Aging: Molecules to Main Street

46th Annual Meeting of the American Aging Association

held in conjunction with the

Nathan Shock Centers'

Pre-Meeting Symposium on

Emerging techniques and technologies for basic aging research **(June 9)**

Marriott at Brooklyn Bridge
Brooklyn, New York
June 9 – 12, 2017

NEW YORK

SCHEDULE AT A GLANCE

Friday *JUNE 9, 2017* *NATHAN SHOCK* *Symposium*	Nathan Shock Centers of Excellence in the Basic Biology of Aging (Salon D) **9:00 AM – 9:15 AM** Introductions **9:15 AM – 9:30 AM** Meeting Overview **9:30 AM – 10:10 AM** University of Washington Nathan Shock Center **10:10 AM – 10:50 AM** The Jackson Labs Nathan Shock Center **10:50 AM – 11:20 AM** Coffee Break **11: 20 AM – 12:00 PM** Albert Einstein School of Medicine Nathan Shock Center **12:00 PM – 12:40 PM** University of Oklahoma Health Science Center Nathan Shock Center **12:40 PM – 1:30 PM** Lunch (Salon C) **1:30 PM – 2:10 PM** University of Texas Health Science Center at San Antonio Nathan Shock Center (Salon D) **2:10 PM – 2:50 PM** University of Alabama Nathan Shock Center **2:50 PM – 3:00 PM** Wrap-up discussion **3:00 PM** Nathan Shock Symposium Adjourns
Friday *JUNE 9, 2017*	**3:30 PM – 4:00 PM** Trainee Chapter Meeting (Salon D) **4:00 PM – 5:30 PM** Career Development Roundtable and Networking Forum

SCHEDULE AT A GLANCE

Friday *JUNE 9, 2017* *46TH ANNUAL MEETING OF THE AMERICAN AGING ASSOCIATION*	**6:30 PM – 10:00 PM** OPENING RECEPTION (Salon D Foyer) Introduction and Welcome: Richard G.A. Faragher, Ph.D., University of Brighton, UK President, American Aging Association
Saturday *JUNE 10, 2017* *46TH ANNUAL MEETING OF THE AMERICAN AGING ASSOCIATION*	**7:00 AM – 8:00 AM** BREAKFAST (Salon D Foyer) **8:00 AM – 8:45 AM** KEYNOTE ADDRESS (Salon D) **8:45 AM – 10:45 AM** SESSION 1: CELL SENESCENCE…AND AFTER **11:00 AM – 12:40 PM** SESSION 2: Concurrent Panels SESSION 2A in Salon D and SESSION 2B in Greenpoint **12:40 PM – 1:30 PM** LUNCH (Salon D Foyer) **1:30 PM – 3:30 PM** SESSION 3: NEW MODEL SYSTEMS (Salon D) **4:00 PM – 4:45 PM** SESSION 4: TRAINEE CHAPTER SYMPOSIUM **4:45 PM – 7:00 PM** POSTER SESSION AND RECEPTION 1 (Salon D Foyer) **7:00 PM – 10:00 PM** STUDENT DATA BLITZ (Salon E) AGE Board of Directors Meeting (Greenpoint)

SCHEDULE AT A GLANCE

Sunday *JUNE 11, 2017* *46TH ANNUAL MEETING OF THE AMERICAN AGING ASSOCIATION*	**8:00 AM – 9:00 AM** BREAKFAST (Salon D Foyer) **9:00 AM – 11:00 AM** SESSION 5: WHAT CAN WE LEARN FROM PROGEROID SYNDROMES? (Salon D) **11:30 AM – 12:30 PM** SESSION 6: Concurrent Panels of Oral Presentations from Selected Abstracts (6A Salon D; 6B Greenpoint) **12:30 PM – 2:00 PM** DENHAM HARMAN AWARD LECTURE AND LUNCHEON (Buffet in Salon D Foyer; lecture in Salon D) **2:00 PM – 4:00 PM** SESSION 7: ETHICS AND COMMUNICATION PANEL (SPONSORED BY THE GLENN FOUNDATION) (Salon D) **4:30 PM – 5:30 PM** AGE GENERAL MEMBERSHIP MEETING **5:30 PM – 8:00 PM** POSTER SESSION AND RECEPTION 2 (Salon D Foyer) **8:00 PM – Midnight** Trainee Chapter Social (off site)
Monday *JUNE 12, 2017* *46TH ANNUAL MEETING OF THE AMERICAN AGING ASSOCIATION*	**7:00 AM – 8:00 AM** BREAKFAST (Salon D Foyer) **8:00 AM – 9:50 AM** SESSION 8: Oral Presentations from Selected Abstracts (Salon D) **9:50 AM – 11:50 AM** SESSION 9: INFLAMMATION, INTERVENTION AND IMMUNOSENESCENCE **12:10 PM – 1:10 PM** SESSION 10: JAMES JOSEPH ADDRESS **1:10 PM – 1:30 PM** AWARDS CEREMONY AND CLOSE OF MEETING

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A C K N O W L E D G M E N T S

The American Aging Association is grateful to the following sponsors for support of this conference as well as grant support from the National Institute of Aging. Their generous contributions have enabled us to continue a tradition of offering an excellent program of pertinent topics presented by speakers renowned in their fields, providing valuable mentoring opportunities for junior investigators and scholarships for students.

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Overview

The National Institute on Aging (NIA), one of the 25 institutes and centers of the National Institutes of Health, leads a broad scientific effort to understand the nature of aging and to extend the healthy, active years of life. In 1974, Congress granted authority to form the National Institute on Aging to provide leadership in aging research, training, health information dissemination, and other programs relevant to aging and older people. Subsequent amendments to this legislation designated the NIA as the primary federal agency on Alzheimer's disease research.

Mission

The NIA's mission is to improve the health and well-being of older Americans through research, and specifically to:

Support and conduct high quality research on:

-aging processes
-age-related diseases
-special problems and needs of the aged

Train and develop highly skilled research scientists from all population groups

Develop and maintain state-of-the-art resources to accelerate research progress

Disseminate information and communicate with the public and interested groups on health and research advances and on new directions for research.

Programs

NIA sponsors research on aging through extramura and intramural programs. The extramural program funds research and training at universities, hospitals, medical centers and other public and private organizations nationwide. The intramural program conducts basic and clinical research in Baltimore, MD, and on the NIH campus in Bethesda, MD.

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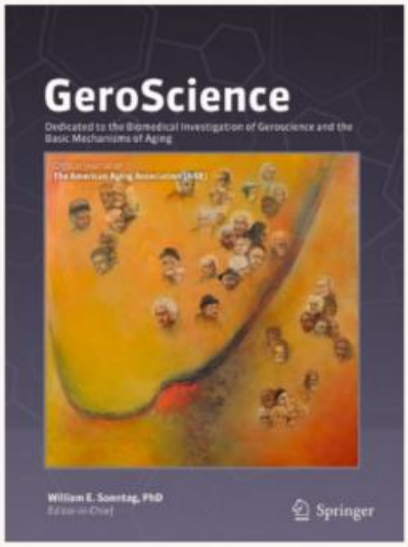

The Official Journal of the American Aging Association

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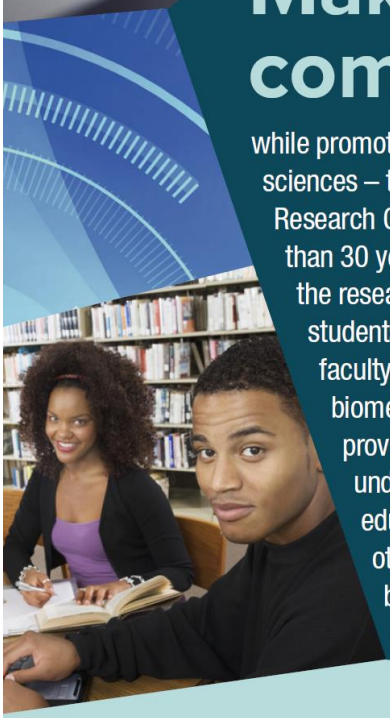

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- A wide array of designated travel awards for students, graduate students and postdoctoral fellows that enable attendance at appropriate FASEB events.
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- Focused career development programs and activities for biomedical researchers and students – sponsored by FASEB MARC during the Experimental Biology, ABRF and ASHG meetings.

FASEB
Federation of American Societies for Experimental Biology

MARC: Maximizing Access to Research Careers

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2017 Nathan Shock Center Symposium:
Emerging techniques and technologies for basic aging research

Friday June 9, 2017

9:00 AM – 9:15 AM	Introductions: Stephanie Lederman, Felipe Sierra
9:15 AM – 9:30 AM	Meeting Overview: Steven Austad
9:30 AM – 9:50 AM	Matt Kaeberlein, University of Washington: Microfluidic technologies to study mechanisms of aging
9:50 AM – 10:10 AM	Daniel Promislow, University of Washington: Metabolomics and the Systems Biology of Aging
10:10 AM – 10:30 AM	George Sutphin, The Jackson Laboratory, Translational Core: Combining Systems and Comparative Genetics to Identify Novel Molecular Targets for Longevity and Age-Associated Disease
10:30 AM – 10:50 AM	Catherine Kaczorowski, The Jackson Laboratory: Systems Genetics Identifies Resilience Factors in Cognitive Aging
10:50 AM – 11:20 AM	Coffee break
11:20 AM – 11:40 AM	Esperanza Arias, Albert Einstein College of Medicine: Mechanism of action of CR in animals
11:40 AM – 12:00 PM	Laura Santambrogio, Albert Einstein College of Medicine: Aging-associated protein carbonylation, glycation, lipoxidation and their effects on cellular proteostasis and immunosenecence
12:00 PM – 12:20 PM	Willard Freeman, University of Oklahoma Health Sciences Center: Use of next generation epigenomic approaches to study the role of DNA methylation in aging
12:20 PM – 12:40 PM	Jonathan Wren, Oklahoma Medical Research Foundation: Transcriptional changes with age - taking advantage of large, public datasets to answer important questions
12:40 PM – 1:30 PM	Lunch
1:30 PM – 1:50 PM	Marty Javors and Al Fisher, University of Texas Health Science Center, San Antonio: Searching for Mechanisms of Aging Interventions Using the Bioanalytical Pharmacology Core

1:50 PM – 2:10 PM	Adam Salmon and Jim Nelson, University of Texas Health Science Center, San Antonio: Testing Rapamycin to Extend Marmoset Longevity Using the Aging Animal Models and Longevity Assessment
2:10 PM – 2:30 PM	Steven Austad, University of Alabama, Birmingham: What can a mitochondrial-nuclear exchange mouse do for me?
2:30 PM – 2:50PM	David Allison. University of Alabama, Birmingham: The Value of Owning Our Statistical Errors in Aging Research: From the Curious to the Humorous
2:50 PM	Wrap-up discussion
3:00 PM	Nathan Shock Symposium Adjourns

3:00 pm – 6:00 pm	**American Aging Association Conference Registration**

2017 American Aging Association Annual Meeting "Aging: Molecules to Main Street"

46th Annual Meeting of the American Aging Association

Friday June 9, 2017

3:30 PM – 4:00 PM	Trainee Chapter Meeting: Chair, R. Michael Anson, Community College of Baltimore County
4:00 PM – 5:30 PM	Career Development Roundtable and Networking Forum:
4:00 PM – 4:30 PM	Chair, Christiaan Leeuwenburgh, University of Florida, Institute on Aging. Outlook for federal and foundation support for biomedical research and research education training and various prospective career opportunities for PhD and Post-Doctoral Students
4:30 PM – 5:30 PM	Panel Participants: Helen Griffiths (Surrey, UK) Felipe Sierra (NIH) Stephanie Lederman (AFAR)
6:30 PM – 10:00 PM	Opening Reception: Richard G.A. Faragher, Ph.D., University of Brighton, UK, President, American Aging Association

Saturday, June 10, 2017

7:00 AM – 8:00 AM	Breakfast
8:00 AM – 8:45 AM	Keynote address: Sarah Harper Ph.D., University of Oxford, Director of the Oxford Institute of Population Ageing (Skype)

Session 1: Cell senescence…and after. Chair: Christian Sell; Co-Chair: Helen Griffiths

8:45 AM – 9:15 AM	James Kirkland, Kogod Center on Aging, Mayo Clinic: Cell senescence and the phenotypes of aging
9:15 AM – 9:45 AM	Judith Campisi, Buck Institute for Research on Aging: Linking Mitochondrial Dysfunction and Senescence
9:45 AM – 10:15 AM	Ittai Ben-Porath, Hebrew University-Hadassah Medical School, Jerusalem, Israel: Effects of p16Ink4a expression and senescence on tissue function
10:15 AM – 10:45 AM	Jesus Gil, Imperial College, London: The SASP: Connecting senescence with inflammation
10:45 AM – 11:00 AM	Coffee Break and Networking

Session 2: Selected oral presentations from submitted abstracts
Concurrent Panels

Session 2A **Chair: Lizzy Ostler (Brighton)**

11:00 AM – 11:15 AM	Jodie Birch, Newcastle University Institute for Ageing: mTOR inhibition ameliorates senescence independently of the SASP in a mouse model of chronic inflammation
11:15 AM – 11:30 AM	Adam Salmon, University of Texas Health Science Center, San Antonio: Targeting mTOR to extend longevity in a non-human primate
11:30 AM – 11:45 AM	Lizzy Ostler, University of Brighton: Resveralogues: a new mechanism for anti-degeneratives?
11:45 AM – 12:00 PM	Jessica Hoffman, University of Alabama, Birmingham: The metabolomic consequences of size and age in the companion dog
12:00 PM – 12:15 PM	Ellen Quarles, University of Washington, Seattle: Short-term rapamycin treatment leads to lasting improvement in cardiac health in aged mice

12:15 PM – 12:30 PM	Panel

Session 2B Chair: Karolina Chocian, Oxford, BSRA
Korenchevsky speaker

11:00 AM – 11:15 AM	Nicolas Martin, University of Wollongong, Australia: Extending maximum lifespan of the honeybee: a new ageing model
11:15 AM – 11:30 AM	Dylan Souder, University of Wisconsin: GSK3β regulates brain energy metabolism
11:30 AM – 11:45 AM	Elena Zambrano, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico: Impaired ischemia induced reperfusion in aged male and female offspring (F1) of obese mothers (MO)
11:45 AM – 12:00 PM	Geoffrey Clarke, University of Texas Health Science Center, San Antonio: Biventricular Cardiac Function Exhibits Sexual Dimorphism in Aging: Magnetic Resonance Imaging (MRI) Results from a Baboon Model
12:00 PM – 12:15 PM	Karolina Chocian, University of Oxford: Dose-dependent functions for chromatin modifiers in regulating lifespan
12:15 PM – 12:30 PM	Panel
12:40 PM – 1:30 PM	Lunch GeroScience Journal Editorial Board Meeting (by invitation)

Session 3: New model systems. Chair: Shelley Buffenstein

1:30 PM – 1:50 PM	Rekha Patel, University of South Carolina: Natural short- and long-lived ecotypes of the microcrustacean Daphnia as models to understand aging
1:50 PM – 2:10 PM	Roger Smith, Royal Veterinary College, University of London: Inflamm-aging and the horse
2:10 PM – 2:30 PM	Dario Valenzano, Max Planck Institute for Biology of Ageing, Cologne: The naturally short-lived African turquoise killifish sheds light on the basis of vertebrate aging
2:30 PM – 2:50 PM	Steven Austad, University of Alabama, Birmingham: Lessons in longevity from *Arctica islandica*

2:50 PM – 3:30 PM	Questions and discussion

Session 4: Trainee Chapter Symposium. Chair: Mark McCormick, Co-Chair: Maria Konovalenko

4:00 PM – 4:45 PM	Trainee talks: Mitchell Lee, The consequences of mutator driven mutagenesis on cellular lifespan. Aref Shahini, NANOG Restores the Myogenic Differentiation Potential of Senescent Myoblasts Ferit Tuzer mTOR inhibition reverses the senescence-associated heterochromatin formation in an Alzheimer's disease mouse model
4:45 PM – 7:00 PM	**Poster Session 1 and Reception** (posters #1 - 28)
7:00 PM – 10:00 PM	AGE Board of Directors Meeting Student Data Blitz - Speakers: Aref Shahini, Mitchell Lee, Yotam Raz, Craig Manning, Jessica Hoffman, Nicolas Martin, Niran Hadad

Sunday, June 11, 2017

8:00 AM – 9:00 AM	Breakfast

Session 5: What can we learn from progeroid syndromes? Chair: Junko Oshima, Co-Chair: Richard Faragher

9:00 AM – 9:20 AM	Junko Oshima, University of Washington/Chiba University: Molecular and Cellular Mechanisms of Progeroid Syndromes
9:20 AM – 9:40 AM	Lorna Harries, University of Exeter, UK: Longevity, genomic plasticity and mRNA processing
9:40 AM – 10:00 AM	Eline Slagboom, Leiden University Medical Center, The Netherlands: Loci and pathways significantly associated with human longevity
10:00 AM – 10:20 AM	Chris Sell, Drexel University *Mitochondria, DNA damage and accelerated aging*
10:20 AM – 11:00 AM	Panel
11:00 AM – 11:30 AM	Coffee Break and Networking

Session 6: Selected oral presentations from submitted abstracts
Concurrent Panels

Session 6A **Chair: David Marcinek, University of Wisconsin**

11:30 AM – 11:45 AM	David Marcinek for Campbell, University of Wisconsin: Intermittent treatment with SS-31 preserves exercise tolerance of aging mice
11:45 AM – 12:00 PM	Niran Hadad, University of Oklahoma Health Science Center: Caloric-restriction Prevents Age-associated Epigenetic Changes in the Aging Brain
12:00 PM – 12:15 PM	Kelly Jin, University of Washington, Seattle: Metabolomics of lifespan extension in calorically restricted fruit flies
12:15 PM – 12:30 PM	Vyacheslav Labunskyy, Boston University: Adaptive aneuploidy as a model to study the role of ER stress resistance in aging

Session 6B: **Chair: Helen Griffiths, Surrey, UK**

11:30 AM – 11:45 AM	Mark McCormick, Buck Institute: Gene-gene interactions in replicative lifespan in *S. cerevisiae*
11:45 AM – 12:00 PM	Sreemathi Logan, University of Oklahoma Health Science Center: Role of IGF-1 in Astrocyte Mitochondrial Metabolism in Brain Aging
12:00 PM – 12:15 PM	Eileen Parks, University of Oklahoma Health Science Center: The impact of reduced neurosteroids in Cognitive Aging
12:15 PM – 12:30 PM	Helen Griffiths, Surrey, UK: Oxidised lipids are increased in patients with dementia and affect miR expression by microvascular endothelial cells

12:30 PM – 2:00 PM **<u>Denham Harman Award Lecture and Luncheon</u>**

Lifetime Achievement Award

Kelvin J. A. Davies, Leonard Davis School of Gerontology, Univ. of So. Calif. (selected by Awards Committee in Spring 2017) "From the Oxygen Paradox to Adaptive Homeostasis: the Ruminations of an Aging Free Radical"

Session 7: Ethics and communication panel (sponsored by the Glenn Foundation) Chair: Richard Faragher

2:00 PM – 2:20 PM	Richard Faragher
2:20 PM – 2:40 PM	Mark R. Collins (Glenn Foundation)
2:40 PM – 3:00 PM	Pat Dade (Cultural Dynamics, UK), Marketer & values based change consultant
3:00 PM – 3:20 PM	Sue Armstrong, Journalist and author
3:20 PM – 4:00 PM	Roundtable discussion
4:00 PM – 4:30 PM	Coffee Break and Networking
4:30 PM – 5:30 PM	**AGE General Membership Meeting**
5:30 PM – 8:00 PM	**Poster Session 2 and Reception** (posters #29 - 56)
8:00 PM – midnight	Trainee Chapter Social

Monday, June 12, 2017

7:00 AM – 8:00 AM	Breakfast

Session 8: selected oral presentations from submitted abstracts Chair: Arlan Richardson

8:00 AM – 8:15 AM	Darcie Moore, University of Wisconsin: Age-specific alteration of the asymmetric segregation of cellular cargoes during mitosis in adult neural stem cells
8:15 AM – 8:30 AM	Willard Freeman, University of Oklahoma Health Science Center: Sexually divergent induction of microglial-associated neuroinflammation with hippocampal aging
8:30 AM – 8:45 AM	Sean Curran, University of Southern California: Mitochondrial ALH-6 is essential for sperm quality and regulates male reproductive senescence

8:45 AM – 9:00 AM	Peter Nathanielsz, University of Wyoming and Texas Biomedical Research Inst.: Mechanisms responsible for the age-related fall in circulating cortisol that begins in early baboon adult life
9:00 AM – 9:15 AM	Reyhan Westbrook, Johns Hopkins: Chronic Inflammation-related Metabolomic Profile Discovery in the interleukin 10tm1Cgn mouse
9:15 AM – 9:30 AM	Arlan Richardson, University of Oklahoma Health Science Center: Role of Necroptosis in Aging and Age-Associated Inflammation
9:30 AM – 9:50 AM	Panel

Session 9: Inflammation, intervention and immunosenescence. Chair: Janko Nikolich-Zugich

9:50 AM – 10:10 AM	Janet Lord, University of Birmingham, UK: Correcting signaling dysregulation in neutrophils and improving immunity in older adults
10:10 AM – 10:30 AM	Megan Smithey, University of Arizona, Arizona Center on Aging: From mice to men: translating discoveries in immunosenescence
10:30 AM – 10:50 AM	
10:50 AM – 11:10 AM	Deborah Dunn-Walters, Surrey, UK: B cell repertoire - what does it tell us about immune senescence
11:10 AM – 11:50 AM	Panel
11:50 AM – 12:10 PM	Coffee Break and Networking
12:10 PM – 1:10 PM	**Session 10: Special Lecture: James Joseph Address.** **Giulio Pasinetti, M.D., Ph.D. Icahn School of Medicine at Mount Sinai (selected by Awards Committee Spring 2017)**
1:10 PM – 1:30 PM	**Awards Ceremony:** **Outstanding Service Award, Nancy Nadon, Ph.D.** **Student Awards and Close of Meeting**
1:30 PM	**AGE Meeting Adjourns**

SPEAKER ABSTRACTS

Natural short- and long-lived ecotypes of the microcrustacean *Daphnia* as models to understand aging.

Rekha C. Patel, Charles A. Schumpert, and Jeffry L. Dudycha. University of South Carolina.

The freshwater microcrustacean *Daphnia* has many attractive qualities as a model for aging research including a fully sequenced genome, adult tissue regeneration, a form of clonal reproduction that allows generating large populations of isogenic individuals, and substantial genetic variation within and among natural populations leading to significant differences in their lifespans. Furthermore, extensive ecological and evolutionary work on *Daphnia* allows our understanding of molecular mechanisms to be integrated with population demography and the evolutionary genetic processes that contribute to differences in aging. In order to lay some groundwork for this model, we first studied the heat shock responses as well as telomere maintenance in both short- and long-lived ecotypes. In addition, we developed a new method for fast and effective RNA interference (RNAi) for use with *Daphnia*. This method is expected to be widely useful for all *Daphnia* biologists, as no method was yet available for RNAi in adult *Daphnia*. Our studies also include the characterization of *Daphnia* Sir2 mRNA levels and activity during life span and examine the effects of RNA interference mediated Sir2 knockdown on the lifespan and survival following proteotoxic stress. Overall, we establish *Daphnia* as a new model organism for research on aging and offer novel insights into mechanisms related to longevity and aging.

Funding: National Institutes of Health grant 1R01AG037969 awarded to RCP and JLD.

Unifying paradigm for the role of aging in the pathophysiology of tendinopathy

Smith, R.K.W.[1], Dakin, S.G.[2], and Dudhia, J.[1]
[1]Dept. of Clinical Sciences and Services, The Royal Veterinary College, Hatfield, Herts. U.K.
[2]Botnar Research Institute, University of Oxford, UK

Tendon overstrain injuries are common in both humans and horses and usually affect highly loaded tendons, most frequently during exercise. It is generally accepted that the most common pathogenesis involves preceding changes to the tendon that predispose it to injury. The horse is an excellent model for the human as it shows an influence of age and suffers naturally-occurring overstrain injuries of functionally similar tendons associated with athleticism. This presentation will focus on the influence of age on the physiological processes in tendon and their relationship to injury using the horse as a model of human tendon disease.

After birth loading induces anisotropy where areas of tendon under compression develop cartilage-like matrix and two types of tendon (positional and weight-bearing) diverge in composition and mechanical properties. Exercise appears to have beneficial effects on tendon growth until skeletal maturity after which this beneficial effect appears to be largely lost in the equine superficial digital flexor tendons (the functional correlate to the human Achilles tendon) where exercise induces deleterious effects on the collagen matrix and a loss of specific non-collagenous matrix components. While these changes have been recorded for the fascicular component of the tendon, other studies have shown changes with age to the interfascicular matrix where most of tendon extension occurs through a sliding mechanism between fascicles and hence may have greater influence on tendon function. While there is little change with age in whole tendon structural properties and composition, there is a loss of interfascicular matrix

elasticity, a reduction in the rotational sliding of fascicles and a decrease in the fatigue resistance of both compartments with age in weight-bearing tendons, giving a potential mechanical mechanism for tendon predisposition to failure.

These changes do not appear to be significantly repaired in adult horses indicating a failure to incorporate new matrix with age. This has been supported by investigations on the half-life of different matrix proteins in adult equine tendon which have demonstrated the half-life of tendon collagen to be almost 200 years, while that of the non-collagenous proteins is in the order of years. Similar absence of functional remodeling has also been shown for human Achilles tendon using nuclear bomb ^{14}C quantification. Our investigations into the mechanism for such loss of reparative ability have shown that, while the tenocytes are alive and similarly metabolically active in the weight-bearing tendons of older horses, they show ~50% reduction in synthetic response *in vitro* to growth factor and mechanical stimuli when compared to tenocytes recovered from young tendon, a concept further supported by proteomic analysis in aged interfascicular matrix. Compounding this poor cellular responsiveness is a reduction in growth factor and gap junction presence which are also needed for a co-ordinated synthetic response.

We hypothesise that his age- and exercise-related change in tendon matrix is induced by cyclical loading, either by direct physical damage or from indirect physical effects on tenocytes such as hyperthermia, and mediated by accumulated enzyme-mediated matrix damage which is greater in older tendon. We believe this process is driven by inflammatory pathways and have recently demonstrated an age-associated decline in pro-resolving pathways ('inflamm-aging'). Uninjured tendon explants from younger, but not older horses, treated with IL-1beta responded by increasing pro-resolving mediators FPR2/ALX, while there was decreased FPR2/ALX receptor expression with concurrent increased PGE_2 levels after injury in older horses, suggesting aged individuals exhibit a reduced capacity to resolve inflammation which results in the development of chronic tendinopathy and re-injury.

Funding: Horserace Betting Levy Board, BBSRC, Japan Racing Association, The Wellcome Trust.

POSTER ABSTRACTS

1. mTOR inhibition ameliorates senescence independently of the SASP in a mouse model of chronic inflammation

Jodie Birch[1], Clara Correia-Melo[1], Edward Fielder[1], Dina Rahmatika[1], Jennifer Taylor[1], Jelena Mann[2], Derek Mann[2], Viktor Korolchuck[1], Joao F Passos[1]

[1]Newcastle University Institute for Ageing, Institute for Cell and Molecular Biosciences, Newcastle University, [2]Institute of Cellular Medicine, Newcastle University

Cellular senescence is characterised by enhanced secretion of pro-inflammatory mediators; the so-called senescence-associated secretory phenotype (SASP). Senescence is causally implicated in ageing and in the development of age-related diseases, including various respiratory disorders. The mechanistic target of rapamycin (mTOR) plays important roles in ageing and inhibition of mTOR with rapamycin extends lifespan in several model organisms and delays onset of age-related pathology. It was suggested that rapamycin may exert these effects by suppressing senescence-associated inflammation. We aimed to determine whether a rapamycin-supplemented diet could ameliorate phenotypes of accelerated senescence and premature ageing in a mouse model of chronic inflammation, whereby NF-κB activity is enhanced due to knockdown of the inhibitory nf-kb1 subunit (*nfkb1-/-*). While no significant

differences in the mean and maximum lifespan were found, overall healthspan was improved in *nfkb1-/-* mice fed with a rapamycin-supplemented diet, including improvements in neuromuscular coordination and long-term memory. Various tissues were also improved by a rapamycin-supplemented diet, including the heart and skin. In the lung, enlargement of alveolar airspaces (indicative of emphysema) observed in *nfkb1-/-* mice was significantly reduced with rapamycin and a number of senescence- associated markers were decreased including telomere-associated damage and p16 expression. Moreover, *in vitro* treatment with rapamycin decreased senescence markers in mouse adult fibroblasts (MAFs) from *nfkb1-/-* mice, including mitochondrial-derived reactive oxygen species (ROS), following X-ray irradiation. However, there were no changes in SASP-associated pro-inflammatory mediators in the serum and lungs of *nfkb1-/-* mice following rapamycin supplementation. Our data suggest that pathways regulating the SASP and cell-cycle arrest are independent when NF-κB activity is enhanced. Beneficial effects of rapamycin may be due to improved mitochondrial function in the *nfkb1-/-* mouse model.

Funding sources:

BBSRC: BB/K017314/1

2. Intermittent treatment with SS-31 preserves exercise tolerance of aging mice.

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Muscle redox status regulates diverse aspects of skeletal muscle function, including mitochondrial energetics and contractile performance. In aging skeletal muscle mitochondrial oxidative stress and contributes to altered redox homeostasis. We have previously demonstrated that reducing mitochondrial oxidant production with acute (1 hr) and 8 week treatment with the mitochondrial-targeted peptide elamipretide (SS-31) can reverse skeletal muscle and mitochondrial dysfunction and reduce redox stress in aging mice. These improvements were also associated with increased exercise tolerance. Despite strong evidence for reversal of dysfunction it is not known whether early treatment with SS-31 can slow age-related decline in muscle function. In order to test the effects of SS-31 on long-term healthspan we treated female C57Bl/6 mice starting at 20 months of age twice weekly with SS-31 (3 mg/kg) for 8 months. Exercise tolerance was tested monthly with a treadmill endurance test. In vivo skeletal muscle and cardiac function were also tested throughout the treatment period. Treatment with SS-31 preserved exercise tolerance throughout the study, while treadmill performance in the saline treated mice declined significantly. There was a non-significant trend toward preservation of muscle force production with direct muscle stimulation and prevention of left ventricular hypertrophy. The greater effect on treadmill performance in aged mice than by independent measures of skeletal and cardiac function suggests that SS-31 may have benefit in multiple systems that is revealed by an integrative test of function such as treadmill endurance. Additionally we tested muscle respiration and H_2O_2 production ex vivo in isolated mitochondria from 28 months old skeletal muscle. We found no significant change in respiration or H_2O_2 production in aged mitochondria in the presence of SS-31 when measuring electron flow in the forward direction. However, reverse flow experiments using succinate as the primary substrate reveal a trend of increased respiration and a concomitant decrease in H_2O_2 production upon treatment with SS-31. These data show that long-term administration of SS-31 can slow

age-related decline in function. Furthermore, the effect on oxidant production with reverse electron flow suggests this may play a more important role in aging mitochondrial dysfunction and physiology than previously assumed. It also reveals a novel possible target for mitochondrial interventions capable of improving function and redox status.

Funding:

T32 AG000057

P01 AG001751

R21 AG042637

3. Targeting Mitochondrial Function to Reverse Cardiac Aging.

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Aging is associated with a higher incidence of cardiovascular disease and a significant decline in cardiac function. In individuals without overt cardiovascular disease, aging results in impaired diastolic function, reduced myocardial performance and increased prevalence of cardiac hypertrophy. Our laboratory has previously shown that transgenic mice expressing mitochondrial catalase (mCAT) have reduced mitochondrial oxidative damage and display delayed cardiac aging. This study aims to determine whether short-term treatments targeting mitochondrial function can reverse cardiac aging in old mice.

The mitochondrial protective SS-31 peptide (elamipretide) has previously been shown to provide similar protection as mCAT in models of pressure overload-induced cardiac hypertrophy and failure. SS-31 binds to cardiolipin and improves the electron carrying function of cyt c, while reducing its peroxidase activity. We administered SS-31 to 24-month-old mice via osmotic minipump for 8 weeks, and found it to reduce cardiac hypertrophy and improve diastolic function and myocardial performance. We observed that SS-31 treatment improved mitochondrial ultrastructure and induced AMPK activation in old hearts.

To investigate whether SS-31 and mCAT protect cardiac aging by overlapping mechanisms, we administered SS-31 to old mCAT mice to study the effects of combined treatments. We found that SS-31 treatment cannot further improve the cardiac function of old mCAT mice. Concordantly, proteomic analysis revealed that changes in the cardiac proteome induced by SS-31 were partially overlapping with changes mediated by mCAT expression, particularly in the mitochondrial proteome, suggesting a convergence of molecular mechanisms of these two treatments. In addition, we showed that mCAT expression transduced by AAV9 in mice at late-life also improved diastolic function, supporting the similar therapeutic effects of short-term mCAT and SS-31. In summary, these results support the therapeutic potential of mitochondrial-targeted interventions to reverse the effects of cardiac aging.

Funding: Glenn/AFAR Postdoctoral Fellowship for Translational Research on Aging, NIA 5T32AG000057 Training Grant and NIA K99 AG051735 to YAC and NIA P01 AG001751 to PSR.

4. Dose-dependent functions for chromatin modifiers in regulating lifespan.

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Following an RNAi screen targeting chromatin factors, we identified H3K27 modifiers as key regulators of lifespan in *C. elegans*. Depletion of the *jmjd-3.2* and *utx-1* H3K27 demethylases, but not *jmjd-3.1* or *jmjd-3.3*, results in significant lifespan and healthspan extension. Furthermore, overexpression of either enzymatically functional or "dead" forms of *jmjd-3.2* both extend lifespan, suggesting that, firstly, both up-regulation and down-regulation of *jmjd-3.2* has similar lifespan consequences, and secondly, that the enzymatic activity is dispensable for improved longevity in worms. In contrast, while overexpression and depletion of *utx-1* also extend lifespan, we show that the enzymatic activity of *utx-1* is absolutely required for lifespan regulation, despite its demethylase-independent role during development (Vandamme et al. 2012). We are currently investigating the molecular targets of *utx-1* and other H3K27 modifiers impacting lifespan.

Funding: Wellcome Trust (grant number: ALRVDK0)

5. Biventricular Cardiac Function Exhibits Sexual Dimorphism in Aging: Magnetic Resonance Imaging (MRI) Results from a Baboon Model.

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Introduction: We have developed a baboon model to study cardiovascular (CV) aging across the life course (PMID: 27988927; PMID: 28439937). We used a 3 Tesla MRI system (Siemens, Malvern, PA) to measure 24 key left ventricular (LV) and right ventricular (RV) parameters in 28 baboons (14F, age range 4.1-22.7 years, human equivalent ~15-85 years). Image analysis was performed using CMR 42 software (Circle Cardiovascular, Calgary, AB). Pearson correlation and principle components analysis (PCA) were performed (significance: p=0.05). As in humans, volumetric data were correlated with body surface area (BSA), so these functional parameters were normalized to BSA.

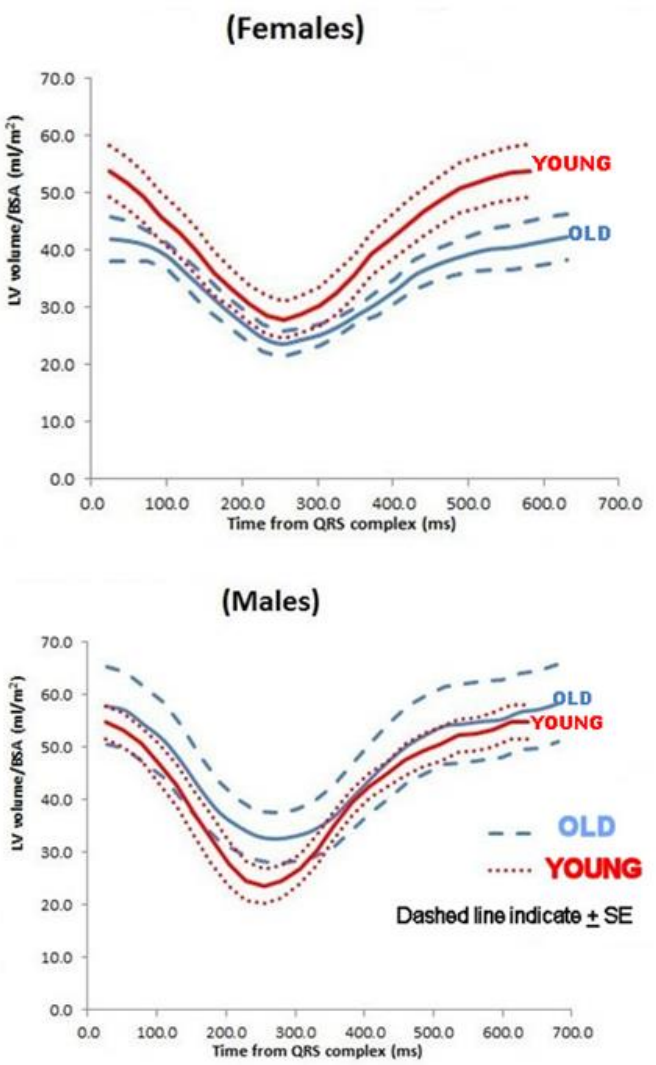

Figure 1. Changes in LV volume throughout the cardiac cycle in male and female baboons with age.

Results: Reduced normalized peak LV wall thickness (r=-0.63, p=0.0003) and increased LV sphericity (r=0.61, p=0.0005) were correlated with age. Additionally, RV % wall thickening (r=-0.51, p=0.006), RV cardiac output (r=-0.41, p=0.029), RV stroke volume (SV, r=-0.38, p=0.045), and RV ejection fraction (EF, r=-0.49, p=0.009) were negatively correlated with age. MRI studies in humans (20-80 yrs) found decreases in normalized LV SV (SV, -9% in M, -13% in F) and RV SV (SV, -7% in M, -9% in F), as well as decreased LV myocardial mass and increased LV sphericity in males only (PMID:16755827, PMID:17088316). We used PCA to distinguish the differences between males and females. The first principal component (PC1) in males showed strong association of age with decreases in LV and RV EF and reduced normalized LV mass. Female PC1 revealed lesser age associations with LV and RV function, as well as somewhat impaired diastolic function with changes in heart rate. Male PC2 showed a strong relationship between ventricular remodeling and impaired diastolic and RV systolic function. Female PC2 was more strongly associated with LV and RV remodeling as well as impaired aortic distension.

Conclusions: These data indicate sexual dimorphisms in both RV and LV function in the baboon which may reflect sex dependent compensation with males compensating by remodeling (increased sphericity) and females by reducing heart rate and extending diastole. We are currently undertaking multiple regression analyses to develop models that incorporate factors associated with CV aging.

Funding: NIH 5P01HD021350- PWN, 5R24OD011183- PWN, 5K25DK089012- GDC, and 1R25EB016631- AHK. OD P51 OD011133 Southwest National Primate Research Center.

6. HAART Drugs Induce Senescence and Metabolic Changes in Astrocytes.

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With the advent of highly active antiretroviral therapy (HAART), survival rates among those infected by HIV have increased. However, even though survival has increased, HIV-associated neurocognitive disorders (HAND) remain, suggesting that these drugs may play a role in its continued prevalence. We propose that one possible mechanism for HAND is HAART-mediated astrocyte senescence since previous results from our group have demonstrated that human astrocytes are able to senesce in response to external stressors and that there is an association of senescent astrocytes, in the human brain with aging and neurocognitive disorders such as Alzheimer's disease. We therefore examined the effects of HAART on the senescence program of human astrocytes. Our results indicate that these drugs, administrated at physiological level, induce growth inhibition, senescence-associated beta-galactosidase and cell cycle inhibitors. HAART treatment is also associated with the induction of reactive oxygen species and upregulation of mitochondrial oxygen consumption. These changes in mitochondria metabolism correlate with increased glycolysis in HAART drug treated astrocytes. Taken together, these results indicate that HAART drugs induce the senescence program in human astrocytes, which is associated with oxidative and metabolic changes that could play a role in the development of HAND.

Funding: 1RO1NS078283, NS78283 and R21AG046943

7. Methionine Restriction Attenuated Kidney Injury in 5/6 Nephrectomized Mice

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Methionine restriction (MR) extends lifespan across multiple species. In rodents, MR prevented obesity and reduced risk for diabetes. We investigated whether MR is beneficial against kidney disease using 5/6 nephrectomized mice. To generate 5/6 nephrectomized mice, male C57BL/6J mice underwent right unilateral nephrectomy with ablation of cranial and caudal poles of the remaining left kidney. Mice were weight matched and fed a control (5/6Nx-CF, 0.86% Met, n = 7) or experimental 5/6Nx-MR (0.12% Met, n = 8) diet for 10 weeks. Levels of urine metabolites such as albumin, cystatin C, lipocalin-2, and clusterin were significantly reduced in 5/6Nx-MR compared to 5/6Nx-CF mice, while creatinine was similar in both groups. Albumin-to-creatinine ratio in 5/6Nx-MR mice was 74% lower than in 5/6Nx-CF mice. In addition, plasma markers for renal injury such as α-2 macroglobulin, cystatin C, lipocalin 2, and clusterin were reduced in 5/6Nx-MR mice compared to 5/6Nx-CF mice. Furthermore, histopathological analyses of kidney sections indicated that capsular focal fibrosis lesions were present in all mice, but the estimated portion of the kidney section affected was < 25% - 50% in 5/6Nx-MR compared to 50% - 100% in 5/6Nx-CF mice. Interstitial fibrosis was observed in 30% of 5/6Nx-MR kidneys while 86% of 5/6Nx-CF kidneys developed lesions. Mild inflammation was observed in 25% of 5/6Nx-MR kidneys but was 86% in 5/6Nx-CF. Mild lesions of basophilia and tubular dilatation were observed in 5/6Nx-MR kidneys, while moderate lesions were found in 5/6Nx-CF. Finally, inflammatory genes such as *Emr1, Nos2*, and *Tnfa*, and fibrosis genes such as *Fn1*, *Serpin*, *Tgfb1*, and *Tgfb2* were reduced in 5/6Nx-MR compared to 5/6Nx-CF. Overall, our data showed that MR attenuated kidney injury in mice due to downregulated inflammatory and fibrosis mechanisms.

Funding: Orentreich Foundation for the Advancement of Science, Inc.

8. Mitochondrial ALH-6 is essential for sperm quality and regulates male reproductive senescence

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3. University of Southern California, Norris Comprehensive Cancer Center

Reproduction is essential to perpetuate life. Mitochondria integrity and functionality has been linked to proper sperm function across multiple species. Most studies have examined the negative impact of the environment and measure the effects of acute stress on sperm function and reproductive output. However, the mechanistic impact that normal cellular metabolism plays in the regulation of sperm quality and activity remains unclear. Here, we show that *C. elegans* with mutations in *alh-6*, a conserved proline metabolism gene, display early reproductive senescence. Loss of proline catabolism results in specific deficits in sperm number, size, and activation. These defects in sperm quality are linked to changes in mitochondria morphology, metabolic output, and ROS generation. Intriguingly, the reproductive defects in *alh-6* mutants are not simply due to reduced flux through the proline catabolism pathway. Instead the premature reproductive senescence in *alh-6* mutants is a result of aberrant ROS homeostasis and loss of energy storing metabolites; however, the relative impact that altered levels of ROS and metabolic intermediates plays in the sperm number, size, and activation phenotypes is remarkably different. Finally, the expression of the mammalian ortholog of *alh-6, Aldh4a1,* is significantly reduced with age in mouse testes suggesting a potential conserved role of *alh-6* in male reproductive fitness. Taken together, we have uncovered a novel role for a conserved and central amino acid catabolism pathway on normal sperm function and our work uncovers a new variable to measure which can predict and alter the rate of aging of the male reproductive system.

Funding: NIH/NIGMS R01GM109028 (S.P.C.); Early work on this project was supported by the American Heart Association (S.P.C.), an Ellison New Scholar Award (S.P.C.), and the American Federation for Aging Research (S.P.C.).

9. Sexually divergent induction of microglial-associated neuroinflammation with hippocampal aging

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The necessity of including both males and females in molecular neuroscience research is now well understood. However, there is relatively limited basic biological data on brain sex differences across the lifespan despite the differential rate of age-related neurological dysfunction and disease in male and females.

Whole genome gene expression of young (3 months), adult (12 months) and old (24 months) male and female C57BL6 mice hippocampus was analyzed. Subsequent bioinformatic analyses and confirmations of age-related changes and sex differences in hippocampal gene and protein expression were performed.

Males and females demonstrate both common expression changes with aging and marked sex differences in the nature and magnitude of the aging responses. Sexually divergent induction of neuroinflammatory gene expression with aging was evident with females demonstrating larger responses. Sex differences in old age were highly over-represented in microglia-specific genes with heightened induction of complement pathway components and other microglial genes in females with aging. Similar patterns of cortical sexually dimorphic gene expression were also evident. Additionally, inter-animal gene expression variability increased with aging in males, but not females.

These findings demonstrate sexually dimorphic neuroinflammation with aging that may contribute to sex differences in age-related neurological diseases such as stroke and Alzheimer's, specifically in the complement system. The increased expression variability in males suggests a loss of fidelity in gene expression regulation with aging. These findings reveal a central role of sex in the transcriptomic response of the hippocampus to aging.

Funding: This work was supported the Donald W. Reynolds Foundation, the National Institute on Aging (R01AG026607, P30AG050911, F31AG038285), National Eye Institute (R01EY021716, R21EY024520, T32EY023202) and Oklahoma Center for Advancement of Science and Technology (HR14-174).

10. Selection on mitochondrial mutations in cell lines.

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Mitochondrial mutations accumulate in somatic cells during aging and are prevalent in cancer. How these mutations increase within cells, starting from a single copy of many mitochondrial genomes, is not well understood. Because mitochondria exchange gene products through fission and fusion, the phenotypes of mitochondrial mutations can be masked by complementation. Despite this, we find that mitochondrial mutations can be positively or negatively selected in cell lines and are working to understand the factors determining the efficiency of this selection.

Funding: Glenn/AFAR Postdoctoral Fellowship for Translational Research on Aging

11. Systems Biology of Human Aging - Network Model 2017.

John D. Furber, Legendary Pharmaceuticals

This network diagram is presented to aid in conceptualizing the many processes of aging, the causal chains of events, and the interactions among them. Contemplation of this network suggests promising intervention points for therapy development. This diagram is maintained on

the Web as a reference for researchers and students. Content is updated as new information comes to light.

[www.LegendaryPharma.com/chartbg.html]

At first glance, the network looks like a complicated web. However, as a conceptual summary, in one view, we can see how the many biogerontological processes relate to each other. Importantly, examination of these relationships allows us to pick out reasonably plausible causal chains of events. Within these chains, we can see age-related changes or accumulations that appear to be promising targets for future therapy development. The many observable signs of human senescence have been hypothesized by various researchers to result from several primary causes. Inspection of the biochemical and physiological pathways associated with age-related changes and with the hypothesized causes reveals several parallel cascades of events that involve several important interactions and feedback loops. This network model includes both intracellular and extracellular processes. It ranges in scale from molecular interactions to whole-body physiology. Effects due to externalities, lifestyle, environment, and proposed interventions are highlighted around the margins of the network.

- **Nuclear mutations**, telomere shortening, chromosome breaks, chromatin alterations, and epigenetic DNA adducts change gene expression.

- **Extracellular proteins** become damaged by glycation, oxidation, crosslinking, and lytic enzymes, resulting in mechanical stiffness, weakness, and inflammation. Altered environmental niches for cells contribute to transdifferentiation, arrested cell division, cell death, cancer, stem cell depletion, tissue wasting, neurodegeneration, and organ malfunction. Stiffer blood vessels promote stroke and heart disease.

- **Lysosomes** accumulate reactive, crosslinked **lipofuscin**, which impairs autophagic turnover of macromolecules and organelles, resulting in accumulation of dysfunctional macromolecules and organelles. This interferes with cell function. When lipofuscin leaks into the cytosol, it can trigger apoptosis of cells, which are not readily replaced.

- **Mitochondrial DNA** mutates. Mutations are copied, resulting in altered cell physiology.

- **Lamin-A** splice-variant, **progerin**, accumulates in the nuclear scaffold, impairing cell division.

- **Nuclear envelope pore** proteins become oxidized, allowing inappropriate traffic of other proteins into and out of the nucleus.

- **Oxidized aggregates** in cytoplasm become crosslinked, resist turnover, inhibit proteasome activity, increase redox poise, and physically interfere with intracellular transport, especially in axons.

- **Proteasomes** get inhibited, reducing turnover of damaged molecules and of expired molecular signals.

- **Increased redox poise** alters signaling and enzyme activities, and erodes telomeres.

- **Inflammatory cascades,** promoted by damaged molecules and sick cells, further damage tissues.

- **Neuroendocrine and immune** systems degrade.

- **ER stress:** Misfolded proteins accumulate in ER.

Funding: Small, crowd-sourced donations

12. Oxidised lipids are increased in patients with dementia and affect miR expression by microvascular endothelial cells.

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Alzheimer's disease (AD) has a complex aetiology involving gene and environmental risk factor interaction. Our recent studies have confirmed that oxidised LDL concentration is higher in vascular dementia patients and is inversely correlated with cognitive function.

We hypothesised that oxidised LDL may contribute to dementia and that endothelial cells of the blood-brain barrier are critical mediators of systemic nutrient effects within the brain. Therefore we have studied the effect of the oxidised lipids 27 hydroxycholesterol and F2alpha isoprostane on microvascular endothelial cell redox state, inflammatory cytokines and regulatory microRNA (miR) profile.

We showed that lipids from patients with dementia or hypercholesterolaemia release directional inflammatory molecular signatures from endothelial cells via a redox state-dependent mechanism. miR expression in endothelial cells with and without oxidised lipid treatment was analysed using an Agilent DNA microarray scanner and microarray data was analysed using GeneSpring GX software. Upregulated miR were predicted to affect oxidative stress and inflammatory pathways. Downregulated pathways included growth factor signalling. Using qPCR, we determined that miR-144 and 146 which are anti-inflammatory and redox regulating modulators were decreased by oxidised lipids. Conversely, a neurotrophic factor-targeting miR was increased in expression by oxidised lipids.

These results suggest that oxidised lipids, which have important regulatory effects on endothelial microvascular cell function, may lead to a secretome that affects the brain compartment.

This work was supported by Alzheimer's Research UK

13. Role of the ULK1 complex-Atg8 interaction in autophagy.

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Protein aggregates and dysfunctional mitochondria are degraded by the lysosomal-dependent process of autophagy. Deficient autophagy causes accelerated aging and age-related pathologies. Although the association between autophagy and aging is well appreciated, the mechanism of autophagy initiation is not completely understood, limiting the development of therapeutics to prevent or treat age-related pathologies. The serine/threonine kinase ULK1 is a key mediator of autophagy initiation and forms a protein complex by interacting with Atg13, FIP200 and Atg101. The ULK1 complex, via the LC3 interacting motif (LIR) in ULK1, Atg13 and FIP200, interacts with Atg8 proteins (Atg8s), which regulate autophagic membrane growth and maturation. In this study, we used reconstitution experiments and CRISPR/Cas9 technique in

human cells to introduce point mutations in ULK1, Atg13, and FIP200 to disable their interaction with Atg8s. The FIP200 mutation caused no autophagy defect, suggesting that the interaction between FIP200 and Atg8s is dispensable for autophagy. Interestingly, the ULK1 mutation caused a decrease in ULK1 expression level, suggesting that binding of Atg8s to ULK1 may be critical for autophagy by promoting ULK1 stability. The Atg13 mutation caused a decrease in the phosphorylation of Atg14 by ULK1, a modification critical for autophagosome formation. Combined, these results support a model in which Atg8s associate with ULK1 and Atg13 to promote ULK1 stability and autophagosome formation.

Funding: NIH (GM097057, AG039758), NIH (T32AG029796)

14. Caloric-restriction Prevents Age-associated Epigenetic Changes in the Aging Brain

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Brain aging is characterized by cognitive decline and increased risk to neurodegenerative disease development. In the CNS, epigenetic mechanisms are vital to proper cellular function and memory formation. Aberrant epigenomic control, specifically in the methylome, is evident with aging and age-related disease. Anti-aging treatments, such as caloric restriction (CR), increase neurogenesis and induce expression of neuroprotective genes in the aged brain. However, the mechanisms underlying these changes remain unknown. In this study, we investigated whether CR, the most proven anti-aging treatment, prevents age-related changes in DNA methylation.

To determine the effect of CR on age-related differential methylation in the brain, hippocampal tissue was collected from young (3M) and old (24M) mice fed *ad lib* diet and 24M old mice calorie restricted from 3M to 24M of age. Hippocampal DNA was extracted and used for bisulfite oligonucleotide capture sequencing to determine DNA methylation levels, genome-wide, at a base-specific resolution.

A large number of differentially methylated CpGs (dmCGs) and CpHs were evident with aging, of which 34% and 40% respectively were prevented by CR. CR specific dmCGs were also evident. dmCGs with both age and CR were enriched in CpG island-shelved and gene bodies. Age-related dmCGs unaffected by diet were enriched in H3K4me1 while CR prevented age dmCGs were enriched in H3K27me3. Age-dmCGs affected genes were over-represented in in inflammatory pathways but this was not observed in age-matched CR animals. Genes affected by CR-dmCGs and age-dmCGs, although being different sets of genes, were often co-enriched in similar pathways.

Our findings demonstrate for the first time that caloric restriction prevents age-induced changes in DNA methylation in the brain. DNA methylation changes induced by CR were also independent of age, suggesting CR may counter-balance the aging process by inducing changes that have a protective effect. The prevention of age-dmCGs by CR highlights the prominent role of DNA methylation as a regulator of the aging process.

Funding: Reynolds Oklahoma Center on Aging, Oklahoma Nathan Shock Center of Excellence in the Biology of Aging (P30AG050911) and OCNS translational seed grant

15. HCN1 Contributes to Sympathetic Relaxation of Mouse Detrusor.

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INTRODUCTION: The Hyperpolarization activated Cyclic Nucleotide gated (HCN) ion channel is the molecular analog of an inward, depolarizing current, I_f. This current has been described in neural, cardiac, and gut tissues, and has recently been identified in human and rat bladders. It serves variously as the feedback current in neural oscillators, membrane potential stabilization, and regulator of susceptibility to excitatory potentials. It is activated by hyperpolarization, and its dynamics enhanced by intracellular cyclic nucleotides. Mouse cystometric evidence suggests enhanced sympathetic-mediated detrusor muscle relaxation with advancing age. This could underpin the loss of bladder volume sensitivity in older humans, associated with an increased risk of urinary dysfunction. We hypothesized that changes in HCN expression would be associated with changes in detrusor relaxation to adrenergic stimulation, in the mouse model.

METHODS: Expression of HCN mRNA and the impact of aging was tested with qRT-PCR using bladder tissue from WT 2-3month (Young, YWT)) and 21-22 month old (Old, OWT) C57Bl/6 mice. HCN1 protein expression was confirmed using Western Blots. The dependence of adrenergic detrusor relaxation on HCN was tested in bladder strips from female YWT and OWT mice and young HCN1 KO mice (YKO) obtained from Dr. Arie Mobley. In these studies, 1 mm mucosa-intact strips taken transversely from the mid-bladder were stabilized at 8-10 mN tension in a Ca+2-based buffer, and loss of tension measured in response to adrenergic stimulation using 1 microM isoproterenol. After re-tensioning the strip with carbachol, the degree of isoproterenol-induced relaxation was again measured in the presence of an HCN blocker, either CsCl or ZD7288. Strip integrity was confirmed at experiment end with carbachol-induced contraction. Tension and spectral power (0.01-0.05 Hz) were compared with 2-way ANOVA across groups and conditions.

RESULTS: YWT mouse bladders express HCN1>HCN2. OWT bladders express significantly less HCN1 but HCN2 levels are similar to YWT. The presence of HCN1 protein was confirmed. Strip tension studies demonstrated isoproterenol-induced relaxation in YWT and YKO strips, significantly inhibited by HCN blockade only in YWT. OWT bladders conversely showed minimal relaxation to isoproterenol in the absence of HCN blockade but significant relaxation in the presence of HCN blockade. Paralleling tension findings, maximum spectral power was increased by isoproterenol in YWT in the absence of HCN blockade, and in OWT in the presence of HCN blockade.

CONCLUSIONS: Aging and/or maturation are associated with a change in HCN expression, away from HCN1 dominance. HCN partially mediates adrenergic detrusor muscle relaxation in young mouse bladders but not in old bladders. Enhanced isoproterenol-induced relaxation is marked by increased spectral power suggesting myocyte coordination and is age- and HCN-status dependent. We conclude that HCN is an age-sensitive determinant of bladder responses to sympathetic stimulation with advancing age. Altered HCN function could contribute to diminished volume sensitivity and detrusor preparation for voiding contraction, leading to disorders of urine storage and voiding.

Funding: UConn IBACS Seed Grant; NIA K76 AG054777

16. The metabolomic consequences of size and age in the companion dog.

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Animal models have long been employed to understand mechanisms of aging and longevity, and none has been more studied with respect to human health and lifespan than the common laboratory mouse. As useful as the mouse is for dissecting the physiological mechanisms involved in aging, the high costs of breeding, maintaining, and aging multiple strains of rodents in varying environments makes experiments to dissect the interaction of genetic background and environment during aging impractical. To this end, studies using companion dogs offer a lower cost alternative source of data. Dogs are supported by and live in their owner's environment, with large genetic heterogeneity across breeds with little genetic variation within breeds. In addition, longevities of dog breeds can vary as much as two-fold with small dogs living significantly longer than large breeds. Little work has been done to understand the natural physiological processes that change as dogs age and how those changes relate to human health. Here, we performed global metabolomics on large and small dogs from the Birmingham, AL, area to understand the metabolic parameters that vary with age and size. We also determined the extent to which different metabolites and metabolic pathways are associated with measures of animal weight and obesity. We find significant variation in metabolites between large and small dogs, suggesting differential regulation of metabolic pathways across different sized dogs. The results of this study suggest the companion dog may be an ideal model to develop new hypotheses about how metabolic regulation impacts aging and longevity.

Funding: This work was funded by a Glenn/AFAR Postdoctoral Fellowship to JMH.

17. Coat thinning is an aging biomarker in baboons (*Papio hamadryas*).

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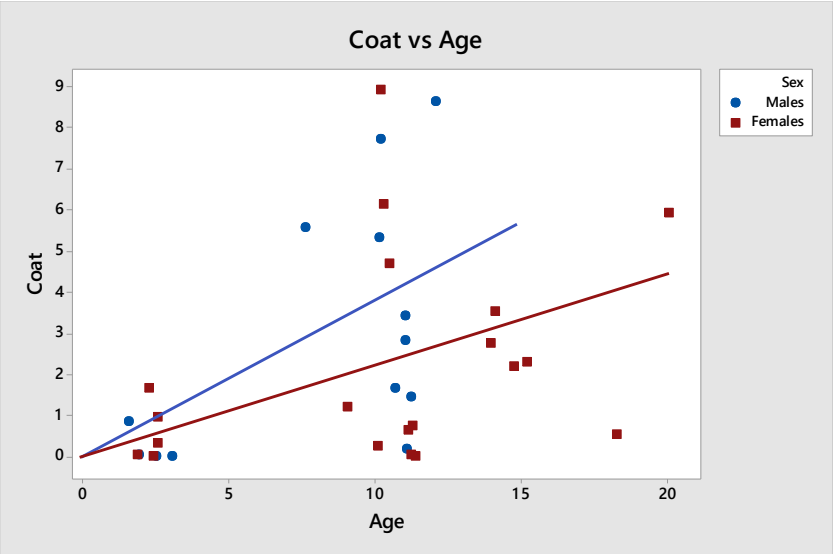
Introduction: Hair loss has been positively associated with age in humans and nonhuman primates. Thus, coat thinning has the potential to serve as an aging biomarker in baboons, an important translational experimental species. There are few data concerning coat thinning in baboons. Availability of biomarkers of baboon aging will enable study of the normative aging process and the aging of animals with different life course histories. We hypothesized that as baboons age, the area of the coat affected by hair loss increases. In addition, based on earlier studies, we hypothesized the relationship would be modulated by cortisol.

Methods: We determined coat thinning in 13 male and 20 female baboons aged 1.5-20 years. A color photograph of the entire back of each individual in a supine position was taken. ImageJ was used to calculate the surface area of the body affected by hair loss from the photographs, yielding the variable coat thinning. Blood was drawn under ketamine sedation. Serum cortisol (ug/dL) was measured by chemiluminescence.

Figure 1. Coat thinning increases as animals age, with variation increasing as the scores increase.



Results: Coat thinning increased with age in a proportional manner. This was consistent with the assumptions of *ratio regression* (Lohr, 1999, *Sampling*, Brooks/Cole, 494 pp), which requires (1) regression through the origin, and (2) that the variation in the response increases as average values increase (Figure 1). For males, the slope was 0.362 (SE = 0.036); for females 0.210 (SE = 0.027). They were each highly significant, and significantly different from each other ($p < 0.005$ in all cases). For males, average coat thinning (CT) can be estimated as CT = 0.362xA, and for females CT = 0.21xA, where A is age in years. Including cortisol in the regression did not add value.

Conclusions: We confirm our hypothesis that as baboons age, the coat thins. The relationship between coat thinning and cortisol was not strong, perhaps because unlike previous studies showing an association (PMID 24827567), hair loss in this sample was minimal not alopecic, and we measured cortisol from serum rather than hair. These data support the use of coat thinning as a noninvasive and objective biomarker of aging in baboons.

Funding: R24 RR021367-01, R24 RR025866-01, R24 RR031459-01, OD P51 OD011133

18. Association of walking speed with age, body size, and serum cortisol in baboons: expansion of speed as a translational aging biomarker.

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Introduction: Walking speed is an important human aging biomarker due to its strong association with morbidity and mortality. Baboons are valuable translational models for aging studies. We previously showed a strong association between age, speed, and body weight in female baboons (PMID: 26411922). Here, we include male data and consider additional biological variables. Our hypothesis was that baboons exhibit age-related decline in walking speed associated with serum cortisol, another potential biomarker of aging, and body size.

Methods: We studied 24 female and 39 male baboons (*Papio hamadryas*) aged 6-18 years by applying an efficient method for measuring walking speed. Landmarks were drawn on the floors of outdoor group cages and distances between all landmarks measured. A single observer used a stopwatch to measure the time each baboon took to walk between landmarks. Morphometric

variables body weight (kg) and body length (cm) were measured and blood samples collected under ketamine sedation. Cortisol (ug/dL) was measured from serum by chemiluminescence. Sex did not add value to predicting speed, so data from the two sexes were pooled.

Results: The strongest regression model included the dependent variable speed (S) log transformed (cm/sec), and the independent variables age (A), body length (L), and cortisol (C), as well as interactions ($F(5, 49) = 9.73$, $P < 0.001$, R^2 49.82%). Baboon walking speed declined with age and cortisol, with effects modulated by body length. Speed declined more rapidly the larger the animal, described by the equation $\ln(S) = -0.22 + 0.227A + 0.043L + 0.034C - 0.0025AxL - 0.00034LxC$. We can see the modulation of length on the effect of age and cortisol by collecting terms associated with age (0.227 - 0.0025L) and cortisol (0.034 - 0.00034L). The shortest length of animals was 105 cm; at that length, the slope for age was -0.03 and for cortisol -0.001. In other words, the fastest individuals were those with shorter body length, lower cortisol, and younger age, while the slowest individuals were those with longer bodies, higher cortisol, and older age.

Conclusions: Walking speed is a valuable baboon aging biomarker given the strong association with age, ease of measurement, and parallel findings in humans. Establishing walking speed and other biomarkers of aging in the baboon model may allow for assessment of biological versus chronological age.

Funding: R24 RR021367-01, R24 RR025866-01, R24 RR031459-01, OD P51 OD011133

19. Metabolomics of lifespan extension in calorically restricted fruit flies.

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Predicting the effects of environmental perturbations on organismal aging is a crucial yet complex problem in the field of aging. Many studies have shown that most organisms live longer when they undergo dietary restriction (DR). However, several studies have found that within the same species, different genotypes respond differently to DR than others. To explore the mechanisms underlying this variation, we exposed 159 inbred fly lines from the *Drosophila* Genome Reference Panel (DGRP) to a calorically restricted (low yeast, DR) or ad libitum (high yeast, AL) diet and recorded the change in lifespan in response to DR (ΔLS). In an effort to further understand the mechanisms underlying DR-mediated lifespan extension, we examined a targeted panel of metabolites measured in each DGRP line in each diet. Initial results indicate highly varied ΔLS responses across different DGRP lines, including some lines that do not respond to or have shortened lifespans as a result of DR. In our analysis, we determine whether there are specific metabolites significantly associated with DR-mediated change in lifespan. Furthermore, we explore the potential for metabolomic profiles to predict how different genotypes respond to DR, which may reveal further insight into the cellular pathways underlying DR-mediated lifespan extension.

Funding: NIH/NIA T32 Genetic Approaches to Aging Training Grant AG000057

20. Immunoproteasome deficiency alters autophagy flux

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A deficiency in cell proteostasis has been associated with multiple neurodegenerative diseases, including age-related macular degeneration (AMD), which is the number one cause of blindness in the elderly. The retinal pigment epithelium (RPE) is one of the primary sites of defect in AMD. These cells are crucial for preserving homeostasis of the retina. RPE are post-mitotic, so maintenance of proteolytic processes is essential for their survival. Two major proteolytic systems, autophagy-lysosomal process (ALP) and ubiquitin-proteasome system (UPS), are the key components of the proteostasis network that is responsible for clearance of intracellular damaged proteins. Although these two proteolytic processes are largely distinct catabolic pathway, recent evidence strongly suggests a common mechanism and cross-talk between these two processes. The current study tests whether there is a connection between autophagy and the immunoproteasome, the inducible form of the proteasome that is upregulated in the retina with AMD. Cultured RPE cells were isolated from wild type (WT) mice and mice deficient in LMP2 (β1i) immunoproteasome subunit. Autophagic flux was tested under two conditions (starvation and rapamycin) that inhibit mTOR activity. Autophagy was evaluated by monitoring the content of LC3 and downstream proteins of the mTOR pathway. We found that LMP2 KO cells showed a significant reduction of autophagy compared to WT cells with both starvation ($p=0.001$) and after rapamycin ($p=0.001$). We also observed a greater amount of autophagy substrate p62, and higher basal levels of ribosomal S6 and cap binding protein 4EBP1 activity in LMP2 KO cells. These results suggest that LMP2 is involved in autophagy regulation. Together, our study uncovered a unique link between the two proteolytic systems that help to maintain proteostasis.

Funding: NIH-NEI R01EY026012, Lindsay Family Foundation, Anonymous Donor for Macular Degeneration, NIH-NIA T32AG029796 (MRK)

Microbial translocation in old monkeys may result from colonic dysbiosis.

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Nonhuman primates (NHP) demonstrate leaky gut with aging, even in the absence of dietary or large differences in the fecal microbiome. We aimed to evaluate the local colonic structural and functional changes that might contribute to reduced mucosal barrier function in old NHPs (*Chlorocebus aethiops*; n=9 middle aged and 10 old female NHPs). Ascending colon was sampled for histology and microbiomic evaluations. Microbial translocation was increased in old monkeys, as portal vein endotoxin levels were more frequently categorized as high ($p<0.05$) and accordingly, toll-like receptor's co-receptor (sCD14) for endotoxin was higher ($p=0.01$). Colonic architecture assessed by histologic measures of crypt depth, wall thickness, goblet cell and tight junction protein occludin-1 abundances, were comparable between age groups.

The colonic mucosal microbiome was distinct from both lumen and fecal microbiome profiles, and overall showed less diversity as expected ($p<0.001$). The colonic microbiome profiles did not show large differences by age grouping with only two genera in the Clostridia class (*Clostridium sensu stricto* and *Anaerobacter*) being significantly higher in samples pooled from the three colonic locations in old monkeys. We did observe higher microbial diversity estimates in the

older animals at the phylum level in feces and samples pooled across the 3 sites (p=0.03 and 0.02, respectively). Old monkeys had evidence of mucosal overgrowth with 90% higher bacterial sequence counts and nearly four-fold greater bacterial gene counts in the colon mucosa (p=0.06). We interpret these findings that ageing leads to lower control over microbial colonization at the mucosal surface and microbial selectivity in the colon. Local innate immune responses, evaluated by the biomarkers circulatory IgA and antimicrobial peptide α-defensin 5, were increased in old monkeys by 16% (p=0.07) and 25% (p=0.02) respectively, which is consistent with greater bacterial exposure.

Our data confirms ageing *per se* increases microbial translocation and suggests that there is a loss in the ability to modulate both the number and diversity of bacterial types on the mucosal surface (dysbiosis). Bacterial overgrowth alone could contribute to higher translocation rates. We posit that deficient immunosurveillance at the mucosal surface is most likely to be causative for greater translocation and contributions to 'inflamm-aging'.

Funding: NIH AG49160, Claude D. Pepper Older Americans Independence Center of Wake Forest School of Medicine (P30 AG21332), T35 OD010946, P40 OD010965, and the services of the primate program funded by NCATS UL1TR001420.

21. Biomarkers of Aging and Age-Independent Mortality Risks Identified in Human Locomotor Activity.

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Several metrics to quantify biological age (bioage), i.e. the "true" age of an organism, have been proposed, based on biological samples of gut microbiota composition [1], human cerebrospinal fluid proteome [2], metabolome [3], DNA methylation patterns in blood and other tissue samples [4]. All these findings indicate that aging manifests at multiple scales and suggest that there is a single biological age parameter. In the present study, we propose that a similar bioage metric can be established in a non-invasive locomotor activity signal to estimate mortality risks. We, however, show that locomotor signal also comprises a signature of age-distinct frailty component, and both these components are required to account for mortality risks.

In the present study, we used one-week long records of human locomotor activity available in the NHANES 2003-2006 dataset (14'000 samples) [5] and in the UK Biobank (74'000 samples) [6]. We introduced a method to quantify locomotor activity in the form of transition probabilities between different activity states. First, we indeed observed aging in a collective parameter (Pearson's correlation with age 0.58), associated with the largest variance in data. Next, Cox hazards ratio model gained additional predictive power when the full locomotor descriptor was used (5-year mortality follow-up prediction ROC AUC improved from 0.59 to 0.70, Cox model adjusted for gender).

Using only cross-sectional data we were able to infer certain parameters of aging dynamics based on the proposed descriptor. The nature of physiological parameter remodeling along both of these directions is quite different. Biological age is associated with a slow deterministic drift during the full lifespan accounting for gradually increasing risks of spontaneous acquisition of the frailty phenotype. Meanwhile, age-independent component describes faster dynamics of responses to external factors such as living environment or lifestyle habits. We found that the bioage-independent locomotor activity parameters are associated with clinical Frailty Index [7]

and lifestyle habits known to impose a devastating effect on life expectancy (smoking and severe obesity, measured with BMI and accompanied by clinically diagnosed type II diabetes mellitus).

In summary, we have shown that the non-invasive locomotor activity signal comprises signature of the biological age and the age-independent frailty component. Due to fundamental differences in nature of biological age and frailty phenotype, the intervention strategies against these two processes may require different approaches. This model may be applied in public health surveillance for health monitoring and disease interception, as well as for robust aging biomarker design and future clinical development of therapeutic interventions.

Funding: The research was funded by Gero LLC.

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22. In Vivo Imaging and Proteomics Reveal Age-related Changes in the Response to a Single Bout of Muscle Contractions.

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Oxidative stress, mitochondrial dysfunction, and a chronic low-grade inflammation, are common characteristics of aging. Exercise remains the most effective treatment for preventing the onset of age and age-related diseases, such as sarcopenia. Despite the positive effects of exercise, many studies indicate that aged muscle is more susceptible to exercise-induced injury. Disruptions in adaptive signaling, mitochondrial function, redox status, and protein turnover can significantly prolong the post-exercise recovery period or exacerbate injury. In this study, we will utilize in vivo technologies to stimulate and visualize contraction-induced injury in young and old CB6F1 (BALB/cBy x C57BL/6) mice. These technologies can overcome the aged rodent non-compliance with standard exercise studies, introduce internal controls, and perform repeated measures on the same mouse to track recovery. In vivo imaging provides insight into the orientation of muscle fibers, the degree of atrophy, sarcomere integrity, fibrosis, and edema. We hypothesize that fatigued muscle from old mice will take longer to recover from contraction-

induced injury and display aberrant adaptive signaling, oxidative injury, inflammation, and dysfunctional protein turnover. To test this hypothesis, we performed a single bout of fatiguing contractions in young and old mice using an in vivo stimulation protocol on the right gastrocnemius, leaving the left limb as a control. High-resolution imaging was performed on the mice in a 14-T magnet. In addition to increased baseline fibrosis and decreased muscle mass, we found that aged muscle resulted in significantly more edema than young muscle after the same stimulation protocol. Injury was observed as late as 48 hours after the fatiguing stimulations, while young stimulated muscle was indistinguishable from the unstimulated limb at the same time. Proteomic analyses revealed a decrease in mitochondrial proteins and antioxidant proteins with age, and elevated inflammatory and apoptosis related proteins. Fatiguing contractions increased proteins associated with inflammation in both young and old mice. Phospho-proteomics revealed a differential signaling profile at baseline and after stimulation with age. Other post-translational modifications were observed within an hour of stimulation. Actin, among other proteins, showed differential protein-S-glutathionylation and ubiquitination with exercise between young and old mice. Fatiguing stimulations reduced the LC3-II/I ratio and increased phosphorylation of P70S6 kinase in young mice, but this was significantly blunted with age. These data demonstrate the aberrant signaling, protein turnover, and inflammation associated with aging may result in the impaired recovery associated with fatiguing contractions. Furthermore, this in vivo model represents an effective experimental strategy to dissect the physiological and biochemical mechanisms associated with contraction-induced injury.

Funding: Genetic Approaches to Aging Training Grant NIH T32 AG000057; Nathan Shock Center Pilot Award P30 AG013280

23. Adaptive aneuploidy as a model to study the role of ER stress resistance in aging.

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Under stress, the yeast genome becomes unstable which often results in adaptive aneuploidy allowing rapid activation of protective mechanisms that restore cellular homeostasis. Here we used a forward genetic screen in *Saccharomyces cerevisiae* to identify genome adaptations that may confer resistance to tunicamycin-induced endoplasmic reticulum (ER) stress. Whole-genome sequencing of tunicamycin-resistant mutants revealed that they acquired extra copies of multiple chromosomes. Despite increased ER stress resistance, most strains disomic for multiple chromosomes were characterized by lengthened doubling time and short replicative lifespan. We find that the ER stress resistance in these mutants is specifically due to chromosome II aneuploidy and is independent of the Hac1 transcription factor, involved in the ER unfolded protein response (UPR). We also demonstrate that constitutive induction of the UPR signaling significantly shortens yeast lifespan. Intriguingly, deletion of *IRE1* and *HAC1*, genes that are involved in sensing ER stress, does not significantly affect yeast lifespan. Analysis of the transcriptome and translatome in *ire1* Δ and *hac1* Δ cells revealed that these mutants compensate for the lack of a major protein homeostasis pathway by activating enzymes involved in dolichol and hexosamine synthesis. These pathways lead to production of UDP-N-acetylglucosamine (UDP-GlcNAc) and N-acetylglucosamine phosphatidylinositol (GlcNAc-PI),

which allow cells to counteract ER stress as a result of the UPR deficiency by increasing export of N-glycosylated and glycosylphosphatidylinositol (GPI)-anchored glycoproteins. Together, our data provide an important insight into the role of the UPR in aging and mechanisms that underlie the link between the ER stress resistance and longevity.

Funding: This work was supported by National Institutes of Health grant AG040191

24. Cell non-autonomous signaling pathways converge on serotonin and *fmo-2*.

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Recent studies in model organisms show that multiple cell non-autonomous signaling pathways play important roles in regulating health and longevity of the organism. These pathways frequently originate in the nervous system and can involve a response to environmental stimulation (i.e. the perception of a stress). We recently discovered a novel cell non-autonomous signaling pathway in the nematode *Caenorhabditis elegans* initiated by the hypoxia-inducible transcription factor, HIF-1. Our data show that stabilization of HIF-1 through genetic (mutation of repressor *vhl-1*) or environmental means (hypoxia) leads to a robust increase in stress resistance and longevity in nematodes. We also find that stabilization of HIF-1 elicits a signal from serotonergic neurons that results in expression changes in peripheral tissues. One of these changes is an increase in the expression of the flavin-containing monooxygenase-2 (FMO-2) protein, a classical phase I detoxification enzyme that we find both necessary and sufficient to increase stress resistance and longevity downstream of HIF-1. Our current studies explore the signaling pathways and downstream mechanisms involved in FMO-2-mediated enhancements in stress resistance and longevity. Interestingly, we find that while serotonin (5-HT) plays a crucial role in FMO-2 induction downstream of HIF-1, FMO-2 can also be induced by a serotonin antagonist, presumably through a different pathway. Our data are consistent with this pathway overlapping with the well-studied dietary restriction pathway, suggesting that HIF-1 and dietary restriction use distinct serotonergic circuits in opposite fashion to achieve the same result, FMO-2 induction and increased longevity. Together, our results suggest that multiple cell non-autonomous signaling pathways utilize similar signaling strategies (serotonin) in distinct ways to modify peripheral tissues and enhance long-term survival.

Funding: NIH grants R00AG045200 and P30AG024824, AFAR Junior Faculty Award, Paul F. Glenn Center for Aging Research

25. Role of IGF-1 in Astrocyte Mitochondrial Metabolism in Brain Aging.

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Decline in mitochondrial function and increase in reactive oxygen species are important determinants of aging. With advancing age there is a concomitant reduction in circulating levels of insulin-like growth factor-1 (IGF-1) that is closely associated with learning and memory impairments and neurodegeneration. Importantly, IGF-1 has been reported to protect many tissues and cell-types against oxidative stress, including neurons and astrocytes. In this study, ***we hypothesized that reduction of IGF-1 signaling negatively impacts astrocyte function, via impairment in energy and redox homeostasis that ultimately contributes to oxidative stress***

and cognitive decline. We used primary neurons and astrocyte cultures from *igfr*$^{f/f}$ mice to knockdown IGF-1 receptor (IGF-1R) by AAV9-mediated CRE expression (AAV-Cre). AAV9-GFP (AAV-GFP) was used as a control. Expression of IGF-1R was quantified by qRT-PCR, which showed a 30% reduction in astrocytes and a 70% reduction in neurons in response to AAV-Cre compared to AAV-GFP. Astrocytes with knockdown of IGF-1R had significantly reduced energy charge (ATP/AMP ratio) compared to controls, while the total mitochondrial copy number was unaffected. Neurons had no effect on mitochondrial copy number or energy charge in response to IGFR knockdown. NADH oxidase (complex 1 subunit) activity was increased in astrocytes with IGF-1 signaling deficiency. Furthermore, mitochondrial sirtuin activity as well as levels and activity of sirtuin substrate, SOD1, were increased in AAV-CRE relative to controls, suggesting compensatory changes in response to IGFR deficiency. These results suggest that IGF-1 positively regulates astrocyte mitochondrial metabolism redox homeostasis in astrocytes. Thus, targeting mitochondrial metabolism in astrocytes may be a potential avenue for therapy to avert or delay cognitive deficits in advanced aging.

Funding sources: Donald W. Reynolds Postdoctoral Fellowship; Oklahoma Nathan Shock Center Award; Grants AG37847 and NS56218 to WES.

26. Extending maximum lifespan of the honeybee: a new ageing model.

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Membrane composition has been related to maximum lifespan in mammals, birds, bivalve molluscs and the nematode *C. elegans*. In all cases, a long maximum lifespan is associated with low polyunsaturated fatty acids (PUFA) in membrane lipids. Female honeybees (*Apis mellifera*) show the same relationship. Female larvae can become either workers or queens. Adult workers typically live for only weeks while adult queens can live for up to 8 years! The membrane lipids of larvae and pupae of workers and queens are similar all having low PUFA levels. Queens are fed mouth-to-mouth by workers throughout adult life with “royal jelly” (has no PUFA) and therefore maintain low PUFA membranes. In contrast, after emergence workers commence eating pollen (with high PUFA content) such that by day 4 of adult life there is a 5-fold increase in proportion of PUFA in their membranes.

To test the hypothesis that this diet-related difference in membrane PUFA is responsible for the much shorter lifespans of workers compared to long-living queens, we fed four populations of newly-emerged adult workers with four different diets; two contained PUFA (honey+pollen; honey+casein+PUFA) while the other two had no PUFA (honey+yeast; honey+casein). The diets with PUFA resulted in membrane lipids with normal worker PUFA levels while worker bees on PUFA-deficient diets had no increase in membrane PUFA. Furthermore, the maximum lifespan (i.e. average longevity of longest-living 10% of population) of honeybees on the PUFA-deficient diets were ~35% greater than those on PUFA-containing diets (p<0.01). This extended maximum longevity of worker honeybees by experimental diet manipulation supports the proposed link between membrane composition and lifespan and will provide a new experimental tool to investigate the processes of aging.

27. Blueberry Health Study 15-Year Report Part I of II: New Power Milestones, Unexpected Gender-Effects and a Healthier Nutrient Balance

Rolf J. Martin, Barrie S. Sachs and Howard A. Raphaelson on behalf of the entire 2002-2017 Blueberry Health Study team: Rolf J. Martin[1], J. Wesson Ashford[2], Ines E. Augie[3], Diana J. Burns[4], Richard J. Coppings[5], David L. Doiron[4], Phil Fichandler[4], Marilyn Gerling[4], Kurt E. Gerstmann[6], Kathy Hull[7], James A. Joseph[8], Amy Casdorph Kokesh[9], Rozlyn A. Krajcik[3], Bruce S Kristal[10], Aaron Lukton[11], Michael Luzi[12], Stephen Massardo[3], Dana Mathew[13], Jessica Norton[14], Norman Orentreich[3], Martin Perez[15], Alec Pruchnicki[16], Howard A. Raphaelson, Sheri A. Raphaelson[17], [4]Barrie S. Sachs[18], Edwin Sanchez[15], Roseanne Schnoll[19], Paul Satkowski[14], Joseph H. Vogelman[3], Anthony Wetherell[20]. [1]Blueberry Health Study / MMT Corp, Sherman, CT, Burke/Cornell Medical Research Institute and Burke Rehabilitation Hospital, White Plains, NY (now retired), [2]Stanford / VA Aging Clinical Research Center, Palo Alto, CA, United States, [3]Orentreich Foundation for the Advancement of Science, Inc., Cold Spring-on-Hudson, NY, [4]Mansfield Senior Center, Mansfield, CT, [5]Lane College, Jackson, TN, [6]New York, NY and Sherman, CT, [7]New Fairfield Senior Center, New Fairfield, CT, [8]Human Nutrition Research Center on Aging at Tufts University, Boston, MA, [9]Raleigh, NC, [10]Weymouth, MA, [11]City University of New York Biochemistry Ph.D. Program, New York, NY, [12]Sherman IGA Supermarket, Sherman, CT, [13] New Fairfield, CT, [14]Foodtown Supermarket, Cold Spring-on-Hudson, NY, [15]C-Town Supermarket, Danbury, CT, [16]Mount Sinai West and Mount Sinai St. Luke's/Mount Sinai Hospital, New York, NY, [17]Midwife Services, Espanola, NM, [18]HR Herbs, Teas and Gifts, Sherman, CT, [19]Health & Nutrition Sciences Dept, Brooklyn College of the City University of New York, Brooklyn, NY, [20]Defence Sci & Tech Lab, United Kingdom (retired),. Email: Blueberrystudy@gmail.com. Web: Blueberrystudy.com and PaulRichterSCICoRE.org.

Main point: After more than a decade of year-after-year memory score increases, our 15th study year was productive because our new hearing page generated more data per minute than any of our other pages, and because an international research effort has enabled us to develop an improved working hypothesis to explain decade-long berry benefits. Daily blueberries evidently activate the Nrf2 (NFE2L2) protective gene center that coordinates and balances serine-methionine-glutathione-tyrosine-phenylalanine-and-lipidomics multi-step longevity pathways, equations for which predict lifespans of genetically-engineered lab nematodes and also primates and humans with nearly perfect 0.99 accuracy coefficients. This global research effort has also produced strong, peer-reviewed evidence that berry-mechanisms are fundamentally important health maintenance systems (see e.g. Shukitt-Hale et al. PMIDs 26392037, 26750735, 27166828, 28212704, 28283823) that can be further strengthened by careful nutrient balancing. Published balance experiments indicate that lifespans can be increased by about 40% by methionine ("MET") restriction (a centrally-important facet of nutrient balancing) (8429371, 8001743, 24847356) and up to 60% by rapamycin and diet restriction, both of which also balance longevity-linked amino acids including MET, serine and glycine according to a University of Texas metabolomics analysis (24304444). The cell division potential of human cells has been more than doubled by MET restriction (24830393).

Background: Our online data collection began in 2002. Short-term decision speed scores improved by 4.2% during the first month after 1/2 to 1 cup/day of berries were consumed, with a significant advantage ($p = 0.025$) for berry-consumers compared to a randomized control group who received balanced "Macarena Pill" antioxidant capsules so named because Dr. Bruce

Ames said his patented nutrient combination (PMID 11854529; US patent 5916912 A) enabled older lab animals to get up and "do the Macarena." Over the next ten years, memory scores among berry-consumers improved steadily with cumulative year-after-year confidence levels far above 95% ($p < 0.01$; see reports at this conference each year through 2016). Unexpectedly, four individuals not enrolled in our study recently notified us that daily berry consumption almost-completely prevented frequent hot flashes and sweating. And a re-examination of our Orentreich collaboration data (Krajcik et al., this meeting, 2008) indicated that four male participants had 20% to 40% increases in serum testosterone after one or two months of berry consumption, suggesting that daily blueberries can in some cases provide much-appreciated benefits to both women and men. These encouraging pilot results together with long-term memory improvements we have seen, merit large-scale follow-up considering other evidence that berries improve our health because they fine-tune and precisely balance fundamental protective and cell-renewal pathways (see 25451098, 27107941, 27847126, 28212704).

Methods: Measurement web sites were constructed in 2002 according to specifications published by A. Wetherell and collaborators (9182033, 9167986). Additional pages were added in 2007 to obtain memory/recall data based on verbs, proper names and two sets of nouns. These measurement pages were not changed thereafter to obtain ten-plus years of consistent cognitive performance and health data. Our measurement pages were formally made available to other research teams in 2006 at this conference even though the methods were covered by US Patent 6712615 B2. A hearing page (also now available to other research teams) was added in 2014 to gather additional Alzheimer's-related data. Initial results were presented at the 2014 Academy of Rehabilitative Audiology meeting (Martin et al; Wannagot et al). Published rapamycin and diet restriction data sets (8001743) were then re-analyzed to develop a refined nutrient-ratio-circadian-senolytic explanation for longevity (Martin et al., this conference, 2016) with high lifespan-prediction coefficients that initially received little attention because predictions without mechanisms have at best questionable implications.

Current results: Our new hearing site, which requires less than one minute for 20 responses to high- or low-pitch sounds, provides results rapidly enough for each participant to measure 5% hearing changes at 95% confidence after approximately 10 minutes of measurement. We believe this is a power-per-minute milestone that allows subtle harmful as well as beneficial changes to be continuously monitored by each person, and may thus help prevent undetected subgroup harm of the kind that became evident only after three years during the NORVIT B-vitamin trial (16531614). We have named this amount of power per minute "1 DB safety unit" and note that Memtrax.com memory measurements of Ashford et al. (see 21908910) also require just several minutes per measurement and can provide a similar amount of safety-related power if measurements are repeated (Ashford, JW, personal communication).

Conclusions and future directions: It appears that anyone wishing to reach age 100 with good health and vigor should balance food combinations quite carefully. Because of these reports and ten-plus years of memory score improvement during our 15-years of data collection, our working hypothesis is that benefits from daily berry and berry-smoothie consumption can be significantly increased if key nutrient ratios are adjusted to reduce circadian imbalance and thereby prevent or reduce metabolite shortfalls and excessive-accumulation (see e.g. 25846330). After receiving recommendations at this meeting we will apply without delay for IRB approval to add synergistic, balanced nutrients to our protocol and begin Stage II of the Blueberry Health Study. We will also work with pet food companies (initial messages already

sent; see PMIDs 26587240, 27078852, 28384169) to extend the healthy lives of our beloved pet cats and dogs and other animal friends.

28. Blueberry Health Study 15-Year Report Part II of II: Evidence that Precise Lifespan Equations with Accuracy Coefficients of 0.99 Correspond to Longevity-Related Nutrient and Metabolite Ratios

Rolf J. Martin[1], Barrie S. Sachs[2] and Howard A. Raphaelson[3] on behalf of the entire 2002-2017 Blueberry Health Study team. [1]Blueberry Health Study / MMT Corp, Sherman, CT, Burke/Cornell Medical Research Institute and Burke Rehabilitation Hospital, White Plains, NY (now retired), [2]HR Herbs, Teas and Gifts, Sherman, CT, [3]Mansfield Senior Center, Mansfield, CT Email: Blueberrystudy@gmail.com. Web: Blueberrystudy.com and PaulRichterSCICoRE.org.

Nutrient/metabolite-based mechanisms that may partly control nematode, primate and human lifespans were identified this past year to add credibility to lifespan-prediction equations discussed at recent American Aging Association meetings, and to fill a large mechanism-gap identified during critically informative post-conference conversations. (i) Cysteine inhibits pro-aging mTOR activation while MET activates it (see PMIDs 27587390, 27727170, 28261376, 25773352, 28314591) explaining how CYS/MET concentration ratios regulate lifespan and why this ratio is central to accurate lifespan prediction. Cysteine, at least in certain concentration ranges and ratios, acts like rapamycin and other rapalogs now known to extend survival and lifespan in many species, with benefits already discussed in over 23,000 U.S. patent applications (http://appft1.uspto.gov). (ii) Glycine and serine both react with MET or MET cycle metabolites and can themselves be removed by MET overconsumption (28337245, 3080429, Brind et al April 2011 The FASEB Journal 25(1) Suppl 528.2) explaining the apparent importance of SER/MET and GLY/MET ratios for species as diverse as cats and dogs, cows, horses, elephants, primates and humans. By reacting with MET or related metabolites, serine and glycine remove major sources of post-translational damage and protein synthesis errors that can be as high as 10% (26709516). Such error rates have apparently been tolerated over billions of years of evolution because of short-term stress-resistance benefits (27672035). However long-term accumulation of MET-error-containing-and-MET-damaged proteins in chromosomes that cannot be repaired or replaced by macro-autophagy can explain why MET levels so closely correlate with very short and long lifespans (24847356, 28108330), why adding dietary glycine to better balance MET increases rat lifespan by about 30% (Brind et al ibid.) and can also explain why Alzheimer's risk is 30% lower for Hispanic-Americans who regularly consume foods with higher SER/MET and GLY/MET ratios and also consume above-average amounts of medium-chain lipids, compared to Caucasian-Americans (Martin et al., this conference, 2016). (iii) Tyrosine/phenylalanine ratios determine how much phenylalanine can be damaged by free radical attack and can thereafter be misincorporated into proteins without noticeably changing missynthesized proteins charge or size (24751667, 25897359, 26785996). (iv) Three human antioxidants, coenzyme Q10 and two forms of vitamin E (α- and δ-tocopherol), were recently measured over a ten-fold coenzyme Q10-concentration range and found to be tightly correlated with one another (r values > 0.95 see PMID 21467235). Evidence has also been published that coenzyme Q10 can selectively kill human breast cancer cells (7908519, 7752835, 20805228, see also 17505263, 24192015, 15188947), suggesting that altering these precise ratios can be toxic to cells and possibly give physicians supplementary approaches to treat some kinds of cancer. (v) Over 30 lipid ratios predict nematode longevity over a ten-fold lifespan range with prediction-accuracy coefficients of 0.99 or higher according to data published in an elegant

paper by Shmookler-Reis et al. (21386131). This report includes RNAi evidence that enzymes governing these nutrient ratios also control lifespan, *by hypothesis* through careful control of lipids and lipid-class proportions for optimum autophagic cell renewal and long-term health. (vi) AA ratios are so tightly regulated and so important to health of "higher" organsisms that even a change of just ***half of one percent*** can cause or can prevent below-normal kitten growth (3351631). Evaluation of cell culture media developed to optimize industrial antibody production also indicates that years of systematic nutrient ratio adjustments have failed to completely balance all AA proportions, with each medium subject to shortfalls or often large (>10-fold) AA excesses during production runs (25846330). The large number of imbalances identified in this study suggest evolution has been unable to develop balancing mechanisms for large numbers of metabolites and pathways, including those involving critically-important amino acids, even after billions of years of natural selection. However we appear to be very fortunate today because (i) careful nutrient adjustments are able to correct imbalances when they are discovered, for example to increase antibody production eight-fold, and (ii) some of the most important lifespan-determining imbalances have already been identified (24847356, 26785996) and responsible scientists may soon be in line for Nobel Prizes that will highlight their work and spur further progress. Important additional data indicating that AA and also AA-subgroup ratios remain constant and are tightly regulated over 50 million-year periods is also available from Davis et al. (8027865). (vii) Published study results demonstrating that amino acid ratios rather than absolute amounts are a matter of life or death were available at least as early as 1951 (see PMID 14912009, special thanks to the NY Academy of Medicine Library for making this paper available). Studies also indicate that both high and low levels of amino acids predicted mortality in humans (28374265). Clinical studies indicating that amino acid supplements and therefore ratio changes can significantly improve human health are also now available in several peer-reviewed publications
(see 25315856, 24201233, 27854233, 22261571, 20190028, 20800897, 24326786) along with lab studies indicating that AA combinations can treat specific health problems (16702321). (viii) Evidence that AAs, methylation-related metabolites and short-chain fatty acid ratios govern human longevity today is also available from studies of Italian and Chinese centenarian populations (23483888, 26678252, 27657115). Hispanic-Americans who consume foods with more favorable nutrient ratios, also have 25% to 35% lower age-adjusted rates of cancer, heart disease, stroke, Parkinson's and Alzheimer's compared to Caucasian-Americans, according to US-CDC mortality and morbidity data, and a higher-than-expected percentage reach 100 years of age.

29. Gene-gene interactions in replicative lifespan in *S. cerevisiae*.

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The genetically tractable single-celled budding yeast *S. cerevisiae* divides asymmetrically, allowing us to track the number of divisions, or replicative lifespan (RLS), of yeast mother cells. We recently completed a screen of 4,698 single-gene deletions for significantly increased RLS. We identified 238 genes whose deletion significantly extended yeast RLS. These genes were clustered into overrepresented known biological processes, and showed significant overlap with orthologous genes whose knockdown or deletion was shown to extend lifespan in the distantly related nematode *C. elegans*.

We have now crossed many of our most longlived single-gene deletion strains with multiple additional gene deletions that are known or suspected to interact strongly with one or more of these lifespan-extending genes. We then measured the RLS of all resulting double deletion yeast strains at high resolution. The RLS of each of the individual double deletion strains and its respective pair of single deletions sheds light on a single gene-gene interaction in terms of replicative lifespan, and may allow strong epistatic inferences in some cases, and suggest new biological hypotheses. Taken as a whole, we have also phenotypically clustered this entire set of RLS information. This will reveal similarities between the various longlived deletion strains, and between the selected interacting single-gene deletions, and allow us to most meaningfully leverage the information in this large set of longlived genotypes by suggesting which of them may share fundamental underlying biology.

Funding: NIH R01AG043080

30. Motoric and cognitive aging are differentially affected by lifelong glutathione deficiency.

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University of North Texas Health Science Center, Institute for Healthy Aging, Center for Neuroscience Discovery

A recent paradigm shift has implicated redox state changes, rather than oxidative damage, as a potential key determinant underlying the aging process. Specifically, it is hypothesized that a pro-oxidizing shift in the ratio of reduced to oxidized glutathione (key substrate in redox status) disrupts cellular signaling and function leading to brain impairments. While acute deficits in glutathione have shortened lifespan in drosophila and have impaired function in mice, it remains essential to test this theory by studying the lifelong effects of chronic glutathione deficiency. Chronic glutathione deficiency is achieved by knockout of glutamate-cysteine ligase modifier (gclm), an enzyme subunit at the rate-limiting step in glutathione synthesis. Glutathione levels in $gclm^{-/-}$ mice are 10-30% of those in $gclm^{+/+}$ mice (17-35% in the brain). Our hypothesis stated that diminished glutathione synthesis is sufficient to produce an accelerated, aging-like pattern effect on motor and cognitive function. To explore the effects of lifelong glutathione deficiency, we subjected $gclm^{+/+}$ and $gclm^{-/-}$ male and female mice (n = 5-12 / sex / age / genotype) to a behavioral battery for motor and cognitive function at 5, 10, and 20 months of age. Overall, age-related declines in function were observed in all tests. In young and adult mice glutathione deficiency did not negatively affect any performance, however it improved coordinated running performance in young females and improved balance, strength, and coordinated running in adult males. In old mice, glutathione derangement improved balance in males and accelerated age-related decline in strength, balance, and coordination in females, yet it had no effect on cognitive function. These data imply that (i) motor and cognitive domains appear to be differentially affected by glutathione deficiency, (ii) females were more susceptible to glutathione depletion leading to further motor impairments. In conclusion, our hypothesis was only partially supported and future research will be needed to determine the underlying cause of this sexual dimorphism in response to glutathione deficiency.

Funding: P01 AG022550, P01 AG027956, T32 AG020494

31. Age-specific alteration of the asymmetric segregation of cellular cargoes during mitosis in adult neural stem cells

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Neural stem cells (NSCs) in the adult brain produce newborn neurons throughout life in a process termed neurogenesis. With age, neurogenesis and stem cell proliferation is dramatically reduced, through mechanisms yet unclear. We have found that during mitosis, NSCs from young mice asymmetrically segregate cargoes such as ubiquitinated proteins and the intermediate filament vimentin to one of the daughter cells, leaving the other daughter cell more “clean.” The consequence of this inheritance is a reduced proliferation rate, suggesting that these cargoes may negatively affect dividing cells. To confirm that the segregation of these cargoes also occurs in a more *in vivo* context, we imaged NSC mitosis in embryonic brain slices and found that cargoes were asymmetrically segregated to the daughter cell which will become the newborn neuron, leaving the stem daughter more “clean.” Further, immunostaining of young adult hippocampal slices revealed that newborn neurons have higher levels of ubiquitinated proteins as compared to the stem cells from which they are derived. Interestingly, we found that dividing NSCs from old mice have a more symmetric segregation of these cargoes both *in vitro* and in hippocampal slices. Taken together, these results suggest a model where young NSCs use an asymmetric segregation of cargoes to maintain a “clean” stem cell, passing on “junk” to the newborn neuron, allowing the stem cells to rejuvenate the population. With age, this process begins to dysfunction, and cargoes are more symmetrically segregated between daughter cells, leading to a decline in function in both daughters. This change in cargo inheritance may contribute to the age-dependent decline in adult neurogenesis. Further understanding the mechanisms behind this segregation may identify important therapeutic targets for improving stem cell aging.

Funding: Swiss National Science Foundation, EMBO Young Investigator Program, the Zurich Neuroscience Center (S.J.), the European Research Council (Y.B.), an EMBO long-term fellowship (G.A.P), and the ETH Fellow and the Human Frontier Science Program long-term fellowship (D.L.M)

32. Age-dependent reduction in aortic distensibility (AD) commences in early adult life and is accelerated in intrauterine growth restricted (IUGR) offspring of undernourished baboons.

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Introduction: Using MRI we have demonstrated altered left ventricular (LV) development and function (PMID: 27988927) in IUGR offspring of moderately undernourished (30% global nutrient reduction) baboons. The LV of IUGR offspring shows fibrosis even in fetal life. AD affects baroreceptor function (PMID: 27122371) and is considered a marker to identify individuals at hypertensive risk (PMID: 27686337). We determined whether adult LV magnetic resonance imaging (MRI) changes are accompanied by impaired AD and increased aortic fibrosis. Since we and others have shown that serum cortisol changes across the lifespan and cortisol levels affect collagen production, we determined the abundance of glucocorticoid receptor (GR) and its active form phosphoGR (pGR) in the abdominal aorta across the life-course.

Methods: Pregnant baboons ate *ad libitum* (CTL) or 70% *ad lib*, which led to IUGR at birth. We studied IUGR (8 male, 8 female; 5.7 yr; human equivalent ~20 yr) and age-matched normal birthweight controls (8 male, 8 female; ~5.6 yr). We also tested a baboon cohort across the life-course from 5-23 years (normal lifespan ~25 years). 3T MRI was performed to quantify distal descending aortic caliber (AC) from average systolic and diastolic lumen cross-section. AD was estimated by lumen size change with contraction divided by diastolic size and pulse pressure. GR and pGR were measured by immunohistochemistry in the aging animals following necropsy but not in IUGR since they were retained for future studies.

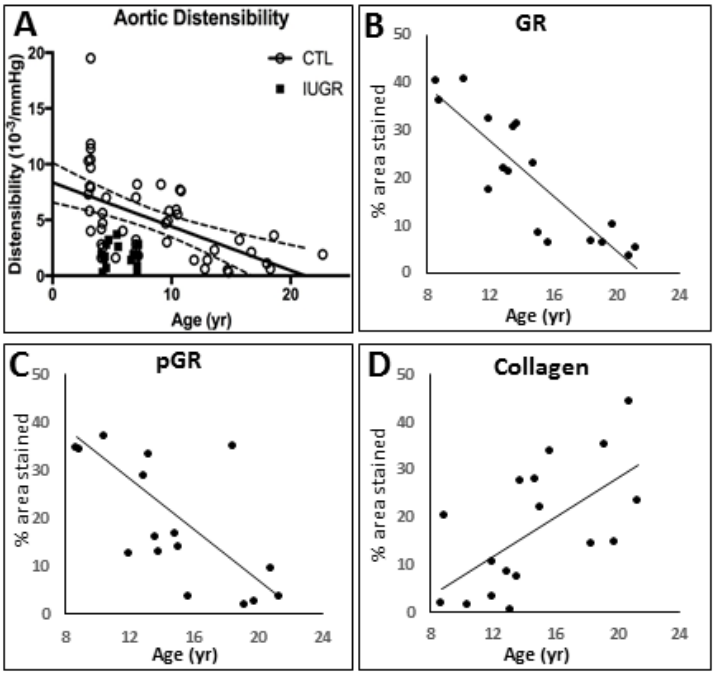

Figure 1 A) AD in CTL baboons (open) and IUGR (closed) offspring - dashed line 95% confidence limits. p<0.001 for IUGR vs CTL; B) GR, C) pGR, and D) collagen in the descending aorta across the life-course.

Results: AC normalized to body surface area was higher in CTL vs. IUGR (0.9 ± 0.05 cm^2/m^2 vs. 1.2 ± 0.06 cm^2/m^2, M ± SEM, p = 0.005). AD was higher in CTL vs. IUGR (3.7 ± 0.5 vs 1.9 ± 0.3 10^{-3}/mmHg, p=0.005) (Fig 1A). No between group differences were seen in pulse pressure (p=0.6). When IUGR AD was evaluated against the life-course regression in aging baboons, all IUGR values fell below the 95% confidence limits for aged animals (p<0.001). Aortic GR and pGR fell (Fig 1B and C) and collagen increased (Fig 1D) across the life-course.

Conclusions: AD decreases from as early as 5 years of age in baboons and is a potential marker for 1) biological aging and 2) aging-programming interactions in this extensively studied nonhuman primate model.

Funding: EC FP7 BRAINAGE; NIH 7P01HD021350; NIH 1R25EB016631; OD P51 OD011133

33. Age-related life-course changes in plasma lipids are accelerated in offspring of poorly nourished baboons enabling assessment of biological versus chronological age.

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Introduction: Life-course changes in plasma lipids (e.g., linear fall in high-density lipoprotein cholesterol- HDL) have been demonstrated in cynomolgus monkeys (PMID: 27342143). Total and HDL cholesterol levels in children are associated with metabolic programming, potentially predisposing to later life cardiovascular diseases (PMID: 25840838). Female but not male offspring of the Dutch Hunger Winter had elevated total cholesterol and low-density lipoprotein (LDL) cholesterol (PMID: 19386743). We hypothesized that poor maternal nutrition in a nonhuman primate model would accelerate offspring life-course lipid changes.

Methods: Pregnant baboons (*Papio hamadryas*) ate Purina Monkey Diet 5038 *ad libitum* (CTL) or 70% *ad lib* diet in pregnancy and lactation, which produced intrauterine growth restriction (IUGR; ~12% decrease in body weight) in offspring. We studied IUGR females (N=8, 8.6 years; human equivalent ~34 years) and age matched CTL (N=9, 8.9 years) as well as 25 females from 7-22 years. Serum lipids were measured on an Alfa Wassermann ACE Clinical Chemistry Analyzer.

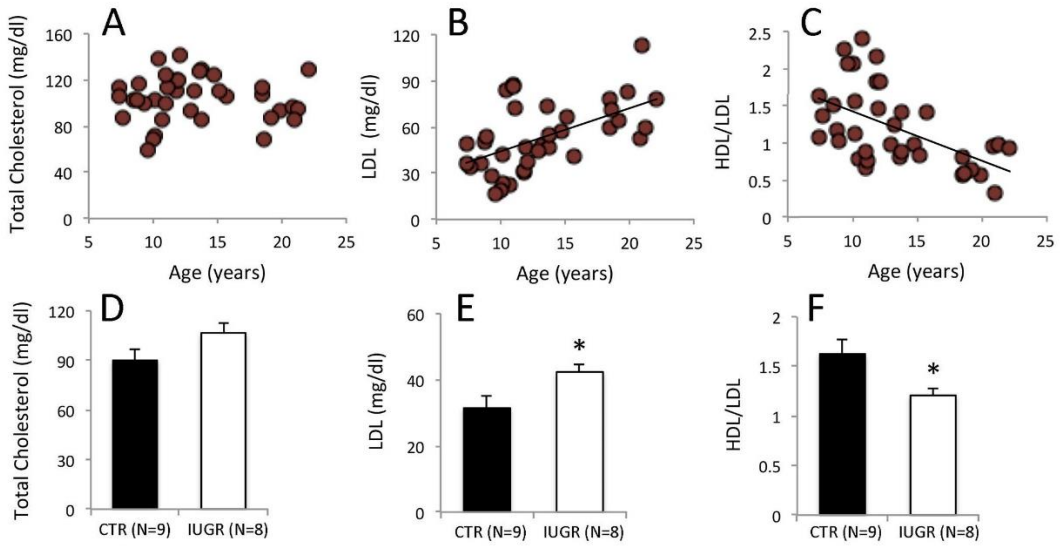

Figure 1. Serum values of total cholesterol, LDL, and HDL:LDL ratio (A-C) across the life course and (D-F) at ages 8.6-8.9 years in CTR (n=9) and IUGR (n= 8) offspring. Mean ± SEM; *P<0.05 CTR vs IUGR.

Results: Figure 1 shows that (A) across the life-course total cholesterol was unchanged but (B) LDL rose (slope=2.8 mg/dl/year) and (C) HDL:LDL ratio fell (slope=-0.067). In IUGR baboons compared with age-matched controls, LDL was increased (Fig 1E) and HDL:LDL decreased (Fig 1F) by amounts equivalent to advancing of aging by 4 and 6 years, respectively.

Conclusions: In female baboons, serum LDL rises 2.8 mg/dl per year and HDL:LDL falls 0.67 units per year across the life-course from 7 to 22 years. Programming by a reduced maternal diet accelerates this process by 4-6 years of life. These data demonstrate the importance of knowing the developmental background of subjects in both human and animal studies when addressing issues of biological and chronological age.

Funding: NIH R24 RR021367-01; HD 21350; OD P51 OD011133

34. Mechanisms responsible for the age-related fall in circulating cortisol that begins in early baboon adult life.

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Introduction: Aging is a multifactorial process involving gene-environment interactions. Disruption of physiological homeostasis leads to premature aging. Hypothalamo-pituitary-adrenal (HPA) activity mediates effects of both normal physiology and stressors. Life-course decreases in circulating glucocorticoids occur in rodents [PMID: 25953670] and baboons [PMID: 25244034]. Here, we had five aims for studies in early adult baboon life (6-13 years, human equivalent ~18-40): 1) confirm the life-course serum cortisol fall in female baboons; 2) measure 11beta hydroxysteroid dehydrogenase (11βHSD) 1 and 2 and hexose 6 phosphate dehydrogenase (H6PD), which together influence local cortisol concentrations and negative feedback; 3) measure hypothalamic paraventricular (PVN) and pituitary glucorticoid receptor (GR), phosphoGR (p-GR), and mineralocorticoid receptor (MR) as markers of negative feedback; 4) measure PVN arginine vasopressin (AVP), corticotropin-releasing hormone (CRH), and pituitary proopiomelanocortin (POMC), markers of HPA drive; and 5) measure aortic GR, a marker of cortisol peripheral receptivity. ***Our hypothesis*** was that the life-course fall in cortisol is accompanied by wider evidence of aging-related decrease in HPA mechanisms and peripheral glucorticoid action.

Methods: Tissues were obtained at necropsy under general anesthesia. Serum cortisol was measured by Immulite 1000 analyzer. Proteins above were measured by immunohistochemistry.

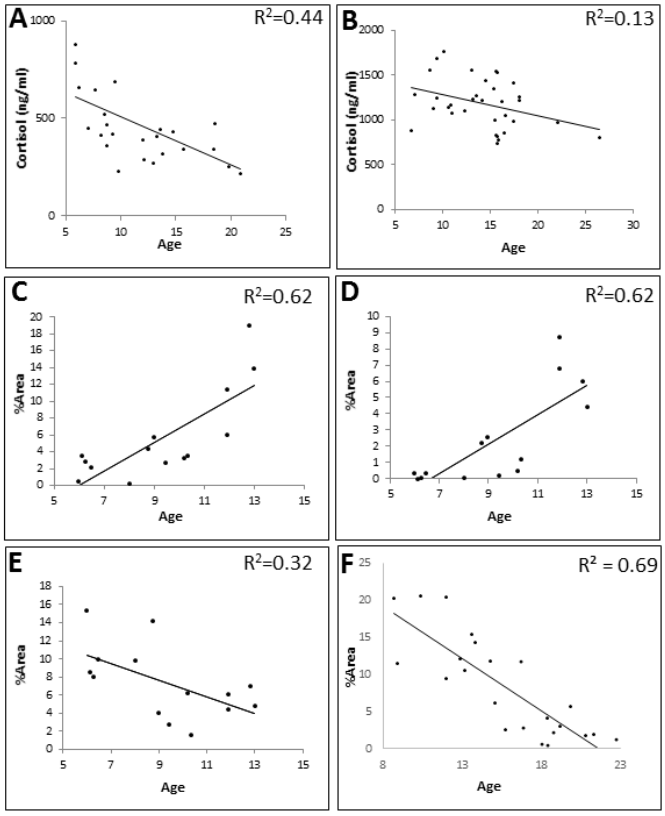

Figure 1: Life-course changes. 1A) our own life-course cortisol; 1B) published Oklahoma primate center data; 1C) increasing PVN GR; 1D) increasing PVN MR; 1E) decreasing PVN AVP; and 1F) decreasing aortic GR.

Results: Life-course cortisol decline was similar between our own data (Fig 1A: cortisol = -24.7*X+735.1 ng/ml – where x is days age; P=0.0007) and published Oklahoma primate center data (Fig 1B: cortisol = -23.7*X+1521; P=0.039) [PMID: 25244034]. Figure 1C-F depicts changes in PVN GR, MR, AVP, and aortic GR across the life-course. For data not in Fig 1, PVN 11βHSD1, 11βHSD2, H6PD, p-GR, CRH, and pituitary POMC, R^2 values were 0.58, 0.53, 0.24, 0.02, 0.01, and 0.40, respectively.

Conclusions: Our data showed 1) a fall in cortisol remarkably similar to the

published study; 2) effects on local PVN cortisol production were equivocal; 3) GR, pGR, and MR increases favor increased negative cortisol HPA feedback; 4) decreased AVP and POMC indicate decreased HPA drive; 5) decreased peripheral tissue GR would decrease peripheral cortisol receptivity. Together, these age-related changes lead to decreased cortisol activity across the life-course as baboons age.

Funding: OD P51 OD011133

35. Maternal obesity induces epigenetic changes in baboon fetal liver that regulate pathways thought to play important roles in regulating life span.

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Introduction: Maternal obesity (MO) increases offspring cardiometabolic disease and shortens lifespan (PMID: 23943697) as a result of developmental programming, defined as *a response to a specific challenge to the mammalian organism during a critical developmental time window that alters the trajectory of development with persistent effects on offspring phenotype*. To anticipate altered aging trajectories it is of interest to determine changes in fetal life capable of altering the rate of aging. We have developed a baboon model of MO in which MO was induced prior to pregnancy by a diet rich in fat and sucrose. Controls (CON) ate normal baboon chow. ***We hypothesized*** that MO would alter fetal baboon liver molecular mechanisms considered key to regulating metabolic pathways that affect longevity.

Methods: Gene array and microRNA (miRNA; small RNA Seq) abundance analyses were performed on near-term fetal baboon livers (CON=6, MO=5) and subjected to pathway analyses to identify coordinated fetal liver molecular response to MO.

36. Resveralogues: a new mechanism for anti-degeneratives?

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There is growing evidence that many of the deleterious processes of aging may be modulatable by simple chemical compounds. Recent work has demonstrated that the removal or alteration of phenotype of senescent cells has the potential to reduce multiple age-related pathologies. The development of orally-available broad-spectrum anti-degenerative medicines is now a realistic goal.

A wide variety of polyphenolic natural products, often isolated from “super-foods”, have long been identified as potential lead compounds for anti-aging therapeutics. It has been suggested that such compounds may exert positive effects via antioxidant activity. However, it should not be forgotten that many of these have multiple other known activities, including oestrogenic effects, and some can even interrupt DNA synthesis and repair via intercalation. Accordingly we

established simple, robust and high-yielding syntheses to facilitate access to a broad range of structural variants of compounds containing the stilbene motif. We have evaluated this panel of "Resveralogues" to determine the specific structural features that underpin differential behaviour in a wide variety of *in vitro* assays, with a particular focus on activities related to cell growth and senescence. A subset of these compounds is able to "rejuvenate" cultures of senescent cells by altering RNA splicing patterns, lengthening telomeres, and enabling a significant fraction of cells to re-enter normal cell cycle. These activities are not dependent on SIRT1 activation or the presence of antioxidant phenolic groups. The structure activity relationships uncovered so far, and implications for the development of anti-degeneratives will be presented.

37. The impact of reduced neurosteroids in cognitive aging

Eileen Parks[1,2], Dr. William E. Sonntag[1,2,3]. [1]Oklahoma Center for Neuroscience, [2]Reynolds Oklahoma Center on Aging, [3]Department of Geriatric Medicine.

Introduction: A large percentage of the population experiences cognitive decline with age, which includes impairments in memory, processing speed, and executive function. Allopregnanolone (ALLO) is a neurosteroid that decreases with age and in various models of neurodegenerative diseases where deficits in cognitive function are evident. However, mechanisms by which reduction in ALLO contribute to age-related cognitive decline has not been investigated. **We hypothesize that the age-related decline in ALLO contributes to reduced neurogenesis and impairments in learning and memory in aged mice**.

Methods: Cortical brain samples from young (3M), middle (12M) and old (24M) male C57BL/6 mice were analyzed by LC/MS for neurosteroids. The effects of ALLO treatment on neurogenesis were assessed in old male mice subcutaneously injected with 10 mg/kg ALLO or vehicle, followed by 100 mg/kg EdU to label proliferating neural stem cells. Hippocampal-dependent learning and memory was tested in the Radial Arm Water Maze (RAWM). Newborn neurons labelled with EdU were visualized by IHC using frozen hippocampal sections.

Results: ALLO levels were significantly reduced at both middle and old age compared to young animals. Performance in the RAWM (Pathlength to target, number of entries (errors) and duration in incorrect arms) decreased with age across all 3 measures and significantly improved in ALLO-treated aged mice. When normalized to young, aged mice had approximately 20% surviving newborn neurons. ALLO-treatment tripled the number of surviving newborn neurons (60%), partially rescuing the age-related deficit in neurogenesis.

Conclusion: The age-related decline in ALLO is likely a contributing factor in learning and memory impairments with age. Importantly, a single injection of ALLO was sufficient to partially restore neurogenesis and improve learning and memory in older animals. Future experiments will elucidate the cellular mechanisms responsible for the decline in ALLO, which is dependent on a network of enzymes that synthesize both ALLO and other steroid metabolites.

Funding sources: Donald W. Reynolds Predoctoral Fellowship; Oklahoma Nathan Shock Center Award; Grants AG37847 and NS56218 to WES.

Table 1: Differentially expressed microRNAs inversely expressed with gene targets in key KEGG pathways.

KEGG Pathway (Direction, z-score)	Gene ID	Gene CON	Gene MO	Fold change	miRNA ID	miRNA CON	miRNA MO	Fold change
Proteasome (Down, 4.7)	**PSMD5**	3.8 ± 0.10	3.4 ± 0.08	-1.30	**miR-185**	31.6 ± 4.16	14.4 ± 5.16	2.20
	PSMC1	4.8 ± 0.09	4.3 ± 0.05	-1.36	**miR-194**	28.4 ± 1.96	13.8 ± 2.36	2.06
	PSMD6	3.3 ± 0.10	3.0 ± 0.07	-1.47	**miR-500a**	13.9 ± 1.54	6.7 ± 1.29	2.10
Oxidative phosphor-ylation (Down, 3.5)	**NDUFB3**	1.9 ± 0.09	1.5 ± 0.10	-1.25	**miR-185**	31.6 ± 4.16	14.4 ± 5.16	2.20
	NDUFB2	5.1 ± 0.09	4.8 ± 0.07	-1.26	**miR-500a**	13.9 ± 1.54	6.7 ± 1.29	2.10
	SDHC	3.3 ± 0.10	3.0 ± 0.07	-1.23	**miR-146**	345.0± 27.05	218.8 ± 7.11	2.20
	COX5A	4.9 ± 0.12	4.5 ± 0.07	-1.32	**miR-362**	20.5 ± 3.50	18.7 ± 2.35	2.56
				-1.32	**miR-885**	135.9 ± 4.82	67.7 ± 7.11	2.00

Results: 933 genes were differentially expressed between control and MO livers: 350 up-regulated and 583 down-regulated. MO fetal livers showed down-regulation of the proteasome and oxidative phosphorylation (Table 1), TCA cycle and glycolytic pathways (not shown), and inversely expressed miRNAs targeting genes in these pathways, supporting MO induction of epigenetic changes that dysregulate pathways central to cellular metabolism in MO fetal livers.

Conclusion: MO fetal livers revealed dysregulation of the proteasome, oxidative phosphorylation, TCA, and glycolysis pathways. There was evidence of epigenetic changes in that miRNAs exhibited inverse expression with genes regulated by the miRNAs in these pathways. This is the first observation of fetal nonhuman primate liver miRNA changes that, if they persist in later life, would result in alteration of mechanisms such as the proteasome and oxidative phosphorylation, which are considered potential candidates driving aging processes.

Funding: OD P51 OD011133 to Southwest National Primate Center; HD 21350

38. Short-term rapamycin treatment leads to lasting improvement in cardiac health in aged mice.

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Even in the context of healthy aging, cardiac morbidity and mortality increases with age in both mice and humans. These effects are evident in the decline of diastolic function, increase in left ventricular hypertrophy, metabolic substrate shifts, and alterations to the cardiac proteome. Previous work from our lab indicated that short-term (2 months) treatment with rapamycin, an mTORC1 inhibitor, improved measures of these age-related changes. Our current work, presented here, demonstrates that the improvement of diastolic function with transient rapamycin treatment persists at about 80% of continuous rapamycin-treatment levels two months after drug removal in both male and female 24^{+} month old mice. There is also a persistent reduction in cardiac hypertrophy. Many of the proteomic and metabolomic abundance changes that occur after 8 weeks of rapamycin treatment revert to resembling control levels after two further months without the drug. However, rapamycin treatment did lead to a persistent increase in abundance of electron transport chain (ETC) complex components, most of which belonged to Complex I. We also present data on the activity level of the different ETC complexes. Our work aim is to find the specific mechanisms by which rapamycin improves cardiac function in old age. By focusing on persistent molecular changes, we hope to find clues to which pathways are most critical for delaying age-related cardiac decline, a problem with no current treatment.

Funding: Genetic Approaches to Aging Training Grant NIH/NIA T32 AG000057

39. **Mild lifestyle changes in older adults lead to skeletal muscle remodeling through activation of myogenesis, protein synthesis and metabolic pathways**

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One of the challenges in aging populations is to unravel aging-associated and life-style determined diseases. Decrease in musculoskeletal functionality during aging hampers mobility and daily functioning, and is known to be affected by poor metabolic health. Moreover, aging-associated metabolic changes in the muscle tissue can lead to changes in whole-body metabolism. While in early adulthood unfavorable metabolic health leads to detrimental metabolic and architectural changes in the muscle tissue, this process is still poorly understood for older adults. We aim to elucidate how improving whole-body metabolic health impacts on elderly human skeletal muscles at molecular and tissue levels.

For this study we investigated muscle biopsies from 87 older adults (mean age 63 years) before and after a mild 13-weeks lifestyle intervention (the Growing Old Together Study, *van de Rest, et al. Aging. 2016*). This intervention was based on 12.5% increase in physical activity and 12.5% decrease in caloric intake, one of the conditions previously applied in the CALERIE study. At baseline and after the intervention, RNA sequencing data were generated and the molecular signature of the lifestyle intervention was explored in the transcriptomes. Additionally, aging-associated metabolic and architectural changes in the muscle tissue were studied by novel procedures of semi-automated quantitative immunofluorescence imaging.

With a paired design, we identified 323 genes that were most prominently (FDR<0.01 and log **fold-change ≥|0.5|) differentially expressed due to the lifestyle intervention (296 upregulated**

and 27 downregulated). The upregulated genes were enriched for extra-cellular matrix genes and catabolism thereof, growth factors and muscle-specific transcription factors. Downregulated genes included fat storage genes. Furthermore, among all significantly differentially expressed genes (FDR<0.01, 2260 upregulated, 2327 downregulated), we found that key muscle developmental and muscle protein synthesis genes were upregulated, while regulators of muscle protein degradation were downregulated. Glycolytic enzyme genes that were earlier found to be upregulated in muscle aging, were downregulated due to the intervention. These results were in part confirmed with quantitative immunofluorescence imaging, showing an increase of extra-cellular matrix deposition, a loss of glycolytic myofibers and an increase in myocytes. In conclusion, we demonstrate that older adults are able to remodel their skeletal muscle by following a mild lifestyle intervention and suggest that this improves their muscle health.

Funding: European Union's Seventh Framework Program (grant number 259679). Netherlands Consortium for Healthy Ageing (grant number 050-060-810) in the framework of the Netherlands Genomics Initiative, Netherlands Organization for Scientific Research (NWO). BBMRI-NL, a research infrastructure financed by the Dutch government (NWO 184.021.007).

40. Mitochondrial Regulation of Aging Metabolism

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Aging is strongly associated with a decline in metabolic function and physical function. Mitochondria are key metabolic organelles with much implication in aging, but it is unclear how they regulate aging metabolism. We previously identified MOTS-c, a peptide encoded in the mitochondrial DNA. MOTS-c reversed age-dependent muscle insulin resistance and prevented diet-induced obesity and insulin resistance in mice. MOTS-c can activate AMPK and improve glucose utilization in mouse skeletal muscle. Here, I show that MOTS-c can impove physical function in mice.

Mice fed a high-fat diet (HFD) and injected with MOTS-c for 10 days were able to run significantly longer and faster on a treadmill compared to the control group. Beyond physical performance, MOTS-c prevented diet-induced obesity. Body composition analysis indicated that mice treated with MOTS-c could maintain relative muscle mass under HFD. These results suggest that MOTS-c may regulate metabolic homeostasis and protect against age-related loss of physical function.

Funding: NIA (1R01AG052558), Ellison Medical Foundation, and American Federation for Aging Research (AFAR) to C.L.

41. Role of Necroptosis in Aging and Age-Associated Inflammation

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Aging is characterized by low-level chronic inflammation, termed sterile inflammation or 'inflammaging'. Inflammaging is an important risk factor for most age-related diseases as well as both morbidity and mortality in older people. Inflammaging is characterized by high levels of circulating proinflammatory cytokines. Although the exact cause of inflammaging is not known, several pathways have been suggested to cause chronic inflammation with age, e.g., immunosenescence, cell senescence, and increased levels of damage associated molecular patterns (DAMPs), which are a strong inducer of inflammation. Necroptosis is a newly identified non-apoptotic form of cell death that plays a major role in inflammation through generation of DAMPs. We hypothesized that necroptosis plays a role in aging and age-associated increase in inflammation. In support of our hypothesis, we found that levels of phosphorylated mixed lineage kinase domain like (P-MLKL), a marker of necroptosis, is elevated by 2.5-fold in epididymal white adipose tissue (eWAT) of 25-month-old wild-type (old-WT) mice, compared to 9-month-old WT (young-WT) mice. Interestingly, only eWAT, not subcutaneous WAT (sWAT), showed an age-dependent increase in necroptosis. Dietary restriction (DR), an intervention known to reduce inflammation and increase lifespan, reduced necroptosis in eWAT of old-DR mice to the level found in young-WT mice. We also measured the level of necroptosis in several tissues of *Sod1*$^{-/-}$ (*Sod1*KO) mice. Sod1KO mice show a 20-30% decrease in lifespan and increased inflammation (e.g., circulating levels of pro-inflammatory cytokines and pathological markers of inflammation). P-MLKL is increased 3.7-fold and 0.4-fold in eWAT and liver of 9-month-old *Sod1*KO mice. Furthermore, markers of M2 macrophages (anti-inflammatory) are reduced and M1 macrophages (pro-inflammatory) are increased in eWAT of Old-WT and young-*Sod1*KO mice. Consistent with the shift of M2 to M1 macrophages, we observed that transcript levels of pro-inflammatory cytokines TNFα, IL-6 and IL-1β are elevated in eWAT of old-WT and young-*Sod1*KO, compared to young-WT mice. In summary, we show for the first time that necroptosis increases with age, is elevated in an accelerated aging model and is reduced by DR suggesting a role of necroptosis in age-associated inflammation and possibly aging.

Supported in part by NIH grants P01-AG051442 and R01 AG045693

42. Skin fibroblasts present a powerful opportunity to study aging: developmental programming interactions in a baboon model of intrauterine growth restriction (IUGR).

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Introduction: Age-related diseases are modified by developmental programming, defined as programming of later life phenotypes following challenges faced while *in utero* or in neonatal life. The mechanisms that persist throughout life are unknown. We developed a cohort of baboon offspring of mothers fed *ad libitum* (control) or a 30% global calorie reduced diet whose offspring were IUGR (birthweight ~12% less than offspring of *ad libitum* fed mothers, PMID 23482706). IUGR offspring show accelerated development of aging-related phenotypes such as early aging of left (PMID 27988927) and right (PMID 28439937) ventricular function and premature brain aging (PMID 28443017). To compare tissue response between control and IUGR offspring we derived primary skin fibroblast lines from adult control and IUGR baboons (ages 7-11 years; human equivalent ~32-50 years) to test effects of programming on selected

"pillars of aging" defined by the Geroscience Initiative (http://dx.doi.org/10.1016/j.cell.2014.10.039).

Methods: A 2mm sterile post-aural punch biopsy was obtained (n = 9 CTR, 7 IUGR). Following enzymatic dissociation, isolated fibroblasts were subcultured using standard techniques to generate sufficient numbers for further testing.

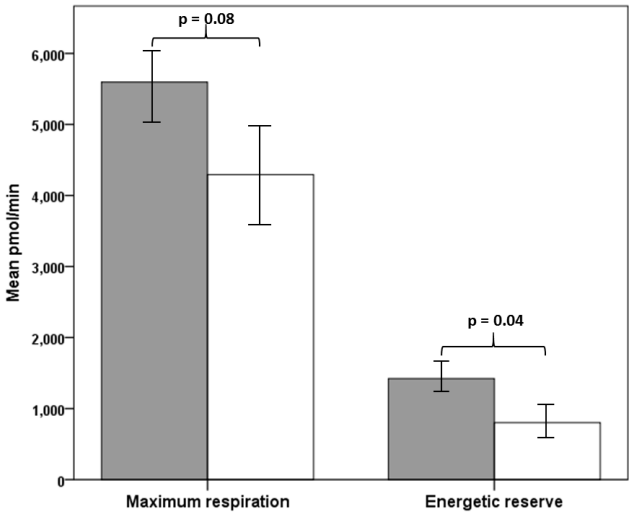

Figure 1. Control (closed bars, n=9) showed greater maximum respiration and energetic reserve compared to IUGR (open bars, n=7). Mean ± SEM.

Results: From explant and isolation, fibroblasts isolated from IUGR baboons required ~50% greater time to reach confluence in culture. Cell numbers at confluence were reduced 25% in IUGR lines compared to control. This delay in growth appeared linked to reduced mitochondrial respiration, suggesting potential functional defects in oxidative phosphorylation that might drive delayed growth. IUGR lines showed 15-20% reduction in oxygen consumption as measured by Seahorse Bioanalyzer (Figure 1). In addition, IUGR-derived fibroblasts displayed reduced cellular resilience to mitochondrial stress in response to the mitochondrial uncoupler FCCP, suggesting reduced energetic reserve in these cell lines (40% reduction in IUGR). IUGR was also associated with reduced resiliency to cellular senescence with earlier and increased levels of senescence-associated β-galactosidase and p16 expression.

Conclusions: Small skin biopsies represent a powerful method of studying molecular mechanisms of aging and programming interactions. Phenotypes are retained *ex vivo* in primary cultures. Our data show that multiple pathways associated with cellular aging are affected by IUGR.

Funding: R24 RR021367-01 A1; OD P51 OD011133

43. Targeting mTOR to extend longevity in a non-human primate.

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Mechanistic target of rapamycin (mTOR) has emerged as a viable pharmaceutical target to lengthen lifespan and healthspan in mice, though the potential for translation is still unclear. Testing whether the inhibition of mTOR extends longevity and improves healthspan in a non-human primate model is a significant step towards the development of translational approaches to delay or reduce age-related diseases in humans through manipulation of this pathway. We have approached this question by utilizing the shortest-lived anthropoid primate, the common marmoset (*Callithrix jacchus*). Here, we outline the design and initial data collected from our

cohort of aging marmosets with mid-life intervention with rapamycin to test whether this treatment will promote healthy aging. A once daily administration with encapsulated rapamycin at a dose roughly equivalent to 1 mg/kg body weight results in clinical concentrations of rapamycin in the blood and inhibition of mTOR in vivo. Both male and female marmosets receive rapamycin (or vehicle) daily over the next 5-6 year period with an ultimate goal of testing the effect of mTOR inhibition on longevity of this primate species. In addition, we will measure longitudinal outcomes of functional assays targeted to five general physiological systems shown to be affected by rapamycin in rodent models: muscle function, cognition and memory, metabolism, immune and inflammation and cardiovascular risk. In our initial assessments of this cohort, chronic rapamycin administration in marmosets reduces both mTORC1 and mTORC2 signaling and has limited to no adverse side effects. In contrast to rodent studies, glucose metabolism is not altered significantly in this non-human primate orally treated with once-daily encapsulated rapamycin. Moreover, despite dramatic reduction in mTOR signaling in vivo there is little evidence for dramatic changes in body weight or composition or in total blood cell counts or specific populations of blood cells. When complete, this study will describe for the first time the potential for pharmaceutical intervention to extend longevity of a primate species with the ultimate goal of significant translational impact to human aging

Funding: R01 AG050797

44. Bridge walking performance is linked to AMP kinase activity in the cerebellum.

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Recent studies of cerebellar function suggest that caloric restriction improves performance of males but the equivalent regimen has no effect on females. The goal of the current study was to address sex differences in bridge walking performance, which is linked to age related decline in cerebellum function.

One of the effects of CR reported in literature is an increase in phosphorylated AMPK. Phosphorylation of AMP kinase (AMPK) is linked to catabolic processes, including autophagy. Autophagy clears toxic metabolites. A decline in efficiency of autophagy function has been linked to aging and neurodegenerative diseases.

Aged male and female mice underwent either caloric restriction (30% CR) or were fed a normal diet ad libitum for 16 weeks. A behavioral battery was administered at 8 weeks of treatment that included tests for bridge walking and other cognitive and motor functions. At the end of 16 weeks, tissue from the periphery and brain was collected for further metabolic marker analysis.

Phosphorylated AMPK was significantly increased in the liver for both males and females indicating increased catabolic activity. However, only aged males under CR had increased phosphorylated AMPK activity in the cerebellum and these mice also had improved bridge walking when compared to their aged matched controls. In contrast, females CR mice and their age-matched control did not differ in bridge walking and nor was there a significant difference in the levels of phosphorylated AMPK in the cerebellum.

Results suggest AMPK phosphorylation to improve motor function. The coupling of AMPK to autophagy processes may underlie sex differences in aging of the cerebellum. Future tests should examine whether different brain regions differ in their metabolic activity thereby affecting motor and cognitive behavior.

Funding: P01 AG027956

45. NANOG Restores the Myogenic Differentiation Potential of Senescent Myoblasts.

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Age-related loss in muscle mass, sarcopenia, is a major problem facing the elderly. Adult skeletal muscle regeneration relies on the activity of resident satellite cells in skeletal muscle niche. However, systemic and intrinsic factors decrease the myogenic differentiation potential of senescent satellite cells. Here we present data showing that late passage myoblasts exhibited significantly impaired the myogenic differentiation potential that was accompanied by impaired expression of myogenic regulatory factors (Myf5, Myod, Myogenin, and MRF4) and members of myocyte enhancer factor 2 family. Notably, ectopic expression of NANOG for at least two weeks preserved the morphology and restored the myogenic differentiation capacity of senescent myoblasts, possibly by restoring the expression level of these myogenic factors. Muscle regeneration was effective in 2D cultures and in 3D skeletal microtissues mimicking the skeletal muscle niche. Interestingly, myoblasts maintained the rejuvenated capacity for 20 days after NANOG withdrawal, suggesting that NANOG might have imparted epigenetic changes. However, the presence of NANOG during differentiation inhibited myotube formation, suggesting that NANOG might have primed the cells for myotube formation but inhibited the differentiation process itself. In conclusion, these results shed light on the potential of NANOG to restore the myogenic differentiation potential of senescent myoblasts and to reverse the loss of muscle regeneration due to aging.

This work was supported by a grant from the National Institutes of Health (R01 HL086582) to Stelios T. Andreadis.

46. GSK3β regulates brain energy metabolism.

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Neurodegeneration produces significant functional decline among the elderly and is the primary factor underlying late-onset Alzheimer's disease (AD). The insulin-sensitive kinase, glycogen-synthase-kinase-3beta (GSK3β) has been directly linked to the principle biochemical features of

AD, tau tangles and beta-amyloid plaques. We previously identified a novel metabolic pathway whereby GSK3β regulates the stability and activity of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), a master regulator of mitochondrial function. Mitochondrial metabolism is significantly impaired with age and PGC-1α targets are thought to mediate the longevity-promoting effects of caloric restriction, a model of delayed aging. Here, we characterize the extent to which GSK3β regulates metabolism at the cellular level, and in the brains of mice treated with lithium, a widely-used GSK3β inhibitor.

Lithium chloride treatment produced substantial upregulation of mitochondrial metabolism in cell culture models of astrocytes (H4) and mature neurons (PC-12). This shift in metabolism extended to increases in basal and maximal oxygen consumption, mitochondrial membrane potential, and lengthening of NAD(P)H fluorescent lifetime *in-situ*, suggesting higher levels of protein bound NAD(P)H. Cellular redox was also impacted by lithium treatment, as indicated by increases in total levels of NAD cofactor. The functional impact of GSK3β inhibition was cell-type specific with neuron-like PC-12 cells displaying a higher magnitude response to treatment. Co-incident with these functional responses was an increase in the stability of PGC-1α protein, which rapidly localized to the nucleus upon lithium administration. Additionally, we observed distinct transcriptional responses with long-term lithium treatment consistent with increases in PGC-1α activity.

Mice fed a diet of lithium carbonate over four months exhibited lengthening of NAD(P)H fluorescent lifetime in key areas of the hippocampus along with alterations in cytochrome c oxidase activity, suggesting that GSK3β operates similarly in regulating metabolism of the whole-brain. Consistent with what was observed in cells, metabolism was non-uniformly impacted by lithium among cell populations in the hippocampus. Histological analysis of lithium treated mice revealed a highly region-specific response to lithium in total levels of PGC-1α protein, phospho-GSK3β, and metabolism. Moreover, these responses were dose-dependent, with the most robust changes being observed at 1.2 mg/kg/d lithium carbonate.

Altogether, these results suggest a role for GSK3β as a driver of metabolic dysfunction with age. Total levels of GSK3β are increased in the aged brain and it is known that GSK3β participates in multiple processes that are associated with neurodegeneration. Furthermore, the factors that contribute to mitochondrial dysfunction in the aged brain are not entirely clear, suggesting that metabolism itself, and the GSK3β/ PGC-1α axis in particular, may be suitable targets for the prevention and treatment of age-related neurodegeneration.

Funding:

Glenn/AFAR Breakthroughs in Gerontology (BIG) Award

Biology of Aging and Age Related Diseases T32 Training Grant (AG00213)

47. 3-Hydroxyanthranilic Acid—A Novel Molecular Target for Lifespan Extension in the Kynurenine Pathway.

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Identifying novel genetic factors that can be targeted to beneficially influence longevity, healthspan, and age-associated disease is an ongoing area of focus in aging science. In a recent

study, we selected 82 *Caenorhabditis elegans* genes based on orthology to 125 human genes differentially expressed with age and conducted an RNAi lifespan screen. The clear outlier was *kynu-1*, encoding the kynurenine pathway enzyme kynureninase. RNAi knockdown of *kynu-1* extended lifespan by >20%. Kynurenine pathway gene expression and metabolite abundance is perturbed in individuals with a number of age-associated diseases, including neurodegenerative disease. Many intermediate kynurenine pathway metabolites have neuroactive or antioxidant properties, and pharmacological interventions targeting kynurenine pathway enzymes are being pursued for Alzheimer's and Huntington's disease. In an expanded survey of the kynurenine pathway, we identified two additional genes for which knockdown results in a similar degree of lifespan extension to *kynu-1(RNAi)—haao-1* and *tdo-2*. Knockdown of *kynu-1*, *haao-1*, or *tdo-2* extended healthspan and delayed pathology in *C. elegans* models of Alzheimer's and Huntington's disease. Knockdown of *haao-1* alone achieved these benefits without impairing reproduction or development. *haao-1* encodes the enzyme 3-hydroxyanthraniate 3,4-dioxygenase, which converts the metabolite 3-hydroxyanthranilic acid (3HAA) into 2-amino-3-carboxymuconate semialdehyde. Worms lacking *haao-1* have highly elevated 3HAA, which is thought to have both direct and indirect antioxidant properties. In ongoing work, we find that treatment of worms with 3HAA phenocopies reduced *haao-1* in the context of aging and neurodegenerative pathology in *C. elegans*, suggesting that it may represent a potent metabolic target for treating age-associated cognitive disease.

Funding: This work was supported by the National Institute on Aging grant AG038070 and the National Cancer Institute Core grant CA034196 to the Jackson Laboratory.

48. Intestinal Function is Impaired by Exposure to the Old Systemic Environment

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Studies utilizing heterochronic parabiosis have demonstrated that aging occurs as a complex interaction of cell autonomous and non-cell autonomous mechanisms, including the transposition of aging phenotypes in cells and tissues. While the importance of intestinal dysfunction is well-recognized in bowel syndromes and cancer, the interplay between the intestine and aging is not well investigated. Thus, we have been intensively focused on charactering intestinal aging phenotypes with normal aging in mice. As compared to young male C57BL/6 mice (4-5 mo old), old male mice (20-24mo) harbor declines in intestinal stem cell (ISC) proliferation, impaired mucosal barrier integrity, and shifts in the gut immune cell composition, including increased CD8+ T cells and dendritic cells (P<0.05). We next generated isochronic (Y-Y, O-O) and heterochronic (Y-O) C57BL/6 male parabionts and observed impaired ISC function in young parabionts exposed to old blood (P<0.05). Rapamycin or salicylate treatment restored ISC function in old mice (P<0.05), without further suppressing mTOR signaling. However, rapamycin reduced plasma cytokines to more youthful levels (P<0.05), suggesting a potential role for inflammation in mediating these effects. *Ex vivo* screening assays confirmed that TNFα and IFNγ potently disrupt ISC proliferation (P<0.05), while *in vivo* treatment with a TNFα- or IFNγ-neutralizing-antibody restored ISC function in old mice (P<0.05). Furthermore, fecal microbiota analysis of the parabionts revealed that the microbial landscape at the phyla level is transposed from old to young (P<0.05). In summary, our data suggest that TNFα and IFNγ represent key pro-geronic factors in driving the transposition of intestinal aging by heterochronic parabiosis.

Funding: American Federation for Aging Research**,** National Institute on Aging (R56AG052981)**,** Prevent Cancer Foundation

49. mTOR inhibition reverses the senescence-associated heterochromatin formation in an Alzheimer's disease mouse model.

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Alzheimer's disease (AD) is the most prevalent neurocognitive disorder in the elderly, and currently afflicts 5.4 million Americans. It is the leading cause of death in the US among people 65 and older, and no cure exists. Aging is the major risk factor for AD, however, the contribution of the aging phenotype to the onset of AD has not been thoroughly examined. Cellular senescence, where cells lose replicative potential and gain a pro-inflammatory secretory phenotype, has been shown to be a component of aging in multiple tissues and organisms. We have shown previously that AD is characterized by an increase in senescent astrocytes in the human brain. The PDAPP mouse is an AD model characterized by amyloid beta (Aβ) deposits, cognitive impairment and hippocampal atrophy. Treatment of this mouse with rapamycin, and inhibitor of the mTOR protein, has previously been shown to reduce cognitive deficits, Aβ levels and loss of vascular integrity in this mouse. We explored whether rapamycin treatment affected senescence associated heterochromatin formation, and pro-inflammatory secretory phenotype in hippocampal slices of control non-transgenic (NTg), PDAPP transgenic (Tg) and PDAPP transgenic mice supplemented with rapamycin in their diet (Tg + Rapa). Our results show that the induction of heterochromatin, as measured by increase in mH2A and decrease in lamin B1 in the Tg mice compared to NTg, is reversed by rapamycin treatment. Tg mice also show an increase in inflammatory proteins, which is not reversed by rapamycin treatment to the same degree as heterochromatin formation. Hence, we suggest that rapamycin treatment possibly influences the cognitive and other pathologic outcomes in the AD mouse model by re-programming chromatin modeling and downstream gene expression. These results offer a mechanistic explanation for the ameliorative benefits of a molecule that can be used in the human context for treating AD in the near future.

Funding:

NIH 1RO1NS078283-01.

Mars Foundation - Aging Initiative Drexel University.

50. What's Up, Dog? Using primary care veterinary data to implement the *One Health* paradigm in geroscience.

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The privately owned domestic dog is an emerging model in the field of geroscience, notably because it shares the human environment including its risk factors, is affected by many of the same age-related diseases, receives comparable medical care, and has excellent veterinary medical data available. The concept that a shared environment with similar risk factors affects health outcomes across species is referred to as One Health and has interesting implications for using non-human animals as models and sentinel organisms for human epidemiology, including the study of aging, age-related disease and age-related mortality.

In this study, we analyzed data from three American primary care veterinary hospitals from a rural, a suburban and an urban setting in order to identify risk factors that determine health outcomes and, ultimately, life span and mortality in a cohort of n = 20,970 privately owned dogs using Kaplan-Meier survival estimators and Cox Proportional Hazards modelling. In doing so, we found significant effects of body size, environment, sex and gonadectomy status, and anatomical features such as brachycephaly on life span and/or causes of death. Our findings regarding body size as well as sex and gonadectomy status reflect previously published data, indicating that our sample was representative; however, urban, suburban and rural living environments, as well as brachycephalic status have not previously been described as having an effect on life span and/or causes of death in dogs. Based on the One Health paradigm, data such as these may have interesting implications for human health in the same rural, suburban and urban environments.

Funding: This work was partially supported by the University of Washington Nathan Shock Center of Excellence in the Basic Biology of Aging (P30AG013280). SU was supported by donations from the Irish Wolfhound Association of New England and the Donner Foundation to the Dog Aging Project. DP and KC received support from NIH Grant R24AG044284.

51. Chronic Inflammation-related Metabolomic Profile Discovery in The interleukin 10^{tm1Cgn} mouse

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The biologic complexity of frailty and the need for greater diagnostic and treatment specificity calls for the use of high throughput technologies and animal models to link biologically relevant pathways to late life decline. The interleukin 10^{tm1Cgn} ($IL10^{tm}$) mouse develops chronic inflammation, a frailty-like phenotype with age, and has increased mortality.

To identify molecular changes that are involved in biological decline in these mice, we used LC/MS technology to profile 188 distinct metabolites from five substance classes including acylcarnitines, amino acids, hexoses, phospho- and sphingolipids and biogenic amines in plasma from $IL10^{tm}$ and C57Bl/6 age and gender matched control mice.

Our initial profiling identified several metabolic pathway alterations in the plasma of the pro-inflammatory $IL10^{tm}$ mouse. One of the most prominent and consistent alterations in the $IL10^{tm}$ mouse was decreased tryptophan with a concomitant increase in kynurenine. Based on these

preliminary findings, subsequent targeted LC/MS measurements within this metabolic pathway showed increased levels of neurotoxic intermediate metabolites in the kynurenine pathway including 3-hydroxykyneurenine and quinolinic acid. Since the kynurenine pathway is essential for *de novo* NAD synthesis, we also profiled related energy substrate pathways and saw decreases in the TCA cycle intermediate *alpha*-ketoglutarate indicating decreased pathway flux. Increased plasma levels of cytotoxic and neurotoxic metabolites, and decreased energy availability may play a causal role in the biological decline in these mice and in frail humans. With this work we have identified a therapeutically targetable molecular pathway which may play a causal role in driving aging and frailty.

Funding:

Glenn/AFAR Postdoctoral Fellowship for Translational Research on Aging

OAIC P-30AG021334 Johns Hopkins Older American Independence Center, Research Supplements to Promote Diversity in Health-Related Research Program (PI Walston) 6/1/13-6/1/15 (expired)

52. Deletion of *Nrip1* extends female mice longevity, increases autophagy and delays cell senescence

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Using age of female sexual maturation as a biomarker, we previously identified nuclear receptor interacting protein 1 (*Nrip1,* also named *Rip140*) as a candidate gene that may regulate aging and longevity. In the current report, we found that the deletion of *Nrip1* can significantly extend longevity of female mice (log rank test, P=0.0004). We also found that *Nrip1* expression is altered differently in different tissues during aging and under diet restriction. Remarkably, *Nrip1* expression is elevated in white adipose tissue (WAT) with aging, but significantly reduced after four months of diet restriction. However, in gastrocnemius, *Nrip1* expression is significantly reduced during aging but upregulated seven times after four months of diet restriction. In the mouse embryonic fibroblasts we found that deletion of *Nrip1* can suppress fibroblast proliferation, enhance autophagy under normal culture or amino acid starvation conditions, as well as delay oxidative and replicative senescence. Importantly, in WAT at 16 months of age, the number of senescent cells is significantly reduced in the *Nrip1* null mice. These results suggest that deleting *Nrip1* can extend female longevity, but tissue-specific deletion may have different effects on healthspan. The deletion of *Nrip1* in WAT may delay the senescence in WAT and extend healthspan.

Funding: NIA grants K01AG046432 and R03AG046605

53. Developmental Programming and Aging Interactions Play a Key Role in Determining Life Course Circulating Corticosterone Levels in Rats.

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Introduction. There is abundant evidence for developmental programming of pituitary adrenal function by the nutritional state of the mother in pregnancy and lactation. However, effects of aging of hypothalamus-pituitary-adrenal function in rats are not clear. We recently demonstrated a fall in rat circulating corticosterone – the rodent glucocorticoid (GC), in males and females between postnatal day (PND) 450 and 650 (1) in offspring (F1) of obese rat mothers fed a high fat diet (MO). GC levels were higher than control (C) in F1 whose mothers ate a high fat diet. Importantly although levels were different the fall occurred within the same age windows in MO and C. (Fig 1 A and B).

Methods and Results. We measured corticosterone to determine life course profiles in archived samples from contemporaneous studies in male and female F1 of mothers fed a low protein (LP) diet (10% casein) compared with C (20%) diet (Fig 1 C and D). In male and female LP F1 there was a fall in corticosterone between 220 and 650 days. Importantly in our colony C and LP survived longer than 850 days whilst MO offspring started to die around 650 days.

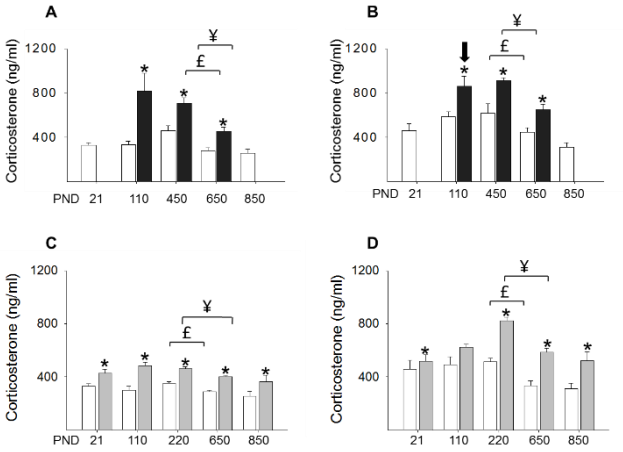

Figure 1. Mean ± SEM, n=5-14. P<0.05 * vs C; timing of the corticosterone fall in £ C and ¥ LP or MO. A and B MO, C and D (LP). C (open histograms), MO (black) and LP (grey). A and C Male, B and D Female. Female corticosterone values were higher than male in all groups at all ages except in offspring of obese group at PND 110 (downward-pointing arrow).

Conclusion. Although the time windows differ in the studies in the MO and LP models the importance of these data are 1) they provide further evidence that when sampled at multiple ages over a wide period of the life course, circulating corticosterone in control animals falls: 2) programming by both LP and MO increases the circulating levels but within the range of ages studied, the fall in corticosterone occurs at similar ages. The life course fall in corticosterone may be a) causing aging, b) protecting against aging, c) responding to aging or d) an epiphenomenon with a related time course to aging.

Funding. RCUK-CONACyT. **References.** 1) Age. 2015;37(3):9774.

54. Maternal exercise intervention before and during pregnancy programs long-term offspring (F1) health benefits.

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Introduction: Epidemiological and animal studies demonstrate that maternal obesity (MO) predisposes aging F1 to metabolic disorders - excessive weight, adiposity and dysregulation of lipid metabolism – that increase with age. There is a need for interventions that slow or even reverse these aging outcomes. Exercise is well established to have numerous health benefits; however the effects of maternal exercise (Mex) on the F1 aging metabolic phenotype are poorly understood. We hypothesized that Mex has preventive effects on adverse age-related outcomes that persist into late life. The aim of this study was to determine if Mex intervention has beneficial effects on long-term metabolic health of male and female F1.

Methods: F0 female Wistar rats ate control (C) or obesogenic diet (MO) from weaning through lactation. From 90 to 120 days (when they were bred) half of each group wheel ran 30 min/day, 5times/week providing four groups: control (C), obese (MO), exercised controls (CEx), and exercised obese (MOEx). After weaning all F1 groups ate C diet. We evaluated serum leptin and triglycerides (TG) in male and females at 110 and 650 d, fat depots excised and weighed. Data M ± SEM, Two-way, ANOVA, post-hoc Tukey test.

Results: With aging, increases in total fat, leptin and TG between 110 and 650 d were greater in MO F1 than in CF1. However, Mex prevented these age related changes in MOF1 at 650 d (Fig. 1A-F). Interestingly Mex even lowered leptin and in CEx males.

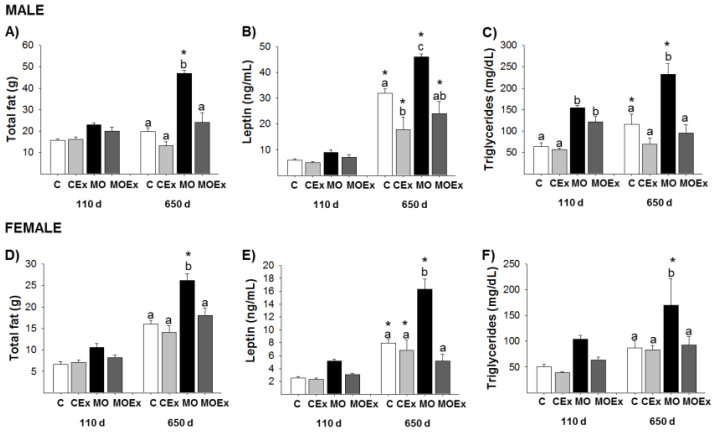

Figure 1. F1 aging metabolic variables in male and female. Mean ± SEM, n = 6-8 rats from different litters. P <0.05 for data not sharing at least one letter among groups at the same age, * P <0.05 650 d vs 110 d within the same group.

Conclusion: MO affects F1 natural aging trajectory predisposing F1 to premature aging and increases the risk of metabolic diseases. F0 voluntary exercise intervention prior and during pregnancy help to prevent negative age-related programming by MO in F1 rats.
Funding: CONACYT-RCUK.

55. Acceleration of metabolic aging in offspring (F1) of obese rats showing interaction between developmental programming and aging mechanisms.

Elena Zambrano, PhD[1], Consuelo Lomas, PhD[2], Luis A. Reyes-Castro, PhD[1], Guadalupe L. Rodríguez-González, MSc[1], Carlos Ibañez-Chavez, MSc[1] and Peter W. Nathanielsz, MD, PhD, ScD, FRCOG[3]. [1]Reproductive Biology, Instituto Nacional de Ciencias Médicas y Nutrición SZ, [2]CONACyT, Biología de la Reproducción, Instituto Nacional de Ciencias Médicas y Nutrición SZ, [3]Animal Science, University of Wyoming.

Introduction: A high maternal BMI predisposes human F1 to a risk of early death, in keeping with the developmental programming hypothesis which can be defined *as a response to a specific challenge to the mammalian organism during a critical developmental time window that alters the developmental trajectory with persistent effects on F1 phenotype*. The emphasis in

programing studies has been on predisposition to chronic disease. This predisposition likely results in shortened life spans –partially due to earlier onset age-related disease onset. We have developed a rat colony that allows us to control the phenotype and past life history of mothers (F0) and, if necessary grandmothers and control the environment and maintain F1 for the full life span. Using this model we have extensively examined effects of maternal obesity on F1. There is considerable evidence that F0 obesity increases F1 insulin resistance (IR) and leptin levels and increased adiposity – common features of aging and obesity. We hypothesized that F1 of obese F0 would 1. have shorter life spans; 2. earlier and augmented age related increases in IR and leptin. **Methods:** F0 female rats ate control (C) or obesogenic diet (MO) from weaning through lactation. After weaning F1 ate C diet. F1 males and females were studied at postnatal day (d) - 36, 110, 450 and 650 serum leptin, glucose and insulin and fat depots excised and weighed to determined adiposity index (AI = total fat (g) x body weight g^{-1} 100^{-1}. Homeostatic model assessment (HOMA) was calculated (HOMA= glucose (mmol-1) x insulin (µU/mL). 22.5^{-1}). Data M ± SEM; n = 5-8 per group – each subject from a different litter. Two-way ANOVA and Tukey test.

Results: In all our studies the life-span of F1 control males and females survive longer than 850 days while no F1 MO of obese mothers are alive at that age. By 450d every variable except glucose was increased in both males and female along the same trajectory as aging in F1C. These increases persisted at 650d and F1 MO had earlier and augmented age related increases in IR and leptin (Fig 1 A-C and G-I).

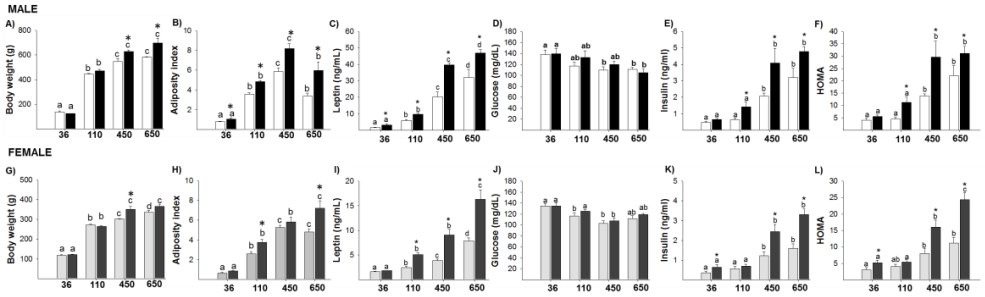

Figure 1. F1 morphometric and metabolic variables in male and female aging. Mean ± SEM, n = 5-8 rats from different litter. P <0.05 for data that do not share at least one letter in the same maternal diet (different age), * p <0.05 vs C, at same age.

Conclusions: The interaction across the life course relation between aging and programming on IR and leptin physiology has not previously been studied. Age related trends occur in a variety of indices as early as 110d.

Funding: CONACYT-RCUK.

56. Impaired ischemia induced reperfusion in aged male and female offspring (F1) of obese mothers (MO).

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Science, University of Wyoming and [4]Texas Pregnancy & Life Course Health Research Center, Southwest National Primate Research Center, Texas Biomedical Research Institute.

Introduction: MO predispose F1 to cardiovascular disease, metabolic dysfunction and shorter lifespan, than F1 of normal BMI mothers (controls – C) (1). The extent to which shorter life spans are due directly to impaired cardiac function is best established by *in vitro* approaches. We hypothesized that isolated, aged rat MO F1 hearts would show impaired function compared with age-matched C F1. **Methods:** We tested *in vivo* and *in vitro* aged F1 hearts of C mothers fed normal chow *ad lib* and mothers fed obesogenic diet (MO) from weaning through pregnancy and lactation. Non-sib male and female F1 were weaned to chow. At postnatal day 550, rats were anesthetized (low dose sodium pentobarbital, 0.3U/100g body weight, i.p.) for echocardiography analysis. 2 d later hearts were removed for 20 min *in vitro* global ischemia with 30 min reperfusion, fat depots dissected and adiposity index (AI = total fat (g) x body weight g^{-1} 100^{-1}), and insulin and glucose measured. Homeostatic model assessment (HOMA) was calculated (HOMA= glucose (mmol-1) x insulin (µU/mL). 22.5^{-1}). Data M ± SEM; t-test. **Results:** Relative heart weight (male C=3.3 ± 0.6, MO= 4.2 ± 0.6*; female C=3.3 ± 0.5, MO=3.4 ± 0.05 g/kg, *p<0.05) adiposity index (male C=2.8 ± 0.3, MO= 6.6 ± 0.6*; female C=8.1 ± 0.4, MO=9.9 ± 1.1, *p<0.05) and mediastinal fat (male C= 0.7 ± 0.1, MO= 2.6 ±0.5*, female C=1.3 ± 0.3, MO=1.9 ± 0.4 g, *p<0.05) were higher in male MO F1 than C; data in females were similar. Thoracic fat were higher only in male MO than in C. Glucose was similar among groups. Insulin and HOMA (fig 1A and B) were higher in male and female MO than C. Resting echocardiogram evaluation was similar between groups. Fig 1 (C and D) shows that left ventricular pressure (LVP) was lower in male MO (41% lower) and female (47% lower) than respective controls. On reperfusion female controls recovered to 90% baseline while MO recovered only to 63%. In males these figures were lower 71% and (38%). Heart rates (HR) were similar between sexes throughout.

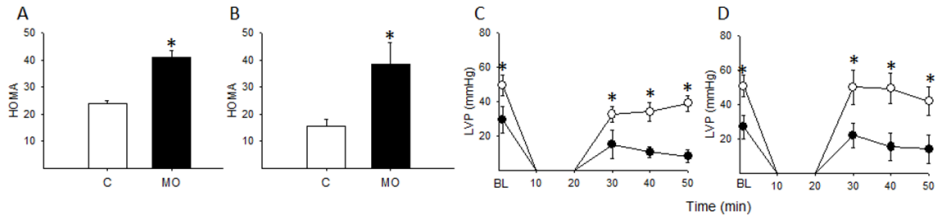

Figure 1. HOMA index in F1 male (A) and female (B); LVP in F1 male (C) and female (D); Mean ± SEM, n=5-8. C (open circles), MO (closed circles); *MO vs C, p<0.05.

Conclusions: MO hearts recovered worse after reperfusion in both sexes. MO showed generalized insulin resistance which impairs cardiac function. These outcomes fit with the principle that aging effects in many physiological systems are absent at rest only emerging when challenged. Increased local fat may decrease cardiac function.

Funding: CONACyT – RCUK.

Reference. 1. The lancet. *Diabetes & endocrinology* 2017; 5(1): 53-64.

SECOND FLOOR