondrial translocation of STAT3(mitoSTAT3), and proinflammatory cytokine production was observed in the T cells from the older adults. STAT3 localization to the mitochondria is known to influence mitochondrial metabolism. We hypothesized that aging-induced increase in mitoSTAT3 fuels an addictive dependence of CD4+ T cells on oxidative metabolism, thus preventing the required activation-induced glycolytic switch, resulting in a pro-oxidative and pro-inflammatory cellular milieu. To test this, we utilized a novel mitochondria-targeted curcuminoid (mitocur1), which prevents mitochondrial translocation of STAT3. CD4+T cells isolated from peripheral blood mononuclear cells were activated with T cell specific Î±CD3/Î±CD28 stimulus for 40h and treated concurrently with Â±3-12 ÂµM mitocur1. Mitocur1 treatment of T cells from older adults prevented age-induced high basal mitochondrial respiration rate, proton leak, and improved the spare respiratory capacity. Lactate production was higher indicating a shift towards aerobic glycolysis. Mitochondrial superoxide and cellular peroxide levels were lower upon mitocur1 treatment, but interestingly, cellular superoxide levels were higher. Most importantly, mitocur1 treatment resulted in a numerical reduction in proinflammatory cytokines; IL-6 and Th-17 cytokine IL-17A. Collectively, our data suggests that preventing age-induced mitochondrial translocation of STAT3 is beneficial in promoting mitochondrial health and preventing inflammation. Funding Acknowledgement: NIA R15AG06895701A1

Age-related impaired polyunsaturated fatty acid (PUFA) synthesis disrupts RPE phagocytosis.

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DNA methylation of several CpGs in the regulatory region of ELOVL2 (Elongation of Very Long Chain Fatty Acids-Like 2) is the most reproducible biomarker of aging. ELOVL2 encodes a transmembrane protein that plays critical roles in the biosynthesis of omega-3 docosahexaenoic acid (DHA) (22:6n-3) and very long-chain polyunsaturated fatty acids (VLC-PUFAs), which are highly enriched in photoreceptors and essential for maintaining healthy visual function. The activity of ELOVL2 declines in the aging retina; however, the molecular role of ELOVL2 in the aging eye has yet to be fully understood. The aim of this study was to investigate the role of ELOVL2 in supporting the phagocytic function of retinal pigment epithelial (RPE) cells. We used small interfering RNAs (siRNAs) directed against ELOVL2, challenged RPE cells with FITC-labelled rod outer segments (FITC-ROS), and quantified the number of internalized ROS particles. In addition, we used a lipidomic approach to independently quantify phagocytosis efficiency. Our data show a ~30% decrease in phagocytosed ROS (p<0.001) in ELOVL2 knockdown RPE cells compared to control in both assays, underlining the role of ELOVL2 products in RPE function. Overall, the findings of this study support our hypothesis and suggest that low levels of ELOVL2 expression and subsequent decline in DHA and VLC-PUFA synthesis result in impaired phagocytic function of RPE cells- a phenotype observed in aging RPE. This study not only provides insight into the molecular mechanisms of RPE phagocytosis but also opportunities to explore therapeutic treatments for pathologic states such as age-related macular degeneration (AMD) using lipid supplementation. Funding: BrightFocus Foundation to DSK and unrestricted grant from Research Preventing Blindness to the Gavin Herbert Eye Institute at the University of California, Irvine.

Regulation of a brain-specific isoform of the mitochondrial regulator PGC-1a in neurons.

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Brain aging is associated with morphological and homeostatic changes, including alterations in brain size, cognitive impairment, and white and grey matter integrity; however, the underlying biology is only beginning to be understood. The ubiquitously expressed transcriptional coactivator PGC-1a (peroxisome proliferator-activated receptor gamma-coactivator 1-alpha) is a master regulator of mitochondrial function, and connections have been established between PGC-1a and metabolic disease. Defects in PGC-1a have also been linked to neurodegenerative disorders. Intriguingly, a specific PGC-1a transcript variant is expressed only in the brain, although its biological significance is unknown. Here we show that the regulatory mechanisms controlling PGC-1a expression are promoter-specific, the dominant isoform is cell type-specific, and the functional outcome of altered PGC-1a expression is not equivalent among brain cell types. Expression profiling reveals that the neuron-specific variant of PGC-1a is turned on during differentiation of progenitor cells and not expressed in astrocytes. Quantitative measures of cellular respiration and redox metabolism reveal marked differences in metabolism between astrocytes and neurons. Analysis of PGC-1a activation and repression demonstrates that the regulatory mechanisms controlling the canonical and alternate promoters are distinct. GSK3b inhibitor lithium induces changes in isoform distribution and abundance of PGC-1a, where expression from canonical and alternate promoters is induced, but expression from the brain-specific promoter is repressed. Importantly, changes in PGC-1a isoform expression are associated with changes in respiration and redox metabolism, indicating that the brain isoform is the functionally dominant form in neurons. The data presented here highlight fundamental mechanisms for neuronal metabolism regulation that are likely relevant to neurodegenerative diseases with a mitochondrial dysfunction component. This work was supported by NIH grant R01AG067330.

microRNA in Hepatic Vascular Aging and Age-Related Endothelial Progenitor Function in Chronic Liver Disease.

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Age is a major risk factor for chronic liver disease. CD34+ endothelial progenitor cells have angiogenic potentials contributing to neovascularization within ischemic sites or to vascular repair during aging associated organ injuries. We aimed to define the aged endothelial progenitor function and liver fibrosis during ch liver injury. Senescence-accelerated mouse-prone 8 (SAMP8), bile duct ligation (BDL) and miR-34a knockout mice were used as the animal models. CD34+ cells were isolated from mouse liver using laser capture microdissection (LCM). In CD34+ cells isolated from SAMP8 and BDL mice liver by LCM, real-time PCR and nCounter single cell analysis demonstrated the enhanced expressions of sinusoidal endothelial dysfunction (SED) markers ICAM1, endothelial marker vWF as well as the inflammatory cytokines TNFÎ±, CCl2, IL-1Î², IFNÎ² and IL-7. The upregulation of miR-34a in EPCs and human liver sinusoidal endothelial cells (HLSECs) led to a time-dependent repression of its target protein Sirt1 levels, and a significant increase of NOS3. SED marker ICAM-1 and endothelial marker vWF were significantly up-regulated in the progressive phases of aging with CLI. Knockout of miR-34a in vivo reversed the serum ALT level, and restored the levels of Sirt1 coupled with decreased NOS3 expression as well as the reduced levels of TNFÎ±, CCl2, IL-1Î², IFNÎ² and IL-7 in LCM isolated CD34+ cells analyzed by nCounter single cell gene expression assay. Depletion of miR-34a in vivo also induced a significant down-regulation of profibrogenic genes and MMPs in total liver tissues and LCM isolated CD34+ cells by senescence PCR array and single cell gene assay from BDL mice liver. Conclusion: Our discovery that CD34 associated endothelial progenitor dysfunction is regulated by miR-34a during aging associated chronic liver injury and liver fibrosis implicates an exciting field with potential therapeutic benefits for related human disorders. Funded by VA Merit Review Award and NIH/NIDDK grants to Dr. Meng.

The effects of a Fasting Mimicking Diet on an E4FAD mouse model of Alzheimer's disease.

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AD is a neurodegenerative disease characterized by the accumulation of amyloid beta (A?) via amyloid-? oligomers (oA?) that can be toxic in their fibrillar form or aggregate to form amyloid plaques and promote the generation of hyperphosphorylated tau protein. This distinct neuropathology can lead to inflammation and oxidative damage, synaptic degeneration and neuronal death, ultimately impacting the learning and memory functions of the cerebral cortex and hippocampus. The EFAD-Tg mouse model has the human APOE genotypes (APOE2, APOE3, APOE4) knocked into the 5xFAD-Tg mice, allowing for investigation of the interactive effects of APOE- and sex-induced risk with the development of AD pathology using E2FAD, E3FAD or E4FAD mice. The efficacy of drugs thus far approved for AD treatment is limited. Dietary interventions such as chronic fasting and caloric restriction have long been studied to understand their effects on ameliorating the symptoms of chronic and age-related diseases such as Alzheimer's disease (AD). As chronic fasting can be a rigorous regimen to sustain for most individuals, our lab developed the low calorie, low protein fasting-mimicking diet (FMD) to provide the benefits of chronic fasting without its rigors. The wide-acting effects of Fasting Mimicking Diets (FMDs) on metabolic, inflammatory and regenerative pathways leading to reduced pathology or risk factors for various diseases in mice and humans, has the potential to be effective against AD. Here, we show that in female E4FAD mice, ~ 4 months of bi-weekly FMD cycles improved spatial memory in the Barnes Maze and reduced hippocampal A? load, enhanced genesis of Type I/II neural stem cells in the dentate gyrus as indicated by immunohistochemistry and immunofluorescence staining, and reduced expression of superoxide-generating NADPH oxidase (Nox2) assessed by western blots. Findings could hold promise for a FMD regimen that is sustainable for many individuals in delaying the onset of AD symptoms.Funding Acknowledgement: This study was funded in part by the NIA T32 training grant AG052374 to P.R. and the NIH/NIA grants AG20642, AG025135 and P01 AG034906 to V.D.L.

Assessing osteoarthritis related pain following long term in vivo senolytic treatment in mice.

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Cellular senescence is a phenotypic state that contributes to age-related osteoarthritis (OA) through the secretion of matrix-degrading and pro-inflammatory molecules collectively referred to as the senescence associated secretory phenotype (SASP). An emerging therapeutic strategy for OA is to eliminate senescent cells by selectively initiating apoptosis. We have shown that Navitoclax is an effective senolytic in murine chondrocytes, reducing the percentage of senescent cells measured by p16tdtomato from 17.9% to 6.1% (average of 13 matched pairs, p<0.001) in an ex vivo cartilage explant model of senescence induction. We then moved to intra-articular injections to assess the effectiveness of senolytic clearance on functional pain outcomes of OA. To increase the dynamic range for in vivo studies, we utilized Jnk2 knockout mice that we had previously shown have higher levels of senescence in the knee joint and increased age-related OA. A total of 40 mice were divided into Navitoclax (11 female, 10 male) and DMSO vehicle control (10 female, 9 male) groups. Mice received 3 intra-articular injections over 1 week in a single limb at 10, 11, 12, and 13 months of age. After the last set of injections, mechanical allodynia (pain to a normally non-painful stimulus) was assessed using Von Frey filaments on each hind paw. A sex difference was observed in the effect of senolytic treatment on Von Frey outcomes. Female mice showed an increase in 50% pain threshold (the force required to elicit a reaction 50% of the time) with Navitoclax as compared to vehicle control (DMSO: 0.27 a.u, Nav: 0.47 a.u, p<0.05). Males required a higher force than females to elicit a response at baseline and showed a trend towards improvement with Navitoclax (DMSO: 0.5 a.u, Nav: 0.70 a.u, p = ns). Ongoing histological analysis will determine whether Navitoclax mitigates the development of joint pathology in addition to the effects on pain. Funding: NIH R56 AG066911 (BOD); NIH R01 AG044034 (RFL)

Using isolated mitochondria to measure age-related ADP insensitivity.

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Background: ADP insensitivity is observed in aged human and mouse muscle fibers and may play a role in aged muscle weakness. ADP insensitivity is when a given concentration of ADP stimulates a lower mitochondrial response such as oxygen consumption (OCR) or production of ATP or ROS. Most measurements of ADP sensitivity have been performed using in vivo techniques or permeabilized muscles fibers. We sought to establish isolated muscle mitochondria as a model for ADP sensitivity and to use this model to compare ADP sensitivity with age and in vitro treatment of elamipretide (ELAM;SS-31). Methods: We isolated mitochondria +/- 10 Î¼M ELAM from young (5-8 mo) and old (26-28 mo) mouse skeletal muscle and measured OCR, ROS production (Amplex Red), membrane potential (TMRM), ATP production (ATP-linked NADPH autofluorescence), and 3H-ADP uptake. We addressed two limitations of isolated mitochondria as a model: (1) Isolated mitochondria quickly use low concentrations of ADP, making it not possible to measure steady state responses, and (2) standard isolation procedures damage the outer-membrane of mitochondria, causing cytochrome c to leak out and limit respiration. To address this, we used a hexokinase clamp system to create an ADP/ATP equilibrium at low ADP concentrations and added saturating cytochrome c. Results: Hexokinase clamp and cytochrome c significantly increased ADP sensitivity and total respiration capacity. We find no difference in kinetics of ADP sensitivity in young and aged isolated mitochondria. Absolute values of mitochondrial function declined as measured by significantly lower OCR, membrane potential, and ATP production rates. While acute treatment of ELAM did not repair these phenotypes in aged mitochondria, it significantly increased uptake of 3H-ADP. Conclusions: ADP insensitivity is observed in both aging muscle fibers and in vivo but not in isolated mitochondria. This study suggests that isolation of mitochondria, which disrupts the mitochondrial network in skeletal muscle, disrupts the ADP insensitivity phenotype. Therefore, ADP insensitivity may occur due to age-related changes at a structural level that is maintained in permeabilized fibers but not isolated mitochondria. Funding sources. P01AG001751, T32AG066574. ELAM was provided by Stealth BioTherapeutics Inc. at no cost.

Extending healthy lifespan with 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 kynurenine pathway genes—kynu-1, tdo-2, and acsd-1—for which decreasing expression extends lifespan in invertebrate models. More recently we discovered 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). Aging mice fed a diet supplemented with 3HAA are similarly long-lived. The mechanism of action liking 3HAA to aging is complex and partially overlaps with multiple pathways previously implicated in aging. In recent work, we have identified activation of the Nrf2/SKN-1 oxidative stress response and alterations to iron homeostasis as key players in the benefits 3HAA. Ongoing work explores the relationship between 3HAA, Nrf2/SKN-1, and iron in C. elegans and mammalian aging, age-associated immune decline, and cancer. This works 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.

Colanic Acid Regulate Mitochondrial Dynamics and Longevity via the Endo-lysosomal Pathway

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Both genetic and environmental factors, including microbiota, influence organism longevity. In our studies, we explore how microbial factors affect host longevity in Caenorahbditis elegans. We discovered that the deletion of lon (?lon), which encodes Lon protease in E. coli, results in the overproduction of exopolysaccharide – Colanic Acid (CA) and consequently promotes host longevity. Interestingly, the treatment of either ?lon or purified CA induces mitochondrial fission in the host, a CA-induced phenotype that is required for the pro-longevity effect in the host. To understand how CA regulates longevity through host mitochondria, we discover that in the host intestine knocking down the key regulators in the endo-lysosomal pathway fully suppresses the CA-induced mitochondrial fission and lifespan extension. Through proteomic profiling, we revealed the enrichment of mitochondrial proteins in the purified lysosomes from the worms treated with ?lon. Together, these findings suggest an increased interaction between lysosomes and mitochondria upon the CA treatment and its importance in regulating longevity. Furthermore, using transcriptomic analysis, we systemically profiled CA-associated gene expression changes and found the up-regulation of heat shock response and F-box protein genes. As a transcription factor required for heat shock response, HSF-1 regulates the effects of CA in both mitochondrial dynamics and lifespan extension. Together, our results suggest the key roles of the endo-lysosomal pathway in facilitating intestinal uptake of CA and affecting mitochondrial dynamics, and HSF-1-mediated transcriptional response involves in these CA-induced phenotypes. Future studies will confirm the induction of lysosome-mitochondria interaction by CA and characterize the roles of F-box proteins in the regulation of mitochondrial dynamics and longevity. Understanding the molecular mechanisms by which bacterial CA regulates host longevity will provide a new therapeutic strategy for improving healthy aging.R01AT009050

Rat Leukocyte Population Dynamics Predicts a Window for Intervention in Aging

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Age-associated changes in human hematopoiesis have been mostly recapitulated in mouse models; but not much has been explored in rats, a physiologically closer model to humans. To establish whether rat hematopoiesis closely mirrors humans', we examined the peripheral blood of rats throughout their lifespan. Significant age-associated changes showed distinctive population shifts predictive of age. A divergence between predicted versus chronological age changes was indicative of fragility; thus, these data may be a valuable tool to identify underlying diseases or as a surrogate predictor for intervention efficacy. Notably, several blood parameters and DNA methylation alterations defined specific leverage points during aging, supporting non-linear aging effects and highlighting a roadmap for interventions at these junctures. Overall, we present a simple set of rat blood metrics that can provide a window into their health and inform the implementation of interventions in a model system physiologically relevant for humans.This research was supported entirely by the Intramural research Program of the NIH, National institute on Aging.

Progression of Sarcopenia in Old and Very Old C57BL/6J Mice

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Aging C57BL/6J mice is a well-established animal model for sarcopenia. Most studies using C57BL/6J mice select mice aged 18-24 months (m) as the “old” group and/or only include one old group. Since human life expectancy is about 77.8 years old in the U.S. (2020), it is also important to understand the animal model at an equivalent older age (~28m in C57BL/6J mice). Therefore, the objectives of this study are to 1) characterize the progression of sarcopenia in C57BL/6J at 24m and older; 2) elucidate the mechanisms that contribute to muscle loss and weakness with aging. Methods: Young (6m), old (24-26m), and very old (27-33m) C57BL/6J mice were evaluated for body composition, muscle mass, and physical function (treadmill test for endurance, grip strength). Skeletal muscles were collected for biochemical analysis. Results: At 24m, muscle mass (all hindlimb muscles) was 15% lower than the young; while grip strength was 27% and endurance was 25% lower. At age of ~30m, both muscle mass and grip strength were 28%, and 33% lower, respectively; whereas endurance was 50% that of young animals (Young vs. 24m vs. 30m: Muscle mass: 470 vs. 400 vs. 337mg; Grip strength: 170 vs. 123 vs. 113g; Endurance: 917 vs. 692 vs. 463sec). Muscle function was positively correlated with AMPK signaling (pAMPK), transcript levels of autophagy markers (p62, LC3, Atg5, and Becn1), mitochondrial biogenesis (PGC1a), and E3 ubiquitin ligases (MuRF1 and Atrogin1), which are decreased with aging. In addition, old and very old mice showed lower mitochondrial fusion and fission, mitophagy (Bnip3) and myogenesis (Pax7, Myog2) than the young mice. Conclusion: Muscle function and mass decline at different rates in old and very old mice and this decline is associated with alterations in the mitochondrial quality control system and myogenesis in very old mice.Funding Acknowledgment: The U.S. Department of Veterans Affairs (BX002807 to JMG). The Congressionally Directed Medical Research Program (PC170059) and NIH (R01CA239208, R01AG061558).

Multi-omics analysis identifies Cth2 as a negative regulator of mitochondrial translation during aging.

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Iron homeostasis is known to be affected during aging, which is associated with a wide range of age-related diseases, such as metabolic disorders, cardiomyopathies, anemia, cancer and neurodegeneration. In eukaryotes, iron utilization and storage are tightly controlled. Iron deficiency leads to coordinated changes in gene expression that activate iron uptake and allow metabolic adaptations to iron depletion. While transcriptional responses to iron deprivation have been extensively characterized, little is known about the role of iron in regulating protein translation. Previous studies have shown that iron deficiency leads to global inhibition of protein synthesis, which is dependent on the Gcn2/eIF2ï• ¡ pathway. However, the mechanisms of translational regulation and specific genes that are translationally regulated in response to iron deficiency are not known. In this study, we performed a genome-wide analysis of gene expression, protein synthesis, and metabolic changes in response to iron deficiency in yeast. We uncovered that a group of genes involved in iron deficiency response is specifically up-regulated at the translation level. Our multi-omics analysis identified Cth2 mRNA-binding protein as a key regulator of mitochondrial translation and revealed rewiring of numerous metabolic pathways affected by iron deficiency. We also demonstrate that expression of Cth2 is increased during aging, whereas deletion of CTH2 extends replicative lifespan. Together, these studies identified an important role of Cth2 in translational regulation and modulating lifespan and suggest that iron homeostasis can serve as a target for potential aging interventions.This work was supported by the NIH Grants AG058713 and AG066704 (to V.M.L.).

Older microbiota promotes early brain aging by promoting leaky gut and inflammation via suppressing butyrate-FFAR2/3 pathway

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Age-related cognitive decline is a debilitating problem in older adults, with a poor understanding of its etiology and no prevention and treatment strategies are available. Multiple emerging evidence indicates that abnormal changes in microbiota, increased gut permeability (“leaky gut”), and chronic inflammation are linked with age-related cognitive decline, however, their causal relationship is still not known. Herein, we demonstrated that older microbiota promotes leaky gut by disrupting mucin barriers, which in turn increase inflammation and cognitive decline. Further, older microbiota characterized with significantly reduced the capacity to produce beneficial metabolites like short-chain fatty acids (SCFAs) specifically butyrate, which compounded with reduced butyrate signaling molecules such as free fatty acid receptors 2 and 3 (FFAR2/3) expression in the older gut wall. Fecal microbiota transplantation of older to young mice recapitulated these phenotypes in recipient mice, while butyrate treatment reversed the leaky gut and inflammation by restoring mucus barriers. Further, intestine-specific knock-out mice of FFAR2 and FFAR3 exhibit higher abnormalities in gut microbiota, leaky gut, and inflammation that are linked with increased cognitive dysfunctions. Overall, our results show that the older gut microbiota induces leakiness by disrupting mucus barriers through suppressing butyrate-FFAR2/3 signaling, resulting in increased cognitive decline, and butyrate treatment reverses these abnormalities, suggesting that butyrate can be a preventive strategy to reduce cognitive decline in older adults.

Body shapes are linked with cognitive decline, microbiome and gut inflammation in older adults - in Microbiome in aging Gut-Brain (MiaGB) Consortium studies

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Microbiome in aging Gut and Brain (MiaGB) Study teamAbstractObesity is a key risk factor for Alzheimer's disease (AD) related dementia (ADRD) in older adults, however, contribution of obesity in age-related cognitive decline and ADRD remains debatable. There is no effective treatment for AD, and while some early interventions help to prevent and delay AD, early risk detection remains a challenge. We have demonstrated that the gut microbiome signature in older adults with mild cognitive impairment (MCI), an early stage of AD, and ADRD differs significantly from healthy. With funding awarded by the Florida Department of Health, we have established multi-site studies across Florida as part of our Microbiome in Aging Gut and Brain (MiaGB) study statewide consortium. We plan to establish connections of body shapes with cognitive decline and determine the microbiota and gut inflammations moderating effects. Outcomes will contribute to better understanding the significance of body shapes and role of unique microbiome characteristics, inflammation, in risk of age-related cognitive decline.

Early-adulthood protein translation spike drives aging via juvenile hormone/germline stem cell signaling

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Protein translation (PT) sharply declines with age in invertebrates, rodents, and humans. It has been implicitly assumed that elevated PT at young ages is beneficial to health and PT ends up dropping as a passive byproduct of aging. However, whether this holds true and how dynamic fluctuation in PT over time impacts aging remain unknown. In Drosophila, we show that a transient PT spike in early-adulthood exerts long-lasting negative impacts on aging trajectories and proteostasis in later-life. Conversely, blocking the early-life PT spike robustly improves life-/health-span and prevents age-related protein aggregations. Further, greater early-life PT rise strongly predicts shorter future lifespan across different fly strains and is observed in neurodegenerative disorders before the onset of symptoms. Proteomics-guided investigations revealed that during the early-adulthood PT rise, juvenile hormone triggers proteostatic dysfunction and drives aging via aggregation-prone large lipid transfer proteins. The early-life PT spike also transcriptionally represses stress responses essential for proteostasis maintenance and drives aging via germline stem cell signaling. Our findings suggest that PT is thereby suppressed after early-adulthood as an adaptive response to alleviate proteostatic burden, slow down aging, and optimize life-/health-span. We propose that the rise and fall in PT over time may impact aging in the opposite direction from what was assumed in the past. Our work provides a novel theoretical framework for understanding how lifetime PT dynamics regulate the onset of aging.Funding Acknowledgement: This research was supported by NIA/NIH R56-AG061051, Glenn Foundation for Medical Research, American Federation for Aging Research (AFAR), Voelcker Young Investigator Award, San Antonio Nathan Shock Center, San Antonio Pepper Center, Barshop Institute T32 Program on the Biology of Aging NIA T32-AG021890, UAB Medical Scientist Training Program NIH T32-GM008361, and South Texas Medical Scientist Training Program NIH T32-GM113896.

Primary data for longevity of nonhuman primate species common to biomedical research

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Nonhuman primates (NHP) are important translational aging models. To begin to clarify the correlation between NHP age and corresponding human age, it is essential to accurately identify average and maximum longevity across NHP species commonly used for biomedical research. Reported NHP lifespans have often failed to investigate the primary source, relevance, and validity of the lifespan data provided. For example, numerous papers list 37.5 years as the Papio hamadryas lifespan, all of which have derived from a report on a single male baboon that died at the Brookfield Zoo in 1972. An important issue is that average and maximum NHP lifespan in captivity may be changing due to improvement in care and maintenance procedures. It is necessary to investigate primary data on NHP lifespan past and present to evaluate. Here, we are developing a central source for primary data regarding average and maximum lifespan of captive NHP species commonly used in biomedical research to test three hypotheses: 1. Lifespan differs by sex. 2. Lifespan increases with length of time the species has been in captivity. 3. Lifespan is positively associated with body size. We seek relevant data from NHP investigators, including data on mean and median lifespan, as well as maximum longevity, with as many of these variables as possible: sex, species, date of birth, weight at birth, date of death, body weight at death or within 3 months of death, and cause of death (e.g., natural, sacrifice for research reasons, sacrifice for humane reasons). The analysis focuses on natural death and sacrifice for humane reasons. Species of interest include but are not limited to Callicebus cupreus, Callithrix jacchus, Cercopithecus aethiops, Macaca fascicularis, M. fuscata, M. mulatta, Pan troglodytes, and Papio sp. Investigators with relevant data or contacts are encouraged to reach out to the authors for collaboration (hhuber@txbiomed.org, jnegrey@wakehealth.edu). The final goal of the project is a data-backed resource where investigators can find captive NHP lifespan information that is organized by both sex and time of study. Funding from NIA 1U19AG057758, NIH P51 OD011133.

Development of an Old World primate resource to study developmental programming-aging interactions

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Baboons–nonhuman primates closely related to humans, with similar physiology, metabolism, and genetics–are excellent models for translational studies of aging comorbidities, e.g. dyslipidemia, hypertension, and diabetes. Maternal nutrient reduction (MNR) and maternal obesity and over-nutrition (MO) predispose offspring (F1) to later life chronic conditions by developmental programming–fetal or neonatal responses to a specific challenge in a critical developmental time window that alter organ development with resulting effects on health that persist throughout life. We conduct fetal and postnatal studies in baboon F1 exposed to two maternal challenges during pregnancy and lactation, along with age-matched controls: 1) MNR, 30% maternal global food reduction. 2) MO, maternal high-fat high-energy diet. We have archived tissues from male and female F1 (frozen, fixed) at 90, 120, 140, 165, and 175 days gestation (term 185 d) to test early life effects on the aging process. We also have living cohorts: n=12 (12-15 yrs) born to MNR mothers and n=20 (6-9 yrs) born to MO mothers. Data include birthweights and longitudinal morphometric, clinical, and metabolic circulating measures. In vivo longitudinal studies are ongoing and include MRI, euglycemic clamp, CANTAB cognitive tests, and gait speed. To date we have shown programming by MNR results in premature aging of heart and brain and alterations in vasculature, potentially reversible with healthy lifestyle. We have cultured cells from skin fibroblasts and are developing astrocyte, heart, and liver cultures. Liver and muscle biopsies are available. In vitro studies include cell energetics and tests of resilience and reserve. Data generation includes transcriptomics, proteomics, and metabolomics, with resources for statistical and bioinformatic methods development. The overall goal is to identify early signatures of aging and molecular changes that drive differential aging rates with MNR or MO. Collaborative noninvasive studies are feasible. Please contact Hillary Huber (hhuber@txbiomed.org) for information.NIA 1U19AG057758, NIH P51OD011133.

Lack of TRIB2 expression contributes to the preferential loss of naive CD8+ T cells with age.

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Aging of the human immune system entails dramatic alterations in the T lymphocyte compartment including loss of naive T cells, which contributes to rendering older individuals more vulnerable to new infections. Naive CD4+ T cells are more resistant to age-related loss than naive CD8+ T cells suggesting molecular mechanisms that preferentially protect naive CD4+ T cells during aging. Here, we discovered that TRIB2 guards naive T cell quiescence via suppressing AKT activation. TRIB2 was more abundant in human CD4+ than in CD8+ T cells; TRIB2 silencing in human CD4+ T cells caused increased AKT activation, accelerated proliferation and differentiation in response to homeostatic cytokines to a level characteristic of human CD8+ T cells. Likewise in mice, CD4+ T cells from Trib2 knockout mice exhibited increased AKT activity, proliferation, and differentiation during lymphopenia conditions. We found that TRIB2 transcription was controlled by lineage-determining transcription factors (TFs) ThPOK and RUNX3 and knockout of both TFs mimicked loss of Trib2-mediated T cell quiescence. Importantly, TRIB2 declined with age, which contributed to accelerated homeostatic CD4+ T cell proliferation and defective quiescence in older individuals. Collectively, these data indicate that TRIB2 via AKT inhibition prevents naive T cell loss with age making it an attractive target for clinically oriented investigations to promote healthy aging.

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Maternal obesity predisposes offspring (F1) to age-related metabolic changes in later life despite maintaining a normal dietary lifestyle

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Maternal obesity (MO) is linked to increased and premature risk of age-related metabolic diseases in F1 but underlying cellular mechanisms are not understood. METHODS: We aimed at identifying metabolic changes in liver and muscle biopsies and plasma from MO F1, n=19 (10 females and 9 males) fed a high energy diet before and during pregnancy and lactation compared to F1 born to mothers fed a standard diet, CON, n=13 (6 females and 7 males). All F1 ate normal chow diet after weaning so differences between groups are due to programming by maternal diet. We used untargeted gas chromatography mass spectrometry–based metabolomics analysis. F1 were 3-6.2 years old at sample collection. RESULTS: A total of 1090 metabolites were identified and quantified across the three tissue sample types. We identified 58 significantly different metabolites in liver, 46 in muscle and 0 in plasma between F1 MO and CON animals, 8 shared between liver and muscle. Eight male-specific (all downregulated) and 11 female-specific metabolites (all upregulated except 2-methylhippuric acid) were significantly different in livers of MO F1. Pathway and enrichment analysis of MO F1 liver rmetabolites revealed D-glutamine and D-glutamate, D-galactose, alanine, aspartate and glutamate, aminoacyl-tRNA biosynthesis, arginine and proline, arginine biosynthesis, glutathione, nitrogen and beta-alanine metabolism. Similarly, citric acid cycle, D-glutamine and D-glutamate, alanine, aspartate and glutamate metabolism and aminoacyl-tRNA biosynthesis were the most impacted pathways in skeletal muscle of MO F1. Interestingly, none of the tissue-specific alterations in metabolite abundances are reflected in plasma samples, and liver and muscle sample analyses reveal different sets of metabolites altered by MO exposure in utero. CONCLUSIONS: We demonstrate early metabolic perturbations in F1 liver and reveal potential novel mechanisms underlying the impact of MO leading to F1 age-related metabolic diseases. NIA U19AG057758, NIH P51OD011133.

An immunotranscriptomic clock measures aging in humans

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As the world population becomes older, unprecedented efforts must be made to improve lifespan and healthspan, and to treat and prevent immunosenescence. Studying aging free of disease and identifying biomarkers of aging from blood transcriptome data is expected to lead to the discovery of genes associated with immunological aging and elucidation of their molecular mechanisms. We hypothesized that leveraging diverse human blood transcriptomic data, by performing cross-platform harmonization, may allow the development of a novel immunotranscriptomic clock that measures aging. Using peripheral blood mononuclear cell transcriptome datasets from the 10,000 Immunomes Project in the NIAID's ImmPort repository, we applied Training Distribution Matching (TDM), an algorithm for making RNA-seq data compatible with microarray data for use with machine learning, which had not been done before in immunological data or biological aging studies. To develop our clock, we applied two machine learning algorithms, elastic net and LASSO, to the microarray dataset and identified a panel of 51 and 17 transcripts, respectively, which we used to develop multiple linear regression models for estimating immunological age. Remarkably, our model with the 51 transcripts achieves high accuracy on both microarray data and TDM-normalized RNA-seq data with only 0.22 and 0.63 years of error, respectively. Similarly, our model with the 17 transcripts is highly accurate with only 0.25 and 0.66 years of error, but using only a third of transcripts compared to elastic net. The high accuracy of our novel immunotranscriptomic clocks with months of error outperforms other available aging clocks, and suggests that the found transcripts may be used as immunological biomarkers of aging in diverse adult populations. Furthermore, our successful microarray--RNA-seq data harmonization indicates that our methodology may be more widely applicable to other tissues, thereby enhancing the discovery and elucidation of pathways and molecular mechanisms related to human aging by using transcriptome signatures. Lastly, findings from our study have the potential to advance precision medicine in geroscience. Funding: 1R03AI151499-01A1

Transcriptional profiling of the aging primate brain: age and neuropathology-associated transcripts in olfactory pathway brain regions of vervets.

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Background: Olfactory dysfunction primarily affects those aged >60 years and is an early symptom of several neurodegenerative diseases. Identification of age-related alterations in transcriptional profiles (TPs) in olfactory pathway brain regions may illuminate processes underlying dysfunction. Methods: Brain regions associated with the olfactory pathway including olfactory bulb (OB), piriform cortex, temporal lobe white matter, and inferior temporal cortex (ITC) were carefully collected and frozen at necropsy from 5 healthy middle-aged (9-11 years) and 5 older (19-26 years) female vervets. Transcriptional profiles were determined for each region using the Human HT-12 v3 Expression BeadChips (Illumina). Results: Analyses of mRNA expression levels across the 4 brain regions using a fold change (FC) cutoff of >1.2 and a false discovery rate (FDR)<0.05 revealed 77 genes that were differentially expressed by age group; 51 of these (66%) were upregulated in older individuals. Differentially expressed genes (DEGs) with the lowest FDRs (all<0.005) included CTSB (Cathepsin B; FC=1.32), LAMTOR3 (Late Endosomal/Lysosomal Adaptor, MAPK And MTOR Activator 3; FC=1.74), and JAGN1 (Jagunal Homolog 1; FC=1.31). CTSB was the only DEG that was consistently significantly associated with markers of neuropathology in all four brain regions (e.g., amyloid beta plaque counts: Pearson râ€™s = 0.6-0.8, all p values<0.05), suggesting that normal CNS aging and neuropathology can be differentiated using this approach. Conclusions: Our results suggest age-related differential gene expression in the olfactory pathway. Several genes differentially expressed by age have been implicated in human neurodegenerative diseases and may be worthwhile targets for research in age-related sensory impairment. Ongoing studies are evaluating gene expression patterns between olfactory-associated brain regions and potential molecular pathways involved in aging vs. neuropathology. Funding Acknowledgment: NIH RF1 AG058829; R24 AG073199; P40 OD010965

Differential morphology of cerebral microhemorrhages originating from arterioles, capillaries and venules in mice

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Cerebral microhemorrhages (CMHs; microbleeds), which are small intracerebral hemorrhages, contribute to the pathogenesis of cognitive decline and dementia in older adults. Clinical and preclinical studies revealed that aging and hypertension are contribute to the prevalence of CMHs, and CMHs can exhibit distinct sizes and morphologies. We hypothesize that this may be due to the differences in the size and intraluminal pressure of the vessels the CMHs originate from. The aim of this study was to establish the relationship between size and morphology of CMHs and characteristics of vessel of origin. To achieve this, in mice equipped with a chronic cranial window, brain vasculature was loaded and visualized with intravenous FITC-dextran tracers. CMHs induced by multiphoton laser ablation in arterioles, capillaries and venules were characterized using 2-photon imaging. The time course of the development of CMHs was recorded, and 3D volumetric reconstruction was done with FIJI/ImageJ and IMARIS for Neuroscientist package. We established a multiphoton laser ablation method that allowed us to induce CMHs in arterioles, capillaries and venules. Microvessels were classified based on their morphology, microvascular architecture and the direction of blood flow. CMHs originating in arterioles were larger, rounder and caused more tissue dislocation. Capillary bleeds were the smallest. Venous CMHs had more irregular shapes and often spread in the perivascular spaces. Development of the CMHs resulted in immediate constriction of the capillaries (likely due to pericyte activation) and constriction of the precapillary arterioles, the time course of which have been simultaneously recorded. Longitudinal imaging of the CMHs allowed us the visualization of reactive astrocytosis around the bleed, as well as the resolution of the bleeds.

The influence of chronological and reproductive aging on resistance to oxidative stress in post-reproductive female mice.

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Reproductive and chronological aging both impose a burden on health and increase disease rates. In the current experiments, we utilized proteins isolated from homogenized liver tissue to assess the oxidative stress response in CBA/J female mice to chronological age and to varied reproductive function through heterochronic ovarian tissue transplantation, both with and without ovarian follicles. Overall, expression of oxidative stress response proteins akr1b1, gpx1, gsta3, gsta4, gstm1, prdx2, prdx3 and txn1 was increased with aging, whereas proteins aldh1a1, aldh2, cat, gsr, gstp1, hsp90b, hspa1a, hspa5, hspa9, nnt, phb1, phb2, prdx1, prdx5, prdx6, txnrd1 and maob were decreased with aging. Expression of superoxide dismutase 1 (sod1) increased 24% from 4.4mo (reproductively cycling) to 12.9mo (the point of cessation of reproductive cycling) and continued to increase past 31 months of age (39%). sod1 levels were not influenced in 22.5mo post-reproductive mice that received transplanted follicle-containing or follicle-depleted young ovarian tissue. In contrast, sod2 decreased 20% from 4.4mo to 12.9mo, but increased 10% from 12.9mo to 26.7mo and 22% from 12.9mo to 31.3mo. sod2 levels were decreased in 22.5mo post-reproductive mice to levels found in 12.9mo mice by exposure to follicle-containing or follicle-depleted young ovarian tissue. While several oxidative stress response proteins were influenced by the loss of ovarian function and by transplantation of young ovarian tissues, sod1 and sod2 displayed a dichotomic response. sod1 appeared to be unresponsive and sod2 appeared very responsive to loss of ovarian function and to exposure to young ovarian tissue. In addition, sod2 was decreased in recipients of young ovarian tissue whether or not the tissue contained follicles, suggesting a somatic ovarian tissue mechanism. This work was supported by the National Institute on Aging of the National Institutes of Health (grant number R15AG061795 to J.B.M.): The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

The Effects of Elamipretide (SS-31) and Nicotinamide Mononucleotide (NMN) Treatment on the Aged Mouse Heart Proteome and Acetylome.

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Posttranslation modification (PTM) of proteins plays an important role in the development of age-related dysfunction that occurs across various tissues. Acetylation is a common PTM that can exist as an important regulatory mechanism or as a damaging non-enzymatic addition to proteins. The latter is especially common among mitochondrial proteins with age, which are subject to high levels of carbonyl stress. We analyzed the effects of aging on protein abundance and acetylation, as well as the ability of the mitochondrial-targeted drugs elamipretide (SS-31) and nicotinamide mononucleotide (NMN) to reverse aging-associated changes, in mouse hearts. 24-month-old mice were treated with the drugs for 8 weeks before heart tissue was harvested for comparison with young and old controls. Acetylated peptides were enriched prior to proteomic analysis using an anti-acetyl-lysine pulldown method. Abundance of proteins and acetylated peptides was determined using DIA proteomics. Both drugs had a modest effect on restoring the abundance and acetylation of proteins that are altered with age, while also inducing additional changes. Age-related increases in protein acetylation were predominantly in mitochondrial pathways such as mitochondrial dysfunction, oxidative phosphorylation, and TCA cycle signaling. We further assessed how these age-related changes associated with diastolic function (Ea/Aa) and systolic function (fractional shortening under higher workload) measurements from echocardiography. These results identify a subset of protein abundance and acetylation changes in muscle, mitochondrial, and structural proteins that appear to be essential in regulating diastolic function in old hearts. Funding for this research was provided by NIH grants T32AG000057, P01AG001751, the UW Nathan Shock Center, P30 AG013280, and AHA 19CDA34660311. Elamipretide was kindly provided by Stealth BioTherapeutics (Needham, MA).

The association of resilience, age, and response to lifespan extending interventions in mice.

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Resilience, the ability of an individual to respond to a physical insult or stressor, has become a topic of interest within the biology of aging field recently, as markers of resilience early in life may be predictive of future health and longevity. Here, we test the response to four physical challenges in mice to determine their potential as markers of resilience. We first discover which challenges show significant age effects, followed by determining if the responses to these challenges improve in response to two commonly studied lifespan extending interventions: caloric restriction and 17ï• ¡-estradiol. We find that three of our challenges show significant age effects (recovery from anesthesia, recovery to anemia, and survival under a pathogen challenge); however, one (wound healing time) did not. Interestingly, our interventions fail to improve our markers of resilience, and both caloric restriction and 17alpha-estradiol may lead to decreases in resilience in response to anesthesia recovery. Overall, our results suggest that resilience is multi-dimensional, and may not be encapsulated by individual challenges. In addition, our markers of health may not be ideal indicators of resilience, and not all health markers may improve with lifespan extending interventions. This work was funded by the National Institute on Aging.

Regulation of Germline Proteostasis by HSF1 and Insulin/IGF-1 Signaling in Maternal Aging

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Gametogenesis is sensitive to nutrient availability and environmental perturbation. We recently show that HSF1, the key regulator of cellular heat shock response (HSR), has important roles in germline progenitor cell proliferation and early meiosis in C. elegans. Using the auxin-inducible degron system, we identified a compact but important transcriptional program of HSF1 for nascent folding and protein conformation maintenance in germ cells. Out data indicate that this HSF1 activity is distinct from that in the canonical HSR, and is dictated by the nutrient-sensing, insulin/ IGF-1 signaling to support proteostasis in rapid germline growth. Proteotoxic stress and maternal aging impair HSF1 activities in germ cells, which destabilizes proteins that are essential for gametogenesis and oocyte quality consequently causing reproductive defects. We found that reduction of insulin/ IGF-1 signaling post development is sufficient to extend reproductive span. This is at least partially through slowing protein synthesis therefore maintaining proteostasis and reproductive health with compromised HSF1 activity during maternal aging. Collectively, we propose that it is essential to couple protein synthesis regulated by insulin/IGF-1 signaling and protein folding capacity controlled by HSF1 to achieve germline proteostasis. This work is supported by the NIH R35GM138364.

Evolution of lifespan investigated with epidemiological modeling.

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The evolutionary origins and mechanisms of aging are a topic of ongoing debate. We use epidemiological models to investigate the evolutionary interactions between infectious diseases and lifespan. The host's lifespan is an essential parameter in epidemiological models. If hosts can recover from the disease and become resistant, the lifespan-dependent persistence of these immune individuals will affect epidemiological dynamics. If the disease is irrecoverable but does not kill, the host's lifespan defines the duration of infection and the pathogen's transmission. If the host's longevity affects the evolution of pathogens, could the epidemics reciprocally influence the evolution of lifespan? Short-lived individuals die earlier and transmit fewer chronic pathogens, thus benefiting the neighbors. In viscous populations with limited dispersal, neighbors are likely kin-structured where individuals are related to each other. Therefore, a sacrifice of the infected individual might be an adaptive strategy to protect its kin. We will demonstrate how our approach might explain the observations made in nature: e.g., with our models, we can find fecundity-longevity trade-off; we can explain the correlation between flight and longevity, differences in castes of eusocial animals, and the ecological role of lifespan extension by caloric restriction. We will discuss the consistency and explanatory power of different evolutionary hypotheses of aging.

Tradeoffs between life extension and quality of life: A psychiatric perspective

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Aim: General human use of FDA approved medications known to increase lifespan (rapamycin and lithium) is potentially limited by tolerability in terms of mood disturbance and maximal cognitive output. Objective: Identification of objective biochemical pathways in the CSF and associated behavioral phenotypes may allow for medication selection and dose finding for acute stabilization, primary prevention, and secondary prophylaxis of progression of age-related mood and cognitive disorders. Methods: Patients, N=29, with depression refractory to current medications provided AM fasting CSF samples with subsequent z-score estimation of approximately 151 metabolites. Chart review provided subsequent response to medication treatment, contemporaneous clinical status with self-reported checklists, and basic demographic information. Hypothesis free multivariate analysis compressed the resultant 5000 item data array into 2 dimensions which collectively accounted for approximately 34% of the variance.Results: Dimension 1 accounted for 17.5% of the variance. One end of the line loaded heavily on self-reported autism scores, male gender, and cognitive fatigue checklists. The other end loaded heavily with bipolar diagnosis, mood stabilizer use, rapamycin response, and ethylmalonate disturbance. Dimension 2 accounts for 15.7% of the variance. One end of the line loads heavily with age and the Frobenius norm of the metabolome matrix. The other end of the line loads heavily with TMHF concentration, homocarnosine elevation, and self-reported checklists of depression (PHQ9) and anxiety (GAD21). Incidental findings are age correlating most highly with GABA metabolites (homocarnosine and 4-acetamidobutanoate) and ketamine response correlating with 2-hydroxybutyrate elevation. Discussion: The caloric restriction literature in Drosophila subspecies strongly cautions against using metabolic manipulation uniformly across populations, given a decrease in longevity observed in 10% of species variants. Multivariate estimates of CSF metabolic gradients can allow for nearest neighbor patient identification independent of genetic kinship or alternative phenotypic expression. Immediate clinical utility is illustrated with examples of PPAR gamma modulation.

Single molecule direct mitochondrial DNA sequencing of human skeletal muscle mitochondrial DNA across the human lifespan

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Mitochondrial DNA mutations are causative in many human diseases and implicated in aging but have been inadequately quantitated and characterized due to limitations in available methods. Structural variants of mitochondrial DNA (mtDNA) were first reported over 40 years ago, but approaches to rigorously map and quantitate them in aged tissues have been hampered by the need for DNA amplification. Thus, the true mtDNA deletion frequency and spectrum of deletion types have been unclear. We hypothesized that single molecule, direct sequencing of human mtDNA across the human lifespan, would yield a higher mutation frequency and broader spectrum of age-induced mutations than previously reported. We employed nanopore Cas9-targeted sequencing (nCATS) to directly sequence, map, and quantitate mtDNA structural variants from total DNA samples of human skeletal muscle and brain to begin developing bioinformatic pipelines for the detection, mapping, and quantitation of mtDNA deletion mutations.

The role of endothelial senescence in age-related blood-brain barrier dysfunction and cognitive decline

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Aging-induced blood-brain barrier (BBB) disruption plays an important role in age-related cognitive decline. Cellular senescence is a fundamental cellular mechanism of aging. Yet, the role of senescent cells in age-related BBB disruption remains elusive. We tested the hypothesis that aging-induced increases in cerebromicrovascular endothelial senescence contribute to BBB disruption. To test this hypothesis young (3 m.o.) and aged (24 m.o.) p16-3MR senescence reporter mice were used. In a sub-group of aged mice senescent cells were eliminated genetically (by treating p16-3MR mice with ganciclovir). Cognitive testing (radial-arms water maze) was performed, and BBB integrity was assessed by measuring the extravasation of fluorescent tracers (intravital two-photon imaging). Endothelial senescence was assessed using flow cytometry and single cell transcriptomics, both of which demonstrated that aging increases the number of senescent endothelial cells in the mouse brain, which could be successfully eliminated by senolytic treatment. Aging was associated with increased BBB permeability and cognitive decline, which could be partially reversed by elimination of senescent cells. Our findings provide additional support to the concept that endothelial senescence contributes to the pathogenesis of age-related cognitive impairment. This work is supported by the American Heart Association Predoctoral Fellowship, as well as the Geroscience T32 trainee fellowship.

Impaired proteostasis, not protein synthesis, limits recovery of aged skeletal muscle after disuse atrophy

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Older persons frequently undergo periods of physical inactivity, causing loss of muscle mass and strength. Aged muscle fails to completely recover following disuse despite having similar levels of muscle loss compared to adults. Efforts to improve aged muscle recovery are commonly aimed at increasing protein synthesis via mTOR stimulation despite evidence that old muscle has chronically elevated mTORC1 activity. We hypothesized that old muscle fails to recover mass due to impaired proteostasis, not reduced protein synthesis. Adult (10 mo.) and old (30 mo.) F344BN rats were hindlimb unloaded (HU) for 14-days to induce atrophy, followed by reloading (RE) to study muscle recovery. During RE rats were labeled with deuterium oxide to determine protein and RNA synthesis rates. We assessed muscle mass, fiber size, mTORC1 signaling, and protein aggregate formation in gastrocnemius. Adult and old muscle had significant losses of mass and fiber size with HU. While adult muscle recovered mass and fiber size by day 15, old muscle did not fully recover by 60 days of RE. Despite incomplete mass and fiber size recovery in old muscle, myofibrillar protein synthesis and mTORC1 signaling were higher in old muscle compared to adult. Additionally, RNA synthesis rates and RNA concentration (markers of translational capacity) were also elevated in old muscle during RE. Old muscle also had higher levels of insoluble protein aggregates (a marker of impaired proteostasis). Lastly, we assessed individual protein synthesis rates of the whole muscle proteome and discovered that old muscle had a larger number of proteins with increased synthesis rates compared to adult. These data suggest that old muscle fails to recover after disuse due to impaired proteostasis, not limitations in protein synthesis. Understanding how proteostasis responds during periods surrounding unloading in old muscle are critical to improve muscle recovery after disuse.Funding NIA Training Grant: 5T32AG052363-04APS Postdoctoral Fellowship MMLR01(NCCIH AT009268)

An anti-steatosis transcriptional response controlled by oleic acid through lipid droplet-induced ERAD enhancement promotes health and longevity

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Although excessive lipid accumulation is a hallmark of obesity-related pathologies, some lipids are beneficial. Oleic acid (OA), the most abundant monounsaturated fatty acid, promotes health in humans and extends lifespan in model organisms. Working in C. elegans, we have discovered a lipid homeostasis pathway through which OA benefits the organism by activating the endoplasmic reticulum (ER)-resident transcription factor SKN-1A (Nrf1/NFE2L1 ortholog). SKN-1A/Nrf1 is cleared from the ER by ER-associated degradation (ERAD) and is canonically activated by proteasome impairment. By contrast, OA increases SKN-1A levels independently of proteasome activity by inducing lipid droplet (LD) biogenesis, which leads to enhancement of ERAD and SKN-1A processing. SKN-1A reduces steatosis by reshaping the lipid metabolism transcriptome, and mediates lifespan extension from OA provided through endogenous accumulation, reduced H3K4 trimethylation, or dietary supplementation. SKN-1A is also important in other contexts of lifespan extension. While previous work has implicated the related protein Nrf2/NFE2L2 in longevity, our new results identify SKN-1A/Nrf1 as an important modulator of lifespan. Our findings also reveal that LDs can transduce lipid signals by modulating ERAD and its effects on proteostasis, and suggest that this SKN-1A/Nrf1 lipid homeostasis pathway provides strategies for opposing steatosis and aging and may mediate benefits of the OA-rich Mediterranean diet.Funding: American Federation for Aging Research (PD18019 and #REBOOT21004 to JIC-Q) and National Institutes of Health (AG54215 and GM122610 to TKB).

17?-estradiol does not adversely affect sperm parameters or fertility in male mice

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17?-estradiol (17?-E2) is a well-established pro-longevity intervention that also elicits strong benefits on healthspan in male mice. Despite these benefits, no study do date as evaluated the effects of 17?-E2 effects on male fertility. To address this gap in the literature, we treated breeding age (3 months) male C57BL/6J mice with standard rodent chow (CON; n=30) or chow + 17?-E2 (14.4 ppm; 17?-E2; n=30) from three to seven months of age. At the end of the treatment period, mice were mated with estrous synchronized females to evaluate pregnancy rates and the number of embryos implanted. After mating, mice were euthanized and blood, adipose tissues, testes, and sperm were collected for evaluation. Plasma estrogens and androgens were evaluated by LC/MS/MS and LH and FSH were assessed by ELISA. Sperm counts and morphology were evaluated, as well as motility using the CASA system. As previously reported, 17?-E2 reduced body mass (p=0.001) and visceral fat mass (p=0.008), thereby confirming responsiveness to the treatment. Testes mass remained unchanged (p=0.108) and no differences were observed for any sperm variables analyzed (p>0.05). Plasma testosterone (p=0.071), 17?-Estradiol (p=0.096), LH (p=0.86), and FSH (p=0.88) were similar to control-treated animals, whereas plasma 17?-E2 was found to be different between treatment groups (p<0.0001). Females mated with CON and 17?-E2 treated males were found to have nearly identical pregnancy rates (p=0.78). The number of embryo implantation sites in these impregnated females were also found to be similar between was similar between groups. We conclude that the lifespan-extending dose of 17?-E2 (14.4 ppm) does not impair male fertility.This work was supported by CAPES (J.V.V.I.), FAPERGS (A.S.) and the National Institutes of Health [R01 AG069742 to M.B.S.].

Establishing ceramide accumulation as a universal driver of aging: A functional lipidomics tale

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Aging is the single greatest risk factor for the development of disease, and thus, understanding the biological mechanisms that modulate aging is critical for the development of health-maximizing interventions for older people. Lipids are small hydrophobic molecules that not only serve as fundamental cellular components, but also act as important cellular signaling molecules, with well-established roles in nutrition, health, and disease. Multiple genetic variants associated with longevity are from genes involved in lipid metabolism (e.g. apolipoprotein E). In fact, the first longevity gene discovered in yeast, i.e. the longevity-assurance gene (LAG1), encodes a ceramide synthase. The long-term objective of aging-related research in my laboratory is to identify and validate lipid metabolism pathways as potential therapeutic targets to extend healthspan/lifespan. Applying an unbiased state-of-the-art shotgun lipidomics platform we search for lipid classes/species that associate with aging in multiple animal models, from C. elegans to non-human primates. We found that ceramides, a class of lipids that play essential roles as signaling molecules and sphingolipid intermediates that participate in a plethora of biological processes but become lipotoxic when in excess, are strongly associated with the aging process. We propose ceramide accumulation as a (novel) universal driver of aging based on the following unpublished data: (1) ceramides consistently accumulate with age systemically (in circulation and in multiple organs) in mice; (2) circulating ceramides are dramatically reduced in long-lived growth hormone-releasing hormone knockout mice; (3) hepatic ceramides accumulate in aged marmosets compared to young controls, a process that is reversed by rapamycin, a well-established anti-aging drug; (4) clinically relevant ceramide-lowering drugs extend lifespan in C. elegans and Drosophila.

Frailty in individuals with mental disorders: longitudinal analyses of all-cause mortality

Julian Mutz and Alexandru Dregan King's College London

Frailty in individuals with mental disorders: longitudinal analyses of all-cause mortality.Julian Mutz 1 and Alexandru Dregan 1.King's College London.Background: Frailty is a medical syndrome that is strongly associated with mortality risk, and an emerging global health burden. Mental disorders are associated with reduced life expectancy and elevated levels of frailty. In this study, we examined the mortality risk associated with frailty in individuals with a lifetime history of mental disorders compared to non-psychiatric controls. Methods: The UK Biobank study recruited >500,000 adults, aged 37–73, between 2006–2010. We derived the two most common albeit distinctive measures of frailty, the frailty phenotype and frailty index. Individuals with lifetime depression, bipolar disorder or anxiety disorders were identified from multiple data sources. The primary outcome was all-cause mortality. We have also examined differences in frailty, separately by sex and age. Results: Analyses included up to 297,380 middle-aged and older adults with a median follow-up of 12.19 (IQR?=?1.31) years, yielding 3,516,706 person-years of follow-up. We observed higher levels of frailty in individuals with mental disorders for both frailty measures. For key comparisons, individuals with a mental disorder had greater all-cause mortality hazards than their controls. The highest hazard ratio (3.65, 95%?CI 2.40-5.54) was observed among individuals with bipolar disorder and frailty, relative to the non-frail controls. Conclusion: Our findings highlight elevated levels of frailty across three common mental disorders. The increased mortality risk associated with frailty and mental disorders represents a potentially modifiable target for prevention and treatment to improve life expectancy.Funding: Biotechnology and Biological Sciences Research Council.

Leukocyte telomere length in individuals with mental disorders

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Individuals with mental disorders have a reduced life expectancy and may biologically age faster. The aim of this study was to characterise telomere length, a biological hallmark of ageing, in individuals with mental disorders and to examine associations between telomere length and polygenic risk scores for mental disorders. Methods: The UK Biobank study recruited >500,000 middle-aged and older adults. Leukocyte telomere length (T/S ratio) was measured using quantitative polymerase chain reaction. Polygenic risk scores (PRS) were calculated for individuals of European ancestry. We estimated differences in T/S ratio between individuals with anxiety disorders, depression or bipolar disorders and people without mental disorders. We also estimated associations between T/S ratio and PRS for these three disorders. Results: The analyses included up to 308,725 participants. After adjustment for confounders, individuals with depression had shorter telomeres than people without mental disorders (? = -0.011, 95% CI -0.019 to -0.004, p = 0.004). There was little evidence of case-control differences in telomere length for anxiety disorders or bipolar disorders. PRS for depression were associated with shorter telomeres (? = -0.006, 95% CI -0.010 to -0.003, p< 0.001). There was no evidence that PRS for anxiety disorders or bipolar disorders were associated with telomere length. Conclusion: Although telomere length is a biological hallmark of ageing, we observed limited evidence that it is a useful marker to quantify accelerated biological ageing in individuals with a lifetime history of anxiety disorders, depression or bipolar disorders.Funding: Biotechnology and Biological Sciences Research Council.

Tom70-based transcriptional regulation of mitochondrial biogenesis and aging

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Mitochondrial biogenesis has two major steps: the transcriptional activation of nuclear genome-encoded mitochondrial proteins and the import of nascent mitochondrial proteins that are synthesized in the cytosol. These nascent mitochondrial proteins are aggregation-prone and can cause cytosolic proteostasis stress. The transcription factor-dependent transcriptional regulations and the TOM-TIM complex-dependent import of nascent mitochondrial proteins have been extensively studied. Yet, little is known regarding how these two steps of mitochondrial biogenesis coordinate with each other to avoid the cytosolic accumulation of these aggregation-prone nascent mitochondrial proteins. Here we show that in budding yeast, Tom70, a conserved receptor of the TOM complex, moonlights to regulate the transcriptional activity of mitochondrial proteins. Tom70â€™s transcription regulatory role is conserved in Drosophila. In addition, Tom70 and its substrates nucleate the stress-induced aggregation of cytosolic proteins on the surface of mitochondria. Our results suggest that Tom70 sits at the crossroad of cytosolic proteostasis and mitochondrial biogenesis by regulating both the synthesis and import of mitochondrial proteins, while nucleating the aggregation of cytosolic proteins and summoning machineries to degrade these misfolded proteins on mitochondrial surface when this balance is disrupted by stresses. The age-related reduction of Tom70, caused by reduced biogenesis and increased degradation of Tom70, is associated with the loss of mitochondrial membrane potential, mtDNA, and mitochondrial proteins. While loss of Tom70 accelerates aging and age-related mitochondrial defects, overexpressing TOM70 delays these mitochondrial dysfunctions and extends the replicative lifespan. Our results reveal unexpected roles of Tom70 in cytosolic proteostasis, mitochondrial biogenesis and aging.

The role of lysyl oxidase and collagen-crosslinking in age-related cardiac fibrosis.

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Myocardial fibrosis and extracellular matrix (ECM) remodeling are important contributors to the age-related decline in cardiac function. Collagen is the main protein component of ECM, and increased collagen deposition leads to cardiac fibrosis. Lysyl oxidase (LOX) is an enzyme responsible for collagen crosslinking, which regulates ECM stiffness and turnover. Although LOX-mediated collagen crosslinking is increased in various heart diseases, its role in cardiac aging remains unclear. The goal of this study was to investigate the role of LOX-mediated collagen crosslinking in age-related ECM remodeling and cardiac dysfunction. We compared collagen accumulation in ventricular tissue of young (4 mo.), middle-aged (14 mo.) and old (>24 mo.) C57BL6/J mice using Mason's trichrome staining and hydroxyproline assay. Trichrome staining showed that collagen deposition trended higher in old mice as compared to young mice (p=0.109). Hydroxyproline assay showed total collagen content elevated by 24% in middle-aged and by 48% in old mice as compared to young mice (p=0.007 and p=0.13, respectively). Next, we evaluated collagen crosslinking by measurements of soluble and insoluble collagen content and investigated expression levels of LOX family (LOX, LOXL1, LOXL2, LOXL3, LOXL4). Insoluble collagen content was higher by 30% in middle-aged and by 60% in old mice compared to young mice (p=0.0058 and p=0.14, respectively) and positively correlated with age (r=0.53; p=0.026), suggesting an age-related increase in collagen crosslinking. Transcript levels of LOX family were not different between age groups. However, protein expression of LOX increased in both middle-aged and old mice (p<0.001 and p=0.0024, respectively) and LOXL2 protein expression was higher in old (p=0.0083) mice, when compared to young mice. These data indicate that age-related cardiac fibrosis is associated with increases in LOX and LOXL2 expression and collagen crosslinking. Further investigation of the causal relationship of LOX-mediated collagen crosslinking and ECM turnover in the aging heart will facilitate the development of novel therapies such as LOX inhibition to improve cardiac healthspan. We acknowledge support from NIH R00 AG051735 (YAC).

Mitochondrial retrograde signaling integrates multiple pathways driving senescence-associated inflammation

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Interventions that suppress or remove senescent cells offer potential therapeutic avenues in the treatment and prevention of age-associated disease. Aging is associated with the accumulation of senescent cells, which contribute to disease through the pro-inflammatory senescence-associated secretory phenotype (SASP). We have shown that the SASP is dependent on a mitochondria-nucleus retrograde signaling pathway, in which mitochondrial ROS triggers nuclear expulsion of damaged DNA as cytoplasmic chromatin fragments (CCF). Once in the cytoplasm, CCF activate the SASP through cGAS/STING-dependent signaling. However, the mechanism of CCF formation is incompletely understood. Here we show that the DNA repair protein 53BP1 acts as a suppressor of CCF and the SASP. The MAPK JNK physically interacts with 53BP1 in senescent cells and directly phosphorylates fragments of 53BP1 in vitro. These same residues are phosphorylated in a JNK1/2-dependent manner in senescent cells, and phosphorylation of 53BP1 alters steady-state maintenance of DNA damage foci in senescent cells. Inhibition of this pathway with small molecule inhibitors of JNK prevents CCF formation and the SASP without affecting cell cycle arrest. Transcriptional profiling of senescent cells with CCF-suppressive interventions reveals a mitonuclear signaling network that integrates proteostasis and metal chaperone pathways required for the SASP. These "senomorphic" approaches that suppress the SASP are an alternative to therapeutic strategies that completely remove senescent cells, which can cause toxic side effects. A better understanding of this pathway can uncover new therapeutic targets in the prevention of senescence-associated inflammation and the treatment of age-associated diseases.This work was supported by F32 AG066459-02 (KNM), R01 AG071861-01 (PDA), and P01 AG031862-13 (PDA).

Adiponectin receptor activation impacts skeletal muscle aging in mice

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The loss of skeletal muscle function with age, known as sarcopenia, significantly reduces independence and quality of life and can have profound metabolic consequences. Currently, there are no pharmacological therapeutic interventions for sarcopenia. Adiponectin is an adipose tissue derived hormone that stimulates mitochondrial metabolism in target tissues and been linked to delayed aging by 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 is still largely unknown. This study investigated 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 metabolic data were collected for analysis against histological assessments of muscle composition (fiber type, atrophy, fibrosis). At advanced age (24-28 months), AdipoRon lowered fasting glucose in both males and females, and exerted similar effects on energy expenditure and metabolism. Age-related declines in functional performance in males was attenuated with AdipoRon treatment but not females. AdipoRon preserved body fat as a function of age in females but not males. These data show sex dimorphism in the physiological effects of AdipoRon in skeletal muscle aging; however, AdipoRon exerted beneficial metabolic effects in both sexes. Altogether, this suggests that 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)

Assessing the Role of BHB in Proteasome Activation Across Age and Ketone Ester Supplementation

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Well-working systems slow with age, and the extent to which normal homeostatic processes slow in older adults can make individuals more susceptible to disease, injury, cognitive decline and eventually loss of independence. Neurodegeneration in particular has a crucial impact on the lives of the growing aging population. Our lab's prior studies have shown lifespan extension and prevention of age-related memory decline in murine models using the ketogenic diet. However, the mechanisms involved in cognitive protection from ketone bodies at the molecular level remain to be explored. Preliminary data in our lab indicates R-BHB, the most abundant ketone body in humans, can identify and bind to misfolded proteins in ex-vivo brain extracts from aging mice. Here, we aim to determine if R-BHB can in turn, signal and activate the proteosome degradation machinery to break down misfolded proteins. Overall, we hypothesize that in addition to supplementing cellular energy with a non-glucose fuel, ketone bodies may help detoxify the cellular environment through a new signaling activity of targeting misfolded proteins for degradation. We isolated proteosomes from C57BL/6 mice and determined an age-related decline in proteosome activity onset a middle age in both the brain and liver. Overall, we found 1 week ketogenic- fed mice increased the overall insolubility of proteins and proteins tagged by ubiquitin. To test their ability to degrade proteins we used 12 month ketone ester- fed mice and isolated their proteasomes. Long term ketone ester diet of 12 months shows proteasomal activity is relatively unchanged in the liver. Results of our ongoing studies may find novel signaling role of ketone bodies via the proteasome, therapeutically relevant to aging and neurodegenerative disease. Funding: NIH grant R01AG067333

DNA hydroxymethylation maintains transcriptional stability during aging

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Methylation at the 5’ position of cytosine (5-methylcytosine, 5mC) is the most well-understood DNA modification with important roles in aging and age-related diseases. Recent evidence suggests that the oxidized 5mC base, 5-hydroxymethylcytosine (5hmC), is a stable epigenetic mark and may play a role in modulating tissue-specific transcriptional outputs. Here, we surveyed global levels of 5hmC in multiple tissues and identified an increase of 5hmC in the aging liver. Genome-wide profiling of 5hmC in the liver showed an age-specific accumulation over genic regions associated with tissue-specific functions, specifically, metabolic- and mitochondrial-related processes. Interestingly, integration of transcriptomics data suggests a previously unreported function for 5hmC in maintaining transcriptional stability during aging. We observed that genes with low or no changes in expression during aging were marked by high levels of 5hmC in the gene body, whereas those with high changes in expression were marked by low levels of 5hmC. The genes marked with high levels of 5hmC included tissue-specific genes, whereas those marked with low levels of 5hmC were related to signaling and inflammatory response. We further corroborated these findings in mouse cerebellum and identified a similar function for 5hmC, suggesting that 5hmC may play a role in maintaining transcriptional stability in multiple tissues. Pending direct investigation of 5hmC interactors over these genes, we predict that 5hmC may recruit proteins that either impede transcription elongation or productive re-initiation. Our data identifies a novel epigenetic regulator of tissue aging. We wish to acknowledge the National Institute on Aging Intramural Research Program, National Institutes of Health, for financial support.

The emerging role of 3-hydroxyanthranillic acid in aging and immune function in C. elegans.

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Select kynurenine pathway interventions extend lifespan in invertebrate models and are of interest for treating age-associated diseases. The kynurenine pathway primarily catabolizes tryptophan to nicotinamide adenine dinucleotide (NAD) and is responsive to inflammatory signaling. We are evaluating the impact of pathway interventions on pathogen resistance and age-associated immune decline in Caenorhabditis elegans and mammals. We found inhibition of 3-hydroxyanthranilic acid (3HAA)dioxygenase (HAAO) or supplementation of its substrate, 3HAA, extends lifespan in C. elegans. 3HAA is rapidly degraded under normal physiological conditions and research on functions beyond intermediate metabolite is limited. 3HAA demonstrates both pro/anti-inflammatory properties in mammals, insinuating a role in immune function. C. elegans lack adaptive immune elements, but recapitulate aspects of innate immune signaling and pathogen response. I hypothesized that elevating 3HAA via HAAO inhibition in C. elegans would improve pathogen resistance and curtail age-associated immune decline. C. elegans haao-1 mutants have elevated 3HAA and fared better when challenged with the bacterial pathogen Pseudomonas aeruginosa at older ages. Labeled bacteria displayed both decreased infection incidence and progression in haao-1 mutants. During young adulthood C. elegans display a tissue specific separation between the enzymes that produce and degrade 3HAA . We observed that older animals lose this separation in tissue-specific expression. Together, these observations suggest that 3HAA may have distinct tissue-specific accumulation that is lost with age. Indeed, haao-1 mutants visibly accumulate 3HAA in gut intestinal granules located at the frontline of host/pathogen interaction. Finally, 3HAA inhibits bacterial growth and sensitizes pathogens to alterations in iron availability in vitro, providing a mechanism for 3HAA immune function via altering metal availability or redox state. Because the kynurenine pathway is largely conserved and associated with immune signaling in mammals, 3HAA may be a potent target to improve age-associated immune decline. This work was supported by NIH 5T32GM8659 and NIH R35GM133588.

Hypothalamic melanocortin-4 receptors on astrocytes mediate hypothalamic and systemic inflammation.

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Inflammation is one of the pillars of aging that contributes to acceleration of an aging phenotype. Previous work identified that inflammation has an inverse relationship with lifespan. While whole body inflammation contributes to its deleterious effects, previous studies have demonstrated that remarkably, inhibition of inflammation in the hypothalamus alone was able to significantly extend lifespan in mice and flies. Although melanocortins have established anti-inflammatory effects in other conditions such as multiple sclerosis, cerebral ischemia, and mouse models of Alzheimer’s disease, previous studies have not examined their effect on inflammation in aging and on hypothalamic inflammation specifically. Our studies examined the effect of deletion of hypothalamic MC4R residing on activated astrocytes on inflammation in young mice. To achieve this cell-type and region-specific deletion in adult mice we used stereotaxic injection of AAV-Cre under a glial fibrillary acidic protein (GFAP) promoter into the hypothalamus of MC4R floxed mice to cause hypothalamic deletion of MC4R on activated astrocytes in young mice. Hypothalamic deletion of MC4R resulted in significantly increased levels of inflammatory markers both in the hypothalamus (GFAP and Iba1) and the periphery (TNF-alpha and IL-6). These results suggest a significant role for MC4R in hypothalamic inflammation and in mediating inflammation in aging. These results along with future studies examining transcriptomic changes induced by MC4R treatment in aged mice will provide rationale for a possible new therapeutic approach.Funding Acknowledgement: These studies were supported by NIH Geroscience CoBRE (P20GM125528) and Presbyterian Health Foundation Seed Grants.

Phosphoproteomic profiling of skeletal muscle after potentiation in aged female mice

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The maintenance of skeletal muscle contractile function is vital to the overall health of women, especially as women live 1/3 of their life in an estrogen deficient state. We have shown that estrogen deficiency contributes to reduced skeletal muscle strength as well as blunted recovery of strength after injury in female mice. Reversible protein phosphorylation plays an important role in signaling pathways. In skeletal muscle, phosphorylation has been shown to contribute to regulating sarcomeric function, excitation-contraction coupling, energy metabolism, and other roles. Previously, we showed that the skeletal muscle phosphoproteome in a basal, non-contracting state is remodeled in estrogen-deficient female mice. Thus, we questioned how the skeletal muscle phosphoproteome is altered during force generation in estrogen-deficient females. Skeletal muscle twitch force potentiation (i.e., enhanced twitch contraction force) can be evoked by repetitive muscle activation from the staircase effect or after a tetanic contraction. To elucidate how estrogen deficiency impacts force generation, we performed a label-free phosphoproteomic analysis of the tibialis anterior muscle after a post-tetanic potentiation protocol in Aged (24 mo) and Young (4 mo) C57BL/6J female mice. We identified 2650 phosphoproteins, 3507 phosphopeptides, and 4365 unique modification sites. Ingenuity Pathway Analysis revealed overrepresented molecules in canonical pathways pertaining to calcium signaling and protein kinase A signaling, and in molecular functions and physiological systems pertaining to skeletal muscle development and functions as well as cellular assembly and organization. More, upstream analysis revealed inhibition of four kinases, MAPK1, PRKACA, CSNK2A1, and MTOR, (Z-score< -2) that may underlie the phosphorylation alterations observed between Aged and Young female mice. Overall, our study suggests that loss of estrogen may alter kinase activity, which may contribute to muscle strength loss in aged females. This work was supported by the National Institutes of Health/National Institute of Aging (R01 AG031743-13 and Training Program in Muscle Research T32 AR007612 grants).

Protein structure, function and complex interactions influencing convergent sequence evolution in long-lived species.

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Humans are remarkably long-lived, but not exceptionally so. There are many instances across the tree of life in which substantial longevity has been achieved, and it may be the case that the evolution of longevity in different species converges on the same genes and molecular pathways. Variation in longevity across taxa can thus be leveraged to identify convergent modifications to biological mechanisms, genes, and protein sequence; shedding light on the underpinnings of the aging process and, importantly, how this process has been naturally manipulated to achieve extensions in healthspan/lifespan.We consider convergent amino acid substitutions (CAAS), which stratify across long-lived and short-lived species, in the context of protein groups, structures and functions, and make three key findings. (1) Genes containing CAAS are over-represented in certain protein activities, which overlap activities enriched in aging-related proteins. (2) CAAS can cluster with mutated cancer residues in the structures of disease-relevant proteins, can overlap cancer residues directly, and these clusters can occur within protein-protein interaction domains, suggesting possible shared molecular targets of cancer and longevity. (3) CAAS tend to occur between interacting proteins and are over-represented within interaction domains of protein pairs, indicating that selection for longevity may also act at the level of complex formation. By integrating existing computational and experimental datasets, we find several compelling examples in which CAAS changes may impact how proteins function and interact with one another. Our results suggest that the evolutionary routes taken to achieve lifespan extension across different species are shaped by protein structures and functions, both at the level of individual proteins, as well as protein complexes, to modify their activities and biological roles in the aging process.

OXR1 maintains the retromer to delay brain aging under dietary restriction.

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Dietary restriction (DR) delays aging and neurodegeneration, but the mechanisms behind this remain unclear. We reared over 150 fly strains from the Drosophila Genetic Reference Panel under ad libitum feeding or diet-restricted conditions and measured lifespan and healthspan to identify new targets for DR-mediated longevity. A variant in mustard (mtd, called Oxidation resistance 1, OXR1, in humans), significantly associated with DR-specific lifespan. We demonstrate that mtd/OXR1 in neurons is necessary for DR-mediated lifespan extension. Neuronal mtd knockdown accelerates sensory decline, arguing for a specific role of mtd/OXR1 in neuroprotection. We show that mtd is essential for maintaining the retromer complex, which traffics transmembrane proteins and lipids for reuse. As a result of OXR1 deficiency, the retromer destabilizes and lysosomes become overused. Retromer overexpression or supplementation with chaperone compound R55 rescues the lifespan defects and neurodegeneration seen in mtd-deficient flies and R55 rescues lysosomal aggregation in cells from humans with OXR1 deficiency. We further show through multi-omic analyses in flies and humans that mtd/OXR1 associates with accelerated transcriptomic aging and proteins involved in neurodegenerative diseases, including Alzheimer's disease (AD). Overexpression of OXR1 or retromer proteins rescued AD-associated phenotypes in a fly model of AD. Thus, mtd/OXR1 enhances protein recycling in response to DR through the retromer, improving neuronal health and lifespan through mechanisms conserved across species. Funding: T32 AG000266.

Metformin mitigates doxorubicin-induced senescence signaling and SASPs secretion in endothelial cells.

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Doxorubicin (DOX) is a cardiotoxic chemotherapeutic agent known to induce senescence in endothelial cells (ECs). The secretion of senescent associated secretory proteins (SASPs) is increased in DOX-exposed ECs which can act in a paracrine manner to induce senescence in adjacent ECs. Increased senescent ECs are 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, the goal of our work is to determine the effects of metformin on cellular signaling and SASPs secretion in DOX-induced senescent EA.hy926 human endothelial-derived cells (EAs) and Human Umbilical Vein Endothelial Cells (HUVECs). EAs and HUVECs were treated with metformin for 24 hours before DOX treatment. Cells were then co-treated with DOX and metformin for 24 hours. After 24 hours of co-treatment, cells were washed and metformin was added back onto the cells for an additional 72 hours. Cells and the media were collected for analysis at the end of the treatments. Protein expression of senescence markers and MAPK signaling were measured using Western blotting. The florescent bead antibody system (Luminex platform) was used for cytokine analysis of the media. DOX-induced senescence in EAs and HUVECs was evident by significant increases in p21 and p-p53 protein expression. Co-treatment with metformin prevented the DOXâ€“induced expression of these markers of cellular senescence in a dose-dependent manor. Furthermore, DOX activated JNK signaling in EA and HUVECs which was also prevented by metformin co-treatment. Pro-inflammatory SASPs levels were measured in the media from HUVECs. Co-treatment with metformin abrogated DOX-induced increased secretion of SASP factors (IL-6, TNF-alpha, and MCP-1). Our current work shows that metformin can act as a modulator of SASP secretion in DOX-exposed ECs which is associated with reductions in senescence and JNK signaling. This research was supported by the National Heart, Lung, and Blood Institute (NHLBI) grant R01HL151740.

Methionine sulfoxide reductase A (MsrA) and Sex-dependent effects of Methionine Restriction on metabolism and longevity.

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Methionine restriction (MR) has been well established to provide metabolic and lifespan benefits in various models, however the mechanisms remain unclear. The essential amino acid methionine plays a central role in many critical cellular functions including translation, methylation, and sulfur metabolism. Methionine’s structure renders it easily oxidized which alters its biochemical properties. We reasoned that proper homeostasis of available methionine is important in the effects of MR and tested the role of methionine sulfoxide reductase A (MsrA), an enzyme which repairs oxidized methionine, in the physiological and longevity benefits of MR. This was investigated using genetic mutant mice lacking MsrA (MsrA KO) and wild-type C57BL/6 control mice to compare the effects of MR when begun in 9-month-old adult mice. Under short-term MR, we observed that MsrA is largely not required for MR-mediated improvements in glucose metabolism including glucose and insulin tolerance, and changes to metabolic hormones. We found that males benefited from MR in contrast to females, suggesting sex-dependent effects on metabolic improvements. In an identical cohort we also tested the lifelong effects of MR on longevity and health span, and found that MR improved late-life metabolic outcomes including fasting glucose and HbA1C primarily in males. We also found that MsrA KO mice tended to respond similarly, and in some cases to a greater degree, to MR than did wild-type mice. We also found that continuous MR through adulthood had little effect on several common markers of health span, including frailty, rotarod, and grip strength, at 24 or 30 months. In this study, we found no significant effect of MR on lifespan nor an effect of MsrA KO. While our studies recapitulated findings on metabolic improvement with MR, we did not find measurable effects of MR on longevity or health span. Our results suggest that: 1) the beneficial effects of MR do not require MsrA but do have complex interactions for some effects, 2) MR has sex-effects where females generally receiving less benefit, and 3) that MR started in adulthood may have reduced effects compared to early life intervention. Funding: T32 Training Grant – 5T32AG021890-15.

A multi-omics analysis reveals maternal obesity and overnutrition (MOB) accelerate liver cardio-metabolic aging in adult offspring (F1).

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MOB and overnutrition in pregnancy adversely impair F1 health and lifespan. However, molecular mechanisms causing F1 developmental programming-aging interactions are unknown. We use multi-omics to study a nonhuman primate model to investigate MOB effects on the adult F1 transcriptome, proteome, and metabolome. Liver biopsies were collected from 13 F1 (6 females 3-6.5y and 7 males 4.4-6.2y) of normal weight and diet dams (CON) and 19 MOB F1 (10 females 3.8-6.4y and 9 males 4.3-5.5y). Adjusting for sex and age as covariates, we identified 212 proteins, 347 genes, and 17 metabolites with differences (p< 0.01) between MOB and CON F1. Pathway analyses of altered proteins, mRNAs, and metabolites were conducted independently and jointly. Networks were identified with STRING and MCODE and annotated with KEGG pathways. Gene clusters were enriched for ribosomal function (FDR = 5.1x10-9), NAFLD (FDR = 1.6x10-11), and diabetic cardiomyopathy (FDR = 3.6x10-12). Protein clusters were enriched for spliceosome function (FDR = 1.5x10-18), cytokine signaling (FDR = 1.7x10-3), and platelet degranulation (FDR = 1.7x10-3). Joint pathway analysis bolstered numbers of molecules and connections in clusters. For example, a cluster of molecules related to mitochondrial function (FDR = 6.0 x 10-8) grew from a cluster of 14 genes found only with the RNA-seq data to a highly connected cluster of 24 molecules (17 genes and 7 proteins). Joint pathway analysis also identified new clusters not identified in either of the independent pathway analyses. These clusters were enriched for AMPK signaling (FDR = 8.0x10-2), insulin signaling (FDR = 8.0x10-2), and amino acid metabolism (FDR = 4.3x10-4). Overall, our analyses identified aging- and cardio-metabolic-related pathways that help explain how maternal diet impacts later life F1 health by accelerating aging. Our analyses underscore the importance of multi-omics to determine biological mechanisms that underly developmental programming-aging interactions. NIA U19AG057758, NIH P51OD011133.

Increased Myelopoiesis and Upregulation of Alarmins in the Aging Bone Marrow: Reversal by Angiotensin-(1-7)

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Chronic nonsterile inflammation is a major causal factor in age-associated cardiovascular disease. Increased myelopoiesis in the bone marrow largely contributes to systemic inflammation in aging. Vascular protective members of renin angiotensin system, Angiotensin converting enzyme-2 (ACE2) and Mas receptor (MasR) are expressed in bone marrow stem/progenitor cells. This study tested if the vasoprotective peptide of RAS and MasR agonist, Angiotensin-(1-7), ameliorates myelopoiesis and systemic inflammation in aging. Young and Old male mice (C57Bl/6) of ages 3–4 and 20–24 months, respectively, were used in the study and Ang-(1-7) was administered by using osmotic pumps (1 ?g/kg/min, subcutaneous, for 4 weeks). Hindlimb ischemia (HLI) was induced by ligation of femoral artery and mobilization of vasculogenic progenitor cells (VPC) along with monocytes into the blood circulation were determined. Blood flow recovery was determined by using laser Doppler imaging system. Vasculature and inflammatory cells in ischemic skeletal muscle was evaluated by immunohistochemistry. Myelopoiesis in the bone marrow cells was determined by colony forming unit-granulocytes-macrophages (CFU-GM) assay followed by flow cytometry of monocytes and macrophages. Expression of pro-myelopoietic factors, S100 proteins and HMGb1 were determined in the bone marrow. HLI induced mobilization of monocytes from bone marrow into the circulation and to the ischemic area, which were higher in the Old group compared to the Young. Ang-(1-7) decreased the mobilization of monocytes and macrophage infiltration to the areas of injury. Blood flow recovery and vascularization of ischemic areas were impaired in the Old that were restored by Ang-(1-7). Bone marrow cells from Old group generated higher number of monocytes and pro-inflammatory macrophages compared to the Young that were associated with increased expression of S100A8, S100A9, HMGb-1 and RAGE. Ang-(1-7) decreased myelopoiesis and alarmin expression in the Old group. These results suggest that Ang-(1-7) is a promising molecule for decreasing the pro-inflammatory tone and to restore vascular regeneration in aging. Acknowledgments: National Institutes of Aging (NIA), AG056881.

Paired analysis of gene expression and DNA modifications in hippocampal neurons between sexes with aging.

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The hippocampus is a key neuroanatomical substrate for learning and memory events and the most common site of origin for dementia and neurodegenerative disorders such as Alzheimer's disease. Due to their long lifespan and sustained firing during events such as learning and memory formation, hippocampal neurons are primed to accumulate DNA damage with aging leading to genomic instability and altered gene expression. However, many aging studies to date examine whole tissue and often overlook the effect of sex on aging. Our goal was to identify age and sex-associated differentially expressed genes (DEGs) and differentially methylated regions (DMRs) in specific cell types of the CNS in a paired fashion, with the focus of this study being hippocampal neurons. The NuTRAP allele, when crossed with an inducible neuronal-specific Cre (Camk2a) and induced with tamoxifen, labels nuclear envelope protein RanGap1 with biotin and mCherry and ribosomal protein L10a with GFP in neurons. This model allows for paired isolation of nucleic acids from the same cell type in vivo, eliminating the need for cell sorting. Male and female 3mo and 22mo Camk2a/NuTRAP mice (n=6, N=24) were used for paired RNA-sequencing and WGoxBS sequencing. We observed enrichment of genes involved in neuron branching, glutamate release, and long-term synaptic depression with age. We have also identified several age-related DMRs that correlate with DEGs. Our studies have identified age and sex-associated DEGs and DMRs in hippocampal neurons correlating with increased glutamate excitotoxicity promoting neuronal degeneration. We plan to continue using this model to identify hotspots for DNA damage in aging hippocampal neurons using ATAC-seq, ?H2AX ChIP-seq, and structural optical genome mapping. Funding: This work was supported by NIH (T32 AG052363; R01 AG059430) and VA Merit Award (I01 BX003906).

Senolytic Therapy: Results from a Placebo Controlled 6-Month Nonhuman Primate Trial

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Senescent cell removal improves adipose tissue (AT) function in rodents and humans. We documented real-world effect sizes of a common senolytic combination, dasatinib and quercetin (D[5mg/kg]+Q [50mg/kg]), in nonhuman primates (NHPs). Preliminary data from 3 diabetic NHPs given a single dose of D+Q indicated efficacy. We then dosed NHPs on 2 consecutive days, monthly for 6 months to understand the safety and efficacy of D+Q. Male and female cynomolgus macaques (D+Q, n=10; vehicle CTL, n=6) matched on age, weight, and glycemic control were studied. Both groups included diverse health statuses, including obese and type 2 diabetic NHPs. Primary outcomes were metabolic syndrome criteria, body composition, and AT senescence markers (p21 and p16 gene expression, AT senescence-associated ?-galactosidase staining, and p21 immunohistochemistry). Evaluations were periodic and outcome data is still being generated and under analysis. To date we have observed reduced AT senescence burden, minor improvements in body composition and glucose handling, and reductions in circulatory inflammatory and cardiac biomarkers. These small changes were coincident with NHPs being weight-stable or gaining after 5 months. For the final and 6th month of study, 10% caloric restriction (CR) was implemented which induced small but equivalent reductions in weight in D+Q and CTL NHPs. This shift towards negative energy balance resulted in significant improvements in glucose and lipid-related measures and body composition in D+Q NHPs. The magnitude of change was large, particularly for hemoglobin A1c (>0.5% reduction), an outcome that takes weeks to month to change. Cardiovascular markers N-terminal brain natriuretic peptide and plasminogen activator inhibitor-1 dramatically reduced, and cardiac MRI analyses are underway to understand D+Q effects on cardiac function. D+Q reduced activated and total monocytes in circulation compared to CTL, suggesting immune benefits. Despite the very positive overall metabolic profile that resulted from D+Q+CR, these co-occurred with increases in AT senescence markers. p21+ immunostained cells clustered around small adipocytes and will be co-localized with macrophage identifiers as this cell type can reversibly be senescent and aid in clearing toxic and apoptotic cells. Biochemistry measures confirmed that D+Q was generally safe and we conclude it improved metabolic health in mid-life NHPs, particularly in the context of mild CR. R01HL142930 T32AI007401

Evaluation of Protectin DX as a therapeutic strategy against frailty in mice.

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Frailty in aging is driven by the dysregulation of multiple biological pathways. Protectin DX (PDX) is a docosahexaenoic acid (DHA)-derived molecule that alleviates many chronic inflammatory disorders, but its potential effects on frailty remain unknown. Our goal is to identify age-related changes in metabolic and musculoskeletal systems and to evaluate the therapeutic potential of PDX on frailty, physical performance, and health parameters. Thus, 23-month old C57BL/6 male and female mice were assigned to vehicle (VEHICLE) or PDX daily gavage treatment for 7 weeks, whereas 6-month old (YOUNG) received only vehicle. Forelimb and hindlimb strength, endurance, voluntary wheel activity and walking speed determined physical performance (PP). Frailty Index Score (FIS), glucose homeostasis and heart rate (HR) were also evaluated. Mice that fell in the 20th percentile cut-off values for each frailty criterion were considered positive for that one marker. Three or more positive frailty markers identified an animal as frail, two positive markers indicated pre-frail and one or zero specified robust. Body weight (BW) and temperature (BT) were monitored weekly. Bone mineral density (BMD) was evaluated by both DEXA and ÂµCT. Our data shows that aged vehicle-treated mice from both sexes had greater BW and lower BT during the intervention period compared to YOUNG. Aged VEHICLE mice were hypoglycemic and had similar HR compared to YOUNG. Overall, VEHICLE-treated animals from both sexes demonstrated worsening PP and FIS, and lower BMD. PDX prevented the age-driven decline in treadmill performance in both sexes. In males, PDX attenuated forelimb and hindlimb strength loss, whereas in females it prevented the decline in walking speed. PDX-treatment in females improved BMD in both trabecular and cortical bones. The 23-month-old VEHICLE females and females showed a greater increase in frailty prevalence compared to sex-matched PDX-treated mice. In conclusion, our data provides evidence of the beneficial therapeutic effect of PDX against features of frailty in mice. Further studies are warranted to investigate the mechanisms of action and the potential for human translation. Funding: NIH K07AG072124, NIH R56AG067724, Travis M. Roy Endowed Professorship.

Dysregulation of FGF21-mediated protein preference during aging.

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Poor nutritional status associated with aging is a multifactorial condition characterized by inadequate food intake, decreased nutrient absorption, and increased nutrient loss. Protein-energy malnutrition is common in aging populations and confers increased risk for sarcopenia and muscle loss, yet the underlying cause of dysregulated macronutrient balance is not fully understood. The peptide hormone fibroblast growth factor-21 (FGF21) is produced from the liver during amino acid imbalance or restriction, serving as a neuroendocrine signal of protein restriction. In young animals, we first reported that FGF21 acts via the nervous system to increase the consumption of dietary protein. Circulating FGF21 is increased during aging, likely reflecting the degree of protein-energy malnutrition, and suggesting that increasing age may blunt FGF21-induced protein appetite. Here, we tested this hypothesis in 24 month-old aging mice and 10 week-old young-adult controls. First, we find that pharmacological treatment with FGF21 elicits an age-dependent effect on dietary protein intake, such that aging blunts FGF21-induced protein appetite [P (treatment x age)< 0.01] . Nutritional stimulation of FGF21 secretion by low-protein feeding was equally effective to increase plasma FGF21 in both young and old mice [P (treatment)< 0.01], but the associated increase in subsequent protein appetite was bunted in aging mice [P (treatment x age)< 0.05]. However, we found the expression of second messengers downstream of FGF-receptor activity was blunted in the brains of FGF21-treated young vs old mice [P (age)< 0.05]. Our results suggest that FGF21-mediated behavioral control of systemic protein homeostasis is blunted during aging, and that responsible neuroendocrine circuits may represent a translational target for restoration of macronutrient balance. This work supported by a National Institute of General Medical Sciences-funded Pharmacology Training Program under Award Number T32GM099608 and the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health under Award Number R01DK121035.

Development of an Old World Primate normative aging resource

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Baboons are an Old World Primate species that separated from humans ~32 MYA and marmosets ~43 MYA. Due to the baboon's extensive physiologic, metabolic, and genetic conservation with humans, it serves as one of the key species for translational studies addressing multiple human diseases that are co-morbidities of aging including naturally occurring dyslipidemia, hypertension, obesity, osteoporosis, and diabetes. We have developed a resource of living animals (n= 91; ~ equal numbers of females and males) and archived specimens from virtually every tissue in the body (n=70 donors; ~ equal numbers of males and females) across the adult age span (5-23y; ~15-81 human equivalent) to study normal aging processes. Cohort data include birth weights, longitudinal morphometric and clinical measures, and metabolic-related circulating measures. In vivo studies, such as functional MRI and MRS, hyperinsulinemic euglycemic clamp, cognitive assessment using CANTAB, and gait speed measurement are ongoing. We have prepared fibroblast cultures from skin and heart, astrocytes from different brain regions, and hepatocytes. Biopsies are sometimes taken from liver and muscle. Collaborative non-invasive studies are feasible. In vitro studies, using freshly collected samples, include cell energetics and tests of resilience and reserve. In addition, data generation using a subset of archived tissues, include transcriptomic, proteomic, and metabolomic data. The resource includes statistical and bioinformatic methods development for integration of omic data. The overarching goal of the study is to identify early signatures of aging and molecular changes that correlate with age in normal aging primates. A wide variety of archived tissues are available for collaborative study (H. Huber, hhuber@txbiomed.org). We also have a parallel resource that addresses developmental programming and aging interactions. We welcome all enquiries to use these unique resources. Funding from NIA 1U19AG057758, NIH P51 OD011133.

Vascular smooth muscle cells and IGF-1 in age-related vascular fragility and cognitive decline

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Microvascular pathologies contribute to cognitive decline and gait defects in the elderly. Hypertension and age are two risk factors for both microvascular defects such as cerebral microhemorrhages (CMH) and changes in larger vessels such as impaired myogenic autoregulation. Circulating levels of the vasoprotective factor insulin-like growth factor 1 (IGF-1) decrease with increasing age in humans and animal models. This decrease in aged animal models is associated with an increased incidence of CMHs and other vascular pathologies. However, the mechanisms that regulate CMH development and microvascular fragility are not well understood. Vascular smooth muscle cells (VSMCs) are critical for maintaining vascular integrity and perform protective functions such as autoregulation. We hypothesize that reduced IGF-1 signaling on VSMCs will promote vascular instability and impaired protective vascular responses to hypertension, thus leading to an increased risk of hemorrhage and promoting cognitive decline and gait defects. To test these hypotheses, we induce hypertension in mice with VSMC-specific IGF-1 receptor deficiency (Igf1r), and then evaluate the occurrence of CMH and neurological issues. We have found that VSMC-specific Igf1r deficiency exacerbates the development of hypertension-induced CMH, impairs gait, and results in reduced autoregulatory capacity of the cerebral vasculature. These studies will improve our understanding of CMH pathogenesis and the role of VSMCs in age-related vascular fragility in order to improve healthspan in elderly patients.Funding Sources: National Institutes of Health (R01-AG047879; R01-AG055395; R01-AG070915; T32AG052363; 1P20GM125528, 5P30GM122744; NINDS; R01-NS056218; R01-NS100782), and the American Heart Association.

Effect of Mitochondrial-nuclear interaction on lifespan regulation.

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Growing body of data from studies on model systems has demonstrated that plasticity of mitochondrial function (e.g. mild changes in respiration) can be targeted to promote healthy aging. However, genes, pathways, and related regulatory networks that causally link these changes to longevity remain unclear. Since mitochondrial function and related pathways are jointly coordinated by genes encoded by mitochondrial (mtDNA) and nuclear DNA (nDNA), the genetics of aging should be controlled by variation in (1) mtDNA, (2) nuclear genes, and (3) nDNA-mtDNA interactions. The role of inter-organelle interaction in regulation of aging has become apparent from studies reporting that changes in the mtDNA may significantly influence lifespan. Since aging and longevity have a significant genetic component, this inter-organelle DNA epistasis might be one of the genetic factors of lifespan variation. Accordingly, this proposal addresses a significant question that has been largely neglected in aging research: how the combination and interaction of two genomes, the nuclear and the mitochondrial, triggers changes in cellular processes that regulate the genotype-dependent lifespan differences. To address this question, we developed a novel mitochondria (mito)-nuclear epistasis model by systematically exchanging mitochondrial mtDNA between wild yeast isolates, characterized with highly polymorphic mitochondrial and nuclear genomes. Our pilot lifespan analysis revealed significant lifespan variation between the strains with same nDNA, but different mtDNA backgrounds, and across all synthetic combinations of mito-nuclear genotypes. We further provide evidence that mtDNA background is a significant modulator of the CR-mediated longevity. Overall, the results reveal that mtDNA sequence variation explains a portion of heterogeneity in cellular processes that affect lifespan variation and these epistatic interactions can be further modified by diet.

The MotrMito project: Aging and the mitochondrial response to exercise training, measured by 31P magnetic resonance spectroscopy, among MoTrPAC participants

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Reduced mitochondrial oxidative capacity in skeletal muscle is linked to aging-related decreases in cardiorespiratory fitness, physical performance, and cardiometabolic health. There are two specific questions about the importance and ramifications of exercise-induced mitochondrial capacity changes: 1) Does exercise improve mitochondrial capacity in older and younger people in the same way? 2) What skeletal muscle molecular markers are associated with gains in mitochondrial capacity in these two groups? The MotrMito study fills in these gaps by: a) objectively assessing the spectrum of mitochondrial capacity responses to exercise in vivo; b) investigating the underlying molecular regulation of exercise responses, and; c) linking mitochondrial responses and molecular factors to clinically relevant outcomes such as exercise-induced improvements in cardiorespiratory fitness (VO2max). The goals are to assess differences in the mitochondrial capacity responses to aerobic and resistance exercise training in a wide age range (18 to 60+) via 31P-MRS. By using MoTrPAC's high-throughput 'omics' technologies, we will compare the exercise-induced physiological mitochondrial responses to the molecular transducers of exercise and thus shed light on the mechanisms underlying the health benefits of physical activity in older persons. A total of 375 participants will be recruited, trained, and assessed at four sites. Following the approach taken by MoTrPAC, all MotrMito data will be released to investigators worldwide. We expect the first of these data releases to occur in 2023. Acknowledgements: The authors would like to thank NIH (R01 AG069476) grant for financial support

SOLUBLE PATHOGENIC TAU ENTERS BRAIN VASCULAR ENDOTHELIAL CELLS AND DRIVES CELLULAR SENESCENCE AND MICROVASCULAR DYSFUNCTION IN TAUOPATHY.

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Amyloid plaques and tau tangles are major hallmarks of Alzheimer's disease (AD). Tau protein stabilizes microtubules. Soluble aggregated pathogenic tau, however, is a mediator of AD that is transmitted trans-neuronally in a prion-like fashion, causing the aggregation of native tau in target cells. Recent studies from our laboratory provided the first evidence of tau deposition in brain microvasculature of AD, suggesting that soluble pathogenic tau released from neurons may be transmitted to microvascular cells. We found that pathogenic tau enters human brain microvascular endothelial cells (HBEC), where it triggers phosphorylation of endogenous tau, decreases microtubule density and stability, and impairs activation of endothelial nitric oxide synthase (eNOS), that requires transport of eNOS to cell membranes. Further, transmission of pathogenic tau to HBEC triggered cellular senescence. Consistent with these observations, we found abundant microvascular pathogenic tau in P301S (PS19) mice modeling tauopathy, together with microtubule instability, decreased eNOS activation, vascular cell senescence, and impaired eNOS-dependent vascular reactivity. Our studies indicate that pathogenic tau is internalized by brain microvascular endothelial cells where it causes phosphorylation and misfolding of endogenous tau, destabilizes the microtubule cytoskeleton and blunts the activation of eNOS. Our data suggest that cerebrovascular tau is a novel mediator of cerebrovascular dysfunction and a novel therapeutic target in AD. 1RF1AG057964, 5I0-1BX002211, 1RF1AG068283, the Robert Bailey and daughter Lisa Bailey Alzheimer's Fund, William & Ella Owens Medical Research Foundation Grant, T32AG021890

Peptidomimetic proteasome activators as novel agents to battle Alzheimer's disease.

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Therapeutic approaches to Alzheimer's disease (AD) need to encompass multiple physiological phenomena. The ubiquitin-proteasome pathway (UPP) is the major guardian of proteostasis in human cells, including neurons. The proteasome is an essential protease and a focal point of the pathway. Consequently, deregulation and deficiencies in proteasome-related proteostasis would be expected to affect multiple neuronal functions and to drive many of the functional and symptomatic deficits observed under AD. Indeed lowered activity of the proteasome has been described in animal models of AD, and artificial impairment of proteasome function can mimic many neurodegenerative phenotypes. Importantly, we found that impaired proteasome function as an early-stage marker of AD, preceding many other markers of the disease. Consequently, augmentation of the proteasome function may prevent the progression of AD. However, the proteasome is a giant, modular enzyme, difficult to precisely control with synthetic molecules. To meet the challenge we rationally designed a set of proteasome-activating peptidomimetics, based on fragments of the viral protein HIV-1 Tat. Our compounds allosterically activate proteasomes in vitro, in cellulo and in vivo. Importantly, they boost performance of not only the 20S catalytic core particle, but also the most physiologically relevant 26S holoenzyme: a unique capability. Our two lead compounds effectively cross the blood brain barrier and reduce or even reverse AD symptom progression in fruit fly and mouse models, including reducing deficits in learning and memory. Here we present selected aspects of design and actions of the compounds. We acknowledge funding from NIH/NIA, William & Ella Owens Medical Research Foundation, SA Nathan Shock & Pepper Centers, Glenn Foundation/AFAR, Perry & Ruby Stevens Parkinson's Disease Center of Excellence, National Science Center and Polish Ministry of Education & Science.

Short-term dietary branched-chain amino acid restriction has persistent metabolic health benefits.

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Approximately 70% of Americans are overweight or obese, leading to an increased risk of developing age-related diseases (ARDs). As a consequence of attempted weight loss, overweight and obese individuals experience

periods of high and low compliances with dietary interventions, resulting in severe bodyweight fluctuations. Little is known about the long-term consequences of short-term periods of dieting and reduced weight. Low protein diets are associated with improved health and reduced risk of diabetes and death, which is replicated by a 67% restriction of dietary branched-chain amino acids (BCAAs; i.e. leucine, isoleucine and valine). The Lamming lab previously demonstrated that a short-term BCAA restriction (BCAA-R) normalized body composition, improved glucose homeostasis and increased energy expenditure in diet-induced obese (DIO) mice. Here, we investigate the persistent effects of short-term BCAA-R in the context of obesity once the diet is abandoned. We placed 6-week-old C57BL/6J male mice on a high-fat, high-sucrose Western diet (WD). Using a dietary regimen with long weight-gain and short weight-loss periods, WD is provided over 12 weeks to induce obesity, after which the animals are fed a cycle of BCAA-R over 3 weeks and WD again over 12 weeks. After 3 weeks, BCAA-R improved metabolic health, replicating previously findings. Critically, once cycling back to WD, animals previously fed BCAA-R demonstrated a persistent increase in energy expenditure and decreased lipid droplet size as well as a significantly lowered fasting blood glucose and slightly improved glucose tolerance. These findings show that short-term BCAA-R can confer lasting effects on metabolic health, which could be a translatable strategy for long-term improvements to metabolic health and potentially reduce the risk of developing ARDs. The Lamming Laboratory is supported by the NIH/NIA (AG056771, AG062328, and AG061635 to D.W.L.), NIH/NIDDK (DK125859 to D.W.L.), and the U.S. Department of Veterans Affairs (I01-BX004031).

Canagliflozin modifies hypothalamic function in aging

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Various aspects of physiological deterioration in aging are controlled by the hypothalamus, a critical brain region that connects the neuroendocrine system to physiological functions. Recently, the Interventions Testing Program (ITP) demonstrated that the anti-diabetes drug, Canagliflozin (Cana), a sodium-glucose transporter 2 inhibitor (SGLT2i), improved glucose metabolism in both sexes, extended lifespan by 14% in males but not in females, and reduced body adiposity in female mice only. Here we show that Cana treatment elicits sexually dimorphic effects on energy expenditure in 30-month-old mice. Carbon dioxide production (VCO2), oxygen uptake (VO2), and respiratory exchange ratio (RER) were all increased in Cana-treated female mice, while no significant differences were recorded between Cana-treated and control male mice. Increased RER in female, but not male mice, was associated with increased heat production. Despite their reduced body weight, Cana-treated male and female mice showed increased food consumption and dramatic elevations in water intake. The age-dependent changes in the hypothalamic neurons controlling metabolism, especially the pro-opiomelanocortin (POMC) and agouti-related peptide/neuropeptide Y (AgRP/NPY) neurons, underlie the imbalance of energy homeostasis with aging. Cana treatment modulated the arcuate nucleus of the hypothalamus (ARC), markedly increasing the formation of both AgRP and POMC projections to the paraventricular nucleus of the hypothalamus (PVH) in females only. Moreover, Cana treatment stimulated the activity of CART/POMC neurons innervating sympathetic neurons in the hypothalamus, suggesting that this pathway may contribute to the increased energy expenditure and decreased body weight in response to Cana treatment in female mice. Additionally, long-term Cana treatment sensitized the hypothalamic response to insulin, as shown by increased levels of cytoplasmic FoxO1 in the ARC of the hypothalamus in response to insulin administration in aged 30-months old males but not in females. These results demonstrate the protective properties of Cana treatment in the hypothalamus in both males and females beyond its effects on peripheral metabolism and suggest its therapeutic potential for age-related diseases. Funding: Impetus, AG022303, AG024824

Tau-induced astrocyte senescence as a driver of neuronal dysfunction in Alzheimer’s disease.

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Alzheimer's disease (AD) remains the only condition among the top 10 causes of death in the US that cannot be prevented or treated. Aging is the greatest risk factor for AD. Age-associated molecular damage leads to cellular senescence, defined as irreversible cell cycle arrest and acquisition of an inflammatory phenotype. Some brain cells such as neurons are post-mitotic, while others such as astrocytes maintain the capacity for cell division and can therefore undergo senescence. Tau protein stabilizes microtubules. Aggregated pathogenic tau, however, is a central mediator of AD that is transmitted trans-neuronally. We found that pathogenic tau can also be transmitted to human astrocytes, where it triggered endogenous tau phosphorylation and induced cellular senescence dependent on endogenous tau. Astrocytes undergoing tau-induced senescence caused neuronal damage. Single-cell RNA-seq revealed large numbers of senescent astrocytes in an AD tauopathy model, suggesting that astrocytes are a major cell type driving senescence in tauopathy brain. Our data provide first evidence of transmission of pathogenic forms of tau into astrocytes, and show that pathogenic tau triggers astrocyte senescence, causing neuronal damage. Pathogenic tau-induced astrocyte senescence may thus be central to the etiology of neuroinflammation in AD. Because drugs that eliminate senescent cells are FDA-approved and immunotherapies to remove tau from brain are advancing to clinical trials, our studies may have rapid translational potential for AD and other tauopathies. 1RF1AG057964, 5I0-1BX002211, 1RF1 AG068283, the Robert L. Bailey and daughter Lisa K. Bailey Alzheimer's Fund, William & Ella Owens Medical Research Foundation Grant, NIA T32AG021890

Predicting maximum lifespan using the coefficient of variation of MIEL-based epigenetic signatures in PBMCs.

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The homeostatic stability of many biological systems decreases as organisms age, but these changes are not always linear over time. When modeled to its extreme, this non-linear divergence of "biological age" and "chronological age" provides an indicator of the maximum lifespan of an organism. Research modeling biological age has constructed 'biological clocks' that rely on using longitudinal measurements of CpG island methylation. Bulk CpG methylation variance also increases with age correlating with organism level behavioral and cognitive measurements. To further investigate maximum lifespan, we analyzed the coefficient of variation (CV) of epigenetic state using Microscopic Imaging of Epigenetic Landscapes (MIEL). MIEL captures an epigenetic "fingerprint" through the multivariate analysis of texture features extracted from multi-channel fluorescent images. Individual nuclei are identified and segmented out, then texture features are extracted from each segmented nucleus. The texture features represent the pattern of expression for acetylation, methylation, and chromatin density at the single-cell level. This technique has added benefits to traditional biological clocks as multiple epigenetic marks, including both methylation and acetylation, may be quickly imaged and analyzed. We observed that MIEL-based changes in the CV of the epigenetic landscape in single cells increased with age. Hyperbolic best-fit approximation of MIEL-generated CV provided a reasonable prediction for the maximum lifespan of a mouse at the single cell level. To determine the predictive power of MIEL-based CV we plan to combine longitudinal measurements with functional readouts of aging in individual mice.Funding for this project was given by grants R21AG068913 and R21AG075483.

Lysosome Lipid Signaling From The Periphery To Neurons Regulates Longevity.

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Lysosomes are key cellular organelles that metabolize extra- and intracellular substrates. Alterations in lysosomal metabolism are implicated in aging-associated metabolic and neurodegenerative diseases. However, how lysosomal metabolism actively coordinates the metabolic and nervous systems to regulate aging remains unclear. Here, we report a fat-to-neuron lipid signaling pathway induced by lysosomal metabolism and its longevity promoting role in Caenorhabditis elegans. We discovered that induced lysosomal lipolysis in peripheral fat storage tissue up-regulates the neuropeptide signaling pathway in the nervous system to promote longevity. This cell-non-autonomous regulation is mediated by a specific polyunsaturated fatty acid, dihomo-gamma-linolenic acid (DGLA) and LBP-3 lipid chaperone protein transporting from the fat storage tissue to neurons. LBP-3 binds to DGLA, and acts through NHR-49 nuclear hormone receptor and NLP-11 neuropeptide in neurons to extend lifespan. These results reveal lysosomes as a signaling hub to coordinate metabolism and aging, and lysosomal signaling mediated inter-tissue communication in promoting longevity.

The Role of PPAR?-driven ?-oxidation in Bone Health During Aging

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Musculoskeletal disorders are one of the most significant complications of aging, leading to increased morbidity and mortality. However, our current understanding of the mechanisms by which aging affects skeletal health is limited. Osteocytes are the most numerous and long-lived cells in bone and play key roles in maintaining bone mass by responding to anabolic signals such as mechanical loading. This response to loading is impaired in aged bone by unknown mechanisms, resulting in disrupted bone homeostasis. Energy metabolism is dysregulated in many cells and tissues with aging, however regulation of energy metabolism in osteocytes and how this is affected during aging and by mechanical loading remains undefined. To investigate this, we first used the IDG-SW3 osteocyte cell line to determine the effects of mechanical loading on osteocytes in vitro by applying fluid flow shear stress (FFSS). FFSS increased peroxisome proliferator-activated receptor delta (Ppar?) and carnitine palmitoyltransferase 1 (Cpt1) mRNA expression, key promoters of fatty acid ?-oxidation (FAO). Pharmacological antagonism of PPAR? or CPT1 in IDG-SW3 cells resulted in dysregulated expression of key bone remodeling genes and impaired ATP release in response to FFSS. In vivo, mechanical loading significantly increased FAO in tibia cortical bone. However, FAO was impaired in the bones from aged mice. To further elucidate the role of osteocyte FAO, we deleted PPAR? specifically in osteocytes (PPAR? cKO), which resulted in decreased FAO and significantly reduced bone volume in female PPAR? cKO mice. Lastly, treatment of aging mice with the PPAR? activator GW0742 for 4 weeks resulted in significantly increased bone mineral content, density and trabecular bone volume. These findings suggest important functions of osteocyte energy metabolism in the effects of aging and mechanical loading on bone and identify PPAR?-driven FAO as a novel therapeutic target for improving skeletal health with aging.

17?-Estradiol Mitigates the Negative Effects of High-Fat Feeding in Both Male and Female Mice

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17?-Estradiol Mitigates the Negative Effects of High-Fat Feeding in Both Male and Female MiceMatthew P. Bubak1, Shivani N. Mann2,3, Matle E. Broomfield1, Michael B. Stout1, Benjamin F. Miller1. 1. Aging & Metabolism

Program, Oklahoma Medical Research Foundation, Oklahoma City, OK2. Department of Nutritional Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK3. Department of Neuroscience, University of Arizona, Tucson, Arizona17?-estradiol (17?-E2) is an endogenous, non-feminizing enantiomer of 17?-estradiol that improves the lifespan of male, but not female mice. Although the effects of 17?-E2 on liver and adipose tissue are well described, potential benefits in skeletal muscle are less known. Our study aimed to determine if 17?-E2 can mitigate the negative effects of a high-fat diet (HFD) on skeletal muscle metabolism and if there are sex differences in these responses. We hypothesized that 17?-E2 supplementation would ameliorate the negative effects of a HFD in skeletal muscle of male, but not female mice. To test this hypothesis, wild type male (n=19) and female (n=15) mice began a HFD (45% fat) at 3 months of age. At 9 months of age, half of the males (n=10) and females (n=8) received diets supplemented with 17?-E2 (14.4ppm) for 3.5 months. 17?-E2 reduced whole-body fat mass (p = 0.001) and whole-body lean mass (p = 0.003) in males. In females, 17?-E2 reduced fat mass (p = 0.0001) but did not alter lean mass (p = 0.99). 17?-E2 improved glucose tolerance in females (p = 0.03) but not in males (p = 0.11). 17?-E2 reduced insulin resistance in males (p = 0.001) and females (p = 0.05). 17?-E2 reduced the expression of IL-6 (p = 0.005), IL-1? (p = 0.002), and TNF? (p< 0.001) in males, but 17?-E2 did not change cytokine expression in females (p > 0.05). 17?-E2 did not change oil red O staining in muscle of the male (p = 0.59) or female (p = 0.68) mice. 17?-E2 supplementation did not change triglyceride concentration in males (p = 0.98). However, females on 17?-E2 had significantly lower quadricep triglycerides compared to HFD (p = 0.03). Contrary to our hypothesis, 17?-E2 supplementation had positive effects in the skeletal muscle of both male and female mice fed a HFD. However, the positive effects differed between males and females. Funding: NIA R01AG070035 and NIH 5T32AG052363-04.

Evidence for preserved insulin responsiveness in the aging rat brain.

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Insulin exerts trophic effects in the CNS and has been trialed in delaying age-related cognitive decline. CNS insulin uptake and signaling are altered in aging, limiting the benefits of centrally acting insulin therapy in prophylaxis against cognitive decline. We characterized brain insulin responsiveness in young (4-5 mo) and aged (25-26 mo) rats to determine the extent to which insulin signaling in the CNS varies with age. Despite no age difference in plasma insulin and IGF-1, nor hippocampal igf1 mRNA, CSF insulin and IGF-1 levels were reduced in aged rats, although relative IGF-1R expression was increased in aged hippocampus, with InsR expression trending upward in aged rats. ICV insulin in aged rats restored glucose infusion rate and hepatic glucose production to levels comparable to that of young rats. Resting-state functional magnetic resonance imaging (rs-fMRI) was leveraged to evaluate age- and insulin-related changes in network connectivity within the default mode network. Seed-based connectivity analysis revealed lower connectivity between mesial temporal region and other ROIs in aged rats, whereas k-medoids clustering revealed an age-related loss of heterogeneity in the frequency domain, an indicator of signal complexity. ICV infusion of insulin provoked distinct brain regional activation patterns in aged rats, with greater BOLD signal seen in septal nucleus and hypothalamus and signal reduction in thalamus and nucleus accumbens relative to insulin stimulation in young rats. Ex vivo stimulation of hippocampus with 10 nM insulin increased phospho-Akt Ser 473 and Thr 308 in young but not aged rats, whereas pMAPK 44/42 was increased with insulin stimulation of cerebral cortex in aged but not young rats, indicating distinct age- and region-related changes in MAPK and PI3K/Akt axes. Our findings of increased InsR and IGF-1R expression and reduced CSF concentrations of insulin and IGF-1, with a BOLD signal highly responsive to ICV insulin, suggest that aged rats retain central insulin responsiveness with age. P30 AG038072; P30 DK020541; P30 CA013330; Einstein Startup Funds to DH.

A novel proteostasis adaptation in the long-lived Caenorhabditis elegans rpn-10 proteasome subunit mutant

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The loss of proteostasis due to reduced efficiency of protein degradation pathways is highlighted in aging and age-related disorders. Paradoxically, we have reported that the C. elegans rpn-10(ok1865) proteasomal mutant exhibits enhanced cytosolic proteostasis, elevated stress resistance and extended lifespan, despite possessing mild proteasome dysfunction. The RPN-10/PSMD4 subunit is a ubiquitin receptor on the 26S proteasome that

targets polyubiquitinated substrates to its catalytic core for degradation. We showed that compensatory activation of autophagy and SKN-1/Nrf-regulated responses only partially underlies the robust rpn-10 mutant phenotype. Hence, in this study we elucidate the endoplasmic reticulum protein quality control (ERQC) in the rpn-10 mutant. We determined that the rpn-10 mutant exhibits higher ER stress resistance and altered ER homeostasis compared to the wild-type. Further, we observed attenuated accumulation of the ER-localized aggregation-prone mutant Î±-1 antitrypsin (ATZ) reporter, which indicates augmented ER proteostasis in the rpn-10 mutant. Seeking novel ERQC regulators, we identified ecps-2, a homolog of the proteasome-associated adaptor protein ECM29/ECPAS, via forward genetics screen for suppressors of decreased ATZ aggregation in the rpn-10 mutant. While ecps-2 does not regulate proteasomal subunit expression or the ER stress response, we found that the loss of ecps-2 in the rpn-10 mutant reduces its proteasomal chymotrypsin-like activity. Altogether, this signifies that the modified proteasomal assembly of the rpn-10 mutant actuates improved cellular proteostasis. Moreover, increased rpn-10 mutant lifespan appears to depend partly on ecps-2 but more strongly on its ERQC status. Therefore, we propose that the ecps-2-proteasome interaction induces a unique ERQC adaptation which supports the superior proteostasis and longevity of the rpn-10 mutant.

Mitochondrial DNA copy number; A molecular marker of human aging implicated in health disparities of prostate cancer.

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Mitochondrial DNA copy number (mtDNAcn) has long been implicated as a biomarker for general mitochondrial dysfunction and response to oxidative stress. More recently, mtDNAcn has emerged as a biomarker of aging. Previous studies have shown that mtDNAcn is positively correlated with telomere length, and show a significant decrease in mtDNAcn with age. Moreover, mitochondrial DNA copy number has been associated several age-related diseases such as cancer, neurodegenerative disease, and diabetes. More specifically, previous research may indicate a role for mtDNAcn as a biomarker of prostate cancer prognosis in regards to health disparities. Prostate cancer (PCa) is the second most common cause of cancer death in US men, however, the incidence rate of PCa is 76% higher and mortality rate is 2.3 times higher in African American (AA) men compared to white men. MtDNAcn has been reported as being increased in prostate cancers compared to normal tissue, and more interestingly, higher mtDNAcn has been associated with worst prognosis in AA PCa patients. Here, we show that mtDNAcn is elevated in AA PCa patients compared to white men, and AA controls exhibit higher baseline levels of mtDNAcn compared to white controls. This finding may further implicate mtDNAcn as a novel biomarker for PCa health disparities in AA men. Funding sources: DODW81XWH, U54CA233465, P30CA014089-45S3.

Activation of NAMPT as a preventive strategy for age-associated diseases

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Nicotinamide adenine dinucleotide (NAD+) is a cofactor essential for multiple cellular functions including energy metabolism, chromatin remodeling, immune signaling, and cellular senescence. Tight regulation of these processes is critical for tissue and cellular function as well as healthy aging. Aging is associated with a decline in tissue and cellular NAD+. This decline is linked to numerous age-associated diseases in multiple organisms including humans. Restoring NAD+ levels in aging models by supplementing with nicotinamide riboside (NR) or nicotinamide mononucleotide (NMN) has been shown to delay multiple age-associated disorders such as diabetes, cardiovascular disease, and neurodegeneration in cellular and mouse models, but translating the activity of NR or NMN to humans has been challenging due to tissue complexity and distribution of specific NR and NMN transporters. Currently, there are multiple clinical trials exploring the effects of NR and NMN supplementation in humans, but efficacy remains unclear, suggesting that other approaches are needed. Here, we characterize SBI-

0797812, a potent and selective activator of NAMPT, an enzyme essential for NAD+ biosynthesis. We show that SBI-0797812 increases NAD+ levels in-vitro and in-vivo and we plan to explore the effects of NAMPT activation in age-associated disease models. We propose that NAMPT activation is a feasible strategy to counteract the age-dependent decline in NAD+ levels and ameliorate age-related disease.

Late-life administration of 4-Phenylbutyrate slows frailty onset in mice of multiple genetic backgrounds.

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The growing aging population presents a systemic problem to healthcare and well-being in developed nations, driving an increased need for treatments that can improve health and slow or even reverse onset of frailty. In this study we investigated the potential of the FDA approved drug 4-phenylbutyrate (PBA) to improve functional health in old age. PBA acts through several mechanisms, however two primary modes of action which may target proposed pillars of aging are its activity as both a histone deacetylase (HDAC) inhibitor and chemical chaperone. To evaluate PBAs effect on health in old age, we used a paradigm in which treatment was begun in aged (26 month) mice of different genetic backgrounds: genetically heterogenous HET3 and inbred C57BL/6. We chose this advanced age as a time at which the impact of sarcopenia and frailty are measurable to test the potential benefits of PBA intervention at the onset of decline. Both cohorts of mice received 1g PBA/1L in drinking water for 3 months while repeated physical assessments including grip strength, frailty index, and QMRI were taken. Over the 3-month period we found that PBA treatment slowed the increase in frailty compared to control mice in both HET3 and C57BL/6 mice. In particular, PBA preserved the body condition score of mice which can be used as an indicator of overall health. At the molecular level, we found notable increases in some proteins involved in mitochondrial dynamics such as OPA1 and MFN2 in skeletal muscle of mice treated with PBA. Ongoing studies to delineate the role of PBA on mitochondrial function and health will help to clarify the benefits of this intervention on aging muscle. Overall, our findings suggest that PBA may act to protect against age-associated frailty onset through multiple mechanisms which may drive improvements to skeletal muscle mitochondrial health. Funding: T32 Training Grant â€“ 5T32AG021890-15

Multi-organ gene therapy efficiently rescues disease in a mouse model of Wolfram Syndrome II

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Progeroid syndromes result from single gene mutations that cause accelerated onset of aging-like features in one or more of the body's organ systems. One such syndrome is Wolfram Syndrome II – a rare human and mouse progeria characterized by sensorineural, metabolic and hormonal defects and caused by genetic disruption of the Cisd2 gene. Cisd2 is of considerable interest in the geroscience community, because its loss or reduction of expression leads to premature aging and has been associated with several prevalent human diseases, while its overexpression protects against age-related diseases and increases lifespan in mice. Gene therapies hold promise for treating progerias, but their application has been hampered by the fact that there are no known gene delivery vectors that have sufficient breadth of tropism and uniformity of expression across tissues to effectively complement disease-causing gene mutations in all of the different cell types that can be affected. Here, we have engineered an adeno-associated virus (AAV) based system that enables efficient and tunable gene expression across multiple organs simultaneously. This system (called DAEUS) combines multiple engineered AAV serotypes and gene regulatory elements with model guided dosing. We find that in Wolfram Syndrome II mice, DAEUS-Cisd2 gene therapy restores near wild-type Cisd2 expression across the major tissues of the body and protects against development of disease in all cohorts tested. Specifically, in mice treated as pups or young adults, DAEUS-Cisd2 therapy prevents the development of frailty, loss of activity, loss of vision and extends post-treatment lifespan by 75% to 140%. Surprisingly, in mice with advanced progeria, DAEUS-Cisd2 gene therapy reverts some disease pathology in multiple tissues, in addition to extending lifespan and protecting against further progression of the disease. These data indicate that DAEUS holds promise as a platform for treatment of multi-organ genetic diseases and that restoration of Cisd2 expression may provide therapeutic benefit to Wolfram Syndrome II patients at any treatment age.Funding: NIH DP1 AG063419 and Glenn Foundation

Investigating the role of sex and strain on the metabolic effects of dietary isoleucine restriction.

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While calorie restriction (CR) is the gold standard for prolonging mammalian health and lifespan, adhering to CR diets is difficult for humans. Protein restriction (PR) replicates some of the theorized CR mechanisms and promotes health and longevity in mice; lower consumption of dietary protein is also associated with positive outcomes in humans. We have found that the key mediators of these benefits are the three branched-chain amino acids (BCAAs), leucine, isoleucine (ile), and valine. Restriction of all three BCAAs promotes metabolic health, fitness, and lifespan in C57BL/6J male mice, and we recently discovered that restriction of ile is necessary and sufficient for the effects of a PR diet in mice. Here, we investigate the long-term metabolic impact of different levels of dietary ile on the response of both sexes of two different strains of mice to a high-fat, high sucrose diet. Our results reveal that improved body composition, enhanced glucose tolerance and reduced fasting blood glucose are

shared in response to ile restriction. Intriguingly, lowered circulating cholesterol and triglycerides, increased energy expenditure, and reductions in respiratory exchange ratio was not fully shared in all sexes and strains. We also did not see further detrimental effects resulting from excess ile. Together, this data indicates that sex and genetic background should be considered when evaluating the efficacy and translatability of dietary interventions. 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.T. 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 the William S. Middleton Memorial Veterans Hospital.

The mitochondrial-derived peptide MOTS-c reprograms monocyte-to-macrophage differentiation in an age-related manner to produce a unique population of macrophages.

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Aging is accompanied by a progressively maladaptive immune system with reduced capability to respond to infection. Monocytes and macrophages play a central role in the initiation and resolution of inflammation during infection and mitochondria are increasingly being appreciated as regulators of immune signaling in macrophages. Mitochondrial impairment is a hallmark of aging; however, the role of â€˜mitochondrial immunityâ€™ in aging is largely unexplored. In this study, we examined the role of MOTS-c (mitochondrial ORF of the 12S rRNA type-c), a mitochondrial-encoded peptide, as a novel immunomodulator in monocyte differentiation. In THP-1 cells and primary human monocytes, endogenous MOTS-c increased in a time-dependent manner upon differentiation or proinflammatory stimulation. Monocytes treated with exogenous MOTS-c during differentiation possessed altered cytokine expression in response to stimulation, increased phagocytosis, and increased bacterial killing, determined by qPCR, flow cytometry for fluorescent E. coli particles, and gentamicin protection assay, respectively. For scRNA-seq analysis, we differentiated bone marrow with and without MOTS-c treatment from young, old, male, and female mice (n=5 per group) into macrophages (BMDMs). MOTS-c-directed BMDMs in young and old mice had an increased proportion of cells enriched for genes involved in innate immune response to bacteria. Our study demonstrates that MOTS-c, a peptide encoded in the mitochondrial genome, regulates monocyte-derived macrophage phenotype to improve bactericidal capacity. MOTS-c improved macrophage function in cells derived from aged mice, suggesting MOTS-c may be therapeutic in age-related immune dysfunction.Acknowledgements: Funding was provided by the USC Leonard Davis School of Gerontology, USC Graduate School, NIA (R01AG052258), and the Hanson-Thorell Family.

Receptor for advanced glycation endproducts signaling impacts on healthspan: Early-life cognitive assessments

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Damage to macromolecules from stressors like hyperglycemia can lead to a transition of proteins and lipids to a category termed advanced glycation end products (AGEs), which are known to exacerbate inflammation and oxidative stress. As individuals age, the extent of AGE accumulation throughout the body is associated with reduced healthspan. It has been suggested that disruption of AGE signaling can avert the onset of cognitive impairment associated with diabetes as well as the bone damaging effects of osteoarthritis and other age-related disease states. Few studies have examined the role of AGEs in â€˜normalâ€™ aging processes in the absence of disease. Thus, our lab is examining the effects of AGE signaling on healthy aging. We hypothesize that reductions in AGE signaling will delay the onset of age-related cognitive and physical impairments, as well as the onset of senescence, inflammation, and other molecular hallmarks of aging. To address this, cohorts of male and female WT and receptor for advanced glycation end products (RAGE) KO mice (C57/Bl6J background) were bred and we are observing phenotypic aging across their lifespan. At 3 months of age, behavioral and physical assessments

were made in a young cross-sectional cohort. Consistent with previous reports, RAGE-KO mice weighed significantly more than age-matched WT controls. Despite this difference in weight, no significant effect on motor coordination was observed on the rotarod. However, assessments of cognitive function in the radial arm water maze revealed sex and genotype differences between the RAGE KO and WT mice. Minimal differences were observed in circulating levels of pro- and anti-inflammatory cytokines/chemokines at this age. The 3-month baseline data from the young mice will allow us to gain a clearer picture of the role that AGEs has on the onset of age-related pathologies in the months to come. Our future studies include locomotor, cognitive, and frailty assessments in middle and advanced age. Moreover, we will be comparing the extent of inflammation, senescence, and oxidative stress in key tissues across these three time points to better understand the contribution of AGEs to biological aging processes.Funding provided by NIH: 1P20GM130460

The microbiome-muscle connection: Native microbiota affect muscle ageing and motility.

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Large-scale human metagenomic sequencing has identified associations between gut microbiome composition and host physiology, including immunity, nervous system function, and ageing. New findings in humans and model organisms suggest that the gut microbiome affects healthy ageing, and that the microbiome could be used to develop interventions to improve the way we age, but underlying mechanisms are not understood. To define host-microbiome interactions affecting ageing we have established a new model system consisting of the nematode C. elegans combined with an experimental microbiome of 11 bacterial isolates representing the most abundant genera of C. elegans in the wild. Cultivation with the experimental microbiome preserves age-related motility, an effect that requires components of the p38 MAP kinase pathway, including nsy-1 and pmk-1. The experimental microbiome also induces mitochondrial fragmentation in body-wall muscle in a non-pmk-1 dependent manner, suggesting multiple routes of communication by which the experimental microbiome may modulate age-related motility. In a transgenic proteotoxicity model expressing human A?42 in muscle, age-associated paralysis is suppressed by the experimental microbiome. Cell-free supernatant from the experimental microbiome suppresses paralysis and reduces A?42 aggregation in vitro, suggesting secretion of microbial bioactive compounds capable of abrogating A?42-associated toxicity. Together these findings show that molecular host-microbiome interactions modulate muscle function, mitochondrial dynamics and proteostasis during ageing to delay age-related decline in motility. Travel bursary care of the British Society for Research on Ageing's Korenchevsky Award.

Stem Cell-Mediated Restoration of Neuromusculoskeletal Function in Naturally Aged Mice.

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Aging is the primary risk factor for many debilitating neuromusculoskeletal diseases. Our previously novel findings show that transplantation of our unique adult multipotent muscle-derived stem/progenitor cells (MDSPCs) from young mice—but not old—delays the onset of aging-related diseases and doubles the lifespan in mouse models of progeria. Notably, young MDSPCs also functionally rescued the intrinsic defects—including proliferation and multi-lineage differentiation—of aged MDSPCs via secreted factors. In addition, induced neovascularization in the muscles and brain—where no transplanted cells were detected—strongly suggests a therapeutic secretome-related mechanism. Translating to a more clinically relevant animal model, our recent findings demonstrated that systemic transplantation of young MDSPCs into 2-year-old naturally aged (NA) mice rejuvenated neuromusculoskeletal tissues. This treatment reversed osteoarthritis in knee articular cartilage, increased skeletal muscle weight and fiber cross-sectional area, and decreased muscle fibrosis compared to saline-injected control NA mice. Importantly, systemic transplantation of young MDSPCs improved gait, increased physical activity, and

reduced anxiety in NA mice. These novel findings strongly suggest that young MDSPCs can modulate the systemic environment of aged animals through secreted rejuvenating factors critical for tissue regeneration. Our recent analysis of factors secreted by young MDSPCs indicates a significantly higher level of pro-angiogenic and anti-inflammatory factors when compared with the secretome profile of old MDSPCs. These findings provide further evidence of young MDSPCs improving aged neuromusculoskeletal health and function and reveal underlying secretory mechanisms. This work was supported by the Christopher L. Moseley Foundation, Lisa Dean Moseley Foundation, and Shirley Ryan AbilityLab Innovative Catalyst Grant.

Discovery of Proteome- and Secretome-Based Senescent Monocyte Biomarker Candidates.

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Cellular senescence is a complex stress response characterized by permanent cell-cycle arrest and the activation of a pro-inflammatory senescence-associated secretory phenotype (SASP). Accumulation of senescent cells and SASP are drivers of aging and many pathological processes including chronic inflammation and cancer. Biomarkers of senescent cell burden may be clinically useful for treatment of age-related pathologies in humans. Here we perform comprehensive and quantitative proteomic profiling of intracellular and secreted proteomes of senescent monocytes to identify biomarker candidates of monocyte senescence burden in human peripheral blood. To model the full range of physiological oxygen exposure experienced by monocytes in circulation, we induced senescence using ionizing radiation in two monocyte cell lines, U937 and THP-1, under hypoxic or hyperoxic oxygen conditions. After 6 days, proliferating and senescent cells were transferred in serum- and phenol-red-free RPMI media for a 24-hour period. Secreted media as well as cell pellets were collected for LC-MS/MS analysis and assessment of a panel of canonical senescence and viability markers. Analysis of all samples was performed on the Q-Exactive HF Orbitrap mass spectrometer using Data-Independent Acquisition (DIA) and analyzed in Spectronaut. We established a model of senescence in two independent monocyte cell lines that is characterized by senescence markers that include reduced proliferation (EdU), and increased expression of p21, p16, DPP4, and IL6 production, and increased senescence-associated Î²-galactosidase activity. We report the first comprehensive and unbiased assessment of the senescent monocyte intracellular proteome and secretomes, with many unique features compared to other senescent cell types. We compared the proteomes of senescent monocytes with existing biomarkers of aging in humans to assess the most promising peripheral biomarkers of senescence in humans. These results may provide clinically useful information for establishing therapeutic targets or biomarkers to aid in the translation of senescence-targeted therapies to treat chronic-inflammation and age-related decline.

Behavior of C. elegans on lifespan-promoting bacterial diets.

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C. elegans is a powerful model to study host-microbe interactions and address experimentally how microbes influence nervous system integration of sensory information. C. elegans respond to changes in food availability and environmental conditions through coordinated behavioral, physiological and metabolic responses controlled by signaling in olfactory sensory neurons. While examining the diet-dependent phenotypes of C. elegans on bacteria found in C. elegansâ€™ natural and laboratory environments, we noticed that despite Methylobacterium providing a longer lifespan to the worms residing on it, the worms are found less often on this food source when another food is present. When worms are given the option of another bacteria, they will leave the Methylobacterium and move to the other bacterial diet present. This response is opposite to that when wildtype worms are presented with the option of another lifespan-promoting bacteria, Sphingomonas, which is also found in their natural environment. Avoidance and attraction behavior have been observed in previous studies, linking neuropathways to dietary response and physiological changes. Among these physiological responses influenced by the sensory neurons is both lipid metabolism and lifespan. Previous studies have demonstrated that loss-of-function and gain-of-function olfaction mutants of Ga proteins affect lipid content and lifespan of C. elegans. In light of these behaviors and

changes in physiology, it will be beneficial to investigate if particular neuropathways are responsible for alterations in fat content and lifespan due to differential dietary exposure. The expansion of the bacterial food options to use in the laboratory will provide a critical tool to better understand the complexities of bacterial diets and subsequent changes in physiology, behavior and gene expression.

The interaction of cell senescence and necroptosis in inflammaging

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Cellular senescence and necroptosis are two cell-fate pathways that have been shown to play a role in inflammation and inflammaging. Senescent cells produce and secrete senescent-associated secretory phenotype (SASP), which can have 'bystander' effects on neighboring cells leading to changes associated with the loss of function and pathology that occurs with aging. Necroptosis is programmed lysis of cells resulting in the generation of damage-associated molecular patterns (DAMPs), which are potent inducers of inflammation. We found that necroptosis increased with age, is reduced by dietary restriction, reduced in tissues of the Ames dwarf mice, and increased in tissues of Sod1-/- mice, a model of accelerated aging. To determine the role of cell senescence and necroptosis in inflammaging, we used pharmacological and genetic approaches to inhibit either necroptosis or cell senescence. We found the following: (1) pharmacological and genetic inhibition of necroptosis reduced senescence in old mice tissues (2) pharmacological inhibition of necroptosis reduced senescence in tissues of Sod1-/- mice; and (3) pharmacological elimination of senescent cells reduced markers of necroptosis in tissues of Sod1-/- mice. Based on our data, we propose that cellular senescence and necroptosis interact in a positive feedback loop resulting in a vicious cycle amplifying both cell-fates leading to inflammaging and the pathology associated with aging. Senescent cells, which accumulate with age, trigger necroptosis in surrounding cells by paracrine actions of the SASP-factors. Because senescent cells are resistant to apoptosis and relatively long-lived, they could be a constant source of factors that push cells to undergo necroptosis in old animals leading to the release of DAMPs and the induction of inflammation. In turn, DAMPs arising from necroptotic cells could increase the burden of senescent cells in two ways: inducing cell senescence and/or reducing the clearance of senescent cells by macrophages. Our preliminary data show that reducing necroptosis in livers of old mice improves the phagocytic activity of liver macrophages. Funding: NIH R01AG059718 (DS), P20GM103648 (YC), and VA Merit grant I01BX004538 (AR).

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

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Skeletal muscle (SkM) comprises approximately 40% of total body mass and plays essential physiological roles in the body such as enabling skeletal movements and regulating metabolism. Age-related muscle loss, sarcopenia is a major medical problem facing the elderly and correlates with loss of metabolic function, falls leading to cranial fractures, type II diabetes, and cardiac insufficiency. Here we investigate the age-related metabolic rewiring that occurs in myoblasts using in vitro and in vivo models of aging and rejuvenation. RNA sequencing and pathway analysis data revealed that several metabolic pathways changed significantly upon senescence. We also provided evidence aged myoblasts have less glycolysis and insulin sensitivity, which leads to utilize a different source of energy to generate ATP. Our results suggested that aged myoblasts preferred to catabolize amino acids mostly methionine for ATP production and this came at the expense of accumulation of ammonium that leads to DNA damage and impaired cellular function, compromising regenerative capacity and myotube formation. Interestingly, we found that expression of the embryonic transcription factor, NANOG, in senescent cells restored insulin sensitivity, Akt2 signaling, glucose uptake and utilization of glucose for ATP production. In addition, NANOG decreased expression of methionine adenosyl-transferase (MAT) 2A and ammonium levels. Interestingly, inhibiting MAT2A using shRNA showed similar results as NANOG, including restoration of insulin sensitivity, Akt2 signaling

and increased glycolysis. Most notably, decreasing methionine catabolism by NANOG expression or MAT2A inhibition led to dramatic improvements in skeletal muscle strength in a mouse model of premature aging. This work was supported by grants from the National Institutes of Health, R01AG052387 and R01AG068250 to Stelios T. Andreadis.

Neurobehavioral outcomes of low-dose methotrexate exposure in C57BL6/J pups

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Chemotherapy, while remaining a life-saving treatment, has been associated with many serious neurotoxic side effects collectively known as chemotherapy-related cognitive impairments (CRCI). CRCI affects 70% of U.S. cancer survivors for up to 20 years after treatment completion and affects many domains of brain function (cognitive, affective, and motoric). Children are particularly vulnerable to chemotherapy and have been impacted in their educational achievements, employment, and even life expectancy. Acute lymphoblastic leukemia is the most commonly diagnosed childhood cancer and is often treated with the folate-inhibitor methotrexate (MTX). Our study aimed at establishing a tumor-free mouse model of MTX-induced long-term brain impairments. We hypothesized that early exposure to MTX would induce an accelerated aging phenotype in brain function. Accordingly, male and female C57BL6/J pups (postnatal day 15) received intraperitoneal injections of saline or MTX (2 mg/kg) once a day for 3 days. Pups were weaned at postnatal day 21 and subsets were behaviorally tested 1.5 or 8 months of age for motor, affective and cognitive functions. Preliminary outcomes support MTX-induced impairment of spatial learning and memory at 8 months in males. Further studies are underway to complete other time points and increase power. Furthermore, many chemotherapeutics induce oxidative damage, epigenetic changes, and cytokine dysregulation. Studies of these markers in different brain regions will provide mechanistic insights into these long-term changes to be used for therapeutic development.Funding: The Feddersen Foundation

Increased lifespan through altered Gcn4 / ATF-4 in S. cerevisiae and C. elegans.

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Research: In a recent whole-genome screen for deletions that increase lifespan in S. cerevisiae, we identified increased Gcn4 signaling as a mediator of increased lifespan. Gcn4 is a nutrient-responsive transcription factor whose entire pathway is functionally conserved from yeast through humans. Accumulation of uncharged tRNAs has been shown to upregulate Gcn4 and its mammalian ortholog, ATF4. Here we demonstrate that chemical inhibitors of tRNA synthetases significantly extend lifespan in both yeast and the nematode C. elegans in a dose- and Gcn4-dependent manner. Funding Acknowledgements: NIH/NIGMS NIH P20GM121176, Glenn / AFAR Junior Investigator Grant, AFAR Reboot Award, Longevity Impetus Grant.

Immunosenescence, acceleration of aging by infection, and the evolution of adaptive suicide.

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The interactions between infection, immunity, and aging are poorly understood. Immunosenescence, or age-related immune function deregulation, has attracted recent attention because of higher mortality in the elderly population during the ongoing COVID-19 crisis. In turn, some infections can modulate aging: for example, it is known that HIV and HCV chronic infections would cause accelerated age advancement in patients. We investigate these effectsâ€™ molecular mechanisms and ecological roles using experimental and theoretical approaches. Here we report our results on immunosenescence and accelerated aging in the Drosophila system. To determine whether immunosenescence manifests in decreased tolerance (the ability of the host to withstand pathogenic effects), or decreased resistance (the hostâ€™s ability to eliminate pathogens), we analyzed virus infections in young and old flies. Paradoxically, our data suggested an age-related decrease in the organismâ€™s tolerance or an increase in resistance to disease. We will present a transcriptomic analysis of old and young flies challenged by different viruses. We introduced virus replicon expression in flies and to found that animals with infection mimetic indeed die faster than their control counterparts. Using transcriptomic aging clocks, we demonstrated that elevated mortality is accompanied by acceleration of aging. We will present the tissue-specific effects of replicon expression. Finally, we rationalize our findings from an evolutionary perspective with mathematical modeling of epidemics. We have constructed a comprehensive theory of adaptive suicide that can explain when hosts might sacrifice their fitness to protect their kin from harmful infections. Our model can encompass the infection-induced adaptive suicide in yeast and bacteria, limited lifespan in higher lifeforms, acceleration of aging by infection, and changes in tolerance and resistance with age. Supported by Longevity Impetus Grants

Age-related changes of neurovascular coupling and global brain network function and its association to cognitive performance in human subjects.

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Aging is a major risk factor of vascular cognitive impairment, a common cause of disability in older adults. Cognitive processes depend on functional connectivity (FC) between brain regions featuring simultaneous hemodynamic responses evoked by neural activity. Either attenuated neurovascular coupling (NVC) responses or the inability of the brain to reorganize its functional connections between task-associated regions can impair cognitive function. To investigate the impact of aging on NVC and brain networks, we assessed cerebrovascular and cognitive function of healthy young (n=21, 33.2Â±7.0 years) and aged (n=30, 75.9Â±6.9 years) participants. We recorded hemoglobin concentration changes with near-infrared spectroscopy from 48 regions of the frontal cortex during cognitive n-back task, which were used to characterize NVC responses with the aid of a general linear model; and brain networks by estimating FC. Compared to young group, NVC responses during 2-back task showed a marked reduction among aged participants (p<0.05, false discovery rate corrected), while global measures of FC were found significantly increased in the frontal network that was however not affected by mental workload. Increased FC significantly correlated with accuracy during 2-back â€“ that was lower among the aged (p<0.05) â€“ and NVC impairment. These observations suggest an age-related impairment of cerebral hemodynamic responses with an accompanying increase in the wiring cost of the frontal brain network that associates with cognitive impairment in older adults.Funding: AHA, NIA (RF1AG072295, R01AG055395, R01AG068295, K01AG073614, K01AG073613), NINDS (R01NS100782), the NCI (R01CA255840), the OSCTR (U54GM104938) with an Institutional Development Award (IDeA) from NIGMS, the Presbyterian Health Foundation, the Reynolds Foundation, the NIA-supported Geroscience Training Program in Oklahoma (T32AG052363), the Oklahoma Nathan Shock Center (P30AG050911), and the Cellular and Molecular GeroScience CoBRE (P20GM125528)

Aging in the adrenal zona fasciculata of female nonhuman primates (NHP): Changes in markers of cell division and cellular activity indicate decreasing glucocorticoid production across the life course in the female baboon.

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Debate exists on life-course blood cortisol concentration trajectories in humans and NHP. Because of the many roles cortisol plays in physiological processes and aging, it is important to determine blood cortisol concentrations across the life course. Published studies show an increase, fall, or no change with age in both humans and NHP. Two independent studies have described a linear fall in baboon serum cortisol from ~5-23 years (y; lifespan ~21 y) with similar slopes: 24.7 and 23.7 ng. ml -1.y-1. Since circulating cortisol is susceptible to changing environmental stressors, experimental studies on conscious animals are very difficult to control. METHODS: To lessen influences from immediate environmentally-induced changes, we used immunohistochemistry to quantify key proteins in zona fasciculata function regulation. This approach averages out recent environmentally induced changes. We studied 28 female adrenals (5-22 y). All changes in zona fasciculata were linear across the life course. RESULTS: The following linear changes were observed (p<0.001) in all regressions. Cell proliferation markers PCNA, CDK4, CDK1,2, and Ki67, proteins that drive cell proliferation, all fall. P21, an inhibitor of cell division, rises. Star, the rate limiting regulator of steroid synthesis, falls. Citrate synthase, an index of mitochondrial number, falls. Nitrotyrosine, a marker of oxidative stress associated with aging, rises. mTOR, central to cell nutrition and metabolism, falls. CONCLUSIONS: These marked changes in proteins central to cell function and steroidogenesis indicate a linear fall in zona fasciculata mechanisms responsible for basal cortisol production. While these data are an important part of overall zona fasciculata control mechanisms, they do not address the zona's ability to respond to

acute environmental stimuli. There is also a need for aging male data. Unfortunately, primate centers maintain far fewer numbers of males than females. Supported by NIA U19AG057758, NIH P51OD011133.

Cell autophagy markers fall linearly from 30% of the life course across life in four female baboon tissues, brain, left ventricle, liver, and skeletal muscle.

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Autophagy is a cellular cytoprotective, clearance process, important in aging, that degrades and recycles damaged cell components to maintain tissue health and determine lifespan. This study was designed to determine if autophagy mechanisms are similar in different aging tissues. We hypothesized a decline in abundance of Atg5 and other autophagy proteins across the life course and that the decline is similar in four different baboon tissues. METHODS: Female baboons (n=26-29: aged 7.4 to 22.8 years; average life span at Southwest National Primate Research Center 23 years) were euthanized under general anesthesia. Brain (frontal cortex), left ventricle, liver, and skeletal muscle were collected and snap frozen at -80ï¾° C until submitted to Western analysis. Data were analyzed by linear regression. All linear regressions with age were statistically significant. RESULTS: Protein abundance of Atg5 and LC3B-II/LC3B-I ratio decreased linearly with age in all four tissues (p< 0.01). Protein abundance of LC3BI increased linearly with age in heart and skeletal muscle (p< 0.01), whereas LC3BII protein abundance decreased linearly with age in brain (p< 0.01) and tended to decrease in liver (p< 0.1). SQSTM1 protein abundance increased linearly with age in brain (p< 0.01), liver (p<0.05), skeletal muscle (p< 0.01) and tended to increase in heart (p = 0.065). CONCLUSIONS: Reduced Atg5 protein abundance is essential for initiation and elongation of autophagosomes, accumulated SQSTM1 an indicator of autophagic degradation, and decreased LC3B-II/LC3B-I ratio, key indicators of autophagy, suggest autophagy mechanisms decreased linearly as age increased in the four organs studied. Insufficient autophagy would accelerate aging. These data also support the view that aging processes become apparent early in the life course (~28 years human equivalent). Early appearance of age-related cellular changes has implications for diagnosis, prevention and treatment of aging mechanisms. NIA U19AG057758, NIH P51OD011133

Reactivation of DNA Damage Response restores blood stem cell fitness during aging.

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Stem cells reside in specialized tissue-specific micro-environments termed ‘niches’ where they receive instructive cues to preserve their regenerative potential. Aging is associated with defects within stem cell-niches that contribute towards age-related decline in stem cell potential. However, causes underlying age-related niche defects, and whether restoring niche function can improve stem cell fitness during aging, remain unclear. Here, we sought to determine whether defects within aged blood stem cells, termed hematopoietic stem cells (HSCs), can be reversed by restoring their supportive niche cells within the bone marrow (BM). We identify axon-guidance cue Netrin-1 as a novel regulator of BM niche cell activity including endothelial cells and Leptin receptor+ mesenchymal stromal cells. Conditional deletions of Netrin-1 within niche cells of young (6 month old) mice induces premature aging of their BM niche including adiposity, vascular leakiness and a decline in HSC engraftment potential typically observed in aged (>18 month) mice. Transcriptomic analysis revealed that Netrin-1 regulates the DNA damage response (DDR) within niche cells, and comet assays confirmed that loss of Netrin-1 results in accumulation of DNA damage within the BM niche. Moreover, niche-cell specific deletions of Netrin-1 result in DNA damage accrual within HSCs, identifying a novel role for niche-derived signals in regulating HSC DDR. We show that aging is associated with decreased niche-derived Netrin-1, and identify that DDR downregulation is a conserved attribute of aged BM niche cells and HSCs. We demonstrate that supplementation of aged mice with Netrin-1 is sufficient to reactivate the dampened DDR, resolve DNA damage, and restore functionality of the aged BM niche and HSCs including suppression of adiposity, improved vascular integrity and HSC serial repopulation ability. We show that Netrin-1 restores regenerative capacity of an aged hematopoietic system to endure serial chemotherapy regimens including accelerated hematologic recovery, preservation of body weight and overall survival. Collectively, our data defines a novel role for Netrin-1 as a central regulator of DDR responses within the BM niche and blood stem cells. Funding: NIA, AFAR.

Chronic thermogenic stimulation improves systemic metabolism and cognition in aged mice.

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Age-related cognitive impairment has become a major public concern as its associated loss of independence confers a substantial global health and economic burden. Age-related changes at the level of cerebral microvascular endothelial cells have been implicated in cerebral blood flow dysregulation and neuroinflammation contributing to cognitive decline. However, we currently lack strategies or interventions to treat or reverse age-related microvascular damage and its negative impact on cognition. Adipose tissue metabolism plays a pivotal role in systemic aging by regulating whole-body energy and glucose metabolism. Emerging evidence links increased thermogenesis in the adipose tissue to longevity and potentially delayed onset of age-related diseases. Activation of the thermogenic program results in remodeling of both white and brown adipose tissue marked by increased fuel utilization and insulin sensitivity. It also results in an overall improvement in systemic inflammatory milieu and a favorable adipokine profile, which could potentially have beneficial effects on the aging cerebral microvasculature. In this study, we hypothesized that chronic thermogenic stimulation using beta-3 adrenergic agonist (beta3-AR) treatment will improve systemic metabolism, microvascular endothelial function and cognition in aged mice. Aged C57BL/6J mice (both sexes) were continuously infused with CL 316,243 (beta3-AR) for 6 weeks at the dose rate of 1mg/kg body weight via osmotic minipumps. Chronic beta3-AR treatment reduced body weight, fat mass and improved fasting glucose and insulin levels. Improved systemic metabolism was associated with improved endothelial function as assessed by neurovascular coupling responses. Behavioral analyses showed improved cognitive performance in chronic beta3-AR treated animals when compared to aged controls. Overall, our results show that thermogenesis positively affects metabolism and endothelial function, and thus can be therapeutically targeted to boost cerebrovascular health and cognitive status in aging. Funding support- NIA K01AG073613.

Characterizing the Effect of Cellular Senescence on iPSC-derived Brain Endothelial-Like Cells

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Characterizing the Effect of Cellular Senescence on iPSC-derived Brain Endothelial-Like Cells. Cellular senescence is a key mediator of aging and age-related diseases. Although the senescent phenotype varies, cellular senescence is generally characterized by irreversible cell cycle arrest with an accompanying pro-inflammatory senescence-associated secretory phenotype. Recent studies in mice and humans suggest that senescent cells contribute to age-associated neurodegenerative diseases and blood-brain barrier (BBB) dysfunction. However, it is unknown how cellular senescence of endothelial cells at the BBB influences their functions. To investigate this, we induced senescence in human induced pluripotent stem cell-derived brain endothelial-like cells (iBECs), which are a robust, model of the BBB that can sustain its functions over weeks in culture. We verified that we could induce senescence in iBECs using treatments that cause DNA damage (doxorubicin) or epigenetic stress (vorinostat), as evidenced by significant increases in the senescence markers p16 and p21. We found that neither doxorubicin nor vorinostat caused significant decreases in transendothelial electrical resistance (TEER), suggesting that senescence induced by these mechanisms does not contribute to paracellular BBB disruption. Our results highlight that iBECs can be used to study processes of cellular senescence, and point to the need for further characterization of the effects of cellular senescence on BBB functions. This work was supported by the Biological Mechanisms of Healthy Aging Training Program NIH T32AG066574

Age-mediated changes in systemic milieu alter physiology and gene expression of blood vessels.

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Aged-related cardiovascular diseases are the most common causes of severe long-term disability and mortality among older adults. Recent studies revealed a significant role of circulating factors in the aging of the liver, kidney, and brain. However, the effects of circulating factors on vasculature were unknown. As blood vessels are directly exposed to circulating factors, we hypothesized that the age-related changes in the systemic milieu lead to physiological and transcriptomic changes in blood vessels. To study the role of systemic factors in vascular aging, we used a mouse model of heterochronic parabiosis (a surgical union between 2 mice). In our experiment, we joined mice of different ages (young-old; 6mo and 20mo, respectively) and of the same ages. Upon 8 weeks of parabiosis, thoracic aortas were collected from each experimental group. We found that aged aortas exposed to young blood show improved endothelial-mediated vasorelaxation, reduced vascular ROS production (p<0.05 and 0.05, respectively), and rejuvenated transcriptome. Using RNA-seq, we identified 212 aging-associated genes whose expression was restored by exposure to young blood. Using pathway analysis, we found that these genes are involved in the mitochondrial rejuvenation and oxidative stress attenuation. Additionally, we identified 528 aging-related genes whose expression in young aortas was changed by aged blood. We found that these genes are involved in pathologic vascular remodeling and might contribute to age-mediated atherosclerosis and aneurysm. In conclusion, our findings provide evidence supporting the existence of rejuvenating and pro-geronic circulating factors that contribute to vascular aging. This work was supported by grants from American Heart Association and the National Institute on Aging among funding from other sources.

Development of Novel Knock-In Mouse Models to Study the Role of Necroptosis in Age-Related Diseases

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Necroptosis is a newly discovered pathway of regulated necrosis,which plays a major role in inflammation.Necroptosis is initiated when necroptotic stimuli (e.g.TNF?) sequentially activate the Ripk1,Ripk3,and Mlkl protein through phosphorylation.pMlkl binds to and disrupts the plasma membrane of cells,releasing DAMPs.The DAMPs released from necroptotic cells bind to cell surface receptors on innate cells, which in turn triggers proinflammatory cytokines leading to inflammation.We found that necroptosis is increased with age and is associated with increased chronic inflammation.Studies shown that blocking necroptosis(e.g. genetic ablation of Ripk3 or Mlkl,which inhibits necroptosis)reduces inflammation in several diseases.Our goal was to develop mouse models in which necroptosis can be induced in specific tissues and to determine the physiological impact of inducing necroptosis on a tissue.To achieve our goal,we have generated Ripk3 knock-in(KI) and Mlkl-KI mouse models in the C57BL/7 background using a transgene containing the cDNA for Ripk3/Mlkl with a 3-flag tag and a stop cassette flanked by loxp sites 5' to the Ripk3/Mlkl cDNA.The transgene is inserted into the Rosa26 locus,and is driven by the Rosa26 promoter.We crossed KI female to albumin-Cre male to produce hRipk3-KI/hMlkl-KI mice.The expression of Ripk3/Mlkl transgene in various tissues of KI mice was measured using flag-tag antibody.Flag-tag was not detected in any tissue of KI mice,and the Ripk3/Mlkl transgene was specifically expressed(4-fold greater)in the livers of hRipk3-KI/hMlkl-KI mice. However,we observed no increase in necroptosis in young,non-stressed hRipk3-KI/hMlkl-KI mice. Therefore,we exposed the mice to two stresses to induce necroptosis in liver:diquat and a western diet (WD).In studying the role of necroptosis mediated inflammation,we found that there was an increase in pMlkl, Mlkl oligomer in diquat treated hMlkl-KI mice.In addition,pMlkl was increased in the livers of hMlkl-KI mice fed WD. Importantly,WD feeding increased inflammation partciularly TNF-?, fatty liver, and fibrosis(desmin).Thus,we have generated the first mouse models that will allow investigators to study the impact of inducing necroptosis in specific tissues on aging and age-related diseases.

Inhibition of haao-1 enhances oxidative stress response via the SKN-1/Nrf2 pathway in C. elegans.

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The kynurenine pathway is the main metabolic pathway to produce nicotinamide adenine dinucleotide (NAD+) from tryptophan (TRP). Inhibition of kynurenine pathway has been shown to increase lifespan in Drosophila melanogaster, Saccharomyces cerevisiae, and Caenorhabditis elegans. 3-hydroxyanthranilate d ioxygenase (HAAO), encoded by haao-1 in C. elegans, is an intermediate enzyme in the kynurenine pathway that metabolizes 3-hydroxianthranilic acid (3HAA) into 2-amino-3-carboxymuconate 6-semialdehyde (ACMSA), a precursor for NAD+. Previous findings in our lab have shown that haao-1 inhibition, as well as 3HAA supplementation, promotes healthy lifespan extension in C. elegans; however, the underlying mechanism remains unknown. In this study, we report that haao-1 inhibition induces oxidative stress resistance against several ROS-inducers by activating the SKN-1/Nrf2 pathway in C. elegans. We demonstrate that haao-1(RNAi) increases skn-1 protein expression and activates expression of SKN-1 target genes. Our results show a substantial increase in survival of haao-1 mutant worms exposed to paraquat, juglone, and arsenic compared to wild type animals. haao-1(RNAi) similarly extended survival under oxidative stress conditions in wild type, but not skn-1 mutant , animals. Furthermore, using an endogenous in vivo reactive oxygen species (ROS) biosensor, we identified an increase in ROS in haao-1(RNAi) treated worms. These results suggest that activation of the SKN-1 pathway is mediated by an upward shift in the balance of ROS, preventing SKN-1/Nrf2 degradation, thus generating a hormetic effect. Our results identify a novel hormetic mechanism in which the endogenous metabolite 3HAA can impart health benefits by activating the oxidative stress response pathway. These results provide the basis for modulating the kynurenine pathway to promote healthy aging and enhanced stress resistance. This work was supported by NIH R35GM133588.

The metabolic benefits of 17?-estradiol occur independently of insulin-signaling in POMC neurons

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Chronic diseases are often preceded by metabolic perturbations, which are also exacerbated by obesity. Although reductions in calorie intake can curtail morbidity, compliance issues remain a hurdle due to declines in quality of life. Therefore, pharmacological interventions that reduce calorie intake and/or modulate metabolic signaling pathways have garnered interest for mitigating disease. Our previous work demonstrated that 17?-estradiol (17?-E2), a naturally occurring enantiomer of 17?-estradiol (17?-E2), reduces food intake by modulating pro-opiomelanocortin (POMC) neuron activity in the hypothalamus. We have also determined that 17?-E2 reverses insulin resistance independent of changes in food intake, indicating that 17?-E2 acts in a multimodal fashion to reverse obesity- and age-related metabolic dysfunction. Given that insulin signaling plays a major role in controlling POMC neuron modulation of satiety, glucose homeostasis, and adipose tissue lipolysis, coupled with the fact that POMC neurons become insulin resistant with obesity, we hypothesized that 17?-E2 improves systemic metabolic parameters by reversing insulin resistance in POMC neurons. To test this, we generated mice lacking insulin receptors on POMC neurons (POMC-IRKO) and evaluated the metabolic effects of 17?-E2 treatment. All mice were subjected to high-fat diet feeding for 6 months prior to study initiation. Metabolic parameters were evaluated at baseline and throughout the 10-week intervention. Surprisingly, we found that POMC-IRKO and WT mice responded identically to 17?-E2 treatment as evidenced by similar changes in body mass, fat mass, fasting glucose & insulin, glucose tolerance, and insulin sensitivity. In contrast to our previous work, these observations were not limited to just males, as females also beneficially responded to 17?-E2 treatment, indicating that metabolically-challenged females can benefit from 17?-E2 treatment. Future studies will be needed to determine if 17?-E2 is acting through a different receptor on POMC neurons, likely ER?, or through other hypothalamic neuronal populations that modulate POMC actions in a downstream fashion. This work was supported by the NIH [R00 AG051661; R01 AG070035].

TNF alpha signaling is a cell non-autonomous mediator of intestinal stem cell aging

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Intestinal stem cells (ISC) are responsible for renewal of the gut epithelium, but their function declines with age. In order to gain a greater understanding of this decline, we employed several approaches in mice to better understand the extent that ISC aging is attributable to cell autonomous versus cell non-autonomous mechanisms. We observed a notable reduction in ISC function, via organoid formation assay (p<0.05), and in vivo BrdU labeling in the small intestine of aged mice. Moreover, an organoid formation assay from FACS sorted young and old Lgr5+ ISCs and paneth cells, respectively, revealed a role for aging of both populations in this effect. Heterochronic parabiosis experiments further revealed a transposition of the ISC aging phenotype from old to young mice, including reduced ISC proliferation and increased lysozyme levels in the gut of young heterochronic pairs, but not in sham-operated, co-housed mice. To further elucidate the possible mediator(s) of this effect, we treated mice with rapamycin and observed a stark improvement in aged ISC function. Interestingly, the aged intestine was mostly devoid of crypt pS6 by IHC and this was unaffected by rapa treatment, suggesting a diminution in mTOR signaling was likely not a mediator of these effects. However, rapamycin led to a notable reduction in several circulating cytokines and chemokines as well as crypt lysozyme staining in aged rapa-treated mice, while rescue of ISC function was phenocopied by salicylate treatment in old mice. An in vitro cytokine screening further implicated TNF alpha and IFN gamma, and in vivo inhibition of these factors was able to partially rescue ISC function in aged mice. Finally, a parabiotic rescue model leveraging young intestinal epithelial-specific TNFR1 knockout mice conferred protection against the old environment on ISC function. In summary, our data suggest that ISC aging is regulated at least in part via cell non-autonomous mechanisms, that function can be rescued via targeting inflammation, and TNF alpha represents a key pro-geronic factor involved in this effect.Funding sources: AFAR and NIA

The aging liver, necroptosis and nonalcoholic fatty liver disease: An inflammatory connection

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Aging increases the prevalence of nonalcoholic fatty liver disease (NAFLD), a spectrum of liver diseases ranging from simple fatty liver to more advanced forms such as nonalcoholic steatohepatitis (NASH) and hepatocellular carcinoma, in humans. Aging enhances the progression to NASH, predisposing to increased mortality in the elderly. Inflammation is a key mediator in the pathogenesis of NAFLD and the inflammaging theory agrees with the development of NAFLD with age. Here we tested whether necroptosis, a regulated mode of cell death that causes inflammation, contribute to age-associated hepatic inflammation and fibrosis (associated with NASH) in mice. We found that markers of necroptosis increase with age in the livers of mice which parallels the increase in hepatic inflammation and fibrosis. Inhibiting necroptosis using Necrostatin-1s reduced hepatic inflammation and fibrosis, supporting a role of necroptosis in age-associated NASH. Hepatocytes and macrophages are identified as the cell types that have increased expression of necroptosis markers and inflammatory cytokine expression with age. Hepatic macrophages are the known source of hepatic inflammation. We found that resident liver macrophages (Kupffer cells) are reduced, whereas monocytes-derived macrophages (MoMï• †) which are highly inflammatory in nature are increased in the livers of old mice. Consistent with this, levels of M1 proinflammatory macrophages, derived from MoMï• †, are increased and Nec-1s treatment reduced M1 macrophages in the livers of old mice further confirming the role of MoMï• † in age associated liver inflammation. Thus, our study identifies necroptosis as a major contributor to age-associated hepatic inflammation and NASH. Our ongoing studies will explore the role of cell-type specific effect of necroptosis on hepatic inflammation and NASH. Funding: NIH/NIA grant R01AG059718 (DS), VA Merit grant I01BX004538

Identification of Functional and Genetic Interactors of TOR in Yeast

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The Mechanistic Target of Rapamycin (mTOR1) is an evolutionarily conserved kinase that regulates diverse cellular processes in response to nutritional and other environmental cues as well as various forms of stress. Both the genetic and pharmacological modulation of the mTOR activity has shown to be a promising intervention of longevity in diverse models from yeast to primates. In addition, mTOR signaling is deregulated in common diseases, like cancer, neurodegeneration, and diabetes. It is important to characterize the additional components of the mTOR regulatory network, and mTORC1 targets, which will further provide a potential therapeutic target to regulate mTORC1 activity. Here, we utilized a genome-wide synthetic lethality screen to identify additional interactors of mTORC1 in yeast. Our findings identified highly conserved, previously unknown genetic components of the mTOR regulatory network with diverse biological functions. Although further studies are needed to directly characterize their function in mTOR-dependent cellular processes, our initial characterization of their function in mTOR-dependent lifespan extension validated some of them as a modulator of aging. Because mTOR dependent pathways are highly conserved across many different phylogenies the lifespan regulating roles of these genes might be also conserved in higher eukaryotes.

Therapeutic benefits of 17?-estradiol in hepatic fibrosis

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Aging is a major risk factor for the development of non-alcoholic steatohepatitis (NASH), the leading cause of liver transplant in the US. Moreover, liver fibrosis is the best determinant of mortality in NASH, therefore the reversal of liver fibrosis is a major focus in the field. There is currently no approved therapy for treating liver fibrosis. Men and postmenopausal women are at a higher risk of developing liver fibrosis compared to premenopausal women. Liver fibrosis also increases proportionally to the postmenopausal period in females, thereby indicating a role of endogenous estrogens in controlling the progression of liver fibrosis. Our previous work demonstrated that 17?-estradiol (17?-E2) can reverse fatty liver disease in obese male mice, but the effects on liver fibrosis have never been examined. In this study, we sought to determine if 17?-E2 can prevent and/or reverse collagen deposition and/or increase collagen degradation in a CCl4-induced liver fibrosis model. The preventive and therapeutic effects of 17?-E2 treatment on collagen turnover rates were evaluated using stable isotope labeling techniques. Compared with control mice, mice receiving CCl4 displayed a robust upregulation of hepatic collagen synthesis rates and declines in collagen degradation in parallel with significant elevations in TGF?1 and lysyl oxidase like-2 protein (LOXL2), which are responsible for hepatic stellate cell activation and collagen crosslinking, respectively. Conversely, mice receiving 17?-E2 demonstrated significantly reduced collagen synthesis rates and greater collagen degradation rates, which was mirrored by declines in TGF?1 and LOXL2 protein, especially in the therapeutic group. These improvements were associated with increased matrix metalloproteinases-2 activity and elevated levels of PPAR? in both the preventive and therapeutic groups, which are established mechanisms related to the regression of liver fibrosis. These findings indicate that 17?-E2 acts in a multimodal fashion to reduce fibrotic burden in the liver. Future studies will be needed to determine the cell-type-specific mechanisms by which 17?-E2 affects collagen deposition and degradation in the liver.This work was supported by the NIH [R00 AG051661; R01 AG070035].

Aging microglia and their impact on neural stem cell function in the SVZ.

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Neurogenesis, the production of new neurons, occurs throughout life in brain regions known as neural stem cell (NSC) niches. There are two niches in the brain, the hippocampus and subventricular zone (SVZ). The niches are specialized to provide support for NSC survival and proliferation. The SVZ is the largest NSC niche in the rodent brain and is responsible for producing neurons that integrate into the olfactory bulbs and producing progenitors that migrate to areas or ischemic or traumatic brain injury. Neurogenesis drastically declines with age which results in a decrease in cognitive capacity and reduced injury repair. The causes of age-related NSC dysfunction are not fully understood but reduced number of NSCs and progenitors in the niche is associated with age. However, apoptosis does not increase significantly in the SVZ during aging. We show that microglia, the immune cells of the brain, are morphologically distinct from neighboring striatum in young and aged mice. During aging, microglia in the SVZ become activated before reduced NSC numbers are observed. This pro-inflammatory activation occurs much earlier in the niche and to a greater degree than microglia in other brain regions. We show that young SVZ microglia have reduced phagocytic capacity compared to whole brain microglia in vitro suggesting that these niche microglia are specialized not to phagocytize stem cells and progenitors. However during aging SVZ microglia exhibit increased phagocytosis becoming more similar to microglia from other brain regions. Furthermore, in young microglia we observe very little evidence of phagocytosis in the niche in vivo. During aging we show that there is a significant increase in the number of microglia that have NSC and progenitor markers within the lysosomes, strongly suggesting that during aging microglia begin to engulf NSCs and progenitors.Funding: NIH-NINDS R01NS102448

Immune signals modulating microglial phagocytosis may contribute to synaptic loss and related cognitive deficits in the aging monkey.

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Normal aging in humans, even without neurodegeneration, is accompanied by varying degrees of cognitive decline. The rhesus monkey model of normal aging provides insight into underlying changes in the brain since monkeys display structural and cognitive changes similar to humans but are spared from neurodegeneration. While neurons are not lost with normal aging in humans or monkeys, we and others have shown myelin damage and synaptic loss in areas critical for cognition, such as the dorsolateral prefrontal cortex (dlPFC). Synaptic loss could be secondary to myelin damage, or result from aberrant synapse elimination by microglia, the resident immune cells of the CNS, that become dysregulated with age. Microglial phagocytosis is modulated by immune "eat me" and "don't eat me" signals that respectively initiate and inhibit phagocytosis. Previous research has focused on the "eat me" signals mediated by complement component C1q, leaving the "don't eat me" signals, such as CD47, largely unstudied. To investigate changes in signals initiating and inhibiting microglial phagocytosis, multilabel immunofluorescence (IF) was used to examine the localization of CD47 and C1q on postsynaptic sites labeled by PSD95 in the dlPFC of 5 male and 6 female rhesus monkeys ranging from 8 to 28 years of age. All monkeys were tested on delayed non-match to sample (DNMS) and delayed recognition span task (DRST) that respectively measure recognition memory and working memory. Results revealed age-related decreases in CD47 IF mean intensity ($p=0.042$) and a concurrent increase in C1q intensity ($p=0.036$). IF showed decreased CD47-PSD95 colocalization ($p=0.077$) and increased C1q-PSD95 colocalization ($p=0.038$) in aged compared to young monkeys. Immuno-EM confirmed the localization of C1q on synaptic membranes. Interestingly, increased C1q-PSD95 colocalization was weakly associated with cognitive decline ($p=0.10$). These results suggest that with age, microglia receive decreased phagocytotic inhibition from CD47 along with increased phagocytotic signals from C1q, providing a possible mechanism for age-related synaptic loss and associated cognitive impairment. (Supported by NIH grants AG062831; AG043640; AG042512)

Susceptibility to cerebral microhemorrhages in an IGF-1 deficient mouse model is associated with imaging signs of vascular defects in the retina

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Age-related cerebrovascular defects contribute to vascular cognitive impairment and dementia and other dementias. There has been great interest in developing biomarkers and tools for studying cerebrovascular disease using more easily accessible tissues outside the brain such as the retina. Decreased circulating IGF-1 levels in aging are thought to contribute to the development of cerebrovascular impairment, a hypothesis that has been supported by the use of IGF-1 deficient animal models. Here we evaluate vascular and other retinal phenotypes in animals with circulating IGF-1 deficiency and ask whether the retina mimics common age-related vascular changes in the brain such as the development of microhemorrhages. Using a hypertension-induced model, we confirm that IGF-1 deficient mice exhibited worse microhemorrhages than controls. The retinas of IGF-1 deficient animals do not exhibit microhemorrhages but do exhibit signs of vascular damage and retinal stress such as patterns of vascular constriction and MÃ¼ller cell activation. These signs of retinal stress are not accompanied by retinal degeneration or impaired neuronal function. These data suggest that the role of IGF-1 in the retina is complex, and while IGF-1 deficiency leads to vascular defects in both the brain and the retina, not all brain pathologies are evident in the retina.This work was supported by the National Institutes of Health (R01-AG047879; R01-AG055395; R01-AG070915, K01AG073614, R01-NS056218, R01-NS100782, T32AG052363, 1P20GM125528) and the AHA.

Marked reduction of spinal cord lipidome in late Alzheimer's disease contributes to neurogenic bladder.

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Neurogenic bladder, a disorder of the lower urinary tract resulted from damage to or diseases of the nervous system, is common among late-stage dementia patients and life threatening if left untreated. However, the causal factors for the development of bladder dysfunction in dementia remain elusive. Using lipidomics measurement, we identified marked reduction of lipidome in the spinal cord tissues of Alzheimer's disease (AD) patients compared to cognitively normal individuals. Among which, we found sulfatide (ST), a class of myelin-specific lipids, is dramatically reduced in the spinal cord of AD patients versus controls. To determine if reduction of ST is sufficient to induce neurogenic bladder, a mouse line of adult-onset myelin ST deficiency was established. We observed substantial enlargement of urinary bladder in knockout mice compared to controls, while no difference was detected on water intake, renal, or muscle function. However, lipidomics analysis showed that ST deficiency induces altered spinal cord membrane lipid profiles resembling those occurred in late stage AD patients. Moreover, NanoString analysis showed that spinal cord of knockout mice exhibited markedly induced inflammatory and immune response with greatly reduced oligodendrocyte and neuronal function. Mechanistically, we found that Plcg2, a recently identified AD risk gene, was upregulated upon ST deficiency and might mediate the inflammatory effects initiated by ST decline. Our study identified ST deficiency in the central nerves system as a causal factor for AD dementia-related neurogenic bladder and highlighted the critical role of spinal cord ST levels in regulating bladder function. Targeting to manipulate ST levels may serve as a promising strategy for the prevention and treatment of AD-related lower urinary track dysfunctions. Funding Acknowledgement: RF1 AG061729, RF1 AG061872.

Age-related brain frontal cortex transcriptome changes in female nonhuman primates.

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Normal aging of the brain is associated with progressive cellular and structural changes and can lead to cognitive decline and increased risk for neurobiological diseases. Neuroimaging studies show age-related changes in the frontal cortex between older and younger adults. However, the underlying molecular basis of changes in the frontal cortex with age is poorly understood. The purpose of this study was to nominate age-associated molecular changes in the frontal cortex of female baboons, a common non-human primate model for study of age-related neurological, cardiovascular, diabetes, hypertension, chronic renal disease cardiometabolic and other diseases. METHODS: Frontal cortex samples were collected at necropsy (n=31, 8-23years) from animals maintained on a healthy chow diet throughout life. RNA-Seq data were generated and analyzed using unbiased weighted gene correlation network analysis (WGCNA). RESULTS: Our results revealed one large module of 1435 genes correlated with age. Pathway analysis showed positive correlation with age for nucleotide excision repair (NER), SNARE, synaptogenesis, and autophagy pathways, and negative correlation of mTOR signaling. Upregulation of DNA repair and neurodegeneration with age may be in part due to compensatory repair of age associated DNA damage and possibly reflects healthy aging in female primates. mTOR signaling functions as a nutrient sensor and regulates cellular metabolism and decrease in mTOR activity is known to play a critical role in age associated neurodegeneration. Network analyses revealed Lymphoid enhancer-binding factor 1 (LEF1) as a central activator of a network of genes that may influence variation in age associated neurodegenerative and cognitive dysfunction. Further studies will be conducted to determine the extent to which these genes connected with nerve function also change in other brain areas. CONCLUSIONS: Identification of mechanisms by which these pathways and networks regulate molecular processes in frontal cortex will advance our understanding of aging and cognitive function in female primates. NIA U19AG057758, NIH P51OD011133.

Intergenerational effects of maternal age on offspring metabolome, healthspan, and lifespan

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Advanced maternal age is associated with a decline in offspring health, lifespan, and stress resistance in a wide range of species, including humans. This is a phenomenon known as maternal age effects. However, the molecular and cellular mechanisms causing maternal age effects are unknown. Brachionus manjavacas, an aquatic invertebrate rotifer, has a two-week lifespan and a high maternal reproductive investment, which makes it a useful model to study aging and maternal age effects. In this study, we found that maternal age affects offspring lifespan, reproduction, and stress resistance in rotifers. Although old-mother offspring have a longer lifespan, they also have lower fecundity and are more sensitive to heat stress in early life and to rotenone in late life compared to young-mother offspring. To try to understand the mechanism causing intergenerational effects of maternal age, we compared the metabolomes of young, middle-aged, and old females; and of offspring born to those mothers. We found that many mitochondria-associated metabolites decrease not only with age, but also with increasing maternal age. In particular, most metabolites in glutathione metabolism and arginine biosynthesis pathways are lower in the early life of old-mother offspring compared to young-mother offspring. Interestingly, spermidine, which links the glutathione and arginine pathways, is higher in old-mother offspring. As spermidine is reported as a geroprotector and autophagy inducer, it may contribute to the longer lifespan of old-mother offspring.
These findings will provide targets for future mechanistic investigation of maternal and metabolic effects on offspring health and lifespan.FundingThis work was supported by pilot project funding from the University of Washington Nathan Shock Center and NIH/NIA R56AG065434.

Cerebrovascular effects of time restricted feeding (TRF) in aged mice.

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The structural and functional integrity of the cerebral microvasculature plays an essential role in maintenance of healthy cognitive function. Clinical and experimental studies have demonstrated that age-related microvascular functional and structural impairments that compromise neurovascular coupling (NVC) responses and increase blood-brain barrier (BBB) permeability play a critical role in the development of vascular cognitive impairment (VCI)

and exacerbate the associated loss of cognitive function. There is strong epidemiological and experimental evidence that lifestyle factors, including nutrition and dietary habits, are easily translatable and significantly affect cerebrovascular health and thereby influence the pathogenesis of VCI. This study is aimed at investigating the cerebromicrovascular effects of time restricted feeding (TRF) on NVC responses and BBB integrity in aged mice to explore potential novel interventions against the age-related loss of cognitive function. To test this hypothesis 18-month-old C57BL/6 mice were placed on TRF regimen where they received ad libitum feeding for 6 hours each day. After 6 months, NVC was assessed by measuring CBF responses (laser speckle contrast imaging) evoked by contralateral whisker stimulation and BBB was measured using intravital two-photon imaging. 6- and 24-month-old mice fed 24-hour/day ad libitum were used as controls. All three groups were then subjected to a battery of cognitive tests including radial-arms water maze (RAWM), Y-maze. We found that NVC responses were significantly impaired in aged mice. 6 months of TRF improved BBB integrity and rescued NVC responses by increasing endothelial NO-mediated vasodilation, which was associated with significantly improved learning and spatial memory.

Comparative genomics of longevity: from rockfish, across mammals, and within humans.

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Longevity is a defining trait that varies dramatically across vertebrates. Knowledge of the mechanisms facilitating this traitâ€”and the included means to maintain health over timeâ€”would have untold medical value. To decipher these mechanisms, we have mined variation in Rockfishes, including several species succeeding well past the human lifespan, up to 205 years. We also capitalize on variation in longevity across mammals, for which diverse genomic resources are available from well-characterized lineages. We analyzed these datasets with TRACCER, a new tool to detect relative evolutionary rate convergence, to identify genes and pathways evolving alongside shifts in longevity. Canonical aging targets such as insulin-signaling pathways, DNA repair complexes, and sirtuins are prominent, but we also identify other, less orthodox gene sets. These include â€œflavonoid metabolismâ€• with rockfish longevity, which is likely a misnomer for aryl-hydrocarbon metabolism, and â€œchromatoid bodyâ€• with pan-mammalian longevity, which may have an unappreciated role outside the germline in suppressing transposons and maintaining genomic stability. Finally, we used these evolutionary gene sets to refine genomic variation associating with longevity in humans. Using the rockfish as a filter, we were able to remove the statistical burden of genome-wide hypothesis testing and identify significant genes and gene sets associating with human longevity. Intriguingly, we identify SNPs in the â€œflavonoid (aryl-hydrocarbon) metabolismâ€• gene set as significantly associated with survival to the 99th percentile, indicating a shared mechanism underlying the evolution of longevity in both rockfish and humans. These genes have broad impacts on detoxification and hormonal signaling. The conservation among vertebrates empowers us to leverage the advantages of each of these datasets: the convergence of exceptional longevity in rockfish, the diversity of longevities across mammals, and the large sample sizes of human populations. Intersecting these approaches produces strong candidates for intervention to modulate the aging process directly. Supported by AFAR Postdoctoral Fellowship and the National Academy of Medicine

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Aged visceral adipose tissue microenvironment mediates enhanced proliferation and accumulation of resident gamma delta (Î³Î´)-T cells in mice.

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Adipose tissue is a major source of inflammatory mediators which drives chronic systemic inflammation and contributes to cardio-metabolic disorders and frailty in advanced age. Our prior studies have shown that accumulation of Î³Î´-T cells in visceral adipose tissue (VAT) of aged mice promotes both local and systemic inflammation. The goal of the present study is to unravel the mechanisms of age-associated Î³Î´-T cell expansion in VAT. We hypothesized that the pro-inflammatory state of the aged VAT microenvironment induces a higher rate of proliferation in tissue-resident Î³Î´-T cells. Using intracellular Ki67 as a proliferation marker, we found that the number of Ki67+ Î³Î´-T cells in VAT is significantly greater in aged (21-25 months old) compared to young (4-6 months old) mice (p=0.013),indicating a higher number of proliferating Î³Î´-T cells in the aged. To evaluate the role of the aged VAT microenvironment in supporting enhanced proliferation, conditioned medium was generated from aged VAT explants (VAT-CM) to mimic the microenvironment. Primary VAT-derived Î³Î´-T cells from young and aged mice were subjected ex vivo to VAT-CM and proliferation rate was assessed by MTT assay. Exposure to VAT-CM induced a similar rate of proliferation in both young and aged Î³Î´-T cells (p<0.01), suggesting that the microenvironment, rather than intrinsic age-related alterations to the cells themselves, is likely the major factor promoting enhanced proliferation of Î³Î´-T cells in aged VAT. Collectively these studies suggest that signaling molecules present within the aged VAT microenvironment increase Î³Î´-T cell proliferation, contributing to age-associated accumulation of Î³Î´-T cells in VAT. 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

Leveraging a systems geroscience approach to identify drug synergy for Alzheimer's Disease

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Aging is the major underlying risk factor for chronic illnesses, including neurodegenerative diseases, such as dementia. Alzheimerâ€™s disease (AD), characterized by amyloid plaques and neurofibrillary tangles, is the leading cause of dementia, affecting ~6M Americans and is expected to double in prevalence by 2060. Age-related neurodegeneration is thought to be driven by cumulative dysfunction in various cellular processes, which have been termed aging â€œpillarsâ€• . Together, they lend to the development of the geroscience hypothesis, which surmises that targeting the impairment in the pillars of aging can either delay or prevent the onset of age-related ailments. However, given the complexity of aging, successful therapies are likely to require a combinatorial approach. Here, we have utilized and collected multi-scale data in the 5XFAD mouse model of cerebral amyloidosis treated with one of five compounds for up to 8.5mo with purported age or AD-delaying potential, including spermidine, metformin, rapamycin, lithium carbonate (Li2CO3), and fasudil. Data collected span from behavior, molecular and pathology to transcriptomic analyses, to be used for predicting the most effective combination. 5XFAD control mice tend to weigh less than WT mice, while body weights are significantly reduced further in 5XFAD mice treated with rapamycin or spermidine (p<0.05). Meanwhile in males, only rapamycin significantly reduced body weight. Interestingly, object placement tests for memory detected marked deficits in female 5XFAD controls vs WT (33% v 75% pref), which was partially rescued by metformin and rapamycin and completely preserved by lithium or fasudil (p<0.05). Moreover, preliminary data suggest that some gerotherapeutics may partially normalize anxiety and depressive-like behavior in 5XFAD males. Brain phenotyping is in progress but early indications suggest potential for treatments to modulate Î²-amyloid load. The next phase of this program will be to perform in vivo validation of our predicted combination strategy. Successful implementation of this approach may serve as an exemplar for future approaches to developing clinical pharmacotherapies in age-related disease. Funding: R01AG067312

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). 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 and shortens lifespan, in part via initiation of the mitochondrial unfolded protein response (UPRmt). Pharmacological or genetic inhibition of the mPTP inhibits the UPRmt and restores normal lifespan. Loss of the putative pore-forming component of F-ATP synthase extends adult lifespan, suggesting that the mPTP normally promotes aging. Our findings reveal how an mPTP/UPRmt nexus may contribute to aging and age-related diseases and how inhibition of the UPRmt may be protective under certain conditions.

Alpha modulation in younger and older adults during distracted encoding

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To successfully encode information into long-term memory, we need top-down control to focus our attention on target stimuli. This attentional focus is achieved by the modulation of sensory neuronal excitability through alpha power. Failure to modulate alpha power and to inhibit distracting information have been reported in older adults during attention and working memory tasks. Given that alpha power during encoding can predict subsequent memory performance, aberrant oscillatory modulations might play a role in age-related memory deficits. However, it is unknown whether there are age-related differences in memory performance or alpha modulation when encoding targets with distraction. Here we show that both older and younger adults are able to encode targets paired with distractors and that the level of alpha power modulation during encoding predicted recognition success. Even though older adults showed signs of higher distractibility, this did not harm their episodic memory for target information. Also, we demonstrate that older adults only modulated alpha power during high distraction, both by enhancing target processing and inhibiting distractor processing. These results indicate that both younger and older adults are able to employ the same inhibitory control mechanisms successfully, but older adults fail to call upon these when distraction is minimal. The findings of this study give us more insight into the mechanisms involved in memory encoding across the lifespan.

IgG subclass specific infiltration in skeletal muscle and their role in sarcopenia

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Age-related declines in muscle mass and strength (sarcopenia) impairs physical function and leads to decreased quality of life. A concerted effort to discover novel biomarkers and understand the mechanisms underlying sarcopenia is required to identify novel interventions. Our recently reported findings in humans and mice suggest that an IgG mediated autoimmune process may contribute to sarcopenia. Given that only IgG1 was found to be inversely associated with physical function (400m walk time or SPPB), knee extensor strength, or lean mass in older adults, our findings suggest that IgG infiltration in aging skeletal muscle may contribute to sarcopenia in a subclass-specific manner. Here, using young and old C57BL/6 mice, we have further examined the IgG subclass specific infiltration/deposition in aging skeletal muscle. By immunoblot, we detected all 4 IgG subclasses (IgG1, 2b, 2c, and 3) in the limb muscle of aged (old, 20-22 months, and very old, 26-28) mice, in parallel with their increase with age in the blood. Notably, immunofluorescence staining further revealed that mainly IgG2b and 2c were infiltrated in the vascular wall, myofiber, or interstitial area. In addition, IgG3 was exclusively enriched at the neuromuscular junction (NMJ) area of aged mice and co-localized with activated caspase 3 and 9, in addition to our previously reported co-localization with cardiac troponin T (cTnT) and complement C3. We also found that IgG infiltrated skeletal muscle of aged mice showed decreased motor activity and increased muscle degeneration (e.g. decreased capillary density and increased myofiber atrophy, and NMJ or sarcolemma abnormality). In conclusion, we show for the first time an IgG subclass-specific infiltration/deposition in skeletal muscle of aged mice and older adults, which may serve as novel biomarkers of sarcopenia. Funding Acknowledgement: This work was supported by the R21AG059180 and R21AG060037 (T.Z.), and Wake Forest Claude D. Pepper Older Americans Independence Center P30-AG21332 (S. K.).

Mitochondrial driven inflammation in aged kidneys is exacerbated by nicotinamide mononucleotide but ameliorated by elamipretide

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Mitochondrial dysfunction is characterized by loss of structural integrity, decreased efficiency of the electron transport chain and has been linked to cellular senescence and inflammation. Understanding the impact of late age mitochondrial dysfunction interventions will contribute to the field of senolytics. Previously, systematic

treatment of old mice with tetrapeptide, Elamipretide (ELAM), which interacts with the inner mitochondrial membrane to improve cristae structure and function, reduced mito-dysfunction and senescence in kidney and heart. We hypothesized that additional intervention with Nicotinamide Mononucleotide (NMN), an NAD+ precursor, which contributes to the efficiency of adenosine triphosphate generation, would enhance the mito-energetic capability thus decreasing mito-dysfunction and senescence in aged kidneys. Liver was also analyzed to clarify intervention responses as global vs. kidney-specific mechanisms. We treated old male mice at 24 months-old (mo) for 8 weeks with either ELAM (osmotic pump, 3mg/kg), NMN (drinking water, 300 mg/kg), or combined. Untreated control mice were 4 mo (young) and 26 mo (old). Contrary to our hypothesis, NMN treatment was detrimental in kidney by increasing mRNA expression of inflammatory cytokine IL-1b, relative to untreated aged mice. Exploration of IL-1b downstream targets showed consistent upregulation of CCL2, an inflammatory chemokine and KIM-1, a marker of proximal tubule injury in NMN treated mice. ELAM, however, provided rescue in the coupled treatment group, by significantly reducing gene expression of IL-1b and CCL2. This suggests ELAM lessens senescent burden by reducing renal inflammation; conversely NMN exacerbates existing inflammation pathways in aged kidneys. This result was not observed in liver, demonstrating tissue specificity. RNA in situ hybridization showed IL-1b localization in the 26 mo NMN-treated proximal tubules. Further work seeks to track NMN metabolite levels in primary tubule cells to better understand NMN induced injury and ELAM protection in aged kidneys. NIA P01 AG001751, NIA K01 AG062757

Calcium handling dysregulation associated with neuromuscular functional decline

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Declining physical function and muscle health predicate major losses in quality of life for older adults. Preclinical investigations are needed to delve into the underlying mechanisms. Previously we developed a composite scoring system, CFAB (comprehensive functional assessment battery), which is comprised of 5 well-validated physical function tests, and used it to assess C57BL6 male mice at 6m (months), 24m, and 28m. NGS (Next Generation Sequencing) RNAseq on tibialis anterior muscle and linear regression determined significant gene expression changes (qval<0.05 and log2fc>|1|) between 6m and 28m associated with CFAB (239 Râ‰¥|0.7|; 739 R>|0.5|). Gene Ontology analysis uncovered high enrichment in genes associated with calcium handling. SLN (sarcolipin, log2fc4.33, R=-0.55 with CFAB) protein regulates SERCA (sarcoendoplasmic reticulum calcium ATPase) by blocking its pumping action while retaining ATP cycling. We hypothesized that SLN upregulation may be associated with impaired muscle contraction during aging. To investigate further we performed rt-qPCR and Western blots to confirm upregulation in multiple muscles, finding SLN gene and protein expression was significantly upregulated (p<0.05) with age also in the gastrocnemius [GAS, +250% protein, gene expression fold change, fc (2-Î”Î”Ct), from 6m GAS control=99] and soleus (+202% protein, 28m fc 367% higher than 6m). During ex vivo isometric twitch, Â½ relaxation time (a measure of SERCA pumping efficiency) increased between 6m and 28m in both the EDL (+40%, p=0.006 ,extensor digitorum longus; 6m n=7, 28m n=17) and SOL (+20%, p=0.07, soleus; 6m n=6, 28m n=22) indicating declining SERCA calcium pumping efficiency. Furthermore, 13 weeks of High Intensity Interval Training restored SLN levels in 26m GAS (n=8, fc=8) closer to 10m sedentary (n=6, fc=2.5) versus 28m sedentary (n=8, fc=99.6), demonstrating reversibility of SLN upregulation with exercise. We conclude the connection between SLN and contractile changes with age warrants further investigation though gain/loss of function to determine potential causation.

Ferroptosis as a senolytic target to clear primary and secondary senescent cells

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Senescent cells (SCs) can spread the senescent phenotype to other cells by secreting factors called the Senescence Associated Secretory Phenotype (SASP). The resulting secondary SCs make a significant contribution to the burden of SCs accumulation with age. Efforts made to characterize secondary SCs have mostly been unreliable due to their analysis based on mixed populations of senescent and non-senescent cells. Here, we used dipeptidyl peptidase-4 (DPP4) as a surface maker to isolate SCs from mixed populations. Using this novel

technique, we enriched the percentage of secondary SCs from 40% to 85%, which is comparable to senescence induction levels in primary senescent cell cultures. We then used this enriched culture to dissect the molecular and phenotypic differences and similarities between primary and secondary SCs. Notably, we found that secondary SCs have distinct prosurvival mechanisms and are substantially less susceptible to current senolytics than are primary SCs.

Functional and Genomic Profiling of Heart, Skeletal Muscle, and Adipose Tissues in a Primate Model of Aging.

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Non-human primates, including African green monkeys, show age-related changes in physical function, body composition, fat distribution, behavior, neuropathology, immunosenescence, and cardiometabolic phenotypes. We assessed characteristics of the heart, skeletal muscle, and visceral and subcutaneous fat in female young adults (age 8-10 years, comparable to 30-40-year-old people) and older (age 21-26 years, comparable to 65-80-year-old people) vervets which had consumed a life-long low fat â€œchowâ€• based diet. Echocardiography and cardiac magnetic resonance imaging showed that 3/5 of the aged females had significant left ventricular hypertrophy. Intraventricular septal thickness was increased in the aged cohort and was positively correlated with systolic blood pressure. Septal mitral annular descent, an index of left ventricular relaxation, was lower in aged monkeys, suggestive of diastolic dysfunction, while factional shortening was similar, a consistent precondition for heart failure with preserved ejection fraction (HFPEF). These age-related cardiopathologies developed despite a life-long consumption of a healthy (13% of calories as fat) chow diet. Transcriptional profiling of myocardial tissue @ fold change of 2 and FDR of <0.05 showed differential expression of 156 genes, with elevations in the expression of natriuretic peptide A and B genes (NPPA and NPPB), complement C6, and matrix gla protein (MGP) in the aged monkeys. GABA receptor was among the down-regulated genes in the aged heart. Gene expression-based unsupervised hierarchical clustering completely distinguished cardiac muscle from skeletal muscle, and subcutaneous adipose tissue (SAT) from visceral adipose tissue (VAT). In SAT, expression of 11ï• ¢-hydroxysteroid dehydrogenase 1, the gene which inactivates cortisol through conversion to cortisone, was over 4 fold lower in the aged cohort. CCL5/RANTES was among genes upregulated 2 fold in the aged VAT. Across all tissues assessed, including brain, a transcript for a mitochondrial protein was universally higher in the aged cohort. The studies provide insights into organ-specific and global alterations in geroscience-related phenotypes. Funding: P40 RR019963; NO1HC95170; P30 AG21332.

The transcriptional repressor Cyc8 undergoes liquid-liquid phase separation in response to hyperosmotic shock

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Many hallmarks of aging mirror the cellular consequences of acute stress. To understand aging at a cellular level, we must understand the constellation of adaptive responses to physical and chemical stress. Here, we describe how two stress response phenomenaâ€”protein SUMOylation and liquid-liquid phase separation

(LLPS)â€”converge in a single substrate in budding yeast. Cyc8 is a transcriptional repressor whose targets include several stress response genes. Cyc8 is the primary protein SUMOylated in yeast exposed to acute hyperosmotic shock. Concomitantly, Cyc8 forms subnuclear puncta. In this study, we found that Cyc8 puncta bear characteristics consistent with membraneless nuclear bodies bound together by LLPS. They are sensitive to the weakly disaggregating agent 1,6-hexanediol and show a strong loss of fluidity compared to non-punctate Cyc8 as measured by fluorescence recovery after photobleaching (FRAP). To identify the composition of these subnuclear bodies, we turned to promiscuous biotin ligase-based proximity proteomics. We used the recently developed TurboID variant of the E. coli BirA biotin ligase to label the contents of stress-induced Cyc8 bodies with biotin in vivo, then quantified them using mass spectrometry. We then confirmed a subset of the Cyc8 body interactome using fluorescence colocalization. We conclude that Cyc8 bodies are a previously uncharacterized nuclear body with a specific protein signature that may play a role in cellular adaptation to stress. This project establishes TurboID as a valuable tool for defining macromolecular assemblages in budding yeast and elucidates the workings of a cellular stress response. Future work will investigate how aging alters the properties and composition of Cyc8 bodies and other known LLPS structures in yeast. Funding: TDM: Environmental Health and Toxicology Training Grant NIH T32ES007032-42, RGG: Maximizing Investigatorsâ€™ Research Award NIH R35GM-136234, MK: Nathan Shock Center of Excellence in the Basic Biology of Aging NIH P30AG013280.

Physiological and adipose tissue transcriptional response to CR mimetics in mice

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Caloric Restriction (CR) without malnutrition delays aging and the incidence of age-related diseases. Although the specific factors responsible are not known, substantial evidence suggests that CR engages metabolic pathways as a primary mechanism. Factors that activate or impinge on metabolism may therefore hold promise as CR mimetics. Here we show that pharmacological agents that act on aspects of metabolism have the ability to mimic some of the CR response. Adult male mice were treated with resveratrol, bezafibrate, and lithium carbonate, and compared to control and CR-fed animals. The physiological, systemic, and tissue specific response to ten months of fixed dietary intake drug treatment was quantified. We demonstrate that lithium carbonate and bezafibrate treated mice had lower body weight than control or resveratrol treated mice and were lower in adiposity, matching the biometric effects of CR. CR sensitive indices including circulating levels of glucose, insulin, and adipokines, were responsive to drug treatment but the impact was not equivalent among all treatments. We report significant differences in circulating lipids with CR, that were mirrored in part with drug treatment. Transcriptional profiling of adipose tissues revealed substantial overlap between CR and bezafibrate and to a lesser extent lithium carbonate treatment, while resveratrol treated animals were indistinguishable from controls. We identified an RNA processing signature in bezafibrate treated mice that overlaps with that found in CR mice; lithium treated mice induced the same mechanism but with a more widespread impact beyond factors altered in bezafibrate/CR. Clustering and gene co-expression analysis identified gene sets correlating with systemic traits and the individual gene candidates likely driving these mechanisms. These data show that targeting metabolism pharmacologically can recapitulate the effects of CR and is therefore worth pursuing as a strategy for addressing aging and age-related disease vulnerability. Funding: NIH R01AG037000, R03AG070686

A?42 oligomers trigger synaptic loss through CAMKK2-AMPK-dependent effectors coordinating mitochondrial fission and mitophagy

A?42 oligomers trigger synaptic loss through CAMKK2-AMPK-dependent effectors coordinating mitochondrial fission and mitophagyAnnie Lee 1,2,4,12, Chandana Kondapalli 1,2,12, Daniel M. Virga 1,2,6,12, Tommy L. Lewis Jr. 1,2,10, So Yeon Koo 5,7, Archana Ashok 5,7, Georges Mairet-Coello 8, Sebastien Herzig 9, Marc Foretz 11, Benoit Viollet 11,Reuben Shaw 9, Andrew Sproul 5,7 and Franck Polleux 1,2,31 Department of Neuroscience, Columbia University Medical Center New York, NY2 Mortimer B. Zuckerman Mind Brain Behavior Institute, New York, NY3 Kavli Institute for Brain Sciences, Columbia University Medical Center, New York, NY4 The Integrated Graduate Program in Cellular, Molecular, and Biomedical Studies, Columbia University Medical Center, New York, NY5 Department of Pathology and Cell Biology, Columbia University Medical Center, New York, NY6 Department of Biological Sciences, Columbia University, New York, NY7 Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University Medical Center, New York, NY8 UCB Biopharma, Braine l'Alleud, Belgium9 Molecular and Cell Biology Laboratory- Salk Institute for Biological Studies, La Jolla, CA10 Aging &

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During the early stages of Alzheimerâ€™s disease (AD) in both mouse models and human patients, soluble forms of Amyloid-Î² 1-42 oligomers (AÎ²42o) trigger loss of excitatory synapses (synaptotoxicity) in cortical and hippocampal pyramidal neurons (PNs) prior to the formation of insoluble AÎ² plaques. In a transgenic AD mouse model over-producing AÎ²42o (J20), we observed a spatially restricted structural remodeling of mitochondria in the apical tufts of CA1 PNs dendrites corresponding to the dendritic domain receiving presynaptic inputs from the entorhinal cortex directly and where the earliest synaptic loss is detected in vivo. We demonstrate that AÎ²42o-dependent over-activation of the CAMKK2-AMPK kinase dyad mediates synaptic loss through coordinated MFF-dependent mitochondrial fission and ULK2-dependent mitophagy in dendrites of PNs. Our results uncover a unifying stress-response pathway causally linking AÎ²42o-dependent structural remodeling of dendritic mitochondria to synaptic loss.NIH | National Institute of Neurological Disorders and Stroke (NINDS) - NS089456 (Polleux)

Increased expression levels of SASP factors and senescence biomarkers in aged non-human primates.

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Aging is a complex process that contributes to the inevitable decline of physiological integrity and function in organisms. A great challenge in aging research is selecting animal models that are tractable. To date, rodent models are most frequently used because their lifespan is short (~3 years). However, the age-related diseases that spontaneously occur in rodents are not a perfect match for humans, suggesting the need for other models. Non-human primates (NHP) are ideal as they share genetic, physiological, and behavioral traits with humans than other model organisms. However, currently, little is known about the expression of senescence biomarkers in NHP tissues. Establishing if senescent cells (SC) accumulate in tissues of NHP would facilitate pre-clinical testing of senolytics in non-rodent models. To address this challenge, here, we measured the expression of senescence genes and SASP factors via qRT-PCR in multiple tissues (kidney, liver, heart, and skin) from Rhesus macaque (Macaca mulatta) using archived samples made available through the NIA Biology Resource Branch. We also performed SA-Î²gal staining to measure senescence-associated beta-galactosidase activity. The results suggest that aged animals have greater senescence and inflammation levels than their young counterparts across the tissues in both genders. As expected, the expression of the SC markers and SASP factors were highly variable in the aged NHP. However, sex differences were not significant. This work begins to illustrate in what tissues we can expect to find SC in humans that might be targeted to alleviate age-related diseases. Funding resources: This work is supported by NIH/NIA R01 AG063543, U19 AG056278, and P01 AG062413

Modulating Alzheimer’s Disease by mTORC1 inhibition to augment lysosomal activity

Lear, Travis 1,2; Lockwood, Karina 1; Boudreau, Ã• ine 1; Camarco, Daniel 1; Larsen, Mads 1; Lin, Bo 1; Rizzo, Stacey 1; Liu, Yuan 1; Finkel, Toren 1,2; Chen, Bill1,21)Aging Institute, University of Pittsburgh2)Vascular Medicine Institute, University of PittsburghPathogenic lesions in the central nervous system, comprised of insoluble protein aggregates such as the microtubule associated protein, tau, are thought to be key in the pathogenesis of Alzheimerâ€™s Disease. Protein aggregates are naturally degraded through the autophagy-lysosomal pathway; promoting the clearance of tau has emerged as a potential therapeutic avenue. Key in control of the auto-lysosomal pathway is the activity of the mechanistic target of rapamycin (mTOR) complex 1 system. The mTORC1 system functions by sensing of nutrient status to control cellular metabolism, and directly inhibits the auto-lysosomal pathway. Importantly, mTORC1 activity is increased in brain tissue of AD patients and is associated with tau level; there is a concurrent decrease in autophagic activity. As direct mTORC1 inhibition results in pleiotropic effects and toxicity, an alternative means to influence its activity comes through mTORC1 Regulators, a collection of 30+ recently characterized proteins directly upstream of mTORC1 that modulate its activity. Here we show upregulation of a key mTORC1 inhibitory protein, KPTN, impairs mTORC1 activity and aids in clearance of tau protein in neuronal cell culture. Through unbiased siRNA screening we identify that KPTN is potently controlled by the E3 ubiquitin ligase PDZRN3, leading to KPTN ubiquitination and degradation. Moreover, KPTN and PDZRN3 protein levels are inversely correlated in aged mouse brains, suggesting a potentially causal relationship. Further, we have developed a class of small molecule KPTN activator compounds, which prevent

mTORC1 activation, increase lysosomal activity, and aid in tau protein clearance. The net effect of KPTN aids in clearance of toxic protein aggregates by activation of autophagy, and suggest an alternative pathway of autophagic regulation for tau clearance.Funding:NIH grants to TL (T32 HL110849), TF (R01 HL142663, R01HL142589, P30 AG024827) BBC (R35HL139860, R01HL133184), and YL (R01HL142777) and University of Pittsburgh seed fund to BBC, TF, YL

Late-Life Mortality GWAS in Flies Identifies Diabetes and Obesity Regulated to Regulate Mortality and Resilience.

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Variations in rate of aging in geneticaly heterogenious populations supports the hypothesis that aging is at least partially genetically regulated. However, genetically identical individuals also vary in their time of death. We have observed in D. melanogaster that this variation is genotype-dependent, as specific genotypes have characteristic survival curve shapes that are largely reproducible. Typical aging studies reduce a strainâ€™s lifespan down to a population-level value, i.e. mean lifespan. While these metrics can represent the trends in a population, they are unable to encapsulate the variation in the aging of individuals from the same distinct population. Instead, we used two values that characterize the logistic fit of a strainâ€™s mortality late in life: the risk of initial mortality (Î±) and the rate of aging (Î²). To identify regulators of the rate of aging, we performed a Genome-Wide Association Study (GWAS) of Î² for 160 different fly strains from the DGRP collection on two different diets late in life. This approach identified the candidate gene Diabetes and Obesity-Regulated (DOR), which has known roles in stress response, autophagy, and senescence, as having a role in the late-life mortality. DOR inhibition leads to a significant increase in late-life mortality that is preceded by a reduction in healthspan-related traits. Further, germline-specific inhibition is sufficient to increase senescence-related factors and shorten lifespan. We conclude that a decrease in DOR, a conserved gene, compromises an organismâ€™s resilience through increased inflammation, senescence, and increased mortality, providing a potential target for bolstering the decline seen in human aging.

Plasma transfer as a model to reverse age-related epigenetic changes

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Epigenetic mechanisms have been proposed to play critical roles in aging and age-related neuronal dysfunction. However, before we can advance to mechanistic approaches to understand the direct role of specific epigenomic patterns in aging, we need to: 1) determine whether existing epigenetic patterns can be reversed and 2) identify specific epigenomic targets that can be manipulated to study their direct effects on aging. The goal of this study was to determine whether exposure of old mice to young plasma can reverse age-related DNA modifications to restore â€˜youthfulâ€™ patterns. In initial heterochronic parabiosis experiment, young (Y-3M) and old male (O-24M) mice were surgically joined for 6 weeks to establish parabiotic pairings of Y-O/O-Y (heterochronic) and Y-Y and O-O (isochronic or same age controls) (n=6/group). Low-coverage DNA methylation analysis of hippocampal DNA isolated from parabionts showed no genome-wide hypermethylation or hypomethylation changes while RNA sequencing showed differential expression with a clear separation of expressed genes between parabionts. To improve delivery of and create a more translational model, we designed a plasma transfer approach to intravenously administer plasma via catheters inserted into jugular vein of the mice. We used a 2x2 design, where old mice received plasma from young/old mice while young mice received plasma from old/young mice

(n=6/group). A separate group of aged and young mice (n=3/group) received saline as a control for surgery and changes in blood volume. RNA-Sequencing of the hippocampus showed a separation of differentially expressed genes by age but not by plasma received, whether from young or old mice. Selected targets (including ITGAX and C4B) for quantitative RT-PCR confirmation revealed marginal effects of plasma treatment and some saline effects although these effects were not significant. Currently, we are optimizing the plasma transfer approach and intend to replicate the effects in a cell-specific manner to identify potential cell-specific targets. Funding: NIH [R01AG059430 and P30AG050911-0]; VA [I01BX003906 (FW)], AFAR and OMRF (AV)

Transcriptional Profiling of the Aging Brain

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Cellular senescence is well established to be a driver of aging and age-related pathologies including neurodegenerative diseases such as Alzheimer's Disease and Parkinson's Disease (PD). While several on-going studies in our lab are focused on deleting the DNA repair gene ercc1 in specific cell types in the central nervous system (CNS) to identify which CNS cell type senesces and to investigate the contribution of these senescent cells to PD, the study described here aims to characterize senescence in different brain regions of naturally-aged wildtype (WT) mice. Towards this end, we used quantitative real-time PCR (qPCR) to measure the expression of senescence markers and senescence associated secretory phenotype (SASP) factors at the transcript level from three-year-old male and female WT (C57BL/6) mice. In comparison to young (9-week-old) WT mice, brain regions (cerebral cortex, brain stem, thalamus, hippocampus, olfactory bulb and midbrain), from old animals had a significant increase in p16INK4A and a decrease in mRNA levels of p21CIP1. Several inflammatory SASP factors, including tnfa and mcp1, were upregulated in all the brain regions from old WT mice. qPCR analysis also revealed that old female mice had increased senescence in comparison to old male mice. Spatial analysis of gene expression was carried out using RNAscope in situ hybridization. Preliminary RNAscope results indicate that the cerebral cortex from old mice had an increase in p16-, tnfï• ¡- and il1ï• ¢-positive cells. Using probes for tmem119 revealed that microglia are not positive for p16 and p21 signals. Studies are currently being performed to identify which cell type in the brain from old WT mice undergo senescence. In conclusion, we have previously shown that whole brain tissue from old mice have increased senescence. With the current study, we are beginning to regionally measure brain senescence in old WT mice and are investigating the identity of cell type(s) that undergo senescence with natural aging.

Role of Gadd45b in HSC aging

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The hypothesis that epigenetic changes regulate aging is being actively researched in hematopoietic stem cells (HSCs) aging. HSCs can both self-renew and differentiate to all blood cells to maintain the hematopoietic system through life, but age-related functional decline of HSCs contributes to decline of the hematopoietic system. Recently, epigenetic changes such as DNA methylation alterations have been linked to the aging process. Growth Arrest and DNA Damage-inducible proteins (Gadd45a, Gadd45b, and Gadd45g) are robustly expressed early in HSC activation and been implicated in stress response. Importantly, Gadd45b can induce DNA demethylation, and thus, we wanted to explore the relationship between Gadd45b and age-related HSC changes.To examine the role of Gadd45b in regulating DNA methylation during aging in HSCs, we performed whole-genome bisulfite sequencing (WGBS) and mRNA-seq on HSCs from 25 month-old (old) Gadd45b-knockout (KO) and wild type C57Bl/6 (WT) mice. DMRs were defined between comparison of old KO and WT HSCS, as well as between old and young KO, and old and young WT. We then correlated these DMRs to transcriptional profiles generated from the HSCs. To further examine the effects of Gadd45b loss and changes in the methylation profiles, we analyzed peripheral blood (PB) and bone marrow (BM) from old KO mice to confirm the role of Gadd45b in the hematopoietic system by flow cytometry. However, there were no significant differences in frequencies of the populations analyzed in the KO compared with WT. Next, we performed HSCs transplants to establish the functional potential of both young and old KO HSCs. We competitively transplanted 200 HSCs and found that old KO HSCs had reduced chimerism compared to young KO HSCs, but there were no significant differences when compared to the potential of aged WT mice. Finally, we performed bone marrow analysis 16 weeks after transplantation, but no significant differences were found in the chimerism of marrow cells derived from KO or WT donors. These results suggest that Gadd45b may be functionally redundant but may still drive epigenetic

alterations. We are currently compiling all data for a comprehensive analysis to establish the relevance of Gadd45b in HSC biology.

Prediction vs. Reality: metabolic life course trajectories and associations with mortality within the study of longitudinal aging in mice.

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There are strong links between metabolic homeostasis and the aging process, as there is evidence that similar biochemical pathways mediate the two. The Study of Longitudinal Aging in Mice (SLAM) offers a unique opportunity to investigate this relationship by providing highly longitudinal measurements of body weight, body fat, and fasting blood glucose within both sexes of two strains of mice. We performed a population-wide and sex/strain specific latent class analysis (LCA) to investigate subject-specific life course trajectory classes of these metabolic parameters in the SLAM population. We found distinct classes of life course trajectories of each of these metabolic parameters. This heterogeneity in the metabolic life course trajectories suggests the existence of biologically distinct populations even among sex/strain specific subpopulations. These findings can profoundly impact intervention studies and potentially explain heterogeneous responses to interventions, such as caloric restriction. Significant survival differences between populations with distinct trajectories of body weight, body fat, and fasting blood glucose were found, which reinforce their biological importance. This relationship suggests that metabolic life course trajectories can serve as biomarkers of disease, mortality, and intervention response. This research was supported by the Intramural Research Program of the National Institute on Aging of the National Institutes of Health.

Low isoleucine dietary intervention in aged mice

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Research: The role of individual amino acids as critical determinants of health is a fascinating facet of nutrition and metabolism that is only recently being explored. A low isoleucine dietary intervention can induce both immediate and long-term health benefits in young and adult mice. Specifically, we have shown that a low isoleucine diet can improve various aspects of healthspan, enhance metabolism, and extend lifespan. This present study expand upon these previous findings by examining how old age interact with these effects. Using naturally aged 20 month+ C57BL/6J mice, we observed that the reduction of dietary isoleucine to 1/3 normal levels remains physiologically transformative in both male and female mice. Immediately following the start of the diet, the animals lose 20-30% of their bodyweight with a significant escalation of food consumption. Metabolic chamber experiments confirmed increased energy expenditure and altered respiratory exchange ratio. After three weeks of the dietary intervention, fasting blood glucose is suppressed and glycemic clearance is improved in response to a bolus injection of glucose. A sex-dependent response was seen in rotarod and inverted cling performances. Interestingly, echocardiogram revealed a female-only cardiac remodeling in response to low isoleucine where a decreased in pump volume and a compensatory increase in heart rate was observed. After tissue collection, heart autophagy markers was found to be increased. Further studies found circulating FGF21 level to be highly induced by the low isoleucine diet. In addition, hepatic cellular senescence markers were selectively modified by low isoleucine in a manner unique from low protein. We remain impressed by the ability of the low isoleucine diet to induce profound physiological effects in aged mice. More studies are currently ongoing to determine the underlying mechanisms of these effects and how we may take advantage of this dietâ€™s benefits.Funding Acknowledgement: NIH R01-AG056771-04 (DWL), T32-AG000213-27 (CY)

Can Astaxanthin Improve Redox Signaling in Older Adults?

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Redox signaling to oxidative stimulus is important because cell signaling pathways drive the expression of cytoprotective genes. Previous work from our laboratory has demonstrated that older individuals have impaired redox signaling response to acute exercise compared to young. Furthermore, we have shown that this is due to chronically elevated basal redox stress. The aim of the present pilot study is testing whether redox signaling can be enhanced using a phytochemical antioxidant intervention. Astaxanthin (AX) is a carotenoid that has been shown to have significant antioxidant effects. We hypothesized that a 2-week AX supplementation would improve the cellular response to an ex vivo oxidative stimulus. Men and women ages >55y participated in this study (n=10, mean age: 61y Â± 3. Subjects completed a screening visit that included health history, lifetime physical activity questionnaire, anthropometric measures, and resting blood pressure. Baseline blood draw after an overnight fast was taken and again after 14-days of AX supplementation. Peripheral blood mononuclear cells (PBMCs) were isolated and redox signaling was induced by stimulating the sample with H2O2 (low dose: 25 ÂµM, high dose: 500 ÂµM) and DMSO as control. The samples were incubated with the respective treatment in media at 37ÂºC for 10 minutes. Changes in gene expression of genes associated with redox cell signaling response (Prx1, Prx2, Prx3, Trx1, Trx2, GCLC, NQO1, and HO1) were measured in response to the treatments and the AX intervention using RT-qPCR. Compliance to the supplementation was 99% Â± 2. The response to the H2O2 treatment (low and high concentration, respectively) was increased in Prx2 (4-fold, 5-fold), Trx1 (1.6-fold, 1.8-fold), and NQO1 (1.3-fold, 1.4-fold) and unchanged for Prx1, Prx3, Trx2, and HO-1 after the AX treatment. There was high individual variability indicating there are responders and non-responders to the treatment. These preliminary results indicate that AX supplementation may improve stimulated cell signaling response. Funded by the NIH NIGMS Research Initiative for Scientific Enhancement (RISE) 1R25GM127199-01 (Y.L.: RISE Fellowship). Astaxanthin supplement provided by AstaReal.

Mitochondrial GTP metabolism regulates reproductive aging through controlling oocyte mitochondrial dynamics.

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Research: As one of the earliest signs of age-associated decline, reproductive senescence has a strong impact on society due to the trend of increased maternal age. Aged women exhibit decreased fertility, increased rates of birth defects and miscarriages, of which oocyte quality decline is a major contributing factor. Several studies suggest that having an active mitochondrial network is essential for the proper functioning of oocytes. However, the detailed mechanism involved in mitochondrial regulation of reproductive aging remains largely unknown. To address this question, our lab utilizes the powerful nematode genetic model organism C. elegans, which exhibits pronounced age-associated fertility decline, and shares many conserved molecular mechanisms with human. We first discovered that germline inactivation of sucg-1, encoding a mitochondrial GTP-producing enzyme, extends reproductive lifespan and improves oocyte quality in C. elegans. Interestingly, it is reported that overexpression of the ortholog of sucg-1 in human cell line alters mitochondrial dynamics. Therefore, we examined the oocyte mitochondrial network by confocal imaging and found the mitochondrial morphology exhibits radical changes toward fusion and perinuclear clustering during aging in control but not sucg-1 knockdown worms. Next, we explored the causal relationship between mitochondrial dynamics and germline aging by genetic approaches. Strikingly, we discovered that germline overexpression of drp-1 which is essential for mitochondrial fission, drastically increases C. elegans reproductive lifespan from 4 to over 8 days. To confirm this GTP-mitochondrial dynamics regulatory axis, we conducted epistatic study and discovered that germline DRP-1 depletion suppresses the reproductive lifespan extension conferred by sucg-1 knockdown, suggesting that GTP works upstream of mitochondrial dynamics to regulate rate of reproductive decline. Together, these findings reveal a novel regulatory mechanism of reproductive aging by GTP-producing enzyme and provide a potential path to alleviate age-associated reproductive decline via tuning oocyte mitochondrial dynamics.Funding Acknowledgement: National Institutes of Health (DP1 DK113644), Howard Hughes Medical Institute

Metformin stimulated Mitochondrial-Derived Peptide (Ms.MDP) a contributor to Metformin's Actions on Longevity and Healthspan

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Metformin is an FDA-approved drug currently used to lower glucose levels in diabetic patients. Furthermore, epidemiological links have shown a correlation with healthspan and longevity in humans, and several animal studies have shown similar positive effects of metformin. This has led to ongoing clinical trials testing whether administration of metformin can delay aging in humans. However, the molecular mechanism by which metformin enhances longevity remains unclear. Despite, the lack of an exact mechanism, several studies have shown metformin modulates several mitochondria processes. Our lab has done work to identify novel mitochondrial-derived peptides (MDPs) from small open reading frames found within the mitochondrial genome. MDPs are biologically active, potent, diverse, and have system-wide activity with correlation to human physiology and pathology. We believe there is a biological mechanistic relationship between metformin and MDPs that leads to the downstream beneficial effects seen with metformin. One such discovery of a novel peptide named Metformin-stimulated Mitochondrial-Derived Peptide (Ms.MDP). We employed a unique and mitochondrially focused bioinformatic tool or the mitochondrial version of RNAseq namely mitochondrial-derived peptide sequencing (MDPSeq), utilizing a human clinical trial dataset, where 14 individuals were randomly assigned to a 6-week crossover metformin dosage. In vitro effects of Ms.MDP mimic metformin in relevant assays, including glucose uptake, p-AMPK activation, and oxygen consumption. In vivo studies showed that Ms. MDP mimics some of the benefits of metformin. Mice injected with Ms.MDP with and without metformin on a high-fat diet for two weeks, reduced weight and chronic inflammation. Our results indicate that these actions of Ms.MDP may contribute to the beneficial effects of metformin on healthspan and lifespan. Hence, there is a possibility of Ms. MDP based interventions to promote healthy aging.

Investigation of TXNIP attenuation as a novel strategy to reduce bed rest induced muscle atrophy

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Maintenance of skeletal muscle is vital to health and wellbeing. Over the course of age there is a progressive decline in muscle mass and function. This decline results in frailty, reduced independence, reduced quality of life and elevated risk of mortality. Lean muscle mass contributes approximately 50% of body mass in a young adult while only 25% in an individual in their 80s. Critically, this decline in muscle mass is not continuous but composed of periods of stability punctuated by rapid declines. The chief driver of such events is loss due to injury related inactivity. Bed rest, is an essential part of recovering from injuries, however, long periods of inactivity can lead to skeletal muscle atrophy. A study of just 10 days bed rest in older adults produced a 6% decline in lower extremity lean muscle mass and a 15% decline in muscle function. Importantly much of the muscle mass lost even from short bed rest events are thought to never be recovered and to accelerate age related muscle declines. For this reason, development of interventions which reduce bed rest induced muscular atrophy would have considerable benefits in maintenance of independence for older adults. In my research project I am investigating a novel genetic target to ameliorate muscle atrophy as a consequence of immobility. We have found the protein TXNIP, a critical driver of apoptosis, to be induced under limb immobilization (a mouse model of bed rest). Using TXNIP knockdown and TXNIP inhibitors I am testing the hypothesis that prevention of TXNIP induction can reduce muscle atrophy. If successful this work will reduce loss of muscle mass as a consequence of injury in older adults and as a consequence help to maintain activity and independence.

Can a simple blood test accurately predict age?

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The need of finding alternatives to measure aging arises from the inability of chronological age to capture the heterogeneity of the process. Biological clocks and multiple molecular biomarkers have been proposed to predict age, including epigenetic clocks based on DNA methylation. However, they are expensive, often rooted in cross-sectional data, and difficult to implement in a clinical setting. Aim: Build a hematology-based clock to predict age and aging acceleration. Methods: Data were drawn from the Study of Longitudinal Aging in Mice (SLAM), a cohort of 1940 C57BL/6 and HET3 mice of both sexes, and from the Jackson Laboratories’ longitudinal study of aging, a cohort of 540 Diversity Outbreed mice of both sexes. Blood samples were collected and analyzed at different timepoints during the life of the animals (12,006 observations) to derive 19 hematological variables and 2 metabolic indices. Hematological age and aging acceleration were predicted using a deep neural network (DNN). Results were compared with the magnitudes attained by computing the same data into other commonly used machine learning algorithms. Age acceleration was evaluated for association with all-cause mortality, using survival analysis. Results: Hematological age was significantly correlated with chronological age in all models tested (r = 0.82 to 0.95). The DNN performed best of all candidate models (r=0.95, RMSE=10.2 weeks). Higher aging acceleration was associated with shorter survival in all models (HR= 1.37 to 2.42). Conclusion: The proposed hematological clock using routinely collected blood measures has the potential to be applied without arduous and expensive data collection to provide a better understanding into the variability of the aging process. Intramural Research Program of the National Institutes of Health

LRP1 knockout in adult neural stem cells causes hippocampal dysfunction with age and loss of CXCR4.

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Neurodegenerative disease causes the death of 1/3 of senior citizens, with a prevalence projected to triple by 2050. This expected public health crisis will be exacerbated by a lack of disease-modifying treatments. As the most common neurodegenerative disorder, Alzheimer’s disease (AD) is characterized by a pathological loss of memory function due to hippocampal degeneration. Preservation of hippocampal neurogenesis protects from memory loss in AD, suggesting that improved understanding of molecular processes underlying neurogenesis could lead to more efficacious therapies. Neural stem cells (NSCs) migrate from the subgranular zone niche to integrate into hippocampal circuitry. The chemokine receptor CXCR4 plays a fundamental role in this migration& loss of CXCR4 activity causes hippocampal memory deficits in mouse models, and CXCR4 polymorphisms were identified as risk factors for several neurodegenerative diseases. Our lab has identified a novel regulator of CXCR4; low density lipoprotein receptor related protein 1 (LRP1), which is also implicated in AD. LRP1 expression is decreased in AD patients and is involved in trafficking ApoE4, amyloid beta, and tau; all of which play a role in AD pathogenesis. We used a Nestin-Cre inducible mouse model to knockout LRP1 in NSCs of adult mice. We found that LRP1 knockout caused a 10-fold loss of CXCR4 expression and deficits in ischemia-stimulated migration from the subventricular zone. We have found that mice lacking NSC LRP1 display a variety of behavioral deficits at 9 months of age (6 months after knockout) that suggest dysregulated hippocampal function. Specifically, mice lacking NSC LRP1 exhibit hippocampal-dependent memory deficits with a suggested loss of pattern separation – a hippocampal neurogenesis-specific behavior. Given this, we hypothesize that LRP1 regulates CXCR4 in subgranular zone NSCs to enhance hippocampal function. Ongoing research will elucidate the mechanistic underpinnings of these behavioral changes and their relation to memory loss during aging. We expect our research to uniquely connect three independently identified effectors of neurodegenerative disease: LRP1, CXCR4, and neurogenesis. Funding: Owen’s Foundation, and Veteran’s Administration CDA2, TL1-TST

Copper hormesis in Caenorhabditis elegans

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Cells encounter various forms of stress including oxidative stress, protein misfolding, and heavy metal exposure. As cells age, many forms of cellular stress increase while our cellsâ€™ ability to respond becomes diminished. Most prior research has focused on how cells respond to specific stressors, which has resulted in a detailed molecular characterization of evolutionarily conserved stress response pathways. In contrast, relatively little is known about how cells respond when challenged with multiple forms of stress simultaneously. Our lab uses the nematode Caenorhabditis elegans to identify novel interactions between different stressors and dissect the molecular mechanisms underlying these interactions. Earlier work identified novel interactions between copper, a heavy metal, and multiple other forms of stress, including endoplasmic reticulum (ER), Golgi, and osmotic stress. Specifically, the presence of copper appears to make C. elegans more resistant to these other categories of stress. Exposure to mild stressors can lead to activation of stress response pathways and confers downstream benefits, including resistance to repeated exposure to that stressor, a phenomenon termed hormesis. In the absence of repeated exposure to the inducing stress, hormesis can result in prolonged survival or enhanced stress resistance other forms of stress, the latter a related concept called cross-adaptation. Here we investigate the potential for copper hormesis to beneficially influence aging and lifespan in C. elegans. We hypothesize that treating C. elegans with a sublethal dosage of copper sulfate will increase lifespan and enhance resistance to multiple forms of stress. Here we explore the optimal dosage and exposure timing to maximize lifespan. This work was supported by NIH R35GM133588.

Fucoidans are novel senotherapeutics that enhance SIRT6 and DNA repair activity

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With age, senescent cells accumulate in various tissues where they contribute to loss of tissue homeostasis, aging, and age-related diseases through their inflammatory senescence-associated secretory phenotypes (SASPs). Senotherapeutics able to selectively eliminate senescent cells, termed senolytics, or suppress the detrimental SASPs, termed senomorphics, have been demonstrated to improve age-associated co-morbidities and aging phenotypes. To discover novel senotherapeutics translatable to promote healthy longevity, we conducted a drug screening of diverse natural products based on the characteristic senescence-associated beta-galactosidase activity. Several fucoidans from different brown seaweeds were found to exhibit potent senotherapeutic activity. The best senomorphic fucoidan was able to suppress senescence in cultured senescent fibroblasts, in ex vivo human tissue explants, and in vivo in mouse models of natural and accelerated aging. Specifically, fucoidan reduced markers of cellular senescence and SASP in senescent mouse and human cells. Acute treatment of the fucoidan in naturally aged mice reduced tissue senescence, especially in the kidney and lung. Chronic treatment of the fucoidan in Ercc1-/delta progeria mice attenuated composite aging symptoms and extended healthspan. Interestingly, preliminary mechanistic studies demonstrated that fucoidan can improve non-homologous end-joining-directed DNA damage repair and increase the mono-ADP-ribosylation activity of SIRT6, suggesting a relationship between cellular senescence, DNA repair, and SIRT6 signaling pathways. Collectively, fucoidans were identified as novel senotherapeutics with translational potential for reducing cellular senescence, ameliorating age-associated phenotypes, and extending healthspan as well as improving DNA repair pathways through modulation of SIRT6 activity.

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Accelerated rat offspring (f1) age-related metabolic changes programmed by maternal obesity (MO) and hyperlipidic, hypercaloric diet (MO) are prevented by short term normalization

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Introduction: There is growing evidence for developmental programming-aging interactions. Several studies showed that programming of rat F1 by MO accelerates MOF1 development of metabolic dysfunction premature aging. Altered ceramide (CER) metabolism is implicated in aging yet no studies address lipid programming-aging interactions. C16:0 CER plays a key role in development of insulin resistance, a characteristic of both NAFLD and aging. We aimed to evaluate F1 liver mechanisms responsible for the predisposition to early NAFLD with lipidomic and transcriptomic approaches in C and MOF1 at postnatal days (PND) 110 and 650. Methods: F0 female rats ate control (C) or obesogenic diet (MO) from weaning through pregnancy and lactation. After weaning all F1 ate C diet so later life differences are due to maternal dietary programming of F1. We determined F1 liver lipidome by mass spectrometry and transcriptome (RNA-seq). Values with age and diet are shown as percentage of C (100%) in MO110, C650, and MO650, respectively. Results: Sphingolipid signaling and metabolic KEGG pathways were upregulated in males and down regulated in females in MO650 vs.C650. Aging and programming increased males C16:0 CER 120, 155, and 191% while CER species containing longer fatty acyl chains (e.g., C24:0) were reduced (9, 1, and 31%). Female values of C16:0 and C24:0 CER showed no change in all groups. Total lysophosphatidylcholines (LPCs) (111, 124, and 176%), triacylglycerol (135, -40, and 256%) and fatty acid (FA) (133, -48, and 259%) increased in MO650 vs.C650 in males, but no changes in female groups. Conclusions. CER alter cellular proliferation, differentiation and cell death and interact with pathways involved in insulin resistance, inflammation and apoptosis, all linked to NAFLD. C16:0 CER increase insulin resistance and hepatic steatosis. In the liver, LPCs downregulate genes involved in FA oxidation. We conclude MO programs F1 liver lipid signaling and metabolic dysfunction and aging trajectory predisposing F1 to premature aging and NAFLD.

Maternal voluntary exercise prior and during pregnancy in rats has beneficial effects on both normal age-related and programming-aging induced offspring lipid metabolic changes.

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Background: Human epidemiological studies show that maternal obesity (MO) results in offspring (F1) metabolic dysfunction, premature aging and death. Carefully controlled animal studies demonstrate that MO augments the naturally occurring adverse age-related metabolic changes in adiposity index (AI), total fat, leptin and triglycerides (TG). Maternal exercise before and during pregnancy has been proposed as a means to prevent F1 aging outcomes. We hypothesized that maternal exercise before and during pregnancy has preventative effects on these augmented adverse, metabolic age-related F1 outcomes in both males and females. Methods: Female Wistar rats ate control (C) or obesogenic, hyperlipidic/ hypercaloric diet (MO) from weaning through pregnancy and lactation. From 90 days (d) to 120 d (when they were bred) half of each group wheel ran 30 min/day, 5 times/week, providing four groups: control (C), obese (MO), exercised controls (CEx), and Ex obese (MOEx). After weaning all F1 ate C diet. We evaluated total body fat, adiposity index serum leptin, triglycerides (TG), glucose, insulin and HOMA at 110 and 650 d. Data MÂ±SEM, n = 6-8. Analyses two-way ANOVA with post-hoc Tukey test. Results: Maternal Ex decreased the normal age-related increase in male control F1 body weight, leptin and TG between 110 and 650 d. The increase in male and female total fat, leptin, and TG between 110 and 650 d was greater in MOF1 than CF1. MOEx prevented these age-related programmed changes in both sexes of MO F1 at 650d. Interestingly, maternal Ex even lowered leptin and TG in male F1 of CEx mothers. Conclusions: In rats, maternal voluntary Ex intervention prior and during pregnancy has beneficial effects on both aging in F1 of normally fed mothers and F1 of MO programming-aging induced lipid metabolic changes. This study adds further evidence that aging mechanisms are significantly influenced by the maternal environment. CONACYT-RCUK.

Accelerated Rat Offspring (F1) Age-Related Metabolic Changes Programmed by Maternal Obesity (MO) and Hyperlipidic, Hypercaloric Diet (MO) are Prevented by Short Term Normalization of Maternal Diet Prior to, and During Pregnancy and Lactation

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Introduction: MO predisposes to accelerated F1 metabolic aging, excessive weight, adiposity, insulin resistance and lipid metabolism dysregulation. F1MO lifespan is shortened. We aimed 1) to determine mechanisms by which these lifecourse changes occur and 2) determine if maternal dietary intervention (DINT) corrects these physiological changes. We have reported benefits of DINT on MOF1 outcomes at 110 days (d). We hypothesize that beneficial effects of DINT on metabolic aging persist into later life. Methods: Female Wistar rats ate control (C) or obesogenic, hyperlipidic/ hypercaloric diet (MO) from weaning through pregnancy and lactation. Dietary intervention: In half of MO rats, diet was changed to C one month before pregnancy until the end of lactation. After weaning all F1 ate C diet. We evaluated male and female fat depot weight, serum glucose, insulin, leptin and triglycerides (TG) at 650d. Data MÂ±SEM, Analysis ANOVA, post-hoc Tukey test. Results: At postnatal day 21, when pups were weaned, maternal body weight (BW) and serum leptin were higher in MO vs C mothers. Maternal BW and leptin levels in DINT were similar to C. In both males and females at 650d F1MO increases BW, total fat, AI, HOMA, leptin and TG vs 650d C. These age-related changes in MOF1 at 650d were prevented by DINT in both sexes. Interestingly, DINT even lowered fat and AI in F1 females to lower levels than 650d CF1 (Fat (g), DINT=10.6Â±2.6< C=16.5Â±0.6< MO=27.2Â±2.0 and AI, DINT=3.1Â±0.8< C=5.0Â±0.3< MO=7.2Â±0.7 (p< 0.05 for all comparisons). Conclusions: Combined with our published data at 110d we conclude that MO accelerates age-related changes in key metabolic parameters in MOF1 and increases the risk of age-related metabolic diseases. Maternal DINT prior to and during pregnancy and lactation prevents negative age-related programming outcomes and appears to have a separate and beneficial effects on F1 female fat depots. These data show the potential of altering maternal behaviors on later life F1 aging. CONACYT-RCUK.

Functional cellular assays to delineate mechanisms of aging in nonhuman primates

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Aging is associated with defects in homeostatic maintenance. Delineating the dynamics of the homeostatic network is challenging in vivo and often limited by endpoint measurements. However, we have developed carefully controlled and well-characterized translatable cellular models to bridge some of these challenges using primary cells obtained from male and female baboons across the life course (4 to 21 years; human equivalent 14 to 73 years). Using live cell monitoring of changes in proliferation, we recently published findings showing that donor age corresponds to loss of cellular resilience to oxidative stress in males but not in females. Responses to thapsigargin-induced endoplasmic reticulum (ER) stress were also age and sex dependent, though with different pattern than that of oxidative challenge. Young females alone were vulnerable to ER stress, while young males and old females were not affected. These data highlight that primary cells retain donor characteristics regarding molecular mechanisms associated with aging including those dependent on sex. To further investigate these mechanisms, we investigated whether the proteostatic machinery contributes to cellular resilience outcome following ER stress by using a model that assays dynamic changes to proteostasis before, during and after challenge. In female donors, we find significant decrease in 20 S proteasome activity as well as expression of its catalytic subunit (PSMB 8) in female- (both young and old) derived fibroblasts under basal or ER stress conditions when compared to males. ER oxidoreductin 1 (ERO1) as well as autophagy marker, LC3-II/I ratio, were significantly higher in young females compared to young males during ER stress. These data highlight the propagation of sexual dimorphisms in cellular resilience at the molecular level and suggest that sex differences in maintenance of proteostasis contributes to the vulnerability of young females to ER stress. We are characterizing cellular resilience across other cell types including those derived from baboon heart (cardiac fibroblasts), brain (astrocytes) and liver (hepatocytes). Funding: NIH U19 AG 1U19AG057758

Rapamycin delays age-related osteoarthritis in the common marmoset.

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Mechanistic target of rapamycin (mTOR) activity is increased in osteoarthritis (OA), and the mTOR-inhibitor rapamycin (Rap) can attenuate experimental OA in young mice. However, it remains unknown if Rap can protect in a model which better recapitulates the age-related OA most commonly seen in humans. We evaluated if common marmosets, a translational non-human primate model of aging, can serve as a novel model of age-related OA, and if chronic Rap can decrease OA severity. Common marmosets (2.6-16.5 years) were orally administered Rap (n=22; 13M/9F; 1mg/kg) or a yogurt vehicle (n=34; 18M/16F) daily from mid-life until natural death. Following necropsy, micro-computed tomography (ÂµCT) and histopathology were performed and graded in a blinded fashion to assign a bone-related OA score and articular cartilage structural damage (ACS) score, respectively. Marmosets were stratified as adult (<8 years), aged (8-12 years), and geriatric (>12 years) for grouped analysis. In both control (R2=0.18, P=0.01) and Rap-treated (R2=0.54, P=0.0001) marmosets, OA score increased significantly with age. Differences between trendline slopes approached significance between groups (P=0.06), suggesting different OA progression rates between groups, and a longer period unburdened by OA early in life with Rap. When stratified into age groups, adult Rap-treated marmosets displayed lower OA scores than control (P=0.03), while there was no treatment effect in aged or geriatric marmosets. In the subset of available marmosets (n=36), ACS score correlates with ÂµCT OA score (R2=0.4; P<0.0001). Whole-joint ACS correlated with age in Rap-treated marmosets (R2=0.38, P=0.02) but not in control marmosets (R2=0.12, P=0.12). When stratified by age, there were no significant differences in ACS score in any age bracket, despite adult Rap-treated marmosets displaying ACS scores 50% lower than control (3.1ï‚±0.4 vs 6.0ï‚±2.5; P=0.49). These preliminary data demonstrate that common marmosets develop bone and cartilage-related hallmarks of OA in an age-related fashion and suggest that treatment with Rap may compress severe OA pathology to late life. Funding: R21 AG067464

Pre-pregnancy maternal (F0) rat metabolic markers correlate with offspring (F1) accelerated metabolic aging

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Maternal obesity (MO) is a known risk factor for premature F1 aging. HYPOTHESIS: maternal (F0) metabolic parameters pre-pregnancy are markers of F1 accelerated aging. We studied young (postnatal days PND 110) and aged (PND 650) F1 of MOF0 rats. METHODS: Litters providing F0 were culled to 10 pups. At weaning (PND 21) female F0 pups were randomly assigned as controls (CF0), fed chow or MOF0 females fed a high energy, obesogenic diet, throughout the study. F1 ate chow. One CF0 and one MOF0 provided pregestational data. F1 provided PND 110 (n=8 per group/sex) and 650 (n=6 per group/sex) data. In F0 and F1, body weight (BW) and metabolic parameters (glucose (Glu), triglycerides (TG), adiposity index (AI), cholesterol, insulin (Ins), HOMA, leptin, corticosterone (cort), DHEA and cort:DHEA) were determined. Pearson correlations were made for pre-pregnancy F0 and F1 parameters. RESULTS: Pre-pregnancy MOF0 Glu, Ins, HOMA, TG, cholesterol, leptin, and cort were higher vs CF0. Male MOF1 at both ages, total fat, AI, serum leptin, Ins, HOMA, TG, cort and cort:DHEA were higher vs male CF1. Female MOF1 at both ages, total fat, AI, serum leptin, Ins, HOMA, cholesterol, TG, cort and cort:DHEA ratio were higher vs female CF1. F0 pre-pregnancy Ins, HOMA, cort, leptin, and cholesterol were the biomarkers that correlate better with F1 metabolic parameters. F0 pre-pregnancy Ins positively correlates with F1 leptin, HOMA and cort in both sexes at both ages (r>0.5; p<0.05), and strong negative correlation with male F1 DHEA (r>-0.7; p<0.05), but not in females. However, BW and Glu in F0 pre-pregnancy correlates less well with F1 metabolic parameters in both sexes. CONCLUSIONS: There is now compelling evidence that MOF0 has harmful effects on MOF1 function. Maternal pre-pregnancy insulin predicts life course F1 metabolic changes. This study shows that pre-pregnancy maternal markers are related to accelerated F1 metabolic aging in a sex specific manner.

Life-course rat blood corticosterone blood concentrations show sex specific developmental-programming-aging interactions.

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Corticosterone plays major roles in rat physiological systems throughout life. Corticosterone regulates factors such as reactive oxygen species which induce aging. Debate exists on both normative life-course serum corticosterone and maternal challenges that developmentally program corticosterone levels in rodents and other species. HYPOTHESIS: In the rat, the commonest species for aging studies 1) sexually dimorphic programming of life-course offspring (F1) corticosterone by maternal protein-restriction (R) and 2) F1 corticosterone concentrations depend on the precise developmental period when F1 experience the R nutritional challenge, fetal or postnatally pre-weaning. Methods: We studied life-course, serum corticosterone in F1 of R mothers (10% protein diet). Controls ate 20% protein (C). Mothers ate Control and R (10% protein) diets in pregnancy (P) and/or lactation (L) producing four F1 groups, first letter maternal P and second letter maternal L diet - CC, RR, CR and RC. Results: Age of peak corticosterone ~ postnatal day (PND), 450 was similar in all groups. Peak female F1 corticosterone (M+SEM) was CC 597 Â± 21a, RR 902 Â± 33b, CR 604 Â± 36a, RC 770 Â± 29c and male CC 440 Â± 27a, RR 643 Â± 50b, CR 429 Â± 18b, RC 494 Â± 29b ng/ml. Within a sex, values with different letters are different (p<0.05). Female F1 corticosterone was higher than male in all groups. Male and female F1 corticosterone were highest in RR. At PND 650 in CC, RR,CR and RC respectively, corticosterone fell 63,75, 68 and 76 (females) 39,64,49,34 (males) (ng/ml/30d). Summary, Glucocorticoids are major regulators of both programming and age-related pathology e.g. obesity and diabetes. Our data indicate important life-course, sexually dimorphic rat corticosterone programming-aging interactions and show the need to consider developmental programming in rat studies of aging mechanisms. Funding Conacyt.

Microtubule Associated Protein Tau Alters Tubulin Expression

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Microtubules (MTs) are a vital part of the cellular cytoskeleton and are composed of ? and ? tubulin subunits. Microtubule associated proteins (MAPs) facilitate tubulin polymerization and MT stability. Tau, a neuronal MAP, has distinct phosphorylation sites that negatively regulate its MT-binding affinity. Persistent tau phosphorylation, referred to as “tau hyperphosphorylation,” is associated with toxic tau aggregation across age-associated neurodegenerative diseases and is believed to contribute to neuronal dysfunction by destabilizing MT. Tau knockout (KO) mice and transgenic mice with regulatable tau expression (rTg) were used to directly explore the interaction between tau and MTs in vivo during steady state (WT vs KO), tau pathology (rTg vs WT), tau-dependent stress response (WT and KO on high fat diet (HFD)) and tau suppression (rTg + doxycycline). The results demonstrate that tau modulates tubulin protein expression. WT mice expressed 42.13% and 87.83% higher levels of ?- and ?-tubulin (p=0.0004 and 0.0015), respectively, than KO mice. Levels of acetylated ?-tubulin, an indicator of MT stability, were significantly higher in WT than KO mice (128.28% difference, p=0.0019). Similarly, we observed significantly lower tubulin expression in the presence of hyperphosphorylated tau by comparing rTg mice with AD-associated tau pathology to WT mice. To investigate the role of the tau stress response on tubulin expression and MT stability, we placed WT and? KO mice on 60% HFD for 8 weeks. Only WT mice upregulated brain tubulin expression on HFD (? tubulin: 97.57% higher in WT than KO mice, p<0.0001) suggesting that tau was required for the HFD-associated change in tubulin expression. To assess the role of tau protein in modulating tubulin expression, we suppressed tau transgene expression in rTg mice. Results indicated a significant reduction in ? (total and acetylated) and ?-tubulin expression in response to tau suppression. Our results demonstrate that tau protein expression directly alters ? (total and acetylated) and ? tubulin protein expression.