44th American Aging Association Meeting

May 29 through June 1, 2015

Marina Del Rey, CA

Aging and Geroscience:

New Approaches to Old Problems

SCHEDULE AT A GLANCE

FRIDAY *MAY 29, 2015* *PRE-MEETING SYMPOSIUM*	***SIERRA I & II*** **8:00 AM – 8:05 AM** OPENING REMARKS **8:05 AM – 9:30 AM** PRE- AND POSTNATAL INFLUENCES ON AGING **9:50 AM – 11:30 AM** AGING PROCESSES THAT UNDERLIE THE EXPONENTIAL INCREASE IN CANCER AND CARDIOVASCULAR DISEASE **11:30 AM – 1:00 PM** LUNCH (BAYVIEW BALLROOM) **1:00 PM – 2:30 PM** DISEASE PROCESSES THAT PROMOTE ACCELERATED AGING **2:30 PM** PRE-CONFERENCE ADJOURNS

FRIDAY *MAY 29, 2015*	***BAYVIEW BALLROOM*** **6:30 PM – 7:45 PM** PRESIDENT'S WELCOME AND PANEL DISCUSSION **8:00 PM – 9:30 PM** OPENING RECEPTION
SATURDAY *MAY 30, 2015* *AGING AND GEROSCIENCE: NEW APPROACHES TO OLD PROBLEMS*	***SIERRA I & II*** **8:00 AM – 9:15 AM** INTRODUCTION AND KEYNOTE ADDRESS **9:15 AM – 10:35 AM** LENGTHENED HEALTHSPAN: A NECESSARY PARTNER OF LENGTHENED LIFESPAN? **10:50 AM – 12:10 PM** FRAILTY – FROM HUMANS TO MICE: NEW AND OLD – MODELS AND METRICS **12:10 PM – 1:30 PM** LUNCH ON YOUR OWN **1:30 PM – 2:45 PM** EMERGING MOLECULAR CONCEPTS IN LONGEVITY DETERMINATION **3:00 PM – 4:40 PM** ***SIERRA I & II*** OXIDATIVE STRESS AND AGING: FRIEND OR FOE ***PROMENADE ROOM*** ORAL PRESENTATIONS OF SELECTED ABSTRACTS **4:45 PM – 7:00 PM** POSTER SESSION I AND RECEPTION **7:00 PM – 10:00 PM** STUDENT DATA BLITZ (PROMENADE ROOM)

SUNDAY *MAY 31, 2015* *AGING AND GEROSCIENCE: NEW APPROACHES TO OLD PROBLEMS*	***SIERRA I & II*** **9:00 AM – 10:20 AM** CELL NON-AUTONOMOUS PROCESSES IN CALORIE RESTRICTION AND AGING **10:35 AM – 11:50 AM** GENETICS OF AGING: NEW MODELS TO MAP COMPLEX TRAITS **12:00 PM – 1:30 PM** ***BAYVIEW BALLROOM*** ***LUNCHEON AND DENHAM HARMAN AWARD LECTURE*** ***SIERRA I & II*** **1:45 PM – 3:20 PM** SEX DIFFERENCES IN AGING AND RESPONSES TO INTERVENTIONS: PROBING THE UNDERLYING MECHANISMS **3:30 PM – 4:30 PM** KORENCHEVSKY SPEAKER, SPONSORED BY BRITISH SOCIETY FOR RESEARCH ON AGEING **4:30 PM – 5:15 PM** AGE GENERAL MEMBERSHIP MEETING **5:30 PM – 7:30 PM** POSTER SESSION II AND RECEPTION
MONDAY *JUNE 1, 2015* *AGING AND GEROSCIENCE: NEW APPROACHES TO OLD PROBLEMS*	***SIERRA I & II*** **8:00 AM – 9:45 AM** PHARMACOLOGIC INTERVENTIONS TARGETING AGING PROCESSES: WORMS TO HUMANS **10:00 AM – 11:35 PM** ***SIERRA I & II*** NATHAN SHOCK CENTER SYMPOSIUM ***PROMENADE ROOM*** ORAL PRESENTATIONS OF SELECTED ABSTRACTS **11:45 PM – 12:30 PM** MARK SMITH LECTURE **12:30 PM – 1:30 PM** AWARDS CEREMONY: STUDENT AWARDS AND CLOSE OF MEETING

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 extramural 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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For 32 years, the American Federation for Aging Research (AFAR) has been at the forefront of this revolutionary approach to the science of healthier aging. AFAR has played a major role in providing and advancing knowledge of aging and mechanisms of age-related disease by providing grants totaling more than $132 million in support of researchers in aging and to encourage the training of new scientists and physicians. To learn more about AFAR, visit our website www.afar.org. We also invite you to visit our web site InfoAging.org for the latest information on the biology of aging, common diseases of aging and healthy lifestyles.

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BSRA British Society for Research on Ageing

The British Society for Research on Ageing

The British Society for Research on Ageing (BSRA) promotes research to understand the causes and effects of the ageing process. BSRA encourages publication and public understanding of ageing research, publishes its own journal, **Lifespan**, a monthly electronic newsletter, and holds an annual scientific meeting.

www.bsra.org.uk/index.html

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The Walt Disney World Swan and Dolphin will host GSA's Annual Scientific Meeting this November. Known for its prime location, the hotel is conveniently located within walking distance of two theme parks, more than 40 restaurants, Disney's BoardWalk, and much more. Expect to participate in over 400 scientific sessions including symposia, paper, and poster presentations in this award-winning hotel.

Registration and housing open June 2015.
For more information, visit geron.org/2015.

THE GERONTOLOGICAL SOCIETY OF AMERICA®

ANNENBERG CENTER FOR HEALTH SCIENCES AT EISENHOWER

This Continuing Educational activity is jointly provided by The Annenberg Center for Health Sciences at Eisenhower and The Gerontological Society of America.

Friday, May 29, 2015 Pre-Meeting

Marina del Rey Marriott Hotel
Location: Sierra I & II

"The Geroscience Initiative: Challenges and New Perspectives"

Organizers: Drs. J. Nelson, UTHSCSA, and Felipe Sierra, NIA

7:30 AM - 8:00 AM Breakfast (Foyer)

Session I. Pre- and Postnatal influences on aging

Chairs: Peter Nathanielsz and James Nelson

8:00 AM - 8:05 AM Felipe Sierra, NIA **Opening remarks**

8:05 AM - 8:10 AM 1. Peter Nathanielsz, University of Wyoming, Laramie, and The Southwest National Primate Research Center, San Antonio: **Introduction to Session I**

8:10 AM - 8:30 AM 2. Elena Zambrano, Instituto Nacional de Ciencias Médicas y Nutrición: **Developmental Programming by Maternal Obesity Accelerates Offspring Aging**

8:30 AM - 8:50 AM 3. Peter Nathanielsz, University of Wyoming, Laramie, and The Southwest National Primate Research Center, San Antonio: **Exposure to Glucocorticoid Levels Higher Than Those Appropriate for Current Perinatal Development Play a Role in the Aging Trajectory**

8:50 AM - 9:10 AM 4. Todd Morgan, Univ. of Southern Calif.: **Prenatal exposure to traffic-related air pollution impacts brain development**

9:10 AM - 9:30 AM Panel Q & A: **Morgan, Nathanielsz, and Zambrano**

9:30 AM - 9:50 AM Coffee Break

Session II. Aging processes that underlie the exponential increase in cancer and cardiovascular disease

9:50 AM - 10:10 AM 5. Steve Horvath, UCLA: **The Epigenetic Clock, Biological Age & Cancer**

10:10 AM - 10:30 AM 6. Luzhe Sun, UTHSC San Antonio: **Dysfunctional Aging Murine Mammary Stem Cells**

10:30 AM - 10:50 AM 7. Roberta Gottlieb, Cedars Sinai Medical Center: **Mitophagy and Biogenesis in the Heart**

10:50 AM - 11:10 AM 8. Mark Entman, Baylor College of Medicine: **Mesenchymal Stem Cell Dysfunction in Aging Heart Disease**

11:10 AM - 11:30 AM Panel Q&A: **Horvath, Sun, Gottlieb, and Entman**

11:30 AM - 1:00 PM Lunch (Bayview Ballroom)

Session III. Disease processes that promote accelerated aging

Chair: Felipe Sierra

1:00 PM - 1:20 PM 9. Caterina Rosano, Univ. of Pittsburgh: **Accelerated Brain Aging in Middle-Aged Patients with Type 1 Diabetes: Neurocognitive and Physical Functional Manifestations**

1:20 PM - 1:40 PM 10. Keri Althoff, Johns Hopkins: **Evidence for and Against HIV Infection Promoting Accelerated Aging**

1:40 PM - 2:00 PM 11. William Murphy, UC Davis: **Impact of Aging and Obesity on Inflammatory Responses following Immunotherapy**

2:00 PM - 2:30 PM Panel Q&A: **Rosano, Althoff, and Murphy**

PRE-CONFERENCE ADJOURNS

3:00 pm – 6:00 pm **AAA Conference Registration**

Friday May 29, 2015 Meeting (MARINA DEL REY MARRIOTT HOTEL)

Aging and Geroscience: New Approaches to Old Problems

Chair: James Nelson, PhD

University of Texas Health Science Center at San Antonio

6:30 PM - 7:45 PM President's Welcome Reception Panel (Bayview Ballroom). Introduction, Panel Discussion: **The Path Ahead: Perspectives on Developing Research Programs to Promote Healthy Aging**

Chair: **Brian Kennedy**, Buck Institute; Panelists: **Pinchas Cohen**, USC; **Rita Effros**, UCLA; **James Kirkland**, The Mayo Clinic; **Nicolas Musi**, Barshop Institute, San Antonio; **Nir Barzilai**, Einstein

8:00 PM - 9:30 PM Opening Reception

Saturday May 30, 2015 Meeting (MARINA DEL REY MARRIOTT HOTEL)

7:30 AM - 8:00 AM Breakfast (Foyer)

8:00 AM - 8:05 AM Welcome Address (Sierra I & II)

8:05 AM - 8:15 AM Caleb Finch, Davis School of Gerontology, USC: **Introduction**

8:15 AM - 9:15 AM 12. Eileen Crimmens, Davis School of Gerontology, USC: **Keynote: Demographics of Aging—Genetic and Environmental Factors Influencing Healthspan and Lifespan of Human Populations**

Session 1: Lengthened Healthspan: A Necessary Partner of Lengthened Lifespan?

Chairs: Heidi Tissenbaum and Victoria Gibbs

9:15 AM - 9:35 AM 13. Heidi Tissenbaum, University of Massachusetts: **Dysynchrony of Healthspan & Lifespan in Long-lived Worm Mutants**

9:35 AM - 9:55 AM 14. Zachary Pincus, Washington Univ., St Louis: **Lifespan, Health-span, and the Rate of Aging in *C. elegans***

9:55 AM - 10:15 AM 15. Hiram Beltrán-Sánchez, Univ. of Wis. Madison: **Demographics of Lifespan and Healthspan of Human Populations**

10:15 AM - 10:35 AM Panel Q & A: **Tissenbaum, Pincus, and Beltrán-Sánchez**

10:35 AM - 10:50 AM Coffee Break

Session 2: Frailty—From Humans to Mice: New and Old--Models & Metrics

Chairs: Susan Howlett and Marjana Sarker

10:50 AM - 11:10 AM 16. Kenneth Rockwood, Dalhousie University: **Measuring Frailty Based on Deficit Accumulation**

11:10 AM - 11:30 AM 17. Susan Howlett, Dalhousie University: **The Rockwood Frailty Index applied to aging mice**

11:30 AM - 11:50 AM 18. LaDora Thompson, University of Minnesota: **The Fried Index Applied to Aging Mice**

11:50 PM - 12:10 PM Panel Q & A: **Rockwood, Howlett, and Thompson**

12:10 PM - 1:30 PM Lunch on your own

12:10 PM - 1:30 PM AGE Journal Editorial Board Meeting (Executive Dining Room)

Session 3: Emerging Molecular Concepts in Longevity Determination

Chairs: Malene Hansen and Laura Corrales-Diaz Pomatto

1:30 PM - 1:50 PM 19. Malene Hansen, Sanford Burnham Medical Research Institute: **Understanding TOR-regulated Processes in *C. elegans* Aging**

1:50 PM - 2:10 PM 20. Pankaj Kapahi, The Buck Institute: **Peripheral Molecular Clocks as Determinants of CR-mediated Longevity Extension**

2:10 PM - 2:30 PM 21. Girish Melkani, San Diego State Univ.: **Time-restricted Feeding Attenuates Age-related Cardiac Decline and Maintains Healthspan**

2:30 PM - 2:45 PM Panel Q & A: **Hansen, Kapahi, and Melkani**

2:45 PM - 3:00 PM Coffee Break

Session 4A: Oxidative Stress and Aging: Friend or Foe (Sierra I & II)

Chairs: Yuji Ikeno and Nicholas Thomas

3:00 PM - 3:05 PM Yuji Ikeno, Barshop Institute, San Antonio: **Introductory Remarks**

3:05 PM - 3:25 PM 22. Ting-Ting Huang, Stanford University: **Role of Oxidative Stress and Redox Regulation on Cognitive Function**

3:25 PM - 3:45 PM 23. Warren C. Ladiges, U. Washington: **Mitochondrial Catalase Mediates Anti-tumor Activity Through Stromal Fibroblasts**

3:45 PM - 4:05 PM 24. Yuji Ikeno, Barshop Institute: **Mechanisms that Extend Lifespan in Sprague-Dawley Rats Overexpressing Cu/ZnSOD**

4:05 PM - 4:25 PM 25. William C. Orr, Southern Methodist Univ.: **Oxidative Stress to Redox Stress, The Evolution of an Aging Hypothesis**

4:25 PM - 4:40 PM Panel Q & A: **Huang, Ladiges, Ikeno, and Orr**

Session 4B: Oral presentations from selected abstracts (Promenade)

Chair: Rochelle Buffenstein

3:00 PM - 3:15 PM 13. Dudley Lamming: **An intermittent treatment regimen mitigates deleterious side effects of rapamycin**

3:15 PM - 3:30 PM 47. Rachel Raynes: **The aging-related decline of 20S proteasome adaptation to oxidative stress is dependent on SKN-1 signaling**

3:30 PM - 3:45 PM 84. Kristin Gribble: **Intraspecific diversity in genetically-mediated lifespan**

	extension under reduced temperature
3:45 PM - 4:00 PM	90. John Lighton: **The Skeptical Researcher's Metabolic and Behavioral Measurement Toolbox**
4:00 PM - 4:15 PM	60. Young Jang: **Restoration of Systemic Growth and Differentiation Factor-11 (GDF11) Levels Reverses Age-Associated Dysfunction in Skeletal Muscle**
4:45 PM - 7:00 PM	Poster Session I and Reception (Pacific I II and III)
7:00 PM - 10:00 PM	Student Data Blitz (Promenade Ballroom): Bita Nakhai, NIH/NIA: Preparing Your Grant Application (Writing Tips and Addressing Reviewers)
7:00 PM - 9:00 PM	AGE Board of Directors Meeting (Executive Dining Room)

Sunday May 31, 2015 Meeting (MARINA DEL REY MARRIOTT HOTEL)

8:00 AM - 9:00 AM Continental breakfast (Foyer)

Session 5: Cell Non-Autonomous Processes in Calorie Restriction and Aging

Chairs: Isao Shimokawa and Marta Valcarcel-Ares

9:00 AM - 9:20 AM 26. Shin-ichiro Imai, Washington University School of Medicine, St. Louis: **Fat Helps the Brain in the NAD World: The Inter-Tissue Communication Mediated by NAMPT/NAD+/SIRT1 in Mammalian Aging/Longevity Control**

9:20 AM - 9:40 AM 27. Isao Shimokawa, University of Nagasaki Medical School: **Hypothalamic NPY is Essential for CR Effects on Lifespan and Healthspan in Mice**

9:40 AM - 10:00AM 28. Matt Kaeberlein, U. Washington: **HIF-1 Promotes Longevity Through A Cell Non-Autonomous Mechanism**

10:00 AM - 10:20 AM Panel Q & A: **Imai, Shimokawa, and Kaeberlein**

10:20 AM - 10:35 AM Coffee Break

Session 6: Genetics of Aging: New Models to Map Complex Traits

Chairs: Robert Williams and Andrew Pickering

10:35 AM - 10:55 AM 29. Gary Churchill, The Jackson Laboratory: **Aging Interventions in Genetically Diverse Mice**

10:55 AM - 11:15 AM 30. Robert Williams, University of Tennessee: **Systems Genetics of Lifespan and the Impact of a High Fat Diet**

11:15 AM - 11:35 AM 31. Cheryl Ackert-Bicknell, Univ. of Rochester: **Aging, Bone and Genetics: Lessons Learned from Inbred and Outbred Mice**

11:35 AM - 11:50 AM Panel Q & A: **Churchill, Williams, and Ackert-Bicknell**

12:00 PM – 1:30 PM Denham Harman Award Lecture and Luncheon (Bayview Ballroom): Introduction by Nathalie Sumien, Univ. of North Texas Health Science Center for award to **Michael Forster, Univ. of North Texas Health Science Center**

Session 7: Sex Differences in Aging and Response to Interventions: Probing the Underlying Mechanisms

Chairs: John Tower and Rashmi Singh

1:45 PM - 2:05 PM 32. John Tower, University of Southern California: **Sex-dimorphic Stress Responses and Aging in Drosophila**

2:05 PM- 2:25 PM 33. Scott Pletcher, University of Michigan: **The Dangers of Social Networking: Shared Mechanisms of Aging and Sex Appeal in the Fly**

2:25 PM -2:45 PM 34. Rafael de Cabo, NIA-Baltimore: **Profound Sex Differences in Response to CR in Inbred and F1 Hybrid Mice**

2:45 PM - 3:05 PM 35. Christian Pike, University of Southern California: **Sex Differences and Sex Steroid Hormones in Alzheimer's Disease**

3:05 PM - 3:20 PM Panel Q & A: **Tower, Pletcher, de Cabo, and Pike**

3:20 PM - 3:30 PM	Coffee Break
	Session 8: Korenchevsky speaker (sponsored by British Society for Research on Ageing)
	Chair: Richard Farragher
3:30 PM - 4:30 PM	36. Kasia Whysall, PhD, Institute of Aging & Chronic Disease, University of Liverpool: **Do MicroRNAs Regulate Sarcopenia Development?**
4:30 PM - 5:15 PM	AGE General Membership Meeting (Sierra I and II)
5:30 PM - 7:30 PM	Poster Session II and Reception (Pacific I II and III)

Monday, June 1, 2015 Meeting (MARINA DEL REY MARRIOTT HOTEL)

7:00 AM - 8:00 AM Continental breakfast (Foyer)

Session 9: Pharmacologic Interventions Targeting Aging Processes: Worms to Humans

Chairs: Randy Strong and Ellen Quarles

8:00 AM - 8:05 AM Opening comments

8:05 AM - 8:25 AM 37. Randy Strong, Barshop Institute, UTHSCSA: **The Mouse Interventions Testing Program: An Update**

8:25 AM - 8:45 AM 38. Gordon Lithgow, Buck Institute: **The Caenorhabditis Intervention Testing Program and Other Invertibrate Screening Approaches**

8:45 AM - 9:05 AM 39. Hazel Szeto, Cornell Medical College: **Mitochondrial Bioenergetics and Aging – A Novel Target for Intervention**

9:05 AM - 9:25 AM 40. Nicolaas E Deutz, Texas A & M: **Obtaining Protein Anabolism in Older Adults with Chronic Disease and Cancer. Metabolic Kinetic Phenotyping in Older Adults**

9:25 AM - 9:45 AM Panel Q & A: **Strong, Lithgow, Szeto, and Deutz**

9:45 AM - 10:00 AM Coffee Break

Session 10A: Nathan Shock Center Symposium (sponsored by Nathan Shock Centers) (Sierra I & II)

Chair: Felipe Sierra

10:00 AM - 10:05 AM Felipe Sierra, NIA: **Introduction**

10:05 AM - 10:20 AM 41. Adam Salmon, Barshop Institute, UTHSCSA: **Co-treatment with Metformin Improves Metabolic Defects Caused by Rapamycin**

10:20 AM - 10:35 AM 42. Fernando Macian, Einstein College of Medicine, New York: **Age-associated Decline of Different forms of Autophagy Underlie T Cell Immunosenescence**

10:35 AM - 10:50 AM 43. Carissa Perez Olsen, Univ. of Washington: **Mechanisms that protect the lipid membrane and how they influence aging**

10:50 AM - 11:05 AM 44. Luanne Peters, The Jackson Labs: **Clinical Phenotyping and Lifespan QTL Studies in Diversity Outbred Mice**

11:05 AM - 11:20 AM 45. Keyt Fischer, U. of Alabama, Birmingham: **Comparative Bioenergetics of Aging**

11:20 AM - 11:35 AM 46. Holly Van Remmen, Oklahoma Medical Research Foundation: **New Tools for Aging Research: An Overview of the Oklahoma Nathan Shock Center**

Session 10B: Oral presentations from selected abstracts (Promenade)

Chair: Matt Kaeberlein

10:00 AM - 10:15 AM 5. Shauna Hill: **Sco2 Deficient Mice Develop Increased Adiposity and Insulin Resistance**

10:15 AM - 10:30 AM 81. Keiva Gilmore: **Correlating cellular proliferation with age, size, and longevity**

10:30 AM - 10:45 AM 86. Victoria Gibbs: **Effect of altered day length on measures of healthspan in male C57BL/6 mice**

10:45 AM - 11:00 AM 11. Ying Chiao: **Rapamycin induces rapid mitochondrial remodeling to rejuvenate energy metabolism and energetics in old hearts**

11:00 AM - 11:15 AM 59. Veronica Galvan: **Non-cell Autonomous Control of Metabolism and Aging by Neuronal mTOR Signaling**

Session 11: Special Lecture - Mark Smith Lecture (selected by Awards Committee Spring 2015)

11:45 AM - 12:30 PM 47. Xiongwei Zhu, Case Western Reserve University: **Abnormal Mitochondrial Dynamics in the Pathogenesis of Alzheimer's Disease**

12:30 PM - 1:30 PM Awards Ceremony: Student Awards and Close of Meeting

1:30 pm **MEETING ADJOURNS**

Speakers' Abstracts

1. Symposium Overview

Peter Nathanielsz MD, Ph.D, Sc.D, FRCOG

Distinguished Professor of Life Course Health,

University of Wyoming.

The concept of developmental programming is now well established as a result of human epidemiological studies such as those of offspring of the Dutch Hunger Winter. In the last ten years or so a compelling body of carefully controlled animal studies in several species has shown that decreased and increased fetal nutrition can program function in multiple offspring organ systems and predispose to chronic diseases and premature aging.. Developmental Programming can be defined as ***Responses to challenges to developing organisms during a critical time window in fetal and neonatal life that can alter the trajectory of development and predispose to conditions which emerge later,*** are now well established. We suggest that it follows that programming can also alter the rate of aging, both beneficially and adversely. A metaphor that can be used is that if the automobile is built with substandard materials and functional parts it will not travel for as fast, as far or as long.

To understand developmental programming it is necessary to determine 1) the exact nature of the challenges to the fetus and newborn, 2) the precise changes in phenotype produced and 3) cellular and molecular gene-environment mechanisms involved.

Several distinct challenges have been identified: maternal under- and over nutrition, maternal stress, excessive perinatal glucocorticoid exposure, and environmental pollutants. Phenotype changes in affected individuals can be due to mechanism as the failure to grow enough cells in a particular organ e.g. renal glomeruli leading to renal disease and hypertension in later life, or specific epigenetic changes that can predispose to emergence of disease in later life e.g. increased expression of the key hepatic gluconeogenic enzyme PEPCK with subsequent predisposition to diabetes. One important metabolic process that is receiving major attention is the influence of the various challenges on the level of oxidative stress during both development and later life.

One well established feature of developmental programming is that the changes that occur as a result of various challenges may lay dormant to emerge later in life when exposed to a "second hit." The second hit may reflect normal development – e.g. puberty, or an environmental challenge – e.g. stress.

This session will provide examples of studies in rodents and nonhuman primates using controlled experimental conditions that provide firm evidence on mechanisms by which aging is affected by developmental programming. This information identifies mechanisms that point the way to future studies to determine mechanisms of programming of reduced health span and development of diagnostic markers and therapeutic interventions in relation to aging.

2. “*Developmental programming by maternal obesity accelerates offspring aging”*

Elena Zambrano,Ph.D

Instituto Nacional Ciencias Medicas y Nutricion SZ, Mexico City.

The Developmental Programming hypothesis states that challenges in critical developmental time windows alter development with persistent effects on offspring phenotype. To date the focus has been on developmental programming of life-time occurrence of non communicable diseases. Few studies address programming of aging. Many aging processes have been shown to be related to increased oxidative stress. We and others have shown that several developmental programming challenges increase oxidative stress across the life span. This presentation reports studies in a well-established model of maternal obesity in which female Wistar rats were fed from weaning through lactation on either chow (C) or high energy, obesogenic diet to induce maternal obesity. Mothers were bred at postnatal day (PND) 120 and ate their pregnancy diet – either C or high energy diet until weaning. Offspring ate C diet from weaning and were euthanized at 110, 450 and 650 PND. Serum, liver and adipose tissue were obtained. Aging related programming in offspring liver and adipose tissue will be reported and related to oxidative stress.

Compared with offspring of control mothers, male and female offspring of obese mothers, body weight, adiposity index, serum leptin, triglycerides, insulin and malondialdehyde (MDA) increased earlier as well as liver fat, MDA, reactive oxygen species, reactive nitrogen species as indicated by nitrotyrosine immunohistochemistry, while liver superoxide dismutase activity decreased. These programming effects showed offspring gender specific outcomes.

One potential mechanism by which glucocorticoid accelerate aging is by increasing reactive oxygen species (ROS). We tested the potential interaction between glucocorticoids and ROS in our maternal obesity model by treating obese mothers with Resveratrol from 30 days before breeding to delivery. Resveratrol prevented at least in part the metabolic and hormonal changes produced in the offspring of the MO mothers including the elevated corticosterone and the shortening of life-span.

Conclusion: Aging related changes in antioxidant systems and oxidative stress are accelerated by exposure to maternal obesity during fetal and neonatal life in a sex specific manner.

3*. Exposure to glucocorticoids levels higher than those appropriate for current perinatal development play a role in the aging trajectory,”*

Peter Nathanielsz MD, Ph.D, Sc.D, FRCOG

Distinguished Professor of Life Course Health,

University of Wyoming.

Data from the Dutch Hunger Winter and other human studies show that poor maternal nutrition and other challenges in development decrease health span and shorten life. This presentation addresses the role of life course glucocorticoid exposures from fetal life and through the aging

process. Glucocorticoids regulate multiple cell types and play critical roles in physiological systems that change across the life course. Although glucocorticoids have previously been associated with aging, available data on the aging trajectory in basal circulating glucocorticoids are conflicting. Some reports indicate a rise in glucocorticoids in later life while others suggest a fall. The reason may be that data are sparse covering only limited periods of the total life course in any one study. It is necessary to evaluate effects of developmental programming on the trajectory of aging. Developmental programming can be defined as the response to a specific challenge in a critical developmental time window that alters development with persistent effects on phenotype resulting from metabolic and gene-environment interactions. These epigenetic changes can predispose to multiple organ dysfunction and adversely affect life course health span and aging trajectory.

We evaluated the profile of basal circulating corticosterone across the life course from late early life postnatal day (PND) 2, weaning (PND 21), and in young adult (PND 110), adult (PND 450), aging adult (PND 650) and aged (PND 850) offspring of control and over nourished, obese mothers. Maternal obesity is known to shorten offspring life. Using this full life course six-point data set, male and female offspring of obese mothers had a shorter life span and higher corticosterone levels. Circulating corticosterone was higher in male and female offspring of obese mothers from PND 110 to 650 and fell between PND 400 and 650 in both sexes in offspring of control and obese mothers. We conclude **that** higher corticosterone in offspring of obese mothers, even during life, may play a role in their shorter life-span but age-associated falls occur at a similar time to control offspring. While a six life-course time-point analysis provides important new information on normative and programmed aging of circulating corticosterone even more life-course time-points would are needed for a full picture.

In a nonhuman primate study, we fed pregnant baboons 70% of the global diet of ad libitum fed mothers, offspring are growth restricted at birth (IUGR). IUGR foetuses have elevated cortisol levels. In an MRI study of control and IUGR baboon offspring across the life course (4 to 20 years of age – human equivalent 16 to 80) we showed age and IUGR related alterations in cardiac function that support the hypothesis that programming accelerates the processes leading to diastolic dysfunction associated with cardiac aging.

4. Todd Morgan, Univ. of Southern Calif. **Prenatal exposure to traffic-related air pollution impacts brain development**

Emerging evidence shows that exposure to traffic-derived air pollution during pregnancy and/or early development can increase the vulnerability to neurodevelopmental alterations with consequences to adult brain health. In particular, pre- and postnatal exposure to urban traffic-derived air pollution is associated with higher risk of autism spectrum disorders, of attention-deficit hyperactivity disorder, of anxiety and depression, of schizophrenia, and of impaired cognitive development. Several labs have developed different rodent models studying the developmental effects of traffic-derived air pollution exposure. Our lab developed a model for mouse exposure to re-aerosolized urban freeway nanoparticulate matter (nPM), a sub-fraction of traffic-derived air pollution, under controlled dose and duration. Several studies examined adult C57BL/6 mice that were exposed at different times of development, including prenatal only, pre- and postnatal plus young adult, or young adult only. Selective behavioral and cellular changes occur in response to nPM exposure in our model, with sex differences: adult male mice exposed to nPM prenatally demonstrated anxiety-like, or depressive behaviors, whereas females did not. Other models confirm

greater male vulnerability to traffic-derived air pollution exposure, especially when combined with a second insult (stress, high fat diets). In our model, adult only exposure has not shown any consistent behavioral effect suggesting that nPM exposure during critical periods of neurodevelopment may have the strongest detrimental outcomes.

5: **Epigenetic clock and biological age**

Steve Horvath, Professor of Human Genetics and Biostatistics, University of California, Los Angeles.

The DNA methylation based biomarker of aging known as the "epigenetic clock" can be used to measure the DNA methylation (DNAm) age of any human (or chimpanzee) tissue, cell type, or fluid that contains DNA with the exception of sperm. DNAm age of blood has been shown to predict all-cause mortality in later life, even after adjusting for known risk factors, which suggests that it relates to the biological aging process. Similarly, markers of physical and mental fitness are also found to be associated with the epigenetic clock (lower abilities associated with age acceleration).

DNA methylation age has the following properties: first, it is close to zero for embryonic and induced pluripotent stem cells; second, it correlates with cell passage number; third, it gives rise to a highly heritable measure of age acceleration; and, fourth, it is applicable to chimpanzee tissues.

Analysis of 6,000 cancer samples from 32 datasets showed that cancer types exhibit significant positive and negative age acceleration. Low age-acceleration of cancer tissue is associated with a high number of somatic mutations and TP53 mutations, while mutations in steroid receptors greatly accelerate DNA methylation age in breast cancer.

I illustrate the utility of this novel biomarker of aging by studying obesity, HIV infection, syndrome X, and supercentenarians.

These results suggest that we are close to achieving a long standing milestone in aging research: the development of an accurate measure of tissue age or even biological age.

Relevant reference: Article: Horvath S (2013) DNA methylation age of human tissues and cell types. Genome Biology 2013, 14:R115 Correction: http://genomebiology.com/2013/14/10/R115/comments Wikipedia: https://en.wikipedia.org/wiki/Biological_clock_(aging)

6. **Dysfunctional Aging Murine Mammary Stem Cells**

Lu-Zhe Sun[1], Qiaoxiang Dong[1,4], Hui Gao[1,4], Danhan Wang[4], Anqi Wu[4], Xiang Gu[1], Abhik Bandyopadhyay[1], Yuanshuo Shi[4], Carolina B. Livi[2], Z. Dave Sharp[2], Christi A. Walter[1], Vivienne Rebel[1], Randy Strong[3], Changjiang Huang[4]

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The number one risk fact for breast cancer is aging. Recent research implicated that adult mammary stem cells (MaSCs) might be responsible for the initiation and progression of certain types of breast cancer. In this study, MaSC-enriched basal cells were utilized for the evaluation of MaSC frequency and function during aging by in vitro mammosphere formation and 3D-ECM sphere differentiation assays and by in vivo cleared mammary fat pad transplantation (IVT) as we reported recently (Dong et al., *Stem Cell Res*. 10:396-404, 2013). We found that the basal-to-luminal cell ratios analyzed with flow cytometry and the frequency of MaSCs analyzed with the *in vitro* assays increased steadily with increasing age in various strains of mice. Subsequent IVT using mammospheres or 3D-ECM structures formed by young (2-4 months) or old (25-32 months) MaSCs derived from C57BL/6 mice showed that the regenerated glands from old MaSCs had significantly higher number of spontaneous atypical ductal hyperplastic lesions than those from young MaSCs. These findings indicate that aged MaSCs can serve as the cell of origin for early neoplastic transformation in breast tissue. Subsequent whole genome transcriptome analysis with the second generation sequencing revealed age-associated differential expression of genes involved in immune, inflammatory, and wounding responses in both mammosphere-forming cells and stromal cells suggesting that these may be the main cellular processes contributing to the dysfunctional MaSC phenotypes. Consistently, treatment of old C57BL/6 mice rapamycin, an anti-inflammation drug, showed that short-term (5-10 days) or long-term (> 2 year) treatment reversed phenotypic changes associated with aged mammary gland. Histological analysis of regenerated glands by aged MaSCs derived from control and rapamycin-treated mice showed a significant decrease of early neoplastic lesions in rapamycin-treated group. Similar results were observed in aged mice with diet restriction. In conclusion, our findings suggest that aging causes MaSC to form early neoplastic lesions, which can be inhibited by rapamycin treatment or diet restriction.

7. Mitophagy and Biogenesis in the Heart

Roberta A. Gottlieb, Phyllis-Jean Linton, Allen Andres, David Sengstock, Salik Jahania, and Robert M. Mentzer, Jr.

The heart possesses endogenous mechanisms for protecting itself from ischemic stress and reperfusion injury. We have shown that autophagy is essential for ischemic preconditioning; in the human heart, we have shown that autophagy is briskly upregulated during cardiac stress induced by heart surgery. The magnitude of this response is inversely associated with surgical risk using the STS Database. Preclinical studies have shown that autophagic flux is downregulated with aging albeit we have some evidence that older patients undergoing heart surgery are capable of mounting a robust autophagic response to cardiac stress. Likewise, mitophagy is important for the cardioprotective effects of ischemic preconditioning and statin therapy, and in the response to pressure overload, in which impaired mtDNA degradation is associated with inflammation and pathological remodeling. Chronic inflammation may arise from mtDNA from damaged mitochondria that accumulate due to age-related reduction in mitophagy. Using cells and animal models we have shown that mitophagy in the heart is mediated by Parkin, a ubiquitin ligase which also regulates Paris, a factor that represses transcription of PGC1alpha, a key driver of mitochondrial biogenesis. We have demonstrated a tight regulatory linkage between mitophagy and biogenesis in the heart involving Parkin and Paris. In a limited study of 10 patients undergoing heart surgery with cold cardioplegia, we find evidence for both mitophagy and biogenesis in response to the ischemic stress. We suggest that both mitophagy and biogenesis are part of the homeostatic intracellular repair response (HIR^2) of the human heart.

8. **Mesenchymal Stem Cell Dysfunction in Aging Heart Disease: A Tale of Two Fibroblasts**
Mark L. Entman, M.D., Baylor College of Medicine, Houston, Texas

A greater population of aging patients has given rise to two cardiac syndromes that result in increased morbidity and hospitalization: 1) patients with normal ejection fraction develop shortness of breath and reduced exercise capacity and impaired cardiac filling and 2) patients with myocardial infarction survive without incident but, over the next several years, develop progressive adverse remodeling as a result of defective scar formation. My presentation describes work in mice suggesting that both of these syndromes arise from dysfunctional resident mesenchymal stem cells with markedly reduced responsiveness to TGFβ.

1) In normal aging mice, this results in increased differentiation of mesenchymal stem cells into fibroblasts which secrete collagen and also produce inflammatory mediators (inflammatory fibroblasts) and further promote fibrosis by attracting macrophages and facilitate their differentiation into myeloid fibroblasts. The genesis of these abnormalities will be discussed and potential therapeutic implications will be discussed.

2) In myocardial infarction, evidence will be presented that the fibroblasts arising from mesenchymal stem cells make adequate fibroblasts and collagen but, because of TGFβ resistance, produce inadequate smooth muscle actin and cannot crosslink and contract the scar.

Potential therapeutic approaches of these models will be presented.

9. **Accelerated Brain Aging in Middle-Aged Patients with Type 1 Diabetes: Neurocognitive and Physical Functional Manifestations.**

Caterina Rosano, MPH, MD

With increasing incidence and survival rates in T1D, the number of patients who is aging is also rapidly increasing. As people with diabetes grow older, they continue to be exposed to chronic hyperglycemia, and experience other medical conditions that accompany very long duration diabetes as well as the normal aging process. A striking feature of people with T1D is that they develop brain abnormalities similar to those observed in much older adults without diabetes. Features of accelerated brain aging include psychomotor slowing, brain small vessel disease and atrophy. These brain abnormalities are known to increase the probability of developing disability and dementia in population without diabetes; therefore, they cannot be considered benign. There is an urgent need to quantify the impact of these abnormalities on measures of cognitive and physical health, including clinically relevant cognitive impairment, physical performance, falls and disability. We review here the findings of our neuroimaging, cognitive and mobility study in a cohort of 109 patients with childhood-onset T1D, with retrospective data on microvascular complications (eye, kidney, nerve) since time of diagnosis in 1989 through 2006-08 (mean age of onset 8.6$\pm$4.2 years, current age 47.6 $\pm$5.9, 46% women). In 2006-2008 these patients received a neuroimaging test, an extensive cognitive evaluation, and measures of gait speed at usual pace. Self-reported measures of falls and difficulties with instrumental activities of daily living were obtained concurrently.

10. Keri Althoff, PhD, MPH
Assistant Professor

Department of Epidemiology
Johns Hopkins Bloomberg School of Public Health

Evidence for and against HIV infection promoting accelerated aging

Similar to diabetes and other chronic conditions, human immunodeficiency virus (HIV) has been proposed as a mechanism for accelerated, or premature, aging. Inflammation from the chronic viral infection that now characterizes the treated history of HIV has been proposed as one of main mechanisms that could underlie HIV as a disease that accelerates aging. The scientific literature is lacking, however, in convincing evidence that age-related diseases and phenotypes occur at younger ages in HIV-infected compared to similar uninfected adults. Methodologic issues, such as the appropriate comparison group, confounding, and competing risks, plague many studies. The objective of this talk is to present biological, clinical, and phenotypic evidence for and against HIV as a disease promoting accelerated aging.

11. Impact of Aging and Obesity on Inflammatory Responses following Immunotherapy

William J. Murphy - Departments of Dermatology and Internal Medicine, UC Davis School of Medicine, Sacramento, CA

Aging has been associated with both a decline in immune responsiveness as well as presence of chronic inflammatory states. Furthermore, the adaptive immune system shifts markedly from a naive to memory phenotype. Cancer primarily is diagnosed in individuals over the age of 55 years. Immunotherapy has recently garnered much attention with significant efficacy in several cancers. The vast majority of preclinical modeling involves the user of healthy, young, inbred mice housed under specific pathogen free (SPF) conditions. We first assessed the impact of age and obesity on inflammatory biomarkers in healthy human subjects and laboratory mice. We observed that basal levels of inflammatory biomarkers such as serum CRP and SAA were elevated in both obese humans and mice. We therefore assessed the impact of age and obesity on cancer immunotherapy regimen outcome where systemic immunostimulation was applied. Using aged (greater than 16 months) mice, we observed that a variety of immunostimulatory regimens that exhibited anti-tumor efficacy in young mice were rapidly lethal in aged recipients regardless if tumors were present. We observed multi-organ toxicities affecting liver, gut and lung in the aged mice following immunotherapy. Furthermore, this pathology was associated with markedly increased proinflammatory cytokines (ie TNF-alpha and IL6) in the serum and tissues of the aged mice indicating a “cytokine storm”. Similar results were obtained when systemic LPS was administered to the mice simulating an acute infectious episode indicating that aged individuals may be at risk for heightened pathology following infection. Macrophages were the principal source of the cytokines as systemic removal of macrophages abrogated both the pathology and cytokine levels. Blockade of TNF-alpha with Enbril also mitigated the toxicities mitigated mice and allowed for successful administration of immunotherapy indicating that TNF plays a significant role in the cytokine storm-induced pathologies. We then observed that normal aged laboratory mice were significantly obese. We therefore assessed the effects of

immunotherapy in young obese mice and observed that similar although somewhat delayed toxicities and cytokine induction was occurring in both diet-induced obese (DIO) and genetically susceptible (ob/ob) obese mice indicating that adipose tissue is a primary source for the inflammation but aging also contributes to the heightened responses. Caloric restricted (CR) aged mice were significantly protected from the toxicities and cytokine induction confirming the impact of obesity on outcome. Finally, we observed that obese mice had markedly increased pathology following allogeneic hematopoietic stem cell transplantation particularly affecting the gut. These data indicate that both aging and obesity can contribute to heightened proinflammatory cytokine responses following immunostimulatory regimens or systemic LPS challenge. As the vast majority of preclinical modeling uses young laboratory mice, caution must be taken when attempting to extrapolate these results to clinical conditions. Furthermore, despite the extensive species differences between mouse and man, commonalities with regard to impact of age and obesity on proinflammatory cytokine induction can be observed. These results also indicate that blockade of TNF can be used to mitigate these toxicities and allow for immunotherapies to be given.

12. Eileen M. Crimmins

Healthspan and Lifespan in Human Populations

The past century was a period of increasing life expectancy throughout the age range which resulted in more people living to old age and to spending more years at the older ages. In recent decades, there have been some reductions in the prevalence of physical disability and in the last decade a reduction in dementia. At the same time, the prevalence of disease has increased markedly, in large part due to successful treatment of the physiological dysregulation that occurs with aging. We have yet to experience much compression of morbidity as the age of onset of most health problems has not increased markedly. This will require an improvement in “delaying aging” or the physiological change that results in disease and disability. This may rest on future scientific breakthroughs; however significant improvement in health and increases in life expectancy could be achieved with behavioral and policy changes.

13. Dysynchrony of Healthspan & Lifespan in Long-lived Worm Mutants.

Ankita Bansal, L. Julie Zhu, Kelvin Yen and **Heidi A. Tissenbaum**

Molecular, Cell and Cancer Biology, Program in Bioinformatics and Integrative Biology, Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA 01605

Research focused on the aging process has been very successful at defining genes, pathways and interventions that result in lifespan extension. The general assumption has been that lifespan extension would also result in an extension of healthy aging or healthspan. However, limited efforts have focused on examining the consequences to extending lifespan or defining healthspan/healthy aging. Here, we present our results of a multi-year analysis to define *C. elegans* healthspan. We first performed a series of assays on wild type to define healthspan. Using these methods, we then tested 4 long-lived *C. elegans* mutants (insulin/IGF-1, dietary restriction, protein translation, mitochondrial) in a similar longitudinal manner as the mutants aged. Our results show that few of the long-lived mutants performed better than wild type when counting the number of days (chronological time). Moreover, we observed that all 4 mutants expanded the proportion of time spent in a frail state. Our studies also provide a framework for future healthspan

studies. Our results support that there are potential consequences to lifespan extension. We further reaffirm the importance of studying healthspan in addition to lifespan in future aging research.

14. Lifespan, health-span, and the rate of aging in C. elegans

Zachary Pincus, Department of Developmental Biology and Department of Genetics, Washington University in St. Louis

Lifespans are surprisingly variable across individuals of the same species, even in genetically identical animals reared in identical environments. To determine the mechanisms of such inter-individual variability, we used a novel single-animal culture system to identify predictive markers of future longevity in individual C. elegans. We have identified several molecular and physiological predictors of lifespan, including expression levels of various microRNAs. We find that a substantial amount of lifespan variability can be explained by these predictors early in adulthood, suggesting that long-lived and short-lived fates are determined early in life. Next, we sought to quantitatively characterize the process of senescent decline in long-lived versus short-lived animals. Surprisingly, we found that longer-lived animals do not stay "healthier" (as measured by our predictive markers) for a larger portion of their lives than short-lived animals. Indeed, variation in lifespan is uncoupled from variation in the fraction of of life spent in good health. Further, we find that longer-lived animals do not appear to have additional molecular or physiological endowments. Instead, long-lived animals age along a health trajectory similar to that of short-lived animals, but at a slower pace. This suggests that differences in the rate of aging, rather than qualitative differences in individual biology, drive lifespan variability.

15. Demographics of lifespan and healthspan of human populations

Hiram Beltrán-Sánchez

University of Wisconsin-Madison

The success of the current biomedical paradigm based on a “disease model” may be limited in the future due to large number of comorbidities inflicting older people. In recent years, there has been growing empirical evidence based on animal models suggesting that the aging process could be delayed and that this process may lead to increases in human life expectancy accompanied by improvements in health at older ages. In this talk we explore past, present and future prospects of healthy life expectancy and examine whether increases in lifespan associated with delayed aging link with additional years lived disability-free at older ages. Trends in healthy life expectancy suggest improvements among older people in the U.S., although younger cohorts appear to be reaching old age with increasing levels of frailty and disability. Trends in health risk factors such as obesity and smoking show worrisome signs of negative impacts on adult health and mortality in the near future. However, results based on a simulation model of delayed aging in humans indicate that it has the potential to increase not only the length of life but also the fraction and number of years spent disability-free at older ages.

16. Measuring age related deficit accumulation across the life course in a frailty index

Kenneth Rockwood, Division of Geriatric Medicine, Dalhousie University, Halifax, NS, Canada.

As people age they are more likely to die, but not everyone of the same age has the same risk of death. Those at an increased risk of death are said to be frail, and those at decreased risk to be fit. Frailty is non-controversially age-related and multiply determined. Operationalization is disputed: frailty can be characterized either as a state or as a syndrome, with undoubted merit to each point of view.

Our group quantifies frailty (as a state) by the number of health deficits present. For any individual, their frailty index (FI) is the ratio of deficits present to the total number of deficits considered – e.g. in a database that evaluated 50 deficits, someone with 10 present would have an FI of 10/50=0.20. The is significantly correlated with mortality, suggesting that, as a first approximation, the reason that some people are at a greater risk is that they have more health deficits. The behaviour of the frailty index is characteristic – it accumulates at ~3% per year and only rarely exceeds a value of ~0.7. Annual change in the FI is modelled by a Markov chain, the output of which is Poisson. Recent data suggest that these same parameters hold in animal models. Similar results obtain in all countries studied to date, although parameter estimates vary. In general, the mortality risk and mean FI change of the fittest people (i.e. those with the lowest FI scores) well approximates the ambient health of a population.

Translational research is addressing frailty measurement using biomarkers alone, laboratory test measures alone and clinically identifiable deficits. In combination (rather than with as single items) biomarker deficits appear first, followed by deficits defined by laboratory test abnormalities, and then by clinically detectable deficits.

A frailty index based on health deficits appears to quantify the risk of adverse health outcomes in humans and other animals.

Key words: frailty, frailty index, aged, biomarkers, translational research, clinical.

17. The Rockwood Frailty Index applied to aging mice

Susan E. Howlett, Departments of Pharmacology and Medicine (Geriatric Medicine), Dalhousie University, Halifax, NS, Canada.

Frailty can be defined as a state of increased vulnerability to adverse health outcomes for older adults of the same age. Relatively little is known about the biology of frailty, in part because the concept of frailty and its quantification had not, until recently, been explored in ageing animal models. One common approach to quantifying frailty in people is to construct a "frailty index" (FI) by counting the accumulation of deficits in health, such as clinically apparent signs, symptoms and diseases. The number of deficits in an individual divided by the total number measured yields an FI score between 0 (no deficits) and 1 (all possible deficits). Employing this approach, we have quantified frailty in naturally-ageing mice. We measured more than 30 variables that reflect different aspects of health (*e.g.* hemodynamics, blood work, activity levels and body composition) and showed that 30 month-old mice had significantly higher FI scores than 12 month-old animals (FI scores=0.43±0.03 vs 0.08±0.02; $p<0.001$; $n=12$). We obtained similar results when FI scores were calculated based on the accumulation of more than 30 clinically apparent signs of deterioration in ageing mice. We showed that mice treated with known longevity interventions (*e.g.* caloric restriction and resveratrol treatment) had lower FI scores than untreated controls. Importantly, the murine FI exhibits many of the same properties that characterize the behaviour of the FI in humans. The exponential relationship between FI scores and age (normalized to 90% mortality) was similar in mice and humans and the highest FI scores recorded were close to the submaximal limit to frailty of 0.67 reported in

humans. This ability to quantify frailty in animals is a major step forward in the effort to understand the biology of frailty, and thereby provides a platform to develop and test new clinical interventions.

18. Clinically relevant frailty index for mice.

LaDora V. Thompson, PhD, PT

University of Minnesota

This presentation outlines the development of a Frailty Index in C57BL/6 mice that match the clinical criteria used in humans by Fried et al., 2001 (weakness, slow walking speed, low activity level, poor endurance, unintentional weight loss). The selected criteria include grip strength, walking speed, physical activity, endurance, and body mass. The criteria in mice are evaluated by the inverted-cling grip test, rotarod test, voluntary wheel running, derived endurance scores, and body weight. Each criterion has a designated cutoff point (1.5 SD below the cohort mean) to identify the mice with the lowest performance. If a mouse presented with three of the criteria scores below the cutoff points, it is identified as frail. Mild frailty is designated if two criteria are below the cutoff points. In this mouse cohort, one mouse was identified as frail and one was mildly frail. This prevalence of 9% frailty is consistent with the prevalence of frailty in humans at the same survival age. This presentation will also present the effects of aerobic exercise on reversing frailty using the Frailty Index identified above. Lastly, in order to determine the effect of treatment on individuals animals, The Frailty Intervention Assessment Value, a composite score will be described. Collectively, our selected criterion, cutoff point, Frailty Index and Frailty Intervention Assessment Value provide a potential standardized definition for frailty in mice that is consistent with the operational definition of frailty in humans.

19. **Understanding the role of TOR-regulated processes in aging and disease**

Malene Hansen

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Multiple conserved signaling pathways have been shown to modulate organismal aging as well as disease states, including the nutrient-sensing TOR pathway. The TOR pathway regulates several central cellular processes, including protein synthesis and the intracellular recycling process autophagy. Both of these biological processes have been shown to play important roles in ensuring longevity in numerous model organisms, yet the precise underlying mechanisms are not fully understood. I will here discuss our lab's recent efforts to address how these two key cellular processes may contribute to aging and disease.

20. Pankaj Kapahi, PhD

Buck Institute

The role of circadian clocks in modulating nutrient responses.
Endogenous circadian clocks orchestrate several metabolic and signaling pathways that are known to modulate lifespan, suggesting clocks as potential targets for manipulation of metabolism and lifespan. Furthermore, disruption of circadian clocks has been associated with accelerated aging and is a risk factor for many age-related diseases including cancer and diabetes. Our preliminary data demonstrates cross-talk between circadian clocks and nutritional changes in in the diet that impact lifespan using D. melanogaster.

Our previous data demonstrate that the protective effects of DR require a metabolic adaptation which necessitates a shift towards increased triglyceride turnover. We have now discovered that flies with a null mutation in *tim* or *per* show reduced lifespan and fat turnover under DR conditions. We demonstrate that DR enhances the amplitude of cycling of most circadian clock genes, including *tim,* in peripheral tissues. Furthermore, the core circadian clock genes, *timeless (tim)* and *period (per)* are required for lifespan extension upon dietary restriction (DR) in *Drosophila*. Furthermore, overexpression of *tim* in peripheral tissues improves its oscillatory amplitude, extends lifespan and enhances triglyceride turnover under*ad libitum* conditions. These findings identify a critical role for specific clock genes in modulating the effects of nutrients on fat metabolism and aging. This work will initiate new awareness of circadian gene expression changes in aging and dietary restriction studies and contribute to the sub-discipline of 'chronogerontology.'

21. **Time-restricted feeding attenuates age-related cardiac decline and maintains healthspan**

Girish Melkani, San Diego State Univ.

Circadian clocks orchestrate rest-activity and feeding-fasting to distinct temporal niches over the course of a 24 h day and maintain homeostasis. While a light-dark cycle is considered to be the principal environmental cue that maintains robustness of daily rhythms, the requirement for a diurnal rhythm in feeding and fasting towards tuning organismal physiology is less understood. To assess whether daily consolidated feeding/fasting cycles can sustain health, we explored the effect of time-restricted feeding (TRF; food access limited to daytime 12 h every day) on neural, peripheral and cardiovascular physiology in *Drosophila melanogaster*. Here, we demonstrate improved sleep, prevention of body weight gain and dramatic deceleration of cardiac aging under TRF, even when caloric intake and expenditure are unchanged, relative to *ad libitum* fed (ALF; 24 h access to food) animals. Combining temporal gene expression profiling of multiple tissues with validation through classical loss-of-function genetics, we identified the TRiC/CCT chaperonin, the mitochondrial electron transport chain complexes and the molecular circadian clock as pathways mediating the benefits of TRF. These findings indicate that a daily feeding-fasting rhythm exerts pleiotropic effects through the circadian oscillator, proteostasis and energy metabolism pathways to maintain organismal homeostasis and increase healthspan in Drosophila.

22. *Oxidative Stress and Aging: Friend or Foe?*

Oxidative stress and redox regulation on hippocampal-dependent cognitive functions

Ting-Ting Huang, David Leu, Phillip Yang, and Yani Zou

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Hippocampal-dependent learning and memory relies on the production of new neurons and maintenance of dendritic structures. However, hippocampal formation is exquisitely sensitive to patho-physiological changes, and conditions such as reduced antioxidant capacity or exposure to low dose irradiation can significantly impede hippocampal-dependent cognitive functions by reducing the production of new neurons and altering dendritic structures. Similar to the hippocampal defects observed in SOD-deficient mice and mice exposed to low dose irradiation, reduced capacity in learning and memory, diminishing hippocampal neurogenesis, and altered dendritic network are universal in the aging brains. Although the mechanism leading to impaired cognitive functions is complex, persistent oxidative stress and reduced production of trophic factors likely play an important role. Given the similarities in cellular and structural changes in the aged, SOD-deficient, and radiation-exposed brains, understanding the shared underlying mechanism will provide more flexible and efficient use of SOD deficiency or irradiation to model specific aspects of age-related change in cognitive functions.

23. **Mitochondrial Catalase Mediates Anti-tumor Activity Through Stromal Fibroblasts**

Warren Ladiges,

Department of Comparative Medicine, School of Medicine, University of Washington, Seattle, WA 98195.

The concept of oxidative stress-induced aging has been supported by a number of groups in the past. In an attempt to address this issue in a preclinical mouse model, transgenic mice expressing the human catalase gene targeted to mitochondria (mCAT) were generated and shown to have an extended lifespan and decreased incidence of age-related disease including cancer. In follow up studies, syngeneic tumor growth was suppressed in mouse recipients expressing mCAT but not in WT mice, suggesting that the tumor microenvironment might be playing a role in mCAT-mediated tumor suppression. mCAT was also shown to suppress tumor progression in naturally occurring lung adenocarcinoma in CB6F1 mice. Lung fibroblasts from old mCAT positive mice had increased labeling of p16, p53, and beta galactosidase. Conditioned medium (CM) from mCAT fibroblasts, but not WT fibroblasts, suppressed growth of KRL-13 mouse lung tumor cells, but there was no difference in activation of either mCAT or WT lung fibroblasts when cultured with CM from the KRL-13 tumor cell line. KRL-13 tumor cell growth was suppressed in immune-deficient mice when co-injected with mCAT fibroblasts but not WT fibroblasts. CM from mCAT lung fibroblasts had increased levels of several cytokines including VEGF compared to CM from WT lung fibroblasts. In conclusion, mCAT appears to mediate anti-tumor activity in fibroblasts by enhancing senescence and secretion of soluble anti-tumor factors, most likely by altering mitochondrial hydrogen peroxide signaling. Whether any of these factors, such as VEGF, are involved in a causal role is not known but a possible mechanism might be the elimination of hypoxia-driven tumor growth.

24. Mechanisms that extend lifespan in Sprague-Dawley rats overexpressing Cu/ZnSOD.

Yuji Ikeno

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Our laboratory recently made the exciting observation that overexpression of Cu/Zn superoxide dismutase (Cu/ZnSOD) in Sprague-Dawley (SD) rats [Tg(*SOD*1-SD)$^{+/0}$] resulted in a significant increase in lifespan and a reduction in various age-related pathologies. Tg(*SOD*1-SD)$^{+/0}$ rats showed lower levels of oxidative damage to DNA and lipids *in vivo* and a higher resistance to oxidative stress *in vitr*o. Both Tg(*SOD*1-SD)$^{+/0}$ and wild-type (WT) rats showed an age-related increase in body fat, a characteristic of this strain of rats. Cu/ZnSOD overexpression did not attenuate adiposity but did reduce the accumulation of senescence-associated beta-galactosidase positive cells in fat tissue compared to WT rats. Interestingly, Tg(*SOD*1-SD)$^{+/0}$ rats showed a significant increase in insulin sensitivity and lower plasma glucose levels at an old age, which were associated with an enhanced insulin signaling pathway in skeletal muscle. To investigate the role of Cu/ZnSOD overexpression under nonobese conditions, we generated transgenic F344 rats overexpressing Cu/ZnSOD. Tg(*SOD*1-F344)$^{+/0}$ rats showed similar levels of Cu/ZnSOD overexpression compared to Tg(*SOD*1-SD)$^{+/0}$ rats. The Tg(*SOD*1-F344)$^{+/0}$ rats also showed lower levels of oxidative damage to lipids *in viv*o. However, neither Tg(*SOD*1-F344)$^{+/0}$ rats nor WT rats showed age-related changes in body fat, insulin sensitivity, or plasma glucose levels. Furthermore, Tg(*SOD*1-F344)$^{+/0}$ rats

showed little increase in lifespan and no differences in age-related pathology compared to WT rats. Therefore, the beneficial effects of Cu/ZnSOD overexpression on aging in Tg(*SOD*1-SD)$^{+/0}$ rats seem to be associated with the age-related increase in adiposity characteristic to this strain of rats. Our results are very exciting because these data indicate that overexpression of Cu/ZnSOD could provide increased protection against oxidative stress, enhance insulin sensitivity, retard aging, and reduce age-related diseases under obese conditions in rats. Currently, we are investigating this possibility using transgenic SD rats overexpressing Cu/ZnSOD only in skeletal muscle and/or adipose tissue, which will provide further information regarding the role of oxidative stress in obesity and aging. (Supported by grants from VA Merit Review)

25. **Oxidative Stress to Redox Stress, the evolution of an aging hypothesis**

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A spate of recent transgenic studies has failed to provide strong support for an Oxidative Stress Hypothesis of Aging based on the age-associated accumulation of structural damage. At the same time it has become widely appreciated that reactive oxygen species not only elicit structural damage, but can directly impact redox-sensitive signal transduction pathways through post-translational modifications. Hence an alternative hypothesis has emerged in which it is postulated that age-associated changes in redox state modulate the redox sensitive components of signal transduction, resulting in a decreased capacity to maintain homeostasis and a broad decline in functional capacity that typifies the aging organism. Indeed, accumulating evidence suggests that severe disruption in thiol homeostasis and redox state represent major changes that accompany aging. In *Drosophila*, the accelerated decrease in redox in middle-age flies is coincident with acceleration of the death rate. Moreover, enhanced production of the major cellular reducing equivalents, GSH and NADPH, in transgenic flies resulted in strong effects on increases in life span in *Drosophila* (>40% increase in longevity).

By virtue of their localized actions on peroxides coupled with oxidation of such key “reducing equivalents” as thioredoxin, the peroxiredoxins are considered to be important players in maintaining redox state and thus controlling redox-sensitive signaling pathways. Orthologs for all six members of the human peroxiredoxin family are present in *Drosophila* and consequently we have initiated a series of studies to explore the potential roles of the peroxiredoxin gene family in modulating life span and health span in the fly model.

In a series of physiological and genetic studies we have established that together, the mitochondrially-localized dPrx3 and dPrx5, play a key role in the maintenance of redox homeostasis, with strong effects on both apoptosis and longevity. Their effects have been implicated in multiple pathways/processes associated with aging and have led us to identify at least one potential target, thioredoxin reductase, mediating these effects. In a similar set of studies, we found that modulating redox state by under and overexpression of dPrx4 affected immune- and stress-response genes as well as longevity. Moreover these effects appeared to be largely mediated through JAK-STAT and NF-κB signaling.

Along with these findings, parallel work revealing the redox sensitivity of multiple pathways associated with aging provides the framework for a rigorous assessment of a ***Redox Stress Hypothesis of Aging***.

26. **Fat Helps the Brain in the NAD World: The Inter-Tissue Communication Mediated by NAMPT/NAD$^+$/SIRT1 in Mammalian Aging/Longevity Control**

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Our major interest is to understand the systemic, hierarchical regulation of aging and longevity in mammals and translate that knowledge into an effective anti-aging intervention that could make our later lives as healthy and productive as possible ("productive aging"). We have previously demonstrated that the mammalian NAD$^+$-dependent protein deacetylase SIRT1 in the hypothalamus plays a crucial role in mammalian aging/longevity control, implicating the hypothalamus as a high-order "*control center of aging*" (Satoh *et al.*, *Cell Metab.* 2013). Particularly in the dorsomedial hypothalamus (DMH), we have identified a specific subset of neurons, called SIRT1/Nkx2-1 double-positive neurons, which also express a new DMH-enriched factor Prdm13, a member of the Prdm family (Satoh *et al.*, *Aging Cell* 2014). DMH-specific *Prdm13* knockdown mice show decreased sleep quality and increased body weight and adiposity, indicating that this distinct neuronal subset plays an important role in mammalian aging/longevity control.

We have recently found that adipose tissue regulates hypothalamic NAD$^+$ levels and SIRT1 function through the secretion of a key NAD$^+$ biosynthetic enzyme, nicotinamide phosphoribosyltransferase (NAMPT) (Yoon *et al.*, *Cell Metab.*, In press). The extracellular form of NAMPT (eNAMPT) is enzymatically much more active than intracellular NAMPT (iNAMPT) and secreted actively by fully differentiated adipocytes. This process is regulated by SIRT1-dependent deacetylation. Whole-body and adipose tissue-specific *Sirt1*-decifient mice are unable to increase plasma eNAMPT in response to fasting. NAMPT point mutants reveal a particular lysine responsible for the SIRT1-dependent regulation of eNAMPT secretion and enzymatic activity. Generation of adipose tissue-specific *Nampt* knockout and knockin mice proves that adipose tissue controls hypothalamic NAD$^+$ levels and function, suggesting its novel role as a critical "*modulator*" for the function of the "*control center of aging*". With these findings, we have reformulated our conceptual framework of the systemic aging/longevity control in mammals, named the "NAD World". I will discuss mechanistic details of the NAD World and a potential anti-aging intervention to promote "productive aging" in our heavily aging society.

27. Neuropeptide Y is essential for CR effects on lifespan and health span in mice

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Knowledge of genes essential for the life-extending effect of calorie restriction (CR) in mammals is incomplete. In this study, we found that neuropeptide Y (Npy), which mediates physiological adaptations to energy deficits, is an essential link between CR and longevity in mice. The lifespan-prolonging effect of lifelong 30% CR was attenuated in Npy-null mice, as was the effect on the occurrence of spontaneous tumors and oxidative stress responses in comparison to wild-type mice. In contrast, the physiological processes activated during adaptation to CR, including inhibition of anabolic signaling molecules (insulin

and insulin-like growth factor-1), modulation of adipokine and corticosterone levels, and preferential fatty acid oxidation, were unaffected by the absence of Npy. These results suggest a key role for Npy in mediating the effects of CR. The putative Npy-associated pathway for longevity does not overlap those already known such as IGF-1 signaling.

28. **HIF-1 Promotes Longevity Through A Cell Non-Autonomous Mechanism**

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Abstract: In nematodes, as in mammals, hypoxia-inducible factor (HIF) proteins play a central role in responding to changes in environmental oxygen. HIF proteins are regulated via oxygen-dependent proteasomal degradation and are stabilized under low oxygen conditions to regulate expression of hundreds of downstream targets, collectively dubbed the hypoxic response. In mammals, constitutive stabilization of HIF through loss of the E3 ubiquitin ligase von Hippel-Lindau (VHL) protein leads to a disease characterized by angiomas and renal carcinomas, while in *Caenorhabditis elegans*, loss of the VHL homolog gene, *vhl-1*, improves proteostasis and increases lifespan. Here we describe a cell non-autonomous mechanism of lifespan and healthspan extension by HIF-1 in *C. elegans*. Stabilization of HIF-1 in a subset of neurons induces a transcriptional response in the intestine that promotes longevity and stress resistance. This intestinal transcriptional response does not require expression of HIF-1 in the intestine, but instead involves activation of other transcription factors. A specific enzymatic activity has been identified that is both necessary and sufficient for this cell non-autonomous longevity pathway. Identification of these downstream mechanisms by which HIF signaling promotes healthy aging in *C. elegans* may yield translational therapies that can provide the beneficial health effects of HIF signaling without the detrimental health consequences.

29. **Aging interventions in genetically diverse mice**

Gary A Churchill

Ron Korstanje & Luanne Peters

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We will present findings from cross-sectional and longitudinal studies of lifespan in the Diversity Outbred (DO) mice - a newly developed mouse population that mimics human genetic diversity.

We evaluated lifespans under caloric restriction, rapamycin treatment and standard chow diet. DO mice treated with rapamycin showed only a modest increase in lifespan, whereas the calorie-restricted mice showed a dramatic lifespan extension. In the cross-sectional cohort we evaluated health-span parameters including rotarod, grip strength, bone density, body composition, hematological function, kidney function and immune function. We obtained kidney tissues for histology, RNASeq and proteomics analysis. Genetic and molecular profiling data will help us to understand how these mice respond to life extending interventions. We will present genetic mapping results that identify genetic variants that increase healthy lifespan in a genetically diverse mammalian model system.

30. **Williams RW, Lu L, Ingels J, Mulligan MK, Mozhui K, Williams EG, Auwerx J**

Systems Genetics of Lifespan and the Impact of a High Fat Diet

Disturbances of cellular metabolism, often linked to mitochondrial dysfunction, critically influence whole body metabolism, health and lifespan. The importance of mitochondrial activity in the pathogenesis of metabolic diseases has been established in studies of humans and mechanistically validated using genetically engineered mouse models (GEMMs). Although GEMMs are highly informative, we need new and integrative genetic resources to efficiently study complex metabolic and mitochondrial networks linked to disease and aging that model genetically admixed human populations. In recent work that we have shown in *C. elegans* and mouse models that mitochondrial ribosomal translational machinery—the MRS and MRL family of nuclear-encoded genes—are critical determinants of oxidative efficiency, mitochondrial-nuclear metabolic balance (so-called mitonuclear proteostasis), and longevity (Houtkooper et al., PMID: 23698443). To validate and extend earlier work, we have now built up a set of ~150 BXD-type recombinant mouse strains that are well suited as a translational, mechanistic, and integrative studies of longevity. This large family incorporates just over 5.2 million common sequence variants (SNPs, indels, CNVs, inversions), and is well suited to study complex diseases and gene-by-environmental interactions (GXE). We have aged cohorts of ~50 BXD strains (females) on either a conventional mouse chow diet (18% calories from fat) or a high fat diet (60% calories from fat). While this work is not yet complete, current data demonstrate that a high fat diet is associated with approximately a 14% reduction in life span—from 660 +/- 11 (SE) days to 570 +/- 9 days—in matched cohorts of ~330 individuals and 50 strains per diet. Heritability is ~34% on the conventional diet and ~43% on high fat, demonstrating moderate control by sequence variants, as well as the expected upward shift in heritability by the more stressful diet. Surprisingly, our preliminary analysis of body weight and weight gain after being placed on the high fat diet do not predict longevity. We are generating mRNA and proteome data for liver, muscle, fat, heart, and hypothalamus for subsets of the same strains matched by diet or sex that will enable us to evaluate correlation and linkage between longevity and early biomarkers. This work supported by NIA R01AG043930.

31. Aging, Bone and Genetics: Lessons Learned from Inbred and Outbred Mice

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Osteoporosis is a metabolic disease in which there is a progressive loss of bone mass, ultimately leading to skeletal fracture. Peak bone mass is achieved in late teenage years. While osteoporotic fractures do not typically manifest until later in adult life, this disease really begins in late adolescence. A failure to achieve sufficient bone mass at a young age increases fracture risk in later life. Up to 85% of the variance in peak bone mass is caused by heritable factors, and the majority of animal studies of the genetic regulation of bone mass have focused on using young mice at peak bone mass. Bone is a dynamic tissue that is constantly remodeled. Imbalances in remodeling can result in net increases or decreases in bone mass, and there are data showing that loss of bone with aging is heritable. Using inbred strains of mice, we have showed that the genetic regulation of bone mass is contingent on age, shedding light on the genetic control of maintenance of bone mass with aging. While studies of the genetic regulation of bone mass have been extremely enlightening with regards to increasing our knowledge of basic bone biology, there is a genuine need to examine the genetic control of bone turnover itself. Genome wide association studies (GWAS) of bone turnover are not practical in humans, as directly assessing bone formation is invasive, costly, and time consuming. Measuring bone turnover in mice in a high throughput manner required the development of new methods and new technologies, which has only recently been accomplished. Using the Diversity Outbred population of mice, which was generated by interbreeding 8 strains of inbred mice, we are identifying subpopulations of mice that are achieving high bone mass via either increased formation or decreased resorption. Further, we are planning to use this population to identify genes associated with maintaining bone mass with aging either by suppressing bone resorption or by compensatory bone formation. In summary, studies in mice have substantially increased our understanding of bone biology, mostly at peak bone mass. New studies are focused on understanding changes in bone mass with aging and are particularly focused on capturing the genetic loci controlling bone turnover.

32. **Sex-specific stress response and aging**

John Tower and Gary Landis

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Chronic inflammation during human aging involves NF-kappa B activation, and is causally implicated in diseases including Alzheimer's disease. Interestingly, Alzheimer's disease is more prevalent in women than in men. Mifepristone is a glucocorticoid type II receptor antagonist and progesterone receptor antagonist with human female contraceptive and abortifacient activities, and is reported to reduce NF-kappa B activity. Drosophila also exhibits inflammation during aging. Conserved NF-kappa B factors activate the innate immune response and the expression of targets including Drosophila anti-microbial peptide genes. In female Drosophila mating accelerates this aging-associated inflammation and decreases life span. We found that mifepristone blocks the effect of mating, delays inflammation and increases life span up to +68%. High-throughput RNA sequencing was used to identify genes up-regulated or down-regulated upon mating, and where the change was reduced by mifepristone. Several candidate positive regulators of life span were identified that are conserved in humans, including dosage compensation regulator Unr and the Dopamine 2-like receptor. The candidate negative regulators included neuropeptide CNMamide and several involved in protein mobilization and immune response, including the anti-microbial peptide gene Drosocin. Analysis of Drosocin-GFP reporters in live flies recapitulated the aging-associated inflammation,

including the effects of mating and mifepristone. These results should facilitate testing of conserved targets for genetic and drug interventions in aging and inflammation.

33. "The dangers of social networking: Shared mechanisms of aging and sex appeal in the fly."

Scott Pletcher, PhD

Associate Professor, Molecular & Integrative Physiology
University of Michigan, Ann Arbor

Sensory perception modulates aging and physiology across taxa, but the environmental cues and neural circuitry that mediate these effects are largely unknown. We find that gustatory perception of pheromones from the opposite sex rapidly and reversibly affects lifespan, fat metabolism, and stress resistance in *Drosophila*. Mutation of a specific pheromone receptor *ppk23*, as well as neuronal inhibition targeted to *ppk23*- or *fruitless*-expressing neurons, rendered flies immune to the effects of pheromone exposure, as did surgical removal of the forelegs where these neurons reside. Targeted activation of these pheromone-expressing neurons was sufficient to mimic the physiological impact of pheromone exposure. Selective inhibition of neuro-anatomical regions revealed that neurons expressing the reward-mediating neuropeptide NPF were required for pheromone effects, and activation of NPF-expressing neurons was sufficient to mimic the impact of pheromone exposure on lifespan. Neither courtship behaviors nor mating itself were responsible for our observations. On the contrary, mating reversed the effects of pheromone perception, through a candidate neuropeptide pathway. These results highlight the impact of sex-specific social interactions and suggest that a lack of congruence between perceived sexual opportunity and subsequent sexual reward that may be responsible for effects on health and lifespan. We propose that lifespan is modulated through neural integration of sensory and reward circuits and that healthy aging may be compromised when the expectations defined by sensory perception are routinely discordant with ensuing experience.

34. **Profound Sex Differences in Response to CR in Inbred and F1 Hybrid Mice**

Rafael de Cabo, Translational Gerontology Branch, NIA, NIH

Calorie restriction (CR) is the only established laboratory intervention that consistently delays the onset of aging and age-related diseases. However, recent evidence suggests that some aspects of CR may not universal and that the response to CR in terms of health and lifespan may be dependent on more than just a reduction of calories without malnutrition. Furthermore, the mechanism through which CR acts remains elusive. In this study we used male and female DBA/2J (D2), C57BL/6J (B6), and their two F1 hybrid strains D2B6 and B6D2 mice, and performed longitudinal assessments of health and survival. Despite a reduction in calories of 20 and 40% relative to *ad libitum* (AL)-fed mice, there was no consistent response to CR. We will discuss the importance of systematically examining the contributions of sex and strain of the mouse vis-à-vis CR actions to further our understanding of normal aging.

35. **Sex Differences and Sex Steroid Hormones in Alzheimer's Disease**

Christian J. Pike

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Normal aging is associated with systemic decreases in the levels of estrogens in women and testosterone in men. In brain, neuropathologically normal men show significant age-related losses in testosterone and its active metabolite dihydrotestosterone but not in estrogens. Men with AD show significantly greater testosterone loss that is apparent even at the earliest stages of pathology, suggesting that testosterone loss contributes to development of AD. Conceptually similar findings are observed in women, except that AD is associated with reduced estrogens not androgens. The link between low brain hormone levels and AD suggests that the loss of activational effects of sex steroid hormones creates neural environments conducive to AD neuropathogenesis. Consistent with the possibility, we observe that experimental depletion of estrogens in adult female 3xTg-AD mice and androgens in adult male 3xTg-AD mice accelerates pathological and behavioral indices of AD, effects that are prevented by hormone replacement treatments. We have identified several mechanisms by which estrogens and androgens regulate AD risk, including reduction of β-amyloid protein and promotion of neuron viability. Emerging evidence suggests that AD risk is associated not only with the loss of activational effects of sex steroid hormones during aging, but also the organizational effects during development. New findings show that women with dementia exhibit evidence of greater feminization, implying that that the female brain may be inherently more vulnerable to AD. Studies with early postnatal 3xTg-AD mice support this position, showing that masculinization of females reduces pathology whereas feminization of males increases pathology. The organizational and activational effects of steroid hormones on AD also appear to interact in sex-specific manners with both genetic (e.g., ApoE) and environmental (e.g., obesity) AD risk factors. Collectively, these findings demonstrate sex-specific roles of organizational and activational effects of sex steroid hormones in AD pathogenesis.

36. **Do microRNAs regulate sarcopenia development?**

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As the ageing population increases, it is essential to determine the mechanisms involved in ageing of the musculoskeletal system. A common characteristic of ageing is loss of skeletal muscle (sarcopenia) leading to a decreased life quality. There is currently little data available examining the involvement of epigenetic mechanisms in musculoskeletal ageing although such mechanisms are undoubtedly involved (Liu et al, Cell Rep. 2013). microRNAs are novel regulators of gene expression. microRNAs control myogenesis, regeneration and ageing (Goljanek-Whysall et al, PNAS 2011, Pincus et al, PloS Genetics 2011, Drummond et al, Amer J Physiol 2008). To determine their role during ageing, we identified changes in microRNA and transcript expression in

muscle of adult and old mice. Based on GO term analysis, we chose 2 microRNAs and their putative targets for further studies of their role in sarcopenia development. Our data show differential expression of miRNAs during muscle ageing, atrophy and regeneration. We validated miRNA targets related to acetylation and metabolism and characterised novel miRNA:target interactions *in vitro*. We manipulated the expression of these microRNAs *in vivo*, in adult and old mice, and established their role in muscle homeostasis through molecular, histological and physiological analyses. We demonstrated that dysregulation of expression of a single microRNA can affect myofibre size and force generation by extensor digitorium longus (EDL) muscle. This could lead to design of novel therapeutics for individuals affected by sarcopenia, effectively improving their lifestyle.

The authors would like to thank University of Liverpool, Wellcome Trust, MRC-Arthritis Research-UK CIMA and BBSRC for funding.

37. **The Mouse Aging Interventions Testing Program: An Update**

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The National Institute on Aging Interventions Testing Program (ITP) evaluates agents that are hypothesized to increase life and/or health span in genetically heterogeneous mice. Each compound is tested in parallel at three sites, and all results are published. We report the effects of lifelong treatment of mice with four agents not previously tested: Protandim, fish oil, ursodeoxycholic acid (UDCA) and metformin – the latter with and without rapamycin; and two drugs previously examined: 17-α-estradiol and nordihydroguaiaretic acid (NDGA), at doses greater and/or less than those used previously. 17-α- estradiol at a 3-fold higher dose than previously reported, robustly extended both median and maximal lifespan of male mice, with an increase in median of 19%, but had no effects on female lifespan even at this higher dose. The effects of 17-α-estradiol were statistically significant for male mice at each of the three test sites. The male-specific extension of median lifespan by NDGA was replicated at the previous dose and also at doses 3-fold lower and higher. The effects of NDGA were dose-dependent, male-specific and without an effect on maximal lifespan. Protandim, a defined mixture of compounds that activate Nrf2, extended median lifespan, but not maximal lifespan, at the dose used. Metformin alone, at a dose of 0.1% in the diet, did not extend lifespan. Metformin (0.1%) combined with rapamycin (14 ppm) resulted in a robust lifespan extension that was suggestive of a small added benefit over rapamycin alone, based on historical comparison with earlier studies of rapamycin given alone. Neither fish oil nor UDCA extended life span. These results underscore the reproducibility of ITP longevity studies and illustrate the importance of dose-response experiments for lifespan studies.

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38. Small molecules that promote proteostasis and the new Caenorhabditis Intervention Testing Program

Gordon Lithgow, PhD

Buck Institute for Research on Aging

We have undertaken screen of synthetic and natural compounds to find agents for aging interventions. Since aging can be considered a causal factor in a number of age-related diseases. We hope such screen could yield useful therapeutics. We focused our search on compounds that maintain protein homeostasis. Collapse of protein homeostasis results in protein misfolding cascades and the accumulation of insoluble protein fibrils and aggregates, such as amyloids. A group of small molecules, traditionally used in histopathology to stain amyloid in tissues, bind protein fibrils and slow aggregation in vitro and in cell culture. We proposed that treating animals with such compounds would promote protein homeostasis in vivo and increase longevity. We previously showed that exposure of adult Caenorhabditis elegans to the amyloid-binding dye Thioflavin T (ThT) resulted in a profoundly extended lifespan and slowed ageing. ThT also suppressed pathological features of mutant metastable proteins and human β-amyloid-associated toxicity. These beneficial effects of ThT depend on the protein homeostasis network. A modified form of ThT (HBX) also extends lifespan. HBX binds aggregating protein but also chelates metals. We have investigated the role of metals in protein homeostasis and have found that metal chelation can have beneficial effects during aging.

39. Mitochondrial bioenergetics and aging – a novel target for intervention

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Aging is associated with alterations in many components of the mitochondrial electron transport chain (ETC), resulting in efficiency of electron transport, inhibition of oxidative phosphorylation, and increased production of reactive oxygen species (ROS). As mitochondria become increasingly dysfunctional over time, many age-related conditions, such as vision loss, sarcopenia, and heart failure, set in. The traditional view is that the accumulation of oxidative damage to DNA, proteins, and lipids results in mitochondrial dysfunction with age. However, recent research has revealed that age-related decline in function can be reversed by improving mitochondrial bioenergetics. This presentation will introduce a new class of compounds (SS peptides) that selectively target cardiolipin on the inner mitochondrial membrane and promote electron flux through the ETC. These compounds have been shown to target the rate-limiting step (cytochrome c) in the ETC. A single dose of SS-31 given to aged mice was capable of improving P/O coupling and ATP synthesis, and reducing ROS production. Short-term treatment with SS-31 has been shown to improve treadmill performance and cardiac contractile function, and reverse spatial vision decline in aged mice, while having no effect in young animals. These results have led to clinical trials with SS-31 for muscle weakness, heart failure, and age-related macula degeneration.

40. Obtaining Protein Anabolism in older adults with chronic disease and cancer

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It is unclear how much protein is needed to obtain anabolism in patients with a chronic disease state and whether intake of more protein leads to more protein anabolism. Methods to measure protein requirements are complicated and laborious and this is probably the reason why protein requirement studies were done in healthy individuals.

I will report the results of several studies in older adults in which we studied the intake of a bolus meal with protein and carbohydrates and the response of whole body protein anabolism using stable isotope methodology. This approach establishes the relation between intake and the anabolic response to the protein meal.

We observed a linear relationship between the intake of the essential amino acids and the net protein synthesis response in patients with COPD and healthy subjects. Modifying the protein meal by using free amino acids or hydrolysates or adding extra amounts of the amino acid leucine did not modify this net protein synthesis response. In patients with lung cancer (Stage 3-4), the response to the intake of essential amino acids was also similar between patients that have lost muscle mass, were weight stable or matched healthy control subjects. We hypothesize that the anabolic capacity of older subjects with certain diseases are maintained.

41. **Co-treatment with metformin improves metabolic defects caused by rapamycin.**

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Inhibition of mTOR extends lifespan in mice and invertebrates and improves some markers of age-related functional decline. There is now intriguing evidence that mTOR inhibition may improve same physiological aspects of aging in humans. However, assessing large-scale translational effects of this approach may be limited due to the increased potential for risks associated with administration of the mTOR inhibitor rapamycin and its analogs. A primary example is a significant increase in the risk for new-onset diabetes or impaired glucose metabolism shown with both clinical administration and in rodent models treated with rapamycin. Working with the NIA-funded Interventions Testing Program, we tested whether these metabolic defects could be prevented by co-treatment with metformin, the most-commonly administered and first-line drug of choice for type 2 diabetes. Beginning at four months of age, genetically heterogeneous HET3 mice were treated with encapsulated rapamycin, metformin, or both simultaneously, with all treatments incorporated into their diet. Metformin did not alter the effect of rapamycin on mTOR inhibition *in vivo,* but did independently activate AMPK signaling in both male and female mice. Over nine months of treatment, rapamycin significantly reduced body weight gain in both males and females, with metformin co-treatment eliminating this weight reduction in males only. Glucose metabolism was impaired by rapamycin beginning as early as one month after treatment and continued throughout the duration of the study. Metformin did not alter this outcome in males, but females treated with

both metformin and rapamycin were indistinguishable from controls showing a clear beneficial effect of metformin treatment. At least partly, these beneficial effects of metformin could be attributed to reduction in hepatic gluconeogenesis caused by this drug, though we also found that metformin altered circulating concentrations of adipokines such as leptin and adiponectin. The extension of lifespan caused by rapamycin has been somewhat paradoxical when viewed in light of its potential negative effects on metabolism. In ongoing studies, the Interventions Testing Program will assess the lifespan of HET3 mice co-treated with rapamycin and metformin and, combined with our findings, will soon test whether there is further extension of rapamycin-mediated longevity by alleviation of its metabolic defects.

42. **Age-associated decline of different forms of autophagy underlie T cell immunosenescence**

Fernando Macian

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As with many other tissues and organs, the immune system is also affected by age. Immunosenescence is characterized by a decreased ability of immune cells to mount a productive response upon exposure to new antigens. Autophagy is a catabolic process that delivers cytoplasmic material to the lysosomes for degradation not only to control protein homeostasis but also to regulate protein levels in response to specific stimuli. Defective autophagy is known to be associated with age in several cell types and tissues, and its dysregulation is related to age-associated diseases. Here we show that in T cells, different forms of autophagy have essential roles in modulating activation-induced responses. Our data also show that the activity of those types of autophagy is severely reduced in T cells with age, which contributes to the diminished T cell function observed in those cells. Furthermore, restoration of activity of one of those autophagic pathways, chaperone mediated autophagy, in T cells isolated from old mice results in improved function, supporting the idea that interventions aimed at enhancing autophagy may constitute useful tools to improve immune function in the elderly.

43. Mechanisms that protect the lipid membrane and how they influence aging

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The membranes found in old animals are more rigid and contain more damaged lipids than the membranes in their younger counterparts. These damaged lipids are detrimental to the function of the membrane, yet we know very little about how the membrane is repaired and how the proper phospholipids are installed particularly over aging. In order to monitor the turnover of the membrane and as well as its composition, we developed mass spectrometry based approaches to compare multiple lipid populations simultaneously in *C. elegans*. Using stable isotope incorporation, we have determined that the turnover of the membrane is very high in young adults but decreases significantly over aging. We are exploring how this rejuvenation can function as a membrane remodeling and repair mechanism. Additionally, we used these tools to look for pathways that may prevent the initial damage to phospholipids. In doing so, we identified ether-

linked lipids as major components of the nematode membrane, and, because these specialized ether-linked lipids have been suggested to play a role as sacrificial antioxidants in cells, we further tested a role for these lipids in membrane protection. RNAi of ether-lipid biosynthesis genes resulted in a profound deficiency in this population of lipids as well as a sensitivity to stress in the animals. Overall, we have developed novel methods for monitoring membrane dynamics that have identified multiple novel facets of membrane maintenance that may impact aging. We are using these tools to further understand how these membrane maintenance and preservation pathways may impact the health of the membrane and ultimately the animals' longevity.

44.

45. **Comparative Bioenergetics of Aging**

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University of Alabama at Birmingham.

The University of Alabama at Birmingham (UAB)'s Nathan Shock Center of Excellence in the Basic Biology of Aging focuses on comparative energetics and aging. Energetics is comprehensively defined for this purpose as the study of the causes, mechanisms, and consequences of the acquisition, storage, and utilization of metabolizable energy. Comparative energetics is the study of metabolic processes at multiple scales and across multiple species, in this case as it relates to health and aging. Nearly a century of aging research has reinforced the link between energetics and aging. In modern terms this link is reified as dysregulation of mitochondrial function, metabolic signaling, and nutrient responsiveness. The twin objectives of the Center will be to explore in greater depth and detail than previously the complex relationship among cellular and organismal energetics and their relationship to health and aging, and to provide quantitative, state-of-the-art technologies and novel methodologies in the assessment and analysis of energetics to the basic aging research community at large. The Center will have three research cores: (1) Comparative Organismal Energetics Core will provide expertise and instruction in cutting edge methodology for determining complete whole animal energy balance (intake, assimilation, expenditure) and body composition, including regional distribution of white and brown adipose tissue, in living animals of various species from flies to fish, mice or other mammals, under a range of temperature or activity regimes; (2) Comparative Mitochondrial Health Assessment Core will provide integrated, quantitative energetics analysis at the level of the organelle, cell, or tissue for both traditional and emerging animal models, including targeted metabolomics, assessment of mitophagy, and oxidative stress. This core can also provide murine mitochondrial-nuclear exchange models to enable experiments that evaluate the contribution of mtDNA variation to bioenergetics; (3) Comparative Data Analytics Core will develop innovative analytic approaches to data sets linking comparative energetics to organismal health and longevity and train researchers in the use of these methods. With these research cores plus the administrative and research development core, we aim to: (i) facilitate hypothesis-driven research and leverage these technologies into new projects, new models, new interactions, and new collaborations nationwide in basic aging research; (ii) foster meaningful novel interactions among investigators within UAB and across the region and country; and (iii) provide resources, education,

training, and mentoring to junior investigators through the intellectual resources and research infrastructure, the Center will develop.

46. **New Tools for Aging Research: An Overview of the Oklahoma Nathan Shock Center.**

Holly Van Remmen*, William Sonntag#, Luke Szweda*, Michael Kinter*, Willard Freeman#, Jonathan Wren* and Arlan Richardson#.

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The focus of the Oklahoma Nathan Shock Center is on the newly-developing field of Geroscience and providing investigators across the nation with unique and innovative assays. Because all the assays that are provided by the three research cores use frozen tissue, investigators across the country have easy access to the services provided by the Oklahoma Shock Center. In addition, the Research Development core will provide pilot funds to investigators at other institutions to use the research core services. The three research cores are as follows: The Multiplexing Protein Quantification Core provides investigators with a novel mass spectrometry method for measuring the abundance of proteins from any tissue/cell from any organism that has its genome sequenced. The Core can quantify panels of 25 to 30 proteins in a single run, thereby allowing investigators to interrogate pathways of importance in aging and Geroscience. By developing panels of proteins for *C elegans*, *Drosophila*, and yeast, as well as exceptionally long-lived animals, this Core will provide investigators, for the first-time, a method for measuring protein abundance in these animal models, which are important to aging. The Targeted DNA Methylation & Mitochondrial Heteroplasmy Core uses novel next-generation sequencing with unique primer sets to study DNA methylation either genome-wide or in specific genes. In addition, the Core has developed methods that will allow investigators to conduct a comprehensive analysis of mitochondrial genome heteroplasmy (variants/mutations and deletions) and copy number from very small samples. The Integrative Redox Biology Core provides investigators with a comprehensive state-of-the-art assessment of the oxidative stress status of a cell, e.g., measures of oxidative damage and redox couples, which require major instrumentation and expertise to obtain accurate and reproducible data. The Discovery Bioinformatics Core will provide traditional support in statistics and bioinformatics for data analysis to the Research Cores as well the Pilot Project Investigators and Center Faculty, but in addition, the Core Leader, Dr. Wren has developed novel software to help discover and interpret biological changes that accompany aging and to allow discovery of new genes and genomic regions relevant to aging using predictive methods.

47. **Abnormal mitochondrial dynamics in the pathogenesis of Alzheimer's disease**

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Research in the past four decades revealed that mitochondrial dysfunction is a prominent feature in the brain of Alzheimer's disease (AD) patients. Recent studies demonstrated that mitochondria are highly dynamic organelles characterized by a delicate balance of fission and fusion. This concept has revolutionizes our basic understanding of the regulation of mitochondrial structure, function and distribution which has far-reaching significance in studies of health and disease. Initial studies revealed an imbalance in mitochondrial

fission and fusion in fibroblasts from sporadic AD patients compared with normal healthy fibroblasts from age-matched control patients. Later it was demonstrated that overexpression of familial AD (FAD)-causing AβPP mutant led to mitochondrial fragmentation and redistribution in neuronal cells along with altered expression of mitochondrial fission/fusion proteins. Overexpression of mitochondrial fission/fusion proteins could rescue certain aspects of mitochondrial dysfunction, suggesting a causal involvement of mitochondrial dynamics in mediating Aβ-induced mitochondrial dysfunction. Furthermore, soluble Aβ oligomers also induced mitochondrial fragmentation in primary hippocampal neurons which likely mediated Aβ-induced mitochondrial dysfunction and synaptic dysfunction. Detailed studies revealed that Aβ causes calcium dyshomeostatsis which in turn affects the phosphorylation and mitochondrial translocation of the mitochondrial fission protein DLP1. Genetic and pharmaceutical methods to rescue mitochondrial morphology and distribution could effectively restore Aβ-induced mitochondrial function and alleviate synaptic dysfunction. Importantly, we demonstrate significant changes in the expression and distribution of mitochondrial fission and fusion proteins in vivo in AD in consistent with a shifted mitochondrial dynamics towards excessive fission. Taken together, we suggest that such a fundamental shift in mitochondrial dynamics negatively impacts all aspect of mitochondrial function such as impaired bioenergetics, increased structural damage and ROS production and loss of mtDNA integrity which causes synaptic dysfunction and neuronal dysfunction that is critical to AD pathogenesis. Therefore, strategies to modify abnormal mitochondrial dynamics may be an attractive therapeutic intervention target for AD.

Poster Abstracts

Free Radicals and Aging

1. The conserved role of the Lon protease in Drosophila melanogaster during aging

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Mitochondria are major sites of free radical generation, making mitochondrial proteins proximal targets for oxidation. To minimize the accumulation of oxidative damage, mitochondria utilize reducing enzymes, repair systems, and proteolysis to maintain function. A key proteolytic mitochondrial-matrix enzyme is the nuclear-encoded Lon protease: an ATP-stimulated protein that degrades oxidized proteins. Prior cell culture work has shown Lon is a stress response protein as measured by protein levels following exposure to multiple stressors: hydrogen peroxide, heat shock, and serum starvation. However, while the rapid protein response is prevalent in low-passage fibroblasts, it diminishes in senescent cells. In addition, fibroblasts with reduced Lon protein levels exhibit an increase in protein damage and reduced mitochondrial function. Due to Lon's role in the maintenance of the mitochondrial proteome, our aim is to study the conserved role of Lon during aging in D. melanogaster. The induction of Lon was characterized by measuring mRNA levels, protein expression, and proteolytic activity following exposure to a mild dose of hydrogen peroxide in young and aged D. melanogaster. In addition, the adaptive response was measured based on survival after hydrogen peroxide pretreatment, followed by a toxic dose. Interestingly, both the

inductive and adaptive responses are robust in young fruit flies, but are abrogated in aged fruit flies. As well, lifespan studies using a Lon overexpression strain showed no change in survival, but a marked decrease in a Lon RNAi strain, implying the necessity of Lon for healthy survival. This work provides a better understanding of Lon's role in overall lifespan and the decline in both the inductive and adaptive responses during aging.

2. Neuronal specific reduction in CuZnSOD initiates neuromuscular junction alterations and weakness but not atrophy in mouse gastrocnemius muscle

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Our previous studies showed that mice lacking CuZnSOD (Sod1KO mice) have a number of neuromuscular changes resulting in dramatic and accelerated muscle atrophy and weakness that is evident as early as 8 months of age and mimics age-related loss of muscle. We have further shown, using a conditional knockout model (Sod1 floxed mice), that loss of CuZnSOD in skeletal muscle alone does not recapitulate the phenotype found in the Sod1KO mice and results only in mild weakness and no muscle atrophy. In contrast, neuron specific overexpression of CuZnSOD in Sod1KO mice (nSOD1-Tg/Sod1KO mice) rescued muscle atrophy and weakness, supporting a key role for motor neurons in the initiation of muscle atrophy. In this study we targeted the deletion of CuZnSOD specifically to neurons (nSod1KO mice) and determined the effect of this targeted deletion on muscle mass and weakness. The nSod1KO mice show a significant loss of CuZnSOD activity and protein level in brain and spinal cord but not in muscle tissue. The mass of the gastrocnemius muscle, a hind limb muscle particularly affect by aging in mice, was not reduced in nSod1KO compared to wild type mice, even at 20 months of age. Masses of the tibialis anterior and extensor digitorum longus muscles also were unaffected in the nSod1KO mice, although the quadriceps and soleus muscles showed small but significant reductions in mass in the nSod1KO mice. Maximum isometric specific force was reduced 8% to 10% in the gastrocnemius and EDL muscle of nSod1KO mice, while soleus showed no loss of force generation. Reduced CuZnSOD in motor neurons did not alter oxidative stress or redox status in either the gastrocnemius or quadriceps muscle as measured by levels of reactive oxygen nitrogen species (RONS) regulatory enzymes, protein nitration, and F2-isoprostane levels as a marker of lipid peroxidation. Despite no change in mass, the gastrocnemius showed altered NMJ morphology and increased expression of genes associated with increased denervation such as acetylcholine receptor subunit alpha (AChRa) and the transcription factors Runx1 and Gadd45a, in support of neuronal loss of CuZnSOD initiating alterations at the neuromuscular junction that modulate muscle gene expression. These results and our previous studies support the concept that deficits in either the motor neuron or muscle alone are not sufficient to initiate a full muscle atrophy phenotype and demonstrate that deficits in both tissues are required to recapitulate the loss of muscle observed in Sod1KO mice.

3. Mechanisms that extend lifespan in Sprague-Dawley rats overexpressing Cu/ZnSOD.

Roman, M.G., Flores, L.C., Cunningham, G.M., Zhang, Y., Salmon, A., Liu, Y., Hubbard, G.B., Kirkland, J., Pirtskhalava, T., Tchkonia, T., Ikeno, Y (P)

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Our laboratory recently made the exciting observation that overexpression of Cu/Zn superoxide dismutase (Cu/ZnSOD) in Sprague-Dawley (SD) rats [Tg(SOD1-SD)+/0] resulted in a significant increase in lifespan and a reduction in various age-related pathologies. This is the first study to assess the effects of antioxidant enzyme overexpression on aging in rats, and our findings support the oxidative stress theory of aging. Tg(SOD1-SD)+/0 rats showed lower levels of oxidative damage to DNA and lipids in vivo and a higher resistance to oxidative stress in vitro. Both Tg(SOD1-SD)+/0 and wild-type (WT) rats showed an age-related increase in body fat, a characteristic of this strain of rats. Cu/ZnSOD overexpression did not attenuate adiposity but did reduce the accumulation of senescence-associated beta-galactosidase positive cells in fat tissue compared to WT rats. Interestingly, Tg(SOD1-SD)+/0 rats showed a significant increase in insulin sensitivity and lower plasma glucose levels at an old age, which were associated with an enhanced insulin signaling pathway in skeletal muscle. To investigate the role of Cu/ZnSOD overexpression under nonobese conditions, we generated transgenic F344 rats overexpressing Cu/ZnSOD. Tg(SOD1-F344)+/0 rats showed similar levels of Cu/ZnSOD overexpression compared to Tg(SOD1-SD)+/0 rats. The Tg(SOD1-F344)+/0 rats also showed lower levels of oxidative damage to lipids in vivo. However, neither Tg(SOD1-F344)+/0 nor WT rats showed age-related changes in body fat, insulin sensitivity, or plasma glucose levels. Furthermore, Tg(SOD1-F344)+/0 rats showed little increase in lifespan and no differences in age-related pathology compared to WT rats. Therefore, the beneficial effects of Cu/ZnSOD overexpression on aging in Tg(SOD1-SD)+/0 rats seem to be associated with the age-related increase in adiposity characteristic to this strain of rats. Our results are very exciting because these data indicate that overexpression of Cu/ZnSOD could provide increased protection against oxidative stress, enhance insulin sensitivity, retard aging, and reduce age-related diseases under obese conditions in rats. Currently, we are investigating this possibility using transgenic SD rats overexpressing Cu/ZnSOD only in skeletal muscle and/or adipose tissue, which will provide further information regarding the role of oxidative stress in obesity and aging. (Supported by grants from VA Merit Review, American Federation for Aging Research, and Glenn Foundation)

4. Overexpression of thioredoxin in mitochondria combined with downregulation in the cytosol alters aging in mice.

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Our laboratory has conducted an aging study using mice overexpressing thioredoxin 2 (Trx2) in mitochondria and/or down-regulating thioredoxin 1 (Trx1) in the cytosol. Trx2 transgenic (Tg) mice showed reduced reactive oxygen species (ROS) production in mitochondria and lower levels of oxidative damage, and there were no compensatory changes in the expression of other antioxidant enzymes. A survival study with male mice showed that the median lifespan of Trx2Tg mice was slightly longer (8.8%) compared to wild-type (WT) mice; however, the maximum lifespan was not extended. When testing the effects of reduced levels of Trx in the cytosol, we expected that these mice might have a shorter lifespan due to reduced resistance to oxidative stress. Trx1 heterozygous knockout (KO) mice showed a slight reduction in tumors compared to WT mice at 22-24 months of age. In spite of the possible benefit on cancer development, the survival curve of Trx1KO mice was similar to control mice. Although overexpression of Trx2 or down-regulation of Trx1 alone showed interesting cellular and physiological changes, there was no significant impact on aging. Overexpression of Trx in mitochondria provided an extension of lifespan only in the early part of life, and maximum lifespan was not extended. On the other hand, down-regulation of Trx in the cytosol showed changes in pathophysiology, e.g., a slightly reduced incidence of cancer, an enhanced apoptotic pathway, and impaired mitochondrial function. However, no changes were observed in lifespan. These results led us to believe that overexpression or down-regulation of Trx in only one compartment of the cells is not enough to change lifespan. Currently, we are conducting an aging study with mice overexpressing Trx2 and down-regulating Trx1 because 1) protection of mitochondria, which generate most of the endogenous ROS, from oxidative stress may play important roles in aging and 2) reduced levels of Trx1 could decrease age-related tumor formation by reducing cell growth and enhancing apoptosis. The median lifespan of Trx2Tg x Trx1KO (947 days) is 19.9% longer compared to WT mice (790 days). The current survival rates of Trx2Tg x Trx1KO and WT mice are 31.4% and 11.4%, respectively. These very intriguing results indicate that aging and cancer could be regulated through common and/or independent mechanisms. (Supported by grants from VA Merit Review, American Federation for Aging Research, and Glenn Foundation)

5. Sco2 Deficient Mice Develop Increased Adiposity and Insulin Resistance

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Cytochrome c oxidase (COX) is an essential transmembrane protein complex in the mitochondrial respiratory electron chain. Mutations in genes responsible for the assembly of COX are associated with Leigh syndrome, cardiomyopathy, spinal muscular atrophy and other fatal metabolic disorders in humans. Paradoxically, mice lacking the COX assembly protein SURF1 show increased longevity associated with upregulation of mitochondrial biogenesis and stress response pathways despite significant reductions in COX activity. Here we asked whether a mouse model of cytochrome c oxidase deficiency due to a mutation in the sco2 gene, a copper chaperone that is required for the activity of COX would have similar molecular and physiologic changes. A complete knockout of the Sco2 gene in mice is embryonic lethal, however mice harboring a Sco2 knock-out allele and a mutated Sco2 knock-in allele (KI/KO) are viable, and have a 30-60% reduction in COX activity. We found that Sco2 KI/KO mice have increased fat mass associated with a

reduction in whole white adipose tissue oxygen consumption. The Sco2 KI/KO mice have increased hepatosteatosis, elevated serum triglyceride and cholesterol levels, and changes in circulating adipokine levels compared to wild-type controls. Interestingly, these alterations are associated with the development of insulin resistance in the Sco2 KI/KO mice. Experiments are in progress to further evaluate the consequences of harboring a Sco2 mutation on adipose tissue mitochondrial function, and the mechanism by which a mutation in the Sco2 gene leads to insulin resistance using an in vitro cell culture model. These findings counter to the metabolic phenotype of Surf1-/- mice, illuminating the complex nature of mitochondrial dysfunction on physiology. Results from this study will further enhance our understanding of the role of complex IV in physiological outcomes due to mitochondrial dysfunction.

Dietary Restriction I

6. Calorie restriction protects against age-related dysregulation of neural stem cells in the murine subventricular zone

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Aging is a major risk factor for increased susceptibility to damage from brain insults like stroke, inflammation, and disease. Calorie restriction (CR) can improve physiological markers of health during aging, including extending lifespan and protecting against age-related damage to the brain. The largest source of neural stem cells in the adult brain is the subventricular zone (SVZ). We sought to determine the effect of long-term CR on neurogenesis and the neural stem cell niche in the SVZ of young and aged mice. Here, we show that aged mice fed standard control chow have fewer SVZ-derived neurons in the olfactory bulb, indicating that aging impairs neural stem cell function. Long-term CR preserved neural stem cell function and resulted in a significant increase in neurogenesis in aged mice compared with ad libitum- fed controls. Paradoxically, we have observed that proliferation of neural stem cells is decreased in aged CR mice. This is in the presence of increased neuroblast formation and increased neurogenesis. These data indicate either a change in cell fate or a change in the survival of transit amplifying cells as they mature into neuroblasts, suggesting an altered regulatory mechanism. Confocal imaging and fluorescent staining of SVZ wholemounts revealed an increase in both the total number and reactivity of microglia in the aged control mouse, suggesting increased inflammation in the neural stem cell niche during aging. Remarkably, these age-related inflammatory markers were not observed in the long-term CR aged mice, which appeared no different from young control and young CR mice included in the study. We have found that the neural stem cell chemoattractant mechanism CXCL12, secreted by endothelial cells, and its receptor expressed on neural stem cells, CXCR4, are dysregulated in the aged mouse fed ad libitum, but not in the aged CR mouse. Further, the recently identified rejuvenation factor GDF11 was found to be decreased in the aged SVZ, but not in the aged CR SVZ. These initial experiments have revealed a protective role for CR in the aging SVZ, and are an important first step in understanding how CR may be an effective therapeutic intervention for the aging or damaged brain.

7. Effect of dietary curcumin and caloric restriction on functional outcomes in middle aged and aged mice

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Curcumin, from Curcuma Longa, has antioxidant and anti-inflammatory effects that are hypothesized to benefit impaired brain function related to normal aging. The following results are from a completed study of dietary curcumin alone and in combination with caloric restriction (CR), testing functional outcomes in late middle-aged (MAG) (15 months) and senescent (AG) (20 months) C57BL/6J male and female mice. Mice were assigned in treatment groups to receive: (i) base diet ad libitum (AL), (ii) weight stable caloric restriction (CR), (iii) curcumin in the base diet (7200 mg/kg diet) (CURAL) or (iv) curcumin plus CR (CURCR). At 8 weeks of treatment, all mice were tested on a behavioral battery for detecting age associated cognitive, motor, and sensory dysfunction. Cognitive flexibility, tested using a serial reversal task, was significantly better for MAG males under CR and CURAL compared to AL but not under CURCR, suggesting an antagonistic interaction of these two interventions in males. On the other hand, all MAG and AG females under CURAL and CURCR did significantly better than age-matched AL. No interaction of CR and CURAL was observed in AG males, and CR failed to improve reversal performance in both AG males and females. None of the treatments had a significant effect on hippocampus- dependent spatial memory performance in MAG or AG. These results suggest that when implemented separately, both CR and CUR treatments have an ameliorative effect on impaired frontal cortical function present in late middle age and senescence. Interestingly, an overall effect of diet and sex was also detected from the locomotion performance wherein both MAG and AG female mice spent more time in the center compared to males, particularly those under CURCR, suggesting a possible anxiolytic affect which may be age, hormone and diet-related. Rotorod performance, a test for motor coordination, was improved by CR and CURCR but not CURAL. In addition, MAG males and females and AG males under CURAL displayed improved visual acuity suggesting a main effect of diet. In summary, these results suggest that curcumin intake during normal aging is associated with a variety of beneficial effects, some of which appear to mimic the effect of short-term CR in the absence of diminished energy intake and weight loss.

8. Nitrogen allocation upon dietary restriction in grasshoppers is inconsistent with the disposable soma hypothesis

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Dietary restriction extends lifespan and reduces reproduction in most animals. The disposable soma hypothesis attributes these effects in part to a shift in the allocation of ingested nutrients to increase investment to and maintenance of the soma. Support for the hypothesis has diminished over the last decade, but there is still only one paper reporting allocate to the soma upon dietary restriction (O'Brien et al. 2008). To further investigate the role of nutrient allocation upon life-extending dietary restriction, tissue-specific nitrogen allocation was tracked in grasshoppers (Romalea microptera) upon a full or restricted diet. In addition, in the same samples, carbonyl assays were performed to address tissue maintenance manifested as protein oxidation. To develop a labeled diet on which grasshoppers could

thrive, a naturalistic diet of hydroponically grown Romaine lettuce was enriched with 15N. Each day, individual grasshoppers were fed 'appetizers' (either high 15N or low 15N lettuce) followed by a low 15N 'meal' (either full or restricted). This allowed quantification of the relative proportions of nitrogen distribution upon a normal or restricted diet (60%). There was a 50% decrease in reproductive investment upon dietary restriction. Simultaneously, while ovary sizes differed, relative allocation of 15N to the ovary did not change. For somatic tissues (e.g., mandibular and femur muscle, fat body, gut, and hemolymph proteins), allocation was similar between restricted and normal diet grasshoppers. Carbonyl assays of mandibular muscle, gut, and hemolymph protein revealed reduced protein oxidation in diet-restricted individuals. Hence, dietary restriction does not alter nutrient allocation but does reduce protein oxidation in grasshoppers. Additional maintenance to the soma was observed in the absence of an additional investment of ingested nutrients, a finding that is inconsistent with the disposable soma hypothesis. Having recently generated transcriptomes for fat body and brain), research continues into nutrient sensing and signaling. Knockdown of neuropeptide F (homolog of NPY) upon a restricted diet regimen will investigate its role in mediating many of the salutary effects of dietary restriction.

9. A Conserved Transcriptional Signature Of Delayed Aging And Reduced Disease Vulnerability Is Partially Mediated By SIRT3

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Aging is the most significant risk factor for a range of diseases, including many cancers, neurodegeneration, cardiovascular disease, and diabetes. Caloric restriction (CR) without malnutrition delays ageing in diverse species, and therefore offers unique insights into age related disease vulnerability. Previous studies suggest that there are shared mechanisms of disease resistance associated with delayed aging, however quantitative support is lacking. We therefore sought to identify a common response to CR in diverse tissues and species and determine whether this signature would reflect health status independent of aging. We analyzed gene expression datasets from eight tissues of mice subjected to CR and identified a common transcriptional signature that includes functional categories of mitochondrial energy metabolism, inflammation and ribosomal structure. This signature is detected in flies, rats, and rhesus monkeys on CR, indicating aspects of CR that are evolutionarily conserved. Detection of the signature in mouse genetic models of slowed aging indicates that it is not unique to CR but rather a common aspect of extended longevity. Mice lacking the NAD dependent deacetylase SIRT3 fail to induce mitochondrial and anti inflammatory elements of the signature in response to CR, suggesting a potential mechanism involving SIRT3. The inverse of this transcriptional signature is detected with consumption of a high fat diet, obesity and metabolic disease, and is reversed in response to interventions that decrease disease risk. We propose that this evolutionarily conserved, tissue independent, transcriptional signature of delayed aging and reduced disease vulnerability is a promising target for developing therapies for age-related diseases.

mTOR and Rapalogs I

10. Persistence of short-term rapamycin treatment benefits to cardiac aging

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The largest risk factor for cardiac morbidity and mortality is aging, leading to progressive structural and functional changes that include left ventricular hypertrophy and diastolic dysfunction. Both humans and mice exhibit intrinsic cardiac aging, allowing the use of mice to model the decline of cardiac function in aging, in including diastolic failure (Heart Failure with preserved Ejection Fraction, HFpEF), for which there is currently no therapy. Previously, our lab has shown that short-term treatment with rapamycin in aging mice reverses some aspects of cardiac aging, including HFpEF, hypertrophy and the proteomic and metabolomic shifts that occur with age. In order to understand how often such treatment might be required in order to sustain enhanced cardiac function in aging, this work focuses on the persistence of these treatment-induced changes. To measure this, we have treated 24 month old male and female C57BL/6 mice with rapamycin or control diet for eight weeks, then removed the rapamycin for a further eight weeks. We monitored cardiac function with echocardiography, proteomics and metabolomic changes, and other functional tests to characterize the persistence of the benefits of short-term rapamycin treatment. Preliminary data suggests that some proteomic changes substantially persist 8 weeks after withdrawal of rapamycin in the old animals. For example, 74% of the changes in the abundance of proteins involved in oxidative phosphorylation were persistent at eight weeks, while some other pathways showed less persistence (e.g., 10% in calcium signaling). Rapamycin treatment did not alter body composition (determined by QMR), as expected, and also did not appear to improve endurance on a treadmill.

11. Rapamycin induces rapid mitochondrial remodeling to rejuvenate energy metabolism and energetics in old hearts

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University of Washington

Recently, our laboratory showed that rapamycin (a caloric restriction mimetic) can reverse age-related cardiac dysfunction in 10 weeks, highlighting its therapeutic potential for cardiac aging. At this time point, rapamycin reversed the age-related decrease in levels of mitochondrial proteins, without elevations of mitochondrial number or biogenesis. To dissect the detailed mechanisms of its cardiac aging benefit, we investigated the time course of cardiac remodeling induced by rapamycin. Immunoblotting confirmed that rapamycin treatment rapidly (within one week) reduced phosphorylation of TORC1 target S6 and TORC2 targets AKT and PKCα in old hearts. The mRNA expression of PCG-1α, a mitochondrial biogenesis marker, increased in the first 2 weeks of rapamycin treatment but returned to control levels at 10 weeks. Autophagy (LC3 II/LC3 I ratio and ATG5 levels) increased at 1 week of rapamycin treatment. Concordantly, proteomics analysis showed a mixture of increased and reduced levels of mitochondrial proteins at 1 week but an overall increase at 2 weeks of rapamycin treatment. These findings suggest that mitochondrial turnover and remodeling in the first 2 weeks of rapamycin treatment replenishes the cardiac mitochondrial proteome. Longitudinal echocardiographic analysis revealed that diastolic function of old mice began to improve as early as 2-4 weeks after treatment was initiated but continued to slowly progress over the

course of 10 weeks. Using 13C NMR spectroscopy in isolated perfused heart extracts, we showed that fatty acid oxidation was reduced by 30% in old control hearts, which was consistent with the proteomic data. Strikingly, 1 to 2 weeks of rapamycin treatment reversed the age-related decrease in fatty acid oxidation. This reversal of the age-related substrate shift was also accompanied by increased PCr/ATP ratio in hearts treated with rapamycin for 2 and 10 weeks. Overall, our results suggest that rapamycin induces mitochondrial remodeling in the first 2 weeks of treatment to rejuvenate energy metabolism and energetics and that this more gradually translates to improve cardiac function in old hearts.

12. Glucose homeostasis, energy expenditure, and lifespan--insights from Lamin A/C-deficient mice treated with rapamycin

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Mammalian TOR (mTOR) signaling regulates many fundamental metabolic and physiological processes. However, the in vivo functions of mTOR signaling that control lipid metabolism are not well understood. Furthermore, the role of rapamycin, which suppresses both mTOR complex 1 (mTORC1) and mTORC2 signaling, in regulation of adiposity is still controversial, albeit the extended lifespan by rapamycin in many genetic backgrounds of mice. Previously we demonstrated that rapamycin reverses elevated mTORC1 signaling and extends lifespan in lamin A/C-deficient mice (Lmna-/- mice). Because Lmna-/- mice are runts and were noted to lack fat, we speculated that signaling pathways in lipid metabolism might be dysregulated and contribute to the short lifespan of Lmna-/- mice. Here, we applied Lmna-/- mice as a model to evaluate the role of mTOR signaling in lipid metabolism mediated by rapamycin in adipose tissues. First, we showed that suppressing mTORC2 signaling is critical for life extension in Lmna-/- mice given that rapamycin increased body weight and fat content and induced “glucose intolerance” in Lmna-/- mice. Interestingly, we found that chronic treatment of rapamycin reverses elevated energy expenditure in Lmna-/- mice. Furthermore, Lmna-/- mice subjected to higher ambient temperature (30 oC) capture the life extension by rapamycin. This life extension in Lmna-/- mice is also associated with maintaining more body weight and fat content, and enhancing uncoupling protein 1 (UCP1) protein expression and fatty acid synthesis. These beneficial effects, however, were not observed in long-lived Lmna-/- mice lacking one copy of S6K1 (Lmna-/- S6K+/- mice), one of the major targets of mTORC1 signaling. Lmna-/- mice overexpressing 4EBP1 (Lmna-/- 4EBP1 mice), the other downstream target of mTORC1 signaling, manifested reduced adiposity and surprisingly an even shorter lifespan. Together, these findings point to aberrant mTOR signaling as a mechanistic component of laminopathies associated with reduced A-type lamin function and lipid metabolism. Given mTOR is a key modulator of aging and age-related metabolic diseases, identification of the ultimate mechanisms downstream of mTOR that regulate lipid metabolism will be vital in safely translating these findings into the clinic.

13. An intermittent treatment regimen mitigates deleterious side effects of rapamycin

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Age-related diseases are the major cause of morbidity and mortality in Western society. Recently, inhibition of the mTOR (mechanistic Target Of Rapamycin) signaling pathway by the FDA-approved drug rapamycin has been shown to promote lifespan and delay age-related diseases in model organisms including mice. Unfortunately, rapamycin has serious side effects in humans, including immunosuppression and glucose intolerance, which may preclude the long-term prophylactic use of rapamycin as a therapy for age-related diseases. Our work suggests that while the beneficial effects of rapamycin are largely mediated by inhibition of mTOR complex 1 (mTORC1), many of the negative side effects are mediated by "off-target" inhibition of a second mTOR-containing complex, mTORC2. As rapamycin is an acute, potent inhibitor of mTORC1, but requires a prolonged treatment to inhibit mTORC2, we hypothesized that an intermittent treatment regimen could be designed that efficiently inhibited mTORC1 signaling while minimizing mTORC2 inhibition, resulting in reduced side effects. We will present our results to date, which suggest that many of the undesirable side effects of rapamycin can be reduced, but not eliminated, by an intermittent treatment regimen.

14. Uncovering the distinct effects of rapamycin in stress-induced cell senescence

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Rapamycin increases longevity in several species and delays cell senescence in many cell types in-vitro. Several studies suggest senescent cells contribute to age-related pathology and loss of function, and we hypothesize that some of the beneficial effects of rapamycin are mediated by inhibition of cell senescence. Nrf2 is involved in adaptive response mechanisms to cellular stresses which are known to cause senescence and its activity decreases with age. In addition, silencing the Nrf2 gene leads to premature senescence. Research was conducted to elucidate Nrf2's mechanistic role in rapamycin inhibition of cell senescence. Data indicate that rapamycin increases the levels of Nrf2 in WT fibroblasts, correlating to decreased levels of senescent cells induced by hydrogen peroxide, measured by β-gal staining and p16 and p21 molecular markers. Data also showed autophagy was activated, measured by interconversion of LC3I/LC3II, beclin and p62 levels. However, in Nrf2 knockout mouse cells, rapamycin was unable to activate the autophagy pathway in absence of Nrf2 protein, but still decreased cell senescence. Senescence associated secretory phenotype (SASP) also corroborated the data—pro-inflammatory cytokines measured using an antibody array showed rapamycin still leads to a decrease in the secretion of pro-inflammatory cytokines in Nrf2 knock out mouse, both basally and after hydrogen peroxide treatment. This suggests that, at least in vitro, the activation of autophagy by rapamycin is dependent on Nrf2 pathway, but rapamycin may activate compensatory pathways/mechanisms that bypass the Nrf2 signaling pathway, and is able to reduce cell senescence even in Nrf2 knock out mouse cells.

Pharmacologic & Nutraceutical Interventions I

15. Aging does not result in a generalized increase in mTOR signaling

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Inhibition of the mechanistic target of rapamycin (mTOR) signaling pathway robustly extends the lifespan of model organisms including mice. While the precise molecular mechanisms and physiological effects that underlie the beneficial effects of rapamycin remain unclear, it is currently thought that rapamycin may act in part by blunting a generalized age-associated increase in mTOR signaling. Surprisingly, the effect of age on mTOR signaling has never been comprehensively assessed. Here, we test this model by determining the age-associated changes in mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) signaling in the liver, muscle, adipose, and heart of C57BL6/J.Nia mice, the lifespan of which can be extended by rapamycin treatment. We find that the effect of age on several different readouts of mTORC1 and mTORC2 activity varies by tissue and sex in C57BL6/J.Nia, as well by the particular phosphoresidue examined. We observed equally variable results in the livers of HET3 and DBA/2 mouse strains, and in liver, muscle and adipose tissue of F344 rats. Our results demonstrate that aging does not result in a generalized increased of mTOR signaling in all tissues, and suggest that rapamycin does not promote lifespan by reversing or blunting such an effect.

16. Cocoa epicatechin extends lifespan and promotes health in aged C57/BL mice fed a standard diet

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In our recent studies screening anti-diabetic compounds, we unexpectedly found that epicatechin, a flavonoid present in cocoa/chocolate, tea and various fruits, promoted general health and survival of obese diabetic mice. However, diabetic mouse is not a good model for aging study. In the present study, dietary cocoa epicatechin (EC) intake (0.25% in drinking water, 33 mice/group for 37 weeks) significantly increased survival rate to 69.7% from 39.2% in the control group in aged C57BL/6 mice fed a standard diet. Notably, epicatechin intake maintained higher body weight (39.2 g/mouse of EC vs. 36.8 g/mouse of control) while consumed less food (27.2 g/mouse/week EC vs. 29.3 g/mouse/week of control) comparing to the control group. Moreover, epicatechin intake significantly increased visceral fat weight (1.98 g of EC vs. 0.82 g of control) and reduced spleen (0.11g of EC vs. 0.17 g of control). Our results also showed that epicatechin intake reduced eight pro-inflammatory markers (IL-2, IL-4, IL-6, IL-17, MCP-1, IFNγ, KC and TCA3) in blood. Moreover, epicatechin changed protein expressions of β-actin, total actin and cytochrome C in skeleton muscle. Interestingly, epicatechin intake fully prevented ulcerative dermatitis, a common idiopathic, spontaneous, debilitating syndrome characterized as a chronic ulceration in C57BL mice. Therefore, dietary epicatechin intake really improved general health, attenuated inflammation and extended lifespan in aged C57BL/6 mice fed a standard diet, suggesting epicatechin may be an effective anti-aging agent although more studies are still needed.

17. 2002-2015 Blueberry Health Study Report: Annual Memory Score Increases Continue; Remain Significant After Correction for Practice Effects

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Background: Since 2004 we have reported at annual American Aging Association meetings that study participants consuming 1/2 to 2 cups/day of wild blueberries obtain higher memory scores during online measurements. Changing words from nouns to verbs or names did not significantly affect scores, and total measurements/year have not correlated with year-to-year improvements, suggesting that measurement practice is not responsible for observed increments. This report describes a within-person analysis not previously performed because earlier data sets lacked needed statistical power. Methods: Since our initial randomized, controlled, open label study during 2002, wild Maine blueberries with relatively high polyphenol content have been made available to participants in exchange for 12+ measurements/year at BlueberryStudy.com. This analysis was performed on a subset of data from over 100 initial participants and does not represent those did not join the long term study. Six long-term data sets were isolated and correlations between days-since-previous-measurement and scores were calculated. Scores were placed in 30 bins with an average of 94 scores/bin. Each bin contained only one person's scores after exactly the same delay or delay range, so delays/practice were held constant within each bin. Year-to-year improvements were quantified by assessment of score slopes in each bin, and slopes for each person were then combined into a single weighted-average slope, with weights equal to number of scores/bin. Weighted-average-slopes were then compared to each person's uncorrected overall score slope. Results: Correlations between days-since-previous measurement and subsequent scores were not significant, and in all but one case were slightly negative. The average correlation was -0.038, median -0.051, range -0.082 to 0.025. Corrected year-to-year slopes were in each case larger than slopes based on raw scores. Average slopes increased 7.04-fold (median 6.26, range 2.17 to 16.05). Average percent correct responses increased during 2008-2014 from 96.1% to 97.15%, with an approximately linear trajectory toward an annual score maximum with a slope of zero next year, over 10 years after regular blueberry consumption began. The R-squared statistic for score changes vs. year was 0.989 for this analysis ($p<0.0001$). Discussion: Slopes are unreliable because they are sensitive to outliers, as are correlations. Nevertheless the steady score trajectory (R-squared=0.989) suggests observed trends are real and warrant further study. The long period of memory improvement is consistent with hearing and self-reported health improvements we have seen ($p<0.05$), the impressively-controlled studies of J.R. Chen et al. [20499363, 21912699, 22555620] and Muraki et al. [23990623] who found that blueberries are associated with larger diabetes risk reductions than all other fruits examined. Our working-hypothesis is that blueberry consumption can lead to senolytic-tissue-cleansing-effects [see 25446976, 25754370] for some but not all people by providing daily quercetin and other polyphenols. Conclusion: Daily consumption of wild blueberries is compatible with and may help

support year-to-year memory improvements lasting approximately 10 years, almost the same proportion of healthspan as reported elsewhere [25754370], with only a minor effect from practice. Acknowledgment: This report is submitted on behalf of co-authors no longer with us whose ideas and contributions continue to guide and inspire us.

18. The Mitochondrial-Derived Peptide Humanin Improves Cognition in Old Mice

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Humanin is the first member of a new class of proteins called mitochondrial-derived peptides (MDPs). These peptides are encoded by small open reading frames in larger genes within the mitochondrial genome. Previous studies have shown humanin to have profound neuroprotective and cytoprotective effects. Many of these effects are reminiscent of the effects of dietary restriction (DR), a known intervention that helps maintain memory and cognition in old animals. This study examined if like DR, humanin could decrease the decline in memory and cognition seen in older animals. 18-month-old, female C57BL/6N mice were given biweekly IP injections with a potent humanin analogue (HNG) for 14 months. Cognitive function (measured by the Barnes and Y-maze tests) was substantially better in humanin treated mice. Additionally, improvement in physical performance as measured by the rotarod test was also observed during humanin therapy. Immunohistochemistry of the brains of the mice are ongoing, but our preliminary data suggests that an increase in neurogenesis, as assessed by BrdU staining, may play a role in this improvement. Our data suggests that like DR, twice weekly humanin treatment can help maintain the cognitive ability of old mice.

19. Germ-Line Cells and Human Milk May Contain Uniquely Protective, Rejuvenation-Compatible Amino Acid Ratios That May Extend Human Lifespan

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MMT Corporation

During the evolution of primates, milk amino acid composition steadily evolved to provide precursor nutrients for the centrally-important antioxidant glutathione. Precursors cysteine, glycine and serine increased from 12 to 20, 14 to 22 and 48 to 61 mg/g milk protein [PMID 8027865]. Serine is required to transfer sulfur from methionine to form cysteine and then glutathione. Analysis of the USDA nutrient database indicates that foods which may resemble germ-line cells [yeast, different kinds of fish eggs] have very similar amino acid profiles as human milk when guesstimate-enhanced weights [see US patent 8,838,511] are used to rank similarity, suggesting that protective glutathione-related ratios evolved in early eukaryotes, were maintained in germinal lineages and then recently became predominant in primate and human milk, with the recent evolution of higher serine/protein ratios uniquely distinguishing humans from other primates. Measurements of elevated serine in Italian centenarians [PMID 23483888] suggest serine

may be an important human longevity nutrient for some individuals as part of a balanced diet, possibly in part because of its role at the start of the transsulfuration pathway where it enables (i) methionine-sulfur to be used to form cysteine and then glutathione and (ii) methionine and homocysteine levels to be reduced thereby preventing or postponing methionine-related illnesses by hypothesis including Alzheimer's and geographically-linked illnesses of aging including multiple sclerosis and Parkinson's. These observations extend the misincorporation measurements and framework of Wolfgang Freist [9689940] and suggest that optimum amino acid ratios [OAARs] may be important determinants of human healthspan. To quantify possible healthspan improvements that may result from OAARs at each meal, data from 8027865 and linear adjustments corresponding to amino acid changes linked with centenarians, Alzheimer's and mild cognitive impairment [23483888, 23571809] were matched with lifespan data compiled by R.G. Cutler [812099] and subjected to SIMCA multivariate analysis [Umetrics, Kinnelon, NJ]. Predictions of human lifespan after OAARs reached 180 years of age for maximum projected lifespan potential. This high projection is consistent with large longevities observed by Orentreich et al. [8429371, 8001743], and others [933560] and additive effects reported more recently [24244480]. Advice from Aaron Lukton is much appreciated.

20. Drosophila Intervention Testing for Longevity, Stress Resistance, and Alzheimer's Disease

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Genetic studies indicate that aging is modulated by a great number of genetic pathways. We have used Drosophila longevity and stress assays to test multipath intervention strategies for longevity, stress resistance, and Alzheimer's Disease. To carry out this strategy, we supplemented the flies with Multipath Herbal Extracts (MHEs) that are predicted to modulate the expression of many genes involved in aging and stress resistance, such as mTOR, NOS, NF-KappaB, and VEGF. When flies were housed in large cages with MHEs added, daily mortality rates of both male and female flies were greatly diminished in mid to late life. Surprisingly, MHEs also stabilized midlife mortality rate increases so as to extend the maximum life span substantially beyond the limits previously reported for D. melanogaster. Under these conditions, MHEs also promoted robust resistance to partial starvation stress and to heat stress. Fertility was the same initially in both treated and control flies, but it became significantly higher in treated flies at older ages as the fertility of control flies declined. The data indicate that MHEs are novel herbal mixes with striking effects on enhancing Drosophila stress resistance and life span in some environments, while minimizing mid to late life mortality rates. More extensive work also showed that the environment and other factors can have transformative effects on both the length and distribution of survivorship, and on the ability of MHE to extend the life span. In a separate set of experiments, transgenic Drosophila with drug-inducible Abeta or Tau mutations were generated. These flies developed Alzheimer's like symptoms with Abeta or Tau induction, which we followed by measuring the decline in crawling speed with age. We tested different MHEs on these Alzheimer's Disease models to identify MHE treatments that minimize the genetic effects of Abeta and Tau. The Optimized MHEs treatment was then tested in a small one year clinical trial, which has given promising results in patients with mild symptoms of Alzheimer's Disease.

21. Remediating Age-Related Susceptibility to Environmental Toxins

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An important factor in cellular toxicological response is the inducible Nrf2-regulated phase II detoxification system. We previously showed a 51% age-related decline in Nrf2 protein in rat liver ($p<0.0001$) with a concomitant loss in transcription site binding. However, the role that basal decline in Nrf2-mediated transcription plays in increased susceptibility to oxidative and toxicological insults with age is not known. In order to evaluate the contribution of age-related Nrf2 loss to increased toxicological vulnerability, isolated rat hepatocytes were treated with increasing concentrations of menadione that is detoxified by the Nrf2-dependent enzyme, NAD(P)H:quinone oxidoreductase 1 (NqO1). Consistent with the age-related loss of Nrf2, hepatocytes from 24-month old rats displayed a 63% decline in NqO1 protein levels ($p=0.0119$), and were markedly less resistant to menadione toxicity than those from 3 month old animals (31% lower LC50 with age, $p<0.01$). The heightened toxicity resulted not only from loss of Nqo1, but also from attenuation of hepatic glutathione detoxification capacity. Levels of glutathione (GSH) and GSH-dependent phospholipid hydroperoxidase 4 (GPX4) declined by 35% ($p<0.05$) and 70% ($p=0.0043$), respectively. These results are consistent with lower age-related Nrf2-mediated transcription as expression of GSH synthesis enzymes and Gpx4 are Nrf2 dependent. As the accentuated susceptibility to menadione at least partly stemmed from lower GSH-dependent detoxification, we hypothesized that remediating the age-related loss of hepatocellular GSH may reduce menadione toxicity. To test this, we pretreated cells from old animals with 400 μM N-acetyl-cysteine (NAC) for 1 hour before adding menadione (300 μM). Pretreatment with NAC, which provides hepatocytes with the substrate-limiting levels of cysteine for GSH synthesis, increased resistance to menadione to that seen in hepatocytes from young animals. Taken together, our results thus suggest that Nrf2-mediated detoxification mechanisms decline with age, which may be partially remediated by providing GSH synthesis substrates. Our data thus have important implications for explaining enhanced drug interactions and susceptibility to environmental xeniobiotics with advanced age.

22. The Mitochondrial-Derived Peptide Humanin Enhances Healthspan and Improves Metabolic and Inflammatory Markers in Aged Mice

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Humanin, a 24-amino acid, mitochondrially-encoded peptide, has been reported to be insulin sensitizing, anti-inflammatory, anti-fibrillogenic, neuroprotective and cardioprotective in vitro and in various young/middle-aged rodent in vivo disease-models, including diabetes and neurodegeneration. Humanin levels fall in elderly humans and rodents; furthermore in animal and human models of altered lifespan, humanin levels correlate with longevity and health, suggesting a role for humanin in healthy aging. Hence, we investigated the protective effects of humanin in a normal middle-aged mouse cohort.

Long term treatment (14-months) with intra-peritoneal biweekly injections of the humanin analogue HNG (4-mg/kg/BW) starting at middle age (18-months) did not affect lifespan, but extended multiple markers of healthspan in C57BL/6N female mice. Treatment with HNG moderately but significantly decreased body weight, and substantially improved metabolic markers such as fasting glucose and insulin levels. The body weight loss was correlated with a significant loss of visceral fat with no change in lean body mass. Our data also indicates that humanin mimics some of the benefits of dietary restriction by reducing insulin-like growth factor-1 (IGF-1), and increasing the fasting-responsive IGF-binding protein-1 (IGFBP-1) without actually affecting caloric intake and this may, in part, explain its benefits.

Aging is characterized by chronic low-grade systemic inflammation even in the absence of chronic disease. Aging is associated with an increase in circulating proinflammatory cytokines, which contributes to multiple diseases, poor physical functioning, and mortality. Therefore, reversing the process of chronic inflammation or, at least, slowing it down may prevent or delay morbidities/mortalities in the elderly. Chronic humanin treatment showed a beneficial change in inflammatory markers including 2-3-fold reductions in IL-6, TNFα, IL-1β, INFγ, and IL-10. Together, these data suggest that humanin has a dietary-mimetic activity that delays the normal metabolic and inflammatory decline of aging.

23. Inhibition of Oxidative Stress-Induced Senescence by the Mitochondrial-Derived Peptide Humanin Attenuates Age–Related Macular Degeneration

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Humanin (HN), a 24-amino acid mitochondrial encoded peptide is a neuro-protective factor in vitro and in various animal models. HN has shown to decline with age in mice and humans, and thus may participate in the age-related disease. Age-related Macular degeneration (AMD) is the leading cause of vision loss among people older than 50. The mechanism of AMD remains unclear and treatment choices are limited. Hence, we investigated the potential protective effects and potential mechanism of HN in oxidative stress (OS)-induced retinal cell death in young and old mice as well as in human retinal pigment epithelial (hRPE) cells. In vivo, a single injection (15 mg/kg or 50 mg/kg) of sodium iodide (NaIO3) caused RPE cell damage that was accompanied by photoreceptor cell degeneration in both young and old mice and demonstrated a dose- and age-dependent effect. Higher doses of NaIO3 induced a more severe progressive degeneration of the neurosensory retina and a more extensive loss of RPE cells in the aged mice group, which was ameliorated by co-treatment with HN. In vitro we showed that HN addition decreased cell death and protected from mitochondrial dysfunction induced by OS in hRPE cells. To decipher the potential mechanism involved in the protective effect of HN on RPE cells damage, we further investigated the role of HN in OS (H2O2 treatment)-induced cellular senescence. OS treatment significantly increased senescence-associated β-gal-positive cells, an effect that was dramatically reduced by co-treatment with HN for 48 h ($P < 0.01$). The induction of mRNA expression of the senescence biomarker Apo-J was significantly reduced by HN addition ($P < 0.01$). We further examined the expression of second senescence biomarker, p16INK4a by immunoblotting and showed that the OS-induced up-regulation of p16INK4a was also significantly

inhibited by HN co-treatment ($P < 0.05$). Thus, HN protects RPE cells from oxidant injury by preventing cell death and restoring mitochondrial function and particularly through the inhibition of cellular senescence. These data suggest a potential role for HN therapy in the prevention and treatment of AMD and other conditions involving senescence.

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24. A multi-translational approach to prevent age-related functional decline

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Over the last ten years our research group has embraced a multi-translational perspective towards investigating the efficacy of various behavioral and pharmacological interventions for the prevention of declining physical function and mobility. Among the most promising classes of compounds we have investigated using this approach are the angiotensin converting enzyme inhibitors (ACEi). The primary clinical use of ACEis is in the control of hypertension. However, beyond its impact on hemodynamic functioning, ACEis have been shown to ameliorate a variety of dysfunctional adaptations inherent to the aging process, such as insulin sensitivity, inflammation, and oxidative damage. Furthermore, the combination of ACEis and exercise may confer additional benefits to physical function and mobility than either treatment alone. This presentation will highlight the translational history of our work in this area moving from epidemiological, to pre-clinical, and ultimately clinical studies. Each phase brings to the question critical knowledge necessary for developing a more refined hypothesis for how these effects are realized and ultimately for developing target interventions for the promotion of health-span in older individuals.

25. Mitochondrial protein SQRD-1 is necessary for protein translation in hydrogen sulfide

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Hydrogen sulfide (H2S) is one of three known endogenously-produced gasotransmitters and has been shown to modulate important cellular pathways involved in controlling lifespan. In C. elegans, H2S extends lifespan and improves protein homeostasis, while in mammals H2S has been shown to modulate dietary restriction regiments that increase lifespan. The regulation of protein translation is a key factor in the effects of dietary restriction and protein homeostasis. We therefore measured the effects of H2S on protein translation in C. elegans. We found no change in global protein translation after exposure to concentrations of H2S shown to increase longevity and stress resistance. If we knock out HIF-1, a key transcription factor in the response to H2S, this same concentration of H2S is lethal. Surprisingly, we observed only a small decrease in translation when hif-1 (ia04) null animals are exposed to H2S. Although these results might suggest that the response to H2S does not modulate pathways that coordinate protein

translation, we do not favor this hypothesis. We found that protein translation was rapidly suppressed in sqrd-1 mutant animals exposed to H2S. SQRD-1 is a conserved mitochondrial protein that oxidizes H2S and forms polysulfides that can modify free sulfhydryl groups of proteins. This is thought to be one mechanism for the biological "detoxification" of H2S. As in bacteria and yeast, C. elegans requires sqrd-1 to survive long term exposure to H2S. We have discovered that SQRD-1 is required to maintain global protein translation by ensuring that eIF2α remains unphosphorylated. We found no evidence that stress responses known to stimulate the activity of eIF2α kinases are activated in H2S. Moreover, depletion of either gcn-2 or pek-1, the only two eIF2α kinases in C. elegans, does not abolish the phosphorylation of eIF2α in sqrd-1 mutant animals exposed to H2S. These data suggest a potentially novel mechanism of stimulating phosphorylation of eIF2α to reduce translation initiation in H2S. We are currently working to delineate the molecular and genetic pathways by which SQRD-1 at the mitochondria coordinates global protein translation in H2S.

26. Age-dependent changes in metabolic processes contribute to microglial priming

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Aging is the single biggest risk factor for the development of neurodegenerative disease. While diseases like Alzheimer's, Parkinson's, and ALS have different triggers their progression involves a chronic inflammatory response. Neuroinflammation is a central component of the degenerative process involving both the innate and adaptive immune system. However, the central effector of inflammation in the CNS is its resident macrophage the microglia. As we age microglia can become "primed" by a number of stimuli including aging. Priming is described as the exaggerated and prolonged response of an immune cell to a secondary stimulus. In addition to an exaggerated response microglia of the aged brain are also resistant to down regulation by anti-inflammatory and immune-modulatory cytokines like IL-4 and IL-10 and show an impaired ability to switch to an M2 phenotype. While the phenomenon of priming has been thoroughly described in the literature there is limited understanding of the mechanisms underlying how it occurs Utilizing silac-labeled mass spectrometry and Ingenuity Pathway Analysis we examine the molecular mechanisms underlying the age-dependent priming of microglia. In this manner we identified significant alterations in metabolic processes that may be involved in the priming process. We also demonstrate that aged microglia have an enhanced glycolytic profile both at baseline and in response to LPS. Because M1 polarization preferentially utilizes glycolysis vs oxidative phosphorylation this finding supports the theory that the metabolic flux of aged microglia favors inflammatory activation. Using Ingenuity's upstream analysis we identified mTORC2 as a likely upstream regulator of this activity. Using siRNA we were we demonstrate that knockdown of mTORC2 in the microglial cell line BV2 replicates the "primed" phenotype. The cross-talk between metabolism and inflammation is well documented. We propose that age-dependent changes in mTOR activity contribute to the age-dependent priming of microglia by enhancing glycolytic gene expression. The age related increase in inflammation is thought to contribute to a number of processes including age dependent declines in neurogenesis and cognitive function. If mTOR signaling is indeed at the center of this phenomenon it would represent a readily exploitable target for addressing a number of age related pathologies.

27. RCAN1 is an Important Link Between Amyloid-B and Tau in Alzheimer's Disease

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The main candidates for causing Alzheimer's disease (AD) are the amyloid-B (AB) peptide and the Tau protein. Dysregulation of the normal functions of both AB and Tau may induce neurodegeneration, and the cognitive deficits characteristic of this disease. AB has an elevated toxicity, and causes oxidative stress, while hyper-phosphorylation of Tau (p-Tau) prevents its normal turnover by the proteasome, and leads to disintegration of the microtubule skeleton and the formation of neurofibrillary tangles. Although it has been extensively studied, the link between the AB and p-Tau has not been clear.

The stress-inducible Regulator of Calcineurin gene (RCAN1) may be one of the bridges from one molecule to the other one. Calcineurin is a serine/threonine phosphatase involved in Tau de-phosphorylation. RCAN1 expression results in synthesis of RCAN1-1L, RCAN1-1S, and RCAN1-4 proteins that inhibit calcineurin. RCAN1 expression also causes an induction/activation of glycogen synthase kinase 3B (GSK3B), which is responsible for Tau phosphorylation. Thus, RCAN1 expression can cause extensive Tau phosphorylation.

In various cell types, including cultured neurons, we have demonstrated that AB induces oxidative stress which, in turn, causes up-regulation of RCAN1; the same studies have also shown concomitant decreases in calcineurin activity, and increasing GSK3B activity levels, which eventually cause formation of p-Tau. In RCAN1 knock-in mice there are increased levels of p-Tau in the brain, but simply stressing the animal can also increase p-Tau. Regarding humans, it is known that the apolipoprotein E E4 allele is a risk factor for developing AD. Persons carrying the apolipoprotein E E4 allele have also been found to express higher levels of RCAN1 than non-carriers, and consequently more p-Tau. In fact, in AD patients the RCAN1-1L isoform is overexpressed, confirming the connection between AD and RCAN1.

Much of the Alzheimer field is divided between two quasi-religious 'camps,' those who believe AB is the main culprit in the disease might be called the 'A-Baptists.' Those who favor hyper-phosphorylation of the Tau protein might be considered 'Tauists.' Since RCAN1 is induced by AB and then, in turn, itself induces Tau hyper-phosphorylation, we suggest that RCAN1 may bridge this ecumenical divide.

28. Postnatal neurogenesis

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Klotho (KL) is a transmembrane protein the absence of which shortens mouse lifespan by ~85%, while over-expression extends life 20-30%. The KL knockout was first described as a model of accelerated aging, as in addition to shortened lifespan loss of KL induces rapid accumulation of many aging phenotypes

including thinning skin, muscle loss/atrophy, emphysema, infertility, arteriosclerosis, and cognitive impairment. Little is known about the function of KL in the brain where it is expressed in both neurons and choroid plexus. Research on the brain KL is primarily limited to descriptive studies at a single time near the end of life. From this work we know that without KL the brain appears mildly neurodegenerative with elevated levels of proteins associated with apoptosis and oxidative stress, decreases in synaptic marker proteins and deficits in axonal transport. Although interesting, the terminal decline of the rest of the body complicate data interpretation. Recent work supports a role for KL in the brain by examining the brain of KL overexpressing mice. These animals, as well as humans with a KL polymorphism, experience a life-long increases in cognitive function with mild overexpression. This effect correlates with an increase in the immature neuron NMDA channel subunit, NR2B. As the absence of KL in skin results in rapid depletion of epidermal stem cells, together these data point to a function for KL in the regulation of postnatal neurogenesis. We used wild-type, KL knockout, and KL overexpressing animals to evaluate whether adult neurogenesis was affected by the expression level of KL in dentate gyrus of the hippocampus. Loss of KL transiently increased synaptic plasticity while decreasing both proliferation and maturation of neural progenitor cells. Proliferation is decreased before the onset of overt pathology. KL appears to alter the maturation program, as protein expression patterns are improperly coordinated and progression through to functional maturity is delayed. Overexpression of KL results in an opposite phenotype. Underlying these phenotypic changes, hyperactivation of multiple signaling pathways may provide evidence of KL as a critical coordinator of neurotrophic signaling to regulate the proliferation and progression of postnatal-derived neurons.

29. Obesity in Aging Exacerbates Blood Brain Barrier Disruption and Neuroinflammation Leading to Synaptic Plasticity Deficit and Cognitive Decline in Mice

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There is growing evidence that obesity promotes vascular cognitive impairment in the elderly. Although obesity affects 38% of individuals aged 65 and older in the U.S., the specific microvascular mechanisms through which aging and obesity interact to promote cognitive decline remain unclear. To test the hypothesis that aging exacerbates obesity-induced microvascular damage we compared young (7 mo) and aged (24 mo) control and high fat diet-fed obese C57BL/6 mice. We found that aging exacerbated obesity-induced blood brain barrier (BBB) disruption and neuroinflammation, as indicated by increased microglia activation, pro-inflammatory cytokine expression and oxidative stress. Obesity-induced neuroinflammation in aged mice was associated with impaired hippocampal-dependent cognitive function and reduced long-term potentiation (LTP) elicited by high frequency stimulation in hippocampal slices. Obesity-induced neuroinflammation in aged mice was associated with decline in hippocampal expression of several genes involved in learning, memory formation and LTP, including Grik1, Grik5, Gap43, Cask and Syngr1 (targeted qPCR array). Collectively, obesity-induced BBB disruption leads to a heightened state of neuroinflammation and significant synaptic plasticity deficit, which likely contribute to the significant cognitive decline observed in aged obese animals.

30. Deficiency of PDE4A reverses memory loss associated with Alzheimer's disease via cyclic AMP signaling

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Phosphodiesterase 4 (PDE4), one of the 11 PDE enzyme families hydrolyzing cyclic nucleotides, is critical for controlling intracellular cAMP concentrations and plays a role in the mediation of cognition associated with Alzheimer's disease. However, the contributions of its subtypes (PDE4A-D) remain unclear. Here we tested PDE4A knockout (4AKO) mice and their wild type (WT) controls for memory using passive avoidance and water-maze in the absence or presence of beta-amyloid peptide 1-42 (Abeta42) infused into the dorsal hippocampus. Compared to the WT controls, 4AKO mice displayed reversal of Abeta42-induced memory deficits. In addition, the levels of cAMP and phospho-CREB were increased in the hippocampus of 4AKO mice, which also showed reversal of Abeta42-induced decreases in pCREB in the hippocampus. These results suggest that PDE4A plays a role in the mediation of memory associated with AD and could be a new target for treatment of cognition deficits in AD [This work was supported by research grants from NIA (AG031687) to H.-T. Z.].

31. Dibutyryl-cAMP (dbcAMP) induces peptidylarginine deiminases (PADs) expression in human astrocytoma U-251MG cells

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Peptidylarginine deiminases (PADs) are post-translational modification enzymes that citrullinate (deiminate) protein arginine residues in a calcium (Ca2+)-dependent manner, yielding citrulline residues. Enzymatic citrullination abolishes positive charges of native protein molecules, inevitably causing significant alterations in their structure and functions. Previously, we reported the abnormal accumulation of citrullinated proteins and increase in the amount of PAD2 in hippocampi from Alzheimer's disease (AD) patients. Moreover, glial fibrillary acidic protein (GFAP), an astrocyte-specific marker protein, and vimentin were identified as citrullinated proteins by using two-dimensional gel electrophoresis and MALDI-TOF mass spectrometry. In this study, we investigated PADs expression by using sodium N 6, 2' -O -dibutyryl adenosine 3', 5'-cyclic phosphate (dbcAMP) in human astrocytoma U-251MG cells. Under normal culture condition, PAD2 and PAD3 mRNA expression was detected by quantitative PCR (qPCR) in U-251MG cells. The mRNA expression and protein levels were significantly increase by dbcAMP in a dose- and time-dependent manner. Moreover, PADs enzyme activity were also significantly increased in a dose- and time-dependent manner. These PAD2 and PAD3 mRNA expression by dbcAMP were inhibited by SP600125

which is a c-jun N-terminal kinases (JNK) inhibitor. Therefore, PAD2 and PAD3 expression by dbcAMP might be mediated in the JNK cascade in human astrocytoma U-251 MG cells. It has been reported that JNK phosphorylated transcriptional factor specificity protein 1 (SP1) under oxidative stress conditions in human astrocyte. Additionally, expression level of transcriptional factor SP1 was abnormally increased in Alzheimer's disease brain. Collectively, these results and previous reports strongly suggested that PAD2 and PAD3 expression were mediated by the JNK/SP1 signal transduction pathway. Increase of citrullinated proteins by PAD2 and PAD3 might be involve in the onset and development of the AD.

Comparative Biology of Aging I

32. Higher levels of the small chaperone HSP25 contribute to longer lifespan

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Studies in long-lived animal models such as the naked mole-rat (NMR) show resistance to a broad spectrum of environmental stressors. Collectively these findings suggest that NMRs possess efficient mechanisms to maintain protein quality in multiple tissues. Our previous work indicated that this is attributed in part to altered changes in molecular chaperone levels that influence the transport and disposal of damaged proteins. We extend this study of chaperones and protein degradation mechanisms that compared NMRs and mice to include eight rodent species of various sizes and lifespans by examining key proteasome-related molecular chaperones, proteasome and autophagy markers in the liver and quadriceps muscles. The small molecular weight chaperone heat-shock chaperone 25 kDa (HSP25) showed as significant correlation with longevity in both tissues, and was highest in the NMR, suggesting that HSP25 may play a key role in age-related maintenance of protein homeostasis in long-lived animals. To examine the role of the NMR HSP25 further, we constructed a transgenic C.elegans worm (HSP25NMR). The HSP25NMR worm shows resistance to heat stress, and preliminary data suggest that this transgenic worm will have an extended lifespan. We continue to elucidate phenotypes of the HSP25NMR worm to determine the contribution of this chaperone to longevity, stress resistance, and health span.

33. Exploring Spinal Cord Neurogenesis in a Long-lived Model: The Naked Mole-rat

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The naked mole-rat (NMR) is the longest-lived rodent known, with a maximum lifespan of over 30 years. Following a 10-week gestation period, NMRs are born precocial with larger brains than newborn mice. Despite prolonged gestation, NMRs exhibit an extended phase of somatic growth and brain growth lasting for more than 6 months. This prolonged developmental period provides a novel opportunity to discriminate key milestones and factors that regulate these processes, which cannot be assessed in mice due to their compressed maturation period (2-3 weeks). As no developmental studies have yet been

conducted on the NMR spinal cord, we examine changes in spinal cord morphology, morphometrics, and neurogenic factors spanning from birth to the end of spinal cord maturity. Moreover, since NMRs are eusocial and reproduction is suppressed in more than 95% of the colony members, we plan to also examine if changes in breeding status, which are known to impact growth of the lumbar spinal vertebrae, impact spinal cord structure, and hope to uncover if neurogenic processes can be naturally reactivated even in “adult” mammals.

34. More Efficient DNA Repair in Response to Genotoxic Stress is Evident in the Long-Lived Naked Mole-Rat and Contributes to Cancer Resistance

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DNA repair commonly declines with increasing age in traditional animal models used in aging research. This can result in the accrual of DNA damage, generally linked to age-associated pathology and diseases, frailty and poor quality of life. By exploiting the natural diversity in species longevity, we may uncover novel evolved mechanisms that provide novel insights into lifespan extension that are not possible when studies are confined to the more typical short-lived laboratory species. The naked mole-rat (NMR; Heterocephalus glaber) is an exceptionally long-living rodent species, with a lifespan of ~30 years. This mouse-sized rodent also displays pronounced resistance to both spontaneous and experimentally induced tumorigenesis. Clearly, NMRs must possess efficient mechanisms to protect and maintain genetic integrity and this most likely is integral to their sustained health span and prolonged longevity. However, very little is known about the cytoprotective mechanisms used by this animal model to resist cancer induction. As the DNA repair machinery has not been previously studied in this species, a DNA repair profile under normal physiological conditions, as well as in response to DNA damage induction, may yield important clues as to how the NMR prevents either the accrual of DNA damage and/or its translation into cancer. Our preliminary data suggest increased expression of key DNA repair genes. Additionally, given its resistance to tumorigenesis upon UV exposure and treatment with DNA-damaging agents, we hypothesize that the NMR exhibits superior somatic and germ cell maintenance in the form of increased DNA repair in response to these agents. To test this hypothesis, I elucidated interspecies differences in the DNA repair profile of NMRs and mice 1) in response to spontaneous DNA damage and (2) in response to exposure to genotoxic agents. Moreover, I assessed differences in mitotic, meiotic and post-meiotic cells by examining both somatic and germ cells. Understanding the DNA repair profile in the NMR reveals important insights about the naturally evolved protective mechanisms employed by long-living species to better handle spontaneous DNA damage and prevent tumorigenesis. These mechanisms may not be evident in short-lived species and could lead to the development of novel therapies to enable robust cell survival, improved organ and organism health, protection against cancer and ultimately a longer life.

Genetics of Aging

35. Depleting Nrip1, a potential aging gene, to extend longevity and reduce the risk of breast cancer in female mice

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We previously identified age of female sexual maturation as a biomarker for longevity among inbred mouse strains, in which mice with delayed age of vaginal patency had extended longevity. Using genetic approaches, we identified nuclear receptor interacting protein 1 (NRIP1) as a candidate gene involved in regulating age of vaginal patency. We further verified that the depletion of NRIP1 could significantly delay the age of vaginal patency. Here, we report that the depletion of NRIP1 could also significantly extend longevity in female C57BL/6 mice (>20%, $P<0.05$). Due to the well-known relationship between an early age of menarche and an increased risk for developing breast cancer in women, we tested if the depletion of NRIP1 could reduce the risk of breast cancer. Our results show that NRIP1 is elevated in human breast cancer cell lines and breast cancer tissues. The depletion of NRIP1 in cancer cell lines could significantly suppress cell viability and induce apoptosis. Importantly, we found that in female mice, the depletion of NRIP1 could significantly delay the onset and reduce the risk of 7,12-Dimethylbenz[a]anthracene (DMBA) induced breast cancer. These results indicate that NRIP1 may be a potent aging gene involved in the regulation of cancer. Our studies may provide a genetic, molecular and pathological approach to understanding the long-standing hypothesis regarding the trade-off relationship between female sexual maturation and aging.

DNA Damage and Repair

36. Effects on lifespan of experimental evolution of tolerance to extreme heat and oxidative stress

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Mutagenesis and RNA interference studies in yeast, worms, flies and mice have revealed dozens of genes whose manipulation can result in substantially increased organismal lifespan. These longevity genes are almost always identified to be within genetic pathways that regulate cellular & organismal stress responses, *e.g.*, resistance to oxidative, heat, pathogen or starvation stress. The apparent relationship between stress resistance pathways and longevity genes suggests support for the idea that cellular resistance to stress controls rates of organismal aging (first proposed by Denham Harman in 1953). Despite the apparent correlation between stress resistance and longevity genes, there are very few studies showing direct evidence that alteration of stress or stress-related damage plays a role in longevity. We used the soil nematode *Caenorhabditis remanei* to test the functional independence of individual components (including longevity genes) within and among stress response networks. After collecting a wild population of *C. remanei* from

Toronto, ON, we applied artificial selection on subsets of that population for resistance to acute and chronic heat and oxidative stress. Artificially selected populations responded dramatically to selection on both oxidative and heat stress resistance, but we find little evidence of correlated responses in longevity, or in resistance to other stressors. Mutations at many stress response genes have previously been shown to have broad pleiotropic effects on multiple stressors and on longevity (*e.g., daf-16/FOXO*). However, our data show that in the context of a functioning genetic network, responses to stressors can be regulated separately from lifespan, and in a stress-specific manner. We are currently localizing genetic responses to selection; low levels of linkage disequilibria in *C. remanei* should allow us to do this at a 20-50bp resolution.

37. Determination of the point-mutation genome instability (PIN) error threshold for decreased lifespan in Saccharomyces cerevisiae

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Organismal aging is characterized by the functional decline, loss of vigor, and increased likelihood of pathology associated with post-developmental life. Genome instability increases as cells age, is an important driver in age-related carcinogenesis, and can be modulated through lifespan-extending interventions such as dietary restriction (DR). The levels of point-mutation genome instability (PIN) that a cell can tolerate before lifespan is impacted have not been systematically investigated, largely due to the low amounts of accumulated mutations incurred during aging in model systems and poor detection of PIN in human tissues using current sequencing methodologies. In order to model the effects of PIN on cellular lifespan, Saccharomyces cerevisiae strains expressing replication-based mutator alleles were used to determine the mutagenic threshold for decreased replicative lifespan, both in terms of mutation rate and accumulated mutation burden. A strong negative correlation exists between median lifespan and mutation rate in haploid mutator yeast. Mutation rates 30-50x greater than WT are necessary before lifespan is significantly decreased in these strains. Elevated mutation rate and decreased lifespan can be attenuated by growth under limited-glucose DR in strains expressing mutator alleles. Transiently-mutated diploid strains show decreased lifespan in the absence of mutator alleles, which suggests that PIN is sufficient to decrease lifespan. Whole-genome sequencing is currently underway to determine mutation burden in these strains. Our results provide a quantitative description of mutagenic thresholds necessary for decreased lifespan and indicate that at least one lifespan-extending strategy attenuates mutator and PIN-induced decreased lifespan. These studies will provide a qualitative and quantitative assessment of the consequences of mutator phenotypes on cellular lifespan. They will also provide a framework for future work to determine the importance of PIN in human aging and age-related disease.

38. Age-related changes in DNA damage repair gene expression in acute myeloid leukemia

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Acute myeloid leukemia (AML) arises from the clonal evolution of hematopoietic stem/progenitor cells (HSC) with a mean age of diagnosis of 67 years. Older age confers a 5-year survival of <10%. Aging leads to functional decline of HSC due to increased replication stress and altered replication fork dynamics from decreased expression of mini-chromosome maintenance (MCM) DNA helicase proteins. We sought to investigate age-related differences in expression of MCM DNA helicase and DNA damage response (DDR) genes in AML patients. We hypothesized that older AML patients would show altered expression of these genes compared to younger adults as older age predisposes to myeloid disorders. 30 AML patient samples were obtained at new diagnosis (n = 24) or relapse (n = 6). Total RNA was extracted from AML cells and gene expression profiling was performed by using the Affymetrix U133 Plus 2 array with natural log transformed intensity values obtained after MAS5 normalization and batch effect correction. Patients were categorized as older (>65 yo, n = 9), middle-aged (50-64 yo, n = 12) or younger (<50 yo, n = 9). Differences in gene expression between each age group were determined using 2-sided Student's t-test. Ingenuity Pathway Analysis (IPA) was performed on differentially expressed genes between each age-group. In older patients, gene expression of the MCM DNA helicase complex (MCM2-7) were significantly increased compared to middle-aged patients along with numerous genes involved in DDR - ATM/ATR signaling, base excision repair, mismatch repair, nucleotide excision repair, homologous recombination and Fanconi anemia. DDR genes from older AML patients were also significantly increased compared to younger patients, however MCM gene expression did not differ and there were fewer differences in DDR gene expression likely due to a predominance of abnormal karyotype from the younger AML patients in our sample. IPA revealed increased JAK-STAT and IL-6 signaling in older AML patients compared to younger patients. The increased expression of the MCM DNA helicase and DDR genes may confer resistance to chemotherapeutic damage in AML patients, particularly in older adults. These results identify MCM DNA helicases, DDR signaling/repair, JAK-STAT and IL-6 signaling as potential targets for synergistic therapy in older patients with AML.

Mitochondria and Mitochondrially Derived Peptides

39. FAT10 – Not Only Metabolism; the Pleiotropic Impact of FAT10 on Aging

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FAT10 is an ubiquitin like modifier having two ubiquitin related sequences arranged in tandem. FAT10 expression is very low in most tissues but can be highly induced by inflammatory agents including: LPS, TNF alpha and IFN gamma. FAT10ylation results mainly in degradation of the targeted proteins by the proteasome. However, in some cases, FAT10ylation may change the cellular function of its molecular targets (e.g – p53 oncogene). Although, the molecular mechanism for these activities of FAT10 is not clear, FAT10 may regulates a wide variety of proteins as was shown in recent proteomic studies. Nevertheless, the lack of numerous phenotypic traits did not support the notion that FAT10 is a pleiotropic gene, per se. Last year, we reported on the impact of FAT10 on the aging phenotype of mice. Briefly, the lifespan of

FAT10 knockout mice was increased by 20% (for both genders); their total white adipose tissue was reduced by 50%; they were found to be insulin sensitive and glucose tolerant; and their metabolism of fatty acids was impaired. Still, it was not clear whether the association between FAT10 and aging is limited to FAT10's impact on metabolic networks or, in addition to that, there are more aging related phenotypes associated with FAT10 ablation. Here we report on several phenotypic characteristics of FAT10 deletion in mice that are on par with the slower aging phenotype of the FAT10 knockout mouse model. Among the significant findings that we obtained, we noticed 2 fold increase in the population of hematopoietic and mesenchymal stem cells from 2.5 year old FAT10 knockout mice. In general, the notion of having more stem cells fits the paradigm of an improved aging phenotype, since stem cells are exhausted during the aging process. We also noticed that the femur bones of these FAT10 knockout had hardly any fat content suggesting that FAT10 ablation prevent mesenchymal stem cells to differentiate into adipocytes. Hence, the reduction in fat mass in this animal model is not only due to the metabolic changes driven by the removal of FAT10, but also due to differential restrictions imposed on the adipogenic lineages. Our studies clearly prove that FAT10 affects aging in a pleiotropic manner. Since the aging process is the sum of multi-cellular/tissue processes, it is our aim to study the role of FAT10 in different cells and tissue during various developmental stages.

40. Investigating the Role of Mitochondrial Unfolded Protein Response in Aging and Health

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Mitochondria, the energy producing organelles in eukaryotic cells, play a critical role in cell metabolism. Mitochondrial dysfunction is implicated in a variety of diseases ranging from severe childhood disorders to age-associated neurodegenerative diseases such as Alzheimer's disease. The goal of this project is to define the mechanisms by which cells sense and respond to mitochondrial dysfunction in order to promote healthy aging, using *Caenorhabditis elegans* as a model system. Knockdown of several nuclear encoded mitochondrial genes, including components of the electron transport chain, increases the lifespan in *C. elegans* while simultaneously inducing a stress response referred to as the mitochondrial unfolded protein response (UPR^{mt}). The UPR^{mt} regulates the expression of several nuclear-encoded mitochondrial genes, including chaperones and other factors that assist in folding and processing of proteins in the mitochondria. It was initially proposed that activation of the UPR^{mt} directly promoted longevity in *C. elegans*; however, work from our group and others has indicated that the UPR^{mt} is neither necessary nor sufficient for lifespan extension. To further characterize the UPR^{mt} and its role in aging, if any, we performed a genome-wide RNAi screen in transgenic animals expressing a mitochondrial chaperone GFP reporter *hsp-6pr::gfp* in order to identify gene knockdowns that attenuate the induction of the UPR^{mt} in the context of mitochondrial stress. We have identified approximately 50 genes that are required for full induction of the UPR^{mt}, including genes that encode ribosomal proteins, RNA binding proteins, transcription factors, and vesicle transport machinery. Ongoing studies are aimed at determining how these genes regulate the UPR^{mt} and their role in aging. Understanding the mechanisms by which mitochondrial stress induces context-dependent effects on health and longevity is critically important for the development of effective interventions to delay aging.

41. The Mitochondrial-derived Peptide Humanin is a Potent Inducer of Autophagy

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Autophagy is a cellular process that degrades abnormal proteins and dysfunctional organelles. The increase of abnormal proteins and dysfunctional mitochondria inside cells has been shown to be a causative role in the functional deterioration of biological systems during aging and age-related diseases. Therefore, a therapeutic strategy that can accelerate the elimination of these toxic molecules via autophagy may slow down the process of aging and age-related diseases. Humanin is a 24 amino acid peptide encoded from the 16S rRNA region of the mitochondrial DNA. Plasma and tissue humanin levels decrease with aging. Humanin is secreted in response to cellular stress and has broad neuroprotective and cytoprotective effects in various diseases. Two cell surface receptors have been identified for humanin: humanin can interact with either FPRL1 or CNTFR/WSX-1/gp130 receptor complex and signal through the JAK/STAT and ERK1/2 signaling pathways that are well known to regulate autophagy in response to starvation. Based on the signaling pathway, here, we demonstrate that humanin induces autophagy in several cell types including HEK293 (normal embryonic kidney cells), SH-SY5Y (neuroblastoma), and B16 (melanoma). We recently demonstrated that humanin inhibits cancer progression and enhances the effects of chemotherapy treatments while reducing the side effects of the agents. However, the mechanisms of the anticancer action of humanin are still being unraveled. This study was designed to determine the synergistic effect of humanin on the efficacy of doxorubicin in B16 melanoma cells in vitro and more specifically, to reveal whether autophagy is involved in this combination strategy. The combined treatment caused an additive effect on the cytotoxicity to the cancer cells versus doxorubicin treatment alone. In addition, inhibiting autophagy by treating cells with autophagy inhibitors, including 3-MA and chloroquine, diminished the additive effect of humanin in doxorubicin treated B16 cells. Taken together, this study suggests that humanin has direct effects on tumor cells that involves induction of autophagy and thus, humanin acts as a chemotherapeutic augmenter and synergistically enhances doxorubicin's anticancer effects. Humanin's role as an autophagy inducing peptide makes it a potential therapeutic drug and this role may be related to its apparent effects of enhancing longevity observed in other systems.

42. Regulating cancer metabolism by a novel circulating tumor suppressor encoded in the mitochondrial genome

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Dysregulated metabolism is a hallmark of cancer that provides tumors with sufficient fuel sources and building blocks to support their rampant growth. Metabolic rewiring has been viewed as both the cause and result of tumorigenesis that involves activation of oncogenes and inhibition of tumor suppressors. Because of its central role in tumorigenesis, maintenance and metastasis, cancer metabolism has gained much attention from both basic research and drug development. Mitochondria are highly evolved organelles that coordinate cellular energy production and distribution, and for the past several decades have been strongly implicated in cancer. Nonetheless, the precise role of mitochondria in tumor biology is

still largely unclear. Recently, we have identified a novel peptide encoded within the mitochondrial genome and named it MOTS-c (Mitochondrial ORF within the Twelve S rRNA). MOTS-c is expressed in diverse tissues and also secreted and found in circulation (as a hormone) in an age-dependent manner. Interestingly, MOTS-c expression is down-regulated in tumors. Its primary target is the skeletal muscle where it acts on the folate cycle (vitamin B9), thereby significantly improves insulin sensitivity, in part, via AMPK activation. Notably, we recently found that MOTS-c has anti-proliferative and pro-apoptotic effects on tumors using in vitro and in vivo cancer models. Because of its novel biology, MOTS-c provides a paradigm-shifting 'mitochondria-centric' approach to drug development that may uncover an entirely new therapeutic target that can be applied to a broad range of tumors.

43. Humanin transgenic mice exhibit a dietary-mimetic phenotype including decreased insulin and IGF-I signaling and protection against chemotherapy side effects

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Humanin is a mitochondrial-encoded 24 AA peptide. Endogenous humanin levels fall with age in humans and rodents. In animal and human models of altered lifespan, humanin levels correlate with longevity and health, suggesting a role for humanin in healthy aging. Studies showed that humanin treatment has protective effects in various rodent models of age-related diseases, including diabetes and neurodegeneration. We noticed a number of shared effects between humanin and caloric restriction (CR). Moreover, similarly to CR, chemotherapy induced side-effects are almost completely ameliorated by co-treatment with humanin. To establish an in vivo model of humanin overexpression, and further elucidate the physiological roles of this mitochondrial peptide, we generated humanin transgenic mice. In two independent transgenic strains, elevations of humanin levels were shown in several organs as well as plasma. Mice harboring the humanin transgene are viable and fertile. These mice exhibit no gross abnormalities and, although body weights of these mice were significantly reduced in both sexes, compared with non-transgenic littermates. The lighter body weights are consistent with the notion that humanin works as a CR mimetic. Furthermore, fasted insulin levels were reduced in transgenic mice and plasma levels of IGF-I were dramatically lower, while circulating IGFBP-1 levels were increased in response to humanin overexpression, while these mice have normal levels of circulating cytokines. Our data suggest that humanin, like CR, negatively regulates insulin and IGF-I signaling in mice. As humanin injections was shown to ameliorate side-effects induced by cyclophosphamide (CP), we investigated whether humanin transgenic mice were protected against CP. Male wild-type and transgenic mice were injected with one dose of either saline or CP (200mg/kg body weight). Blood count was done and plasma was collected after 48 hours. Humanin transgenic mice displayed a more pronounced suppression of IGF-I and insulin in response to CP treatment. Importantly, the suppression of lymphocyte count by CP was 2-fold less severe in the transgenics. Taken together, these data suggest that humanin exhibits CR-mimetic effects by suppression of the insulin/IGF-I signaling pathway which leads to protection against chemotherapy side effects. The humanin transgenic mouse also proves to be a valuable model for further studies of aging and age-related diseases.

44. Regulation of the Novel Age-dependent Mitochondrial-derived Peptide MOTS-c

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Metabolic regulation and the role of mitochondria have been strongly implicated in aging and age-related disease research. Neurodegenerative conditions, diabetes mellitus, and cancer all occur increasingly with age and develop concomitantly with mitochondrial and metabolic dysfunction; however, the role of mitochondria in disease onset is still unknown and remains largely unexplored despite its critical cellular role. We have recently identified a short open-reading-frame (sORF) in the mitochondrial DNA that encodes a 16-amino acid peptide, which we have named MOTS-c (mitochondrial open-reading-frame of the 12S rRNA-c). Using in vitro and in vivo models, we show that MOTS-c regulates metabolic homeostasis, promotes insulin sensitivity, and protects mice from diet-induced obesity. Additionally, MOTS-c is detected in rodent and human cells and plasma, lending evidence as a circulating regulator of metabolism. Notably, MOTS-c in rodent tissue and plasma decline with age. Here we show in vitro that MOTS-c is secreted into cell culture media from HEK293 cells transiently transfected with EGFP-tagged MOTS-c (MOTS-c-EGFP). Furthermore, low levels of ER stress induced by Brefeldin A can increase the secretion of MOTS-c-EGFP; interestingly, this simultaneously reduces intracellular levels of endogenous MOTS-c. Understanding the mechanisms regulating MOTS-c expression and secretion, as well as those by which MOTS-c affects cellular metabolism, may provide novel 'mitochondrial-centric' interventions to target aging and age-related diseases.

45. Deciphering the Molecular Signature for Humanin Signaling

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The mitochondria is more than just a "power house" and regulator of cell death. In fact it generates multiple signaling peptides called mitochondrial-derived peptides (MDPs). These MDPs are speculated to have major roles in the human body. One major player is Humanin (HN), which is found in the plasma, secreted from cells, and is also bound to cellular membranes. Humanin is a small peptide consisting of 24 amino acids and has cytoprotective actions against various stress and age-related disease models. It is encoded in the mitochondrial genome within an open reading frame in the 16s rRNA gene. Humanin has been correlated with lifespan and has been shown to decrease with age in both humans and rodents. HN binds to the tripartite receptor composed of CNTFR, WSX-1, and gp130 and humanin also has been shown to bind the FPRL1 receptor. Additionally, a number of different signaling pathways such as JAK/STAT/Apollon and ERK are induced by HN. Although there have been many pathways implicated in humanin signaling, a definitive humanin pathway is still under investigation. In this study an in vitro cell culture assay was performed to determine the phosphorylation sites activated by Humanin. Using SH-SY5Y (human neuroblastoma) cells that were treated for 30 minutes with humanin, we ran these lysates on a phospho-antibody array (Full Moon Biosystems' Phospho Explorer Antibody Array). We then analyzed the resulting data to examine the major pathways activated by humanin stimulation. To validate the results of our antibody array, we ran western blots using antibodies against the phosphorylation sites that were

predicted to change by our previous analysis. The majority of the phosphorylation changes that we tested corroborated the antibody array, although not all of them. We were able to confirm the previously published ERK activation and found additional possible pathways such as AMPK, mTOR, and EGF that are activated during humanin signaling. These new pathways may help determine how humanin protects against age-related diseases and may offer novel insights into these pathways.

Proteostasis, Homeostasis & Aging

46. AMPK mediates proteostasis in response to hypoxia and fasting in *C. elegans*

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In order to survive in changing environmental conditions, organisms must be able to successfully sense and integrate diverse environmental signals and respond appropriately. We are interested in how the energy sensor AMP-activated protein kinase (AMPK) integrates environmental cues regarding oxygen and nutrient availability to regulate protein homeostasis. We have found that specific concentrations of low oxygen (hypoxia) cause a disruption of protein homeostasis in *C. elegans*, as measured by increased aggregation of polyglutamine proteins in the body wall muscles. Here, we show that nutritional cues regulate the effect of hypoxia on proteostasis. Animals that are fasted develop dramatically fewer protein aggregates compared to their fed counterparts when exposed to hypoxia. The effects of hypoxia and nutrient deprivation on protein aggregation are mediated through AMPK-activated protein kinase (AMPK), a cellular nutrient sensor that regulates energy balance. We discovered that, when fed, AMPK mutant animals do not display increased protein aggregation in hypoxia. Moreover, fasting does not protect against hypoxia-induced aggregation in these mutant animals. Polyglutamine aggregation in body wall muscles is thought to be cytotoxic, resulting in uncoordination and eventual paralysis. In support of this, fed wild-type animals exposed to hypoxia have an accelerated rate of paralysis compared to controls maintained in room air. However, neither fasted wild-type animals nor fed AMPK mutants have decreased paralysis rates, despite their reduced aggregate burden, suggesting a complex relationship between hypoxia, protein aggregation, and cytotoxicity. Taken together, our results underscore AMPK's role in modulating cellular pathways that maintain proteostasis in response to a complex interaction of environmental cues.

47. The aging-related decline of 20S proteasome adaptation to oxidative stress is dependent on SKN-1 signaling

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Aging is marked by a collapse of protein homeostasis and deterioration of adaptive stress responses that often lead to aging-related diseases. Cellular stress response pathways are evolutionary ancient

mechanisms adapted to manage the oxidized, misfolded, and damaged proteins that result from denaturing stress. Adaptation to acute stress allows cells and organisms to cope with severe stress by triggering the production of cytoprotective genes. During aging, these stress responses decline along with the proteolytic capacity of the proteasome machinery. We have recently demonstrated that induction of the proteasome during oxidative stress is dependent on Nrf2 and that this mechanism is conserved in the C. elegans ortholog SKN-1. To our knowledge, there are currently no studies to address how the adaptive capacity to induce expression of the proteasome may change with age. We have found that the ability to mount an adaptive response by pretreatment with minor oxidative stress is abrogated in aged C. elegans. By utilizing chromatin immunoprecipitation of SKN-1, quantitative PCR to SKN-1 mRNA targets, and substrate assays for proteolytic activity, we have found that SKN-1 signaling and adaptive proteolysis decline with age. Furthermore, using a transgenic model, we have shown that increased activation of SKN-1 results in increased stress resistance and proteolytic activity, but is unable to restore the aging-related decline of proteasome-dependent adaptation. These results demonstrate that the aging related decline in SKN-1 signaling negatively impacts adaptation of the 20S proteasome in response to oxidative stress, suggesting that rescue of SKN-1 signaling may improve protein homeostasis defects during aging.

48. The Decline of Adaptive Homeostasis in Aging

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The classical view of homeostasis considers a single range of biological capacities, extending above and below a mean value. Thus, we may consider mean blood pressure for a 'normal' 20 year old female to be approximately 110/70, with a high end ranging up to 130/80 and a low end ranging down to 100/60. Over the past two decades, however, studies from this laboratory (and several others) have demonstrated that cells, simple organisms, and even mammals, can temporarily expand the homeostatic range by undergoing transient adaptation. Such adaptive responses depend on altered gene expression and are orchestrated by signal transduction pathways, such as the Nrf2-Keap1 system. Such adaptive pathways allow cells and organisms to cope with transient changes in (internal or external) environments, including many forms of stress. Thus, in addition to the 'normal' range of homeostatic capabilities, there is an additional range of adaptive capacity that I propose should be called, 'Adaptive Homeostasis.' Importantly, several studies from this laboratory now show that Adaptive Homeostasis declines with age in cells, worms, flies, and rodents; in other words, a decline in Adaptive Homeostasis appears to be a 'normal' age-dependent phenomenon. Declining Adaptive Homeostatic capacities may make older organisms (and people?) more susceptible to multiple stresses, and to disease. On the other hand, declining Adaptive Homeostasis may be protective against cancer. While the full explanation for age-dependent declining Adaptive Homeostasis is still under study, our research indicates that diminishing Nrf2 responsiveness, and increasing levels of (competitive?) Nrf1, Bach1, and c-Myc may all play important roles.

Human Aging

49. TRIANGLE NET: tools for fair & non-discriminated healthcare of elderly people

Emel SARDOHAN YILDIRIM

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In this study, within Civil Society in the between of EU and Turkey –III Political Criteria Grant Programme(CSD-III/PC), be aimed to adjust the best implementation modules in England and Germany about elderly care to Turkey with Project of TRIANGLE NET. In determining the module adapted primarily semi-structured interviews conducted with 28 aged care staff; aged care staff, revealed the difficulties faced in the process of providing services to the elderly. Subjects which elderly care personnel slog during the service to elders: To have information about individual's health and psychology, to cope with challenge behavior, recreation of elders, to find proper stance position to elderly people, to promote their socialization. According to these arising requirements, it has targeted to adapt these modules; abuse and neglect of the elderly individuals, dealing with challenge behavior, participation of elderly individuals in the cultural activities and old-age and disability. 6 elderly care center manager, 3 project expert and 12 elderly care personnel who work in nursing homes and elderly care centers in Turkey's cities; Ankara, Konya, Eskiehir, will participate to training and the best examinations in the UK and Germany

50. Restoration of oxidative stress resistance following an 8-week exercise intervention

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Aging is associated with significant impairment in tolerance to acute stressors such as ischemia/reperfusion injury, which appears to contribute to the increased rate of morbidity and mortality of older adults. We have previously shown that fit older adults have greater resistance to oxidative stressors than their sedentary peers suggesting that resistance to oxidative stress may be modifiable through fitness. Additionally, we have shown that a single bout of exercise confers protection against a subsequent oxidative challenge in young, but not older adults. Given these data, we hypothesized that repeated bouts of exercise, as experienced in a regular exercise program, could restore resistance to an acute non-exercise oxidative challenge in older adults through repeated transient shifts in systemic redox balance. We recruited sedentary older men and women (58±4y, n=10) to participate in an 8-week aerobic exercise intervention. Maximal aerobic capacity was determined by VO2max on a stationary cycle ergometer. The exercise intervention consisted of 3 exercise sessions per week, each lasting 45 minutes at an intensity corresponding to 60–75% VO2max. Resistance to oxidative stress was measured by the F2-isoprostane response to a forearm ischemia/reperfusion (I/R) trial. Forearm I/R, induced by inflation and deflation of a blood pressure cuff on the upper arm, is an effective method to temporarily increase oxidative damage, allowing for quantification of response and recovery to the I/R by changes in plasma levels of F2-isoprostanes. A lower F2-isoprostane response to the I/R trial indicates greater resistance to oxidative stress. Each participant underwent the I/R trial twice: once prior to, and once post-exercise intervention. Eight participants have completed the study to date, and two are currently in the exercise intervention phase. In the eight completed participants, the exercise intervention elicited a significant increase in aerobic fitness as measured by VO2max, with a mean increase of 14% ($p<0.001$). Baseline levels of F2-

isoprostanes pre and post-intervention did not differ, but the F2-isoprostane response to the I/R trial was significantly lower following the exercise intervention ($p = 0.055$). These data suggest that resistance to an acute non-exercise oxidative stressor in older adults can be improved in response to regular physical activity. Future studies are necessary to determine the mechanisms of restored oxidative stress resilience.

51. An Association between Frailty and Pulmonary Function in Patients with Chronic Obstructive Pulmonary Disease (COPD) – Application of a Routine Frailty Assessment Approach using Wearable Technology

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Background: Frailty screening among elders with chronic obstructive pulmonary disease (COPD) may help identify risk of poor medication inhalation, exacerbation, and early mortality, enabling better targeted therapy. We previously developed and validated (using the Fried frailty index) an upper-extremity frailty (UEF) assessment method among community dwelling older adults. The purpose of the current study was to determine the association between UEF parameters and respiratory muscle strength in older adults with COPD. Methods: Participants were screened for COPD and a pulmonary function test (PFT) was performed. Participants with known neuromuscular diseases were excluded. PFTs included routine spirometry, and maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP) as indices of respiratory muscle strength. We measured kinematic and kinetic UEF parameters using two wireless inertial sensors attached to the upper-arm and forearm, and subjects performed repetitive elbow flexion for 20 seconds on each side. Using a previously validated model, we extracted slowness, weakness, and exhaustion indicators of frailty. Linear regression-analysis of variance models tested associations between UEF parameters and MIP and MEP, while adjusting for age, body mass index, and sex. Results: We recruited 32 participants with confirmed COPD (53% male, age: 71±7 years, BMI: 28.2±5.3 kg/m2). Strong correlations were observed between UEF parameters and both MIP and MEP. Speed of elbow flexion, an indicator of the slowness Fried frailty feature, ($p = 0.02$; $r = 0.70$), and power of movement ($p = 0.03$; $r = 0.69$) and elbow moment ($p < 0.01$; $r = 0.73$), indicators of Fried muscle strength and weakness frailty features, demonstrated significant correlation with MIP. UEF parameters that were related to the exhaustion Fried frailty feature included variation of elbow moment ($p = 0.04$; $r = 0.50$), speed ($p < 0.01$; $r = 0.57$), and power ($p = 0.04$; $r = 0.50$), which were significantly correlated with MEP. Conclusions: Results from the current study demonstrate a moderate to high correlation between UEF features with MIP and MEP indices of respiratory muscle strength. Associations between MIP and weakness and slowness in COPD patients may result in air-trapping, hyperinflation, and poor diaphragmatic movement, which may affect both MEP and MIP. Frailty may result in inefficient inhaler use in elders with COPD. Further study of Frailty, MEP and MIP on COPD outcomes is warranted.

Dietary Restriction II

52. Attenuation of aging-related bradykinesia in rats by calorie restriction intervention at 18 months of age

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The likelihood of at least two of the cardinal symptoms Parkinson's disease (notably bradykinesia) diminishing the locomotor capabilities of the elderly is extremely high. By retirement age, 15% of the population will be affected by aging-related Parkinsonism, and this percentage doubles each decade of life thereafter. Therefore, lifestyle strategies are needed to reduce the risk of aging-related Parkinsonism. Preclinical studies can not only reveal if such strategies could prove beneficial, but also provide insight into the neurobiological mechanisms associated with locomotor impairment or improvement. Lifelong calorie restriction (CR) has been shown to attenuate aging-related bradykinesia (ARB). Recently we have reported evidence that 30% CR initiated at 12 months of age in male Brown-Norway Fischer 344 F1 hybrid rats (BNF344) can also attenuate ARB for at least 6 months. However, it is not known if such CR effects could still occur if initiated at an even more advanced age , akin to an age of a human retiree. In this study, 30% CR was initiated in 18 month old BNF344 rats after establishing a baseline level of activity for each test subject. Locomotor assessment was conducted every 6 weeks out to 24 weeks after CR initiation when the rats reached 24 months of age. The results revealed that CR intervention compared to ad libitum fed controls attenuated ARB from 18-24 months old. However, notably, the rats in lower 50th percentile in activity at baseline responded to CR such that there was no decrease in activity at any 6-weekassessment during the 6-month study. These results indicate that CR intervention could be an effective non-invasive lifestyle strategy to reduce the risk of ARB at advanced age, particularly in subjects with less than average activity that may be most vulnerable to ARB. Neurochemical analyses are being conducted to identify mechanisms underlying the behavioral observations.

53. Variable responses to dietary restriction among Daphnia genotypes

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Genotypic diversity within species may lead to variable responses to dietary restriction via genotype-environment interactions. We examined the response to dietary restriction in six isolates of the short-lived freshwater crustacean Daphnia pulex and in six isolates of the long-lived Daphnia pulicaria. Daphnia are cyclic parthenogens, so an individual isolated from the field can be maintained in the lab as an isogenic clonally-reproducing line. Daphnia pulex and D. pulicaria are not separate species; rather they are ecologically distinct subspecies that interbreed frequently in the wild. D. pulex inhabits temporary ponds, which favor the evolution of short lifespan. In addition, ponds have rich, abundant resources for Daphnia, so D. pulex is unlikely to have adapted to periods of dietary restriction. In contrast, D. pulicaria lives in permanent lakes, where long lifespan is favored by evolution. However, lake environments are often resource-limited for Daphnia and therefore D. pulicaria is expected to have adaptive responses to dietary restriction. Our results show that the genotypes display a gradient of lifespans, with long-lived D. pulex genotypes being similar to short-lived D. pulicaria genotypes. With respect to lifespan, there is substantial genotypic variation in the response to dietary restriction, but we found no support for the hypothesis that

D. pulex and D. pulicaria differ in their response. However, reproductive responses were stronger in D. pulicaria, the ecotype adapted to naturally-occurring dietary restriction.

54. Deletion of the dopa decarboxylase, BAS-1, in C. elegans may decrease lifespan extension induced by intermittent fasting

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Dietary restriction is the only pro-longevity intervention known to work across all organisms tested. An alternate dietary restriction regime, also known as intermittent fasting, has been shown to result in increased lifespan in many different model organisms. Furthermore, it has been well-proven that many neurotransmitters decrease with age, and it is hypothesized that some neurotransmitters play a role in lifespan extension. Here we show that in C. elegans deletion of the dopa decarboxylase, BAS-1, an enzyme involved in the synthesis of the neurotransmitters serotonin and dopamine, ameliorates the lifespan extension induced by intermittent fasting. Therefore, BAS-1, and potentially serotonin and dopamine, may play a role in mediating intermittent fasting induced lifespan extension.

55. Caloric Restriction Confers Anti-Oxidative, Pro-Angiogenic, and Anti-Inflammatory Effects and Promotes Anti-Aging miRNA Expression Profile in Cerebromicrovascular Endothelial Cells

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Caloric restriction (CR) exerts cerebrovascular protective effects, but the underlying mechanisms remain elusive. Primary cerebromicrovascular endothelial cells (CMVECs) were isolated from young (3 mo old), aged (24 mo old) and aged CR F344xBN rats. Cellular and mitochondrial oxidative stress was increased in aging and prevented by CR. Expression and transcriptional activity of Nrf2 were reduced in aging, which was prevented by CR. Expression of miR-144 was upregulated in aged cells, and its overexpression decreased Nrf2 expression in young and aged CR cells. Overexpression of a miR-144 antagomir in aged cells significantly decreased expression of miR-144 and upregulated Nrf2. CR prevented age-related impairment of angiogenic processes (cell proliferation, adhesion to collagen, formation of capillary-like structures) and inhibited apoptosis. CR also exerts significant anti-inflammatory effects (decreased the transcriptional activity of NF-κB aged cells and the pro-inflammatory shift in endothelial secretome). Collectively, we find that CR confers anti-oxidative, pro-angiogenic, and anti-inflammatory effects, preserving a youthful

phenotype in rat CMVECs. CR may improve cerebrovascular function and prevent vascular cognitive impairment.

mTOR and Rapalogs II

56. Chronic rapamycin treatment attenuates age-related motor deficits in sex-dependent manner in UM-HET3 mice

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Our lab has reported that dietary supplementation with rapamycin increased lifespan in UM-HET3 mice (Harrison et al., 2009; Miller et al., 2011) consistently more in females than males. However, whether this sex-dependent increase in lifespan by rapamycin is accompanied by improvement in motor performance with age in a sex-specific manner is unknown. We hypothesized that if rapamycin treatment slows aging it should also prevent or delay age-related motor deficits in UM-HET3 mice of each sex. Our results showed that age-related decline in coordinated running was more in old female mice. Interestingly, rapamycin treatment attenuated this decline in motor coordination significantly more in females compared to males. In females, rapamycin reduced basal anxiety levels and depressive-like behavior. Age-related decline in locomotor activity in females was also prevented by rapamycin. Rearing was found to be reduced with age in both sexes. However, rapamycin prevented this decline significantly in females than males. Using an FTC-based proteomic approach, we measured the protein carbonyl content in different brain regions. Protein carbonyl content was significantly increased with age in striatum, midbrain, hippocampus, cortex and cerebellum regions of both sexes. More protein carbonylation was observed in the detergent-soluble protein fraction as compared to cytosolic fraction with age. Rapamycin reduced protein carbonyl content in both fractions in each sex. Interestingly, we found more protein carbonylation with age in the insoluble protein fraction of the striatum region in females compared to males. The decline in locomotor function in aged females compared to males suggests a possible association between age-related motor deficits and increase in protein carbonylation. Effect of rapamycin on GFAP expression with age in brain was also evaluated. Calorie restriction is known to reduce the expression of GFAP in the striatum. To elucidate whether rapamycin treatment mimics calorie restriction, we measured GFAP expression in the striatum of both sexes. Rapamycin treatment increased the GFAP expression in old mice of both sexes suggesting that rapamycin does not mimic calorie restriction. Altogether, our findings reveal that the increase in lifespan resulting from rapamycin supplementation is accompanied by improvements in age-sensitive behavioral traits, reduced protein carbonylation and increased astrocyte proliferation.

57. Substantial sex-, age- and diet-dependent life-improving effects of mTOR inhibitor.

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The nutrient-sensing mTOR (mammalian Target of Rapamycin) pathway regulates cellular metabolism, growth and proliferation and is frequently deregulated in various pathological conditions. The pharmacological or physiological inhibition of mTOR by rapamycin, or calorie restriction, respectively, has been shown to extend lifespan and suppresses tumorigenesis and other age-related diseases in several experimental models. Recent findings also indicate that rapamycin can slow down aging in obese mice fed by high-fat diet. Here we tested if life extending effect of pharmacological inhibition of mTOR depends on (a) animal age and gender, and (b) whether it is accompanied by improvements in health, physical performance, and delay in development of age-associated frailty. To address these questions, we assessed a physiological decline associated with frailty by measuring a number of health-related variables in large cohort of NIH/Swiss mice of different age and gender and maintained under different dietary conditions. These parameters include total blood cell analysis (CBC), physical performance (grip strength), hemodynamic parameters (blood pressure), blood biochemistry, levels of circulating pro-inflammatory cytokines and others, which were used to create Frailty Index (FI) for each individual mouse. A substantial increase in FI associated with maintenance on a high-fat diet was found to be specific for males. Although female mice gain weight similar to males, their overall health-related parameters were not changed. Prolonged systemic administration of mTOR inhibitor Rapatar (nanoformulated soluble form of rapamycin) in drinking water completely reverted deteriorating effects of high-fat diet by preventing development of obesity, extending lifespan and decreasing the FI in male mice. When administered to females, Rapatar increased their longevity but had no effect on FI. Moreover, positive effect of Rapatar in obese males was most prominent when treatment started later in life (>90 weeks old). Taken together, these data refine potential use of mTOR inhibition as an anti-aging and life-improving approach and emphasize the importance of its gender- and age-specificity.

58. Co-treatment with metformin improves metabolic defects caused by rapamycin in mice

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Inhibition of mTOR extends lifespan in mice and invertebrates and improves some markers of age-related functional decline. There is now intriguing evidence that mTOR inhibition may improve same physiological aspects of aging in humans. However, assessing large-scale translational effects of this approach may be limited due to the increased potential for risks associated with administration of the mTOR inhibitor rapamycin and its analogs. A primary example is a significant increase in the risk for new-onset diabetes or impaired glucose metabolism shown with both clinical administration and in rodent models treated with rapamycin. Working with the NIA-funded Interventions Testing Program, we tested whether these metabolic defects could be prevented by co-treatment with metformin, the most-commonly administered and first-line drug of choice for type 2 diabetes. Beginning at four months of age, genetically heterogeneous HET3 mice were treated with encapsulated rapamycin, metformin, or both simultaneously,

with all treatments incorporated into their diet. Metformin did not alter the effect of rapamycin on mTOR inhibition in vivo, but did independently activate AMPK signaling in both male and female mice. Over nine months of treatment, rapamycin significantly reduced body weight gain in both males and females, with metformin co-treatment eliminating this weight reduction in males only. Glucose metabolism was impaired by rapamycin beginning as early as one month after treatment and continued throughout the duration of the study. Metformin did not alter this outcome in males, but females treated with both metformin and rapamycin were indistinguishable from controls showing a clear beneficial effect of metformin treatment. At least partly, these beneficial effects of metformin could be attributed to reduction in hepatic gluconeogenesis caused by this drug, though we also found that metformin altered circulating concentrations of adipokines such as leptin and adiponectin. The extension of lifespan caused by rapamycin has been somewhat paradoxical when viewed in light of its potential negative effects on metabolism. In ongoing studies, the Interventions Testing Program will assess the lifespan of HET3 mice co-treated with rapamycin and metformin and, combined with our findings, will soon test whether there is further extension of rapamycin-mediated longevity by alleviation of its metabolic defects.

59. Non-cell Autonomous Control of Metabolism and Aging by Neuronal mTOR Signaling

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The mechanistic target of rapamycin (mTOR) is a major regulator of cellular and organismal metabolism. Reduction of TOR signaling by rapamycin is known to alter organismal metabolism and increase lifespan and healthspan. In invertebrate models, selective reduction of function of TOR in the nervous system is sufficient to extend life. We hypothesized that attenuating mTOR signaling in mature mammalian neurons would extend lifespan by altering critical aspects of metabolism non-autonomously. To test this hypothesis we knocked down the mTOR complex 1 (mTORC1)-specific protein, Raptor, in neurons of adult mice. Reduction of mTORC1 complex formation in neurons of mice by 35 or 60% did not affect body weight but increased lean mass while reducing metabolism. This was associated with enhanced exercise endurance and absent post-exercise hypoglycemia, even though glucose and insulin tolerance were unchanged. To determine cell-autonomous effects of mTORC1 knockdown in neurons we measured spatial learning and memory. While 60% mTORC1 knockdown impaired cognitive plasticity, 35% reduction in mTORC1 complex formation resulted in enhanced spatial memory. Consistent with these observations, 60% neuronal mTORC1 KD reduced brain glucose metabolism and cerebral blood flow, while 35% KD increased brain glucose uptake with no changes in cerebral blood flow. Taken together, our data suggest that reduction of neuronal mTORC1 may have significant non-cell autonomous effects on basal and exercise metabolism. Furthermore, the relationship between levels of mTORC1 in neurons and spatial memory is not linear. Rather, and in agreement with prior studies using rapamycin, spatial memory may be maximal when neuronal mTORC1 levels are slightly lower than WT, but will decrease with further reductions in mTORC1, mirroring the effects of mTORC1 KD on brain glucose metabolism. Our data suggest healthspan effects of neuronal mTORC1 reduction. Ongoing studies will determine whether neuronal mTORC KD also affects lifespan. Funding NIA5T32-AG021890, Ellison Medical Foundation, UTHSCSA Research Council Award, Owens Foundation, Bailey Family Alzheimer's Fund

Pharmacological & Nutraceutical Interventions II

60. Restoration of Systemic Growth and Differentiation Factor-11 (GDF11) Levels Reverses Age-Associated Dysfunction in Skeletal Muscle

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Recent studies involving heterochronic parabiosis indicate that impaired regeneration in aged animals is in part systemically controlled and reversible by exposure to a young circulation. While prior studies have identified a handful of systemic "aging" factors, discovery of the humoral "rejuvenating" factors that act on tissue stem cells to restore regenerative function has been relatively more elusive. Here, we demonstrate that the circulating hormone Growth and Differentiation Factor 11 (GDF11) is a rejuvenating factor for skeletal muscle. Supplementation of systemic GDF11 levels, which normally decline with age, using either heterochronic parabiosis or systemic delivery of recombinant protein, is sufficient to reverse functional impairments and restore genomic integrity in skeletal muscle stem cells (satellite cells). Augmentation of GDF11 levels further improved structural and functional features of resting skeletal muscle, resulting in increased strength and enhanced endurance exercise capacity. Taken together, these data reveal critical mechanisms in the systemic regulation of aging and identify a promising candidate therapeutic for the reversal of age-related skeletal muscle and stem cell dysfunction.

61. The mitochondrial protective peptide SS-31 has persistent effects in improving cardiac function after drug removal

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Heart disease is the number one killer worldwide, and is poised to maintain that title for the foreseeable future. Recent work, reported by our lab and others, has demonstrated that SS-31 reverses the effects of cardiac and skeletal muscle aging. SS-31 is a tetra-peptide, thought to interact with cardiolipin in the mitochondria to maintain healthy structure and bioenergetics. Old mice given SS-31 have decreased cardiac hypertrophy, improved Ea/Aa ratios by Tissue Doppler Imaging (TDI), and have proteomic abundances that more closely parallel those of young mice. Recently, we have begun to investigate how long it will take before the heart will return to a less functional state after treatment is removed. We have found that mice continue to exhibit improved diastolic function (Ea/Aa ratios) six weeks after SS-31 is removed. Proteomics analyses continue to highlight the differences between different functional groups, giving an indication of both the first and last proteomic pathways that change with treatment. These novel results give insight into possible future short-term treatments for cardiac dysfunction.

62. The effect of resveratrol on beta amyloid-induced memory impairment involves inhibition of phosphodiesterase-4 related signaling

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Resveratrol, a natural polyphenol found in red wine, has wide spectrum of pharmacological properties including antioxidative and antiaging activities. Beta amyloid peptides 1-42 (Aβ 42) are known to involve learning and memory impairment, neuroinflammatory and apoptotic processes in Alzheimer's disease (AD). Activation of cyclic AMP (cAMP) and/or cGMP activities can improve memory performance and decrease the neuroinflammation and apoptosis. However, it remains unknown whether the memory enhancing effect of resveratrol on AD associated cognitive disorders is related to the inhibition of phosphodiesterase 4 (PDE4) variants and subsequent increases in intracellular cAMP and/or cGMP activities. The present study investigated the effect of resveratrol on Aβ-induced learning and memory impairment and the participation of PDE4 variants related cAMP or cGMP signaling. Mice microinfused with Aβ 42 into bilateral CA1 subregions displayed learning and memory impairment, as evidenced by reduced memory acquisition and retrieval in the water maze and retention in the passive avoidance tasks; it was also significant that neuroinflammatory and pro-apoptotic factors were increased in Aβ 42-treated mice. Moreover, PDE4A, 4B and 4D expression were significantly increased in Aβ 42-treated mice, which induced decreased cAMP activity. These effects were reversed by treatment with resveratrol at doses of 20 and 40 mg/kg. Resveratrol also reversed Aβ 42-induced decreases in phosphorylated cAMP response-element binding protein (pCREB), brain derived neurotrophic factor (BDNF) and anti-apoptotic factor BCl-2 expression. These findings suggest that resveratrol reverses Aβ 42-induced learning and memory disorder may involve the regulation of neuronal inflammation and apoptosis via PDE4 variants related cAMP-CREB-BDNF signaling.

63. Dipeptidyl peptidase-4 (DPP4) inhibitor, sitagliptin, increases active incretin levels in both plasma and CSF of nonhuman primates

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Dipeptidyl protease-4 (DPP-4) inhibitors – also known as gliptins - are widely used in the effective treatment of type 2 diabetes to safely regulate blood glucose levels. DPP-4 is the key enzyme responsible for the metabolism of the endogenous incretins, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) whose elevated levels in brain, were hypothesized to provide neurotrophic/neuroprotective actions in cellular and rodent models of Parkinson's disease (PD). On evaluating several DPP-4 inhibitors, brain and plasma incretin levels were, indeed, substantially elevated in rodents, and this resulted in amelioration of Parkinsonism and elevations in brain dopamine levels in a well-characterized rodent PD model. In the current study, we evaluated sitagliptin in nonhuman primates to assess whether translational doses elevate systemic (plasma) and central (CSF) incretin levels, as achieved in rats, to (i) cross-validate our studies across species and (ii) de-risk clinical translation of this

drug as a new treatment for PD. Twenty adult rhesus monkeys (Macaca mulatta) were given 1 of 4 drug conditions (Control, 5, 20, or 100 mg/kg) daily for 6 days. On day 6, an oral glucose load was given to stimulate incretin release. Blood and cerebral spinal fluid samples were collected at specified time points during the subsequent 24 hours. Active incretin levels were observed in both plasma and CSF with all 3 doses. The intermediate dose (20 mg/kg) induced the greatest response. DPP4 inhibition in the plasma was dose-dependent. An inverted U dose-response relationship on incretin levels in both plasma and CSF was evident with the high dose providing less activity—likely due to compensatory mechanisms at the level of incretin synthesis/secretion. The current results validate those from rat studies and lay the groundwork for dose selection for repositioning sitagliptin in a potential clinical trial in PD. Research supported by the Intramural Research Program, National Institute on Aging, NIH, and the Michael J. Fox Foundation.

64. Dietary Selenium Deprivation Deteriorates Healthspan but Extends Lifespan

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A role of selenium (Se), particularly, at nutritional levels of intake, in aging is not fully understood. In particular, what is lacking is an appropriate aging model of dietary Se deprivation relative to human physiology displaying features of normal aging and age-related degenerations. Telomere attrition provokes DNA damage response and, subsequently, replicative senescence. Because the chromosomes of mice carry longer telomeres than those of humans, we propose that lengthy telomeres preclude mice deprived of Se to display aging phenotypes and age-related disorders. To test this hypothesis, weanling late generation Terc-/- mice were fed a Se-deficient diet or the diet supplemented with selenate (0.15 ppm) throughout their life. As evidenced by changes in metabolic markers (body weight, glucose intolerance, insulin resistance and bone structure) and aging phenotypes (grey hair, alopecia and wound healing), our data strongly indicate healthspan deterioration by dietary Se deficiency in the short telomere mice. MicroRNAs (miRNA) have been proposed as biomarkers for a variety of diseases and physiological conditions, including aging. To identify the key circulatory mediators during the aging process, we used a high-throughput platform, TaqMan low density array to profile more than 800 miRNAs in plasma to identify circulating miRNA signature and reveal pathway shift towards metabolic dysregulation. Altogether, we have established a very interesting model of aging by deprivation of dietary Se that decouples healthspan and lifespan.

65. Chronic administration of the 12/15-lipoxygenase inhibitor Baicalein protects age-related decline in peripheral motor nerve and muscle function in C57BL/6 female mice

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Aging is accompanied by the loss of skeletal muscle mass and function, termed as sarcopenia. Recent studies in older humans have found a strong association between decline in peripheral nerve function and decrease in muscle power. Structural changes in peripheral nerves during aging include the loss of axons and demyelination. Myelin sheath produced by Schwann cells that surround the large myelinated nerve fibers is highly enriched in both lipids and proteins, leaving the peripheral nerve highly vulnerable to oxidative damage. Here we show that sciatic nerves from 30-mo-old C57BL/6 mice (with a significant decline in motor nerve function and degenerative changes in both axon and myelin structure) exhibit significant increases in 12/15-lipoxygenase (12/15-LO) pathway derived oxidized fatty acid, 12-hydroxyeicosatetraenoic acid (12-HETE, ~2.5 fold vs 8-mo-old) and detergent-soluble protein carbonyls (containing the myelin proteins, ~20% over 8-mo-old). In addition, mechanisms that protect and/or repair oxidative damage (e.g. Nrf-2, HSP's) are significantly reduced in sciatic nerves from aging mice. In 30-mo-old mice, lifelong dietary-restriction (that protects peripheral motor nerve function) attenuated sciatic nerve 12-HETE and detergent-soluble protein carbonyls to levels exhibited by young ad libitum fed mice, suggesting that 12/15-LO pathway mediated oxidative damage may contribute to peripheral nerve dysfunction during aging. Targeting 12/15-LO chronically with Baicalein (10 mg/kg body weight, i.p.) in 24-mo-old female mice for 4 mo completely attenuated the age-related increase in 12-HETE and protein carbonyls in sciatic nerves. Importantly, the inhibition was accompanied by protection of age-related decline in peripheral motor nerve and muscle function (~ 40-50%), providing strong support for the role of 12/15-LO pathway mediated oxidative damage in peripheral neuropathy during aging. Our future studies will use a Cre lox system to directly address the role of peripheral nerve 12/15-LO in age-related peripheral neuropathy and its impact on muscle function.

66. Synthesis and bioevaluation of Resveralogues: a multidimensional approach to developing targeted geroprotectors.

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There is increasing evidence that a few major mechanisms maintain health in higher eukaryotes and that the action of these may be modulatable by simple compounds, including some present in the diet. Resveratrol, a plant polyphenol found in many dietary components probably serves as the exemplar of this class of molecules. Although resveratrol clearly has beneficial effects both in vivo and in vitro, the predominant mechanisms by which these occur remain unclear. One reason for this lack of clarity is that resveratrol has multiple activities (including acting as an antioxidant and an oestrogen analogue) as a result of its interaction with multiple cellular pathways. We have proposed that modulation of the deleterious phenotype of senescent cells is a primary mechanism by which resveratrol and its synthetic analogues (resveralogues) exert their the beneficial effects. To test this hypothesis, and to generate improved therapeutic compounds, the diverse bioactivities of resveralogues require dissection via quantitative structure-activity relationship (QSAR) studies. To that end we have established simple, robust and high-yielding syntheses to facilitate access to a library of "resveralogues", incorporating a broad range of structural variants of resveratrol. Our syntheses utilise simple and rapid (<24h) reactions without the need for air sensitive reagents or complex purification strategies. More than 40 resveralogues, many of which are completely novel have been produced to date. The in vitro activity of these compounds has been characterised with regard to proliferation (as determined by viable biomass measurement and pKI67

staining), induction of senescence (by senescence associated β galactosidase staining), and suppression of inflammatory cytokine release. Our results show that small structural modifications of resveratrol can produce a wide variety of biological effects.

Neurodegeneration & Cognitive Aging II

67. Role of Reduced Synaptobrevin 2 and IGF-1 Levels in Age-Related Cognitive Decline

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University of Oklahoma HSC

One of the major disabilities that affect the aging population is age related cognitive decline. Synaptic dysfunction is emerging as the major cause of cognitive impairment and dementia. Our laboratory and others have identified synaptobrevin-2 (syb2), a SNAP Receptor (SNARE) protein as a key player in synaptic transmission and plasticity. Syb2 is highly expressed in the cerebral cortex and hippocampus. Syb2 protein levels have been shown to decrease with age but the functional effects of this are still elusive. Insulin-like growth Factor (IGF)-1 is a major neurotrophic hormone which stimulates cell growth and proliferation. IGF-1 levels have also been shown to decrease with age. A positive link of IGF-1 levels with cognitive function was reported in the elderly. We hypothesize that reduction of syb2 levels mediates age related cognitive decline and IGF-1 can rescue this decline. We are using heterozygous (het) syb2 knock-out mice which express syb2 at about the same level at 6 month-of-age as old (24 mo) wild-type animals. This is 50-60% of the protein levels found in young wild-type littermates ($p<0.01$). We performed behavioral tests for spatial learning and memory using a radial-arm water maze (RAWM) and a complex cohabitate environment called Intellicage. Neuronal plasticity was assessed by long-term potentiation (LTP) assays on hippocampal slices. We used live fluorescence microscopy (FM assays) to measure the synaptic vesicle (SV) release rate in neuronal cultures. The effects of both acute (10 minutes) and extended (180 minutes) application of des3-IGF-1 on cultured neurons was determined using FM assays with FM1-43 dye to measure the synaptic release rates. The behavioral tests with syb2 het resulted in maintained basic spatial memory acquisition for simple place finding (RAWM) but impaired learning in complex tasks in Intellicage. This impaired spatial memory was reflected in reduced CA1 hippocampal LTP and Syb2 het neurons have reduced SV release rates (~35% in 15 seconds, $p<0.01$ compared to wild-type controls). Acute or extended application of IGF-1 showed an increase in the SV release rate comparable to WT levels with FM assays To our best knowledge, this is the first study to demonstrate that syb2 levels have a causative role in synaptic failure and dementia, and our results suggest syb2 reduction with age mediates age related cognitive decline. Our results also indicate that IGF-1 may partially rescue impaired synaptic neurotransmission.

68. Pharmacologically-induced neurovascular un-coupling is associated with cognitive impairment in mice: implications for cerebromicrovascular aging

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There is increasing evidence that vascular risk factors, including aging, hypertension, diabetes mellitus and obesity, promote cognitive impairment, however, the underlying mechanisms remain obscure. Cerebral blood flow (CBF) is adjusted to neuronal activity via neurovascular coupling (NVC) and this mechanism is known to be impaired in the aforementioned pathophysiological conditions. To establish a direct, causal relationship between impaired NVC and cognitive decline, we induced neurovascular uncoupling pharmacologically in mice by inhibiting the synthesis of vasodilator mediators involved in NVC. Treatment of mice with the epoxygenase inhibitor MSPPOH, the NO synthase inhibitor L-NAME and the COX inhibitor indomethacin decreased NVC by 75% mimicking the aging phenotype, which was associated with significantly impaired spatial working memory (Y-maze), recognition memory (Novel object recognition) and impairment in motor coordination (Rotarod). Blood pressure (tail cuff) and basal cerebral perfusion (arterial spin labeling perfusion MRI) were unaffected. Thus, selective experimental disruption of NVC per se leads to significant impairment of cortical function, including cognitive decline, recapitulating neurological symptoms and signs observed in brain aging and pathophysiological conditions associated with accelerated cerebromicrovascular aging.

69. Reliability and Validity of a New Behavioral Scale to Measure Behavioral and Psychological Symptoms in Dementias (BPSD): Luthra's Behavioral Assessment and Intervention Response (LuBAIR) Scale

Luthra, A. S.

Homewood Health Centre

Background: There are twelve newly formed behavioral categories to classify behaviors in moderate to advanced dementia. These categories were used to develop a new behavioral assessment scale titled LuBAIR. The objective of this study is to establish the reliability and validity of LuBAIR Scale. It is hypothesized LuBAIR will be less labor intensive, more comprehensive as well as offer improved categorization of behaviors into clinically meaningful categories. Methods: Seven long term care facilities in Ontario, Canada, were selected for the study. 120 residents with a diagnosis of dementia were recruited for the study. Sixty residents exhibiting BPSDs were included in the study group and sixty participants not displaying BPSDs were in the control group. Pittsburg Agitation Scale was used to screen for presence of BPSDs. Two registered nurses (RN) completed LuBAIR, BEHAVE-AD, and Cohen-Mansfield Agitation Inventory (CMAI) for each participant in the study group. This was done to establish inter-rater, Construct and Criteria Validity. Fourteen days later, the same RN completed LuBAIR Scale again for each participant for intra-rater reliability. A Clinical Utility Survey (CUS) was developed to evaluate the nurses' viewpoints on the usefulness of LuBAIR on three variables: less labor intensive, more comprehensive and better categorization of behaviors in clinical meaningful categories. Results: Intra-rater reliability was established for 8 of the 12 behavioral categories. Inter-rater reliability was established for 10 of the 12 behavioral categories. LuBAIR had comparable Construct and Criteria Validity. CUS findings showed 23% of nurses found LuBAIR to be less labor intensive, 77 % found LuBAIR to be more comprehensive and an overwhelming majority, 98%, agreed the LuBAIR helps understand behaviors in a clinically meaningful way. Conclusions: LuBAIR Scale has acceptable inter- and intra-rater reliability and Construct and Criteria Validity. It is more comprehensive and is better able to categorize behaviors in clinically meaningful

categories. Keywords: Behavioral and Psychological Symptoms in Dementias (BPSDs), Luthra's Behavioral Assessment and Intervention Response (LuBAIR)

70. Classification of Behaviors in Dementias Based on Principles of Compliance and Aggression

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Objectives: There is vast heterogeneity in use of terminology and classification of behaviors in dementia with no universally accepted classification system. Methods: Criteria proposed by Davis, Buckwalter and Burgio (1997) were identified as the basis for classification of behaviors in dementia. A review of literature was done with a view to identify the "Specification of the Theoretical Construct" (STC) to justify aggregation of similar behavioral symptoms into clinically meaningful categories. Results: STC identified for these behavioral categories are theories on compliance and aggression. Behavioral categories emanating from this construct are; Oppositional Behaviors (OB) and Physically Aggressive Behaviors (PAB). Discussion: OB is the result of non-compliance to the directions being given by the care provider. The types of OB are determined by the level of developmental sophistication or conversely by the degree of cognitive impairment in patients with dementia. PAB are the result of perceived impediment by the patient in goal attainment. This results in the emergence of negative emotions. These emotions are 'out of proportion' to the stimulus. The purpose of this behavior is to warn the care provider of the noxious nature of their involvement in the present situation. Keywords: Dementia, Behaviors, Opposition, Aggression, Classification

71. Role of HIV-1 proteins in Learning and Memory Deficit: Mechanisms and Intervention

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It is estimated that by 2015, about half of all HIV-positive individuals will be older than 50. Yet those over 50 also progress to AIDS faster than adults in their 20s or 30s. Those in the younger age bracket, even those responding well to antiretroviral therapy (cART), still exhibit illnesses and clinical conditions commonly associated with older people, such as HIV-associated neurocognitive disorders (HAND), certain cancers, heart, liver and bones diseases. Regarding neurocognitive disorders, HIV/AIDS patients complained from working memory impairment such as easy forgetfulness and slowness in action, difficulties in concentration, planning, and multitasking in the condition of having a relatively uneventful and well-controlled clinical course with low HIV viral titers. Neuropsychological studies may disclose cognitive impairment in a substantial (15–50%) proportion of patients, including learning and working memory deficits which may affect their quality of life, adherence to treatment and ultimately result in increased comorbidity. It has been shown that the loss of cAMP responsive-element binding (CREB)-1 protein expression and phosphorylation plays a major role in the development of these impairments. Recently, CREB (regulator of long term memory and synaptic plasticity) and PGC-1α (key regulator of

energy metabolism) proteins were shown to play a key role in delaying/ reversing aging process when young mice blood was added to old mice. We showed that HIV-1 Tat and gp120 proteins disrupted mitochondrial DNA, telomeres length, and cellular senescence all are hallmarks of aging. Interestingly, gp120 and Tat caused mitochondrial dysfunction through inhibition of CREB expression and phosphorylation. Effects of these two proteins were reversed in the presence of Rolipram, an inducer of CREB protein expression and phosphorylation. We demonstrated that HIV-1 proteins altered mitochondrial bioenergy hence causing neuronal dysfunction. Our data were confirmed using transgenic mice and human brain tissues prepared from HIV-1 patients. Restoring the effect of CREB protein might help in preventing the cognitive impairment such as, learning deficit and working memory loss that are commonly observed in HIV-1 patients as well as in aged persons. Findings from our results will pave the gap in our understanding between HIV-1/AIDS and brain aging.

Comparative Biology of Aging II

72. The Primates and the Proteasome: A Story of Protein Damage, Disease and Lifespan

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There is large variation in lifespan among different species, and there is evidence that modulation of proteasome function may contribute to longevity determination. Comparative biology provides a powerful tool for identifying genes and pathways that control the rate of aging. Here, we evaluate skin-derived fibroblasts and demonstrate that among primate species, longevity correlates with an elevation in proteasomal activity as well as immunoproteasome expression at both the mRNA and protein levels. Immunoproteasome enhancement occurs with a concurrent increase in other elements involved in MHC class I antigen presentation, including β-2 microglobulin, TAP1 and TAP2. Fibroblasts from long-lived primates also appear more responsive to IFN-γ than cells from short-lived primate species, and this increase in IFN-γ responsiveness correlates with elevated expression of the IFN-γ receptor protein IFNGR2. Elevation of immunoproteasome and proteasome activity was also observed in the livers of long-lived Snell dwarf mice and in mice exposed to drugs that have been shown to extend lifespan, including rapamycin, 17-α-estradiol, and nordihydroguaiaretic acid. This work suggests that augmented immunoproteasome function may contribute to longevity differences in mice and among primate species.

73. High Proteasome Function and Expression of Molecular Chaperones in the Naked Mole-rat Heart

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The greatest cause of death worldwide is cardiovascular disease, many types of which include proteinopathies in their progression. The largest risk factor for diseases of the heart is advanced age, which is often accompanied by the accumulation of toxins and stressors such as oxidative stress. These insults can cause increased protein dysfunction and misfolding. Efficient removal of damaged proteins by the

proteasome and its associated molecular chaperones (HSPs) may thus be crucial to maintaining heart health and staving off cardiovascular disease with age. Here we examine proteasome function in the heart of the longest-lived rodent known, the naked mole-rat (NMR). This species shows pronounced resistance to many toxins, including protein unfolding stressors. Strikingly, NMRs also maintain cardiovascular structure and function well into their last quartile of life, at ages equivalent to >90 year old humans. We therefore hypothesized that the NMR heart would exhibit high levels of HSPs and proteasome function. Compared to mice, NMRs had nearly threefold higher chymotrypsin-like activity in their hearts, a protease function that cleaves after oxidatively damaged residues on proteins. Basal ATP concentrations in NMR hearts were more than 40 fold higher than in mouse hearts, likely contributing to the greater NMR proteasome activity. Furthermore, NMR hearts displayed high expression of proteasome subunits α7 and Rpt5, components of the 20S and 26S proteasome, respectively, in comparison to mice ($p < 0.005$). Similarly to that observed in liver cytosolic extracts, proteasomes in NMR cardiac cytosolic extracts were resistant to inhibition with 20 μM MG132 ($p < 0.0001$). Mice had no significant difference in inhibition by MG132 between cytosolic fractions and total heart lysates. High proteasome function and expression were accompanied by enhanced expression of molecular chaperones. When compared to mice, NMRs expressed higher levels of HSPs 25 and 70 as well as HSF1, the master regulator of HSPs. In summary, the high levels of proteasome function, ATP, and HSPs all support the hypothesis that NMRs have mechanisms in place to rapidly remove damaged proteins. If this level of protein turnover is sustained with age, it could aid NMRs in avoiding heart disease and maintaining cardiovascular structure and function. Further studies will assess if the heart proteasome is critical to the NMR's ability to handle toxins and oxidative stress.

74. Maintained neonatal levels of neurogenic potential as a mechanism for preserved brain health in the longest-lived rodent, the naked mole-rat.

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The naked mole-rat (NMR; Rodentia: Bathyergidae, Heterocephalus glaber) has emerged as an exceptional model to study healthy aging due to its maintenance of healthspan throughout its extremely long life (maximum lifespan >31 years). In addition to its long life, NMRs also exhibit a gestation period 3.5 times longer than that of similar sized mice. Despite their protracted gestation, and being born with larger brains, adult NMR brains exhibit a protein expression profile reminiscent of mammalian neonates and we question whether this youthful phenotype reflects an extended developmental period. To evaluate the age at which these rodents reach hallmark milestones in brain maturation we tracked neural stem cell proliferation, axonal and dendritic maturation, synaptogenesis and myelination throughout their life. We found that NMR brain development more closely tracks with that of humans than of other rodents. Specifically, like humans but not mice or rats, the NMR dentate gyrus development occurs primarily in utero and newborn NMRs contain myelinated axons. Axonal stability, myelination and synaptogenesis all plateau at ~6 months of age in NMRs indicating this age as a hallmark in NMR brain maturation. Further, adult NMRs (>6 months), like adult humans, express both three-repeat and four-repeat tau isoforms instead of exclusively expressing four-repeat tau like adult mice and rats. Strikingly, we found that NMRs maintain neonatal levels of neurogenic potential in the ventricular zone into adulthood, which greatly contrasts to short-lived rodents that rapidly deplete their neural stem cell pool in the first weeks of life. Collectively, our study

reveals 6 months as a major milestone in NMR brain development, and presents the NMR as an exciting new model to comprehensively examine developmental processes relevant to humans. Further, we reveal ventricular zone neurogenesis as a potential mechanism contributing to sustained brain integrity in these extraordinarily long-lived rodents.

Growth Hormones & Aging

75. IGF-1 deficiency impairs neurovascular coupling in mice: implications for cerebromicrovascular aging

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Aging is associated with marked deficiency in circulating IGF-1, which has been shown to contribute to age-related cognitive decline. Impairment of moment-to-moment adjustment of cerebral blood flow (CBF) via neurovascular coupling is thought to play a critical role in the genesis of age-related cognitive impairment. To establish the link between IGF-1 deficiency and cerebromicrovascular impairment, neurovascular coupling mechanisms were studied in a novel mouse model of IGF-1 deficiency (Igf1f/f-TBG-iCre-AAV8) and accelerated vascular aging. We found that IGF-1 deficient mice exhibit neurovascular uncoupling and show a deficit in hippocampal-dependent spatial memory test, mimicking the aging phenotype. IGF-1 deficiency significantly impaired cerebromicrovascular endothelial function decreasing NO mediation of neurovascular coupling. IGF-1 deficiency also impaired glutamate-mediated CBF responses, likely due to dysregulation of astrocytic expression of metabotropic glutamate receptors and impairing mediation of CBF responses by eicosanoid gliotransmitters. Collectively, we demonstrate that IGF-1 deficiency promotes cerebromicrovascular dysfunction and neurovascular uncoupling mimicking the aging phenotype, which are likely to contribute to cognitive impairment.

76. Growth Hormone Modulates Hypothalamic Inflammation in Long-Lived Pituitary Dwarf Mice

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Mice in which the genes for growth hormone (GH) or GH receptor (GHR-/-) are disrupted from conception are dwarfs, possess low levels of IGF1 and insulin, have low rates of cancer and diabetes, and are extremely long-lived. Median longevity is also increased in mice with deletion of hypothalamic GH-releasing hormone (GHRH), which leads to isolated GH deficiency. The remarkable extension of longevity in hypopituitary Ames dwarf mice can be reversed by a six-week course of GH injections started at the age of 2 weeks. Here

we demonstrate that mutations that interfere with GH production or response, in the Snell dwarf, Ames dwarf or GHR-/- mice lead to reduced formation of both orexigenic agouti-related peptide (AgRP) and anorexigenic proopiomelanocortin (POMC) projections to the main hypothalamic projection areas: the arcuate nucleus (ARH), paraventricular nucleus (PVH) and dorsomedial nucleus (DMH). These mutations also reduce hypothalamic inflammation in 18-month- old mice. GH injections, between 2-8 weeks of age, reversed both effects in Ames dwarf mice. Disruption of GHR specifically in liver (LiGHRKO), a mutation that reduces circulating IGF1 but does not lead to lifespan extension, had no effect on hypothalamic projections or inflammation, suggesting an effect of GH, rather than peripheral IGF1, on hypothalamic development. Hypothalamic leptin signaling, as monitored by induction of pStat3, is not impaired by GHR deficiency. Together, these results suggest that early life disruption of GH signaling produces long-term hypothalamic changes that may contribute to the longevity of GH deficient and GH resistant mice.

77. IGF-1 REGULATES VERTEBRAL BONE AGING THROUGH SEX-SPECIFIC AND TIME-DEPENDENT MECHANISMS

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Advanced aging is associated with increased risk of bone fracture, especially within the vertebrae, which exhibit significant age-related reductions in trabecular bone structure. Aging is also associated with a reduction in circulating levels of insulin-like growth factor (IGF-1). Some studies have suggested that the reduction in IGF-1 compromises healthspan, while others report that loss of IGF-1 is beneficial for aging and can increase both healthspan and lifespan. To date, the effect of decreases in circulating IGF-1 on vertebral bone aging has not been thoroughly investigated. In the current study, we delineate the consequences of a loss of circulating IGF-1 on vertebral bone aging in both male and female Igf f/f mice in a C57Bl/6 background. Unlike previous studies, IGF-1 was reduced at specific time points during the mouse lifespan- early in postnatal development (crossing albumin-Cre mice with Igf f/f mice), or early adulthood, and late adulthood using hepatic-specific viral vectors (AAV8-TBG-Cre). Vertebrae bone structure was analyzed in aged mice at 27 months of age using microCT and quantitative bone histomorphometry. Consistent with previous studies, both male and female mice exhibited age-related reductions in vertebral bone structure. In male mice, reduction of circulating IGF-1 induced at any age did not diminish vertebral bone loss. Interestingly, early-life loss of IGF-1 in females resulted in a 67% increase in vertebral bone volume fraction, as well as increased connective tissue density and increased trabecular number when measured at 27 months of age. The maintenance of bone structure in the early-life IGF-1-deficient females was associated with increased osteoblast perimeter in the vertebrae and an increased ratio of osteoprotegerin/receptor-activator of NFκB-ligand levels in circulation. Our studies demonstrate for the first time that the age-related loss of vertebral bone density in females can be reduced by modifying circulating IGF-1 levels at specific time points during the lifespan.

Immunosenescence & Inflammation

78. Systemic inflammation due to lamin-B loss in old Drosophila fat body causes gut immune repression and hyperplasia

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Aging of immune organs, immunosenescence, is suspected to promote systemic inflammation and age-associated diseases, including cancer. Yet, the cause of immunosenescence and how it promotes disease remains poorly understood. We report that Drosophila fat body, the major immune organ, undergoes immunosenescence and mounts a strong systemic inflammation. This leads to the de-regulation of the immune deficiency (IMD) signaling in the old midguts. The inflamed old fat bodies secrete circulating peptidoglycan recognition proteins (PGRPs), which repress the IMD activity in the midguts, thereby leading to gut hyperplasia. We show that fat body immunosenescence is caused by age-associated lamin-B reduction specifically in fat body cells, which contribute to heterochromatin loss and de-repression of genes involved in immune response. Since lamin-associated heterochromatin domains (LADs) are enriched for genes involved in immune response in mammalian and Drosophila cells, our findings open the door to decipher the cause and consequence of immunosenescence in aging mammals.

79. Pilot Study to Examine Relationship of Frailty Syndrome, Cytomegalovirus (CMV) and Immune Parameters

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Aging is associated with defects in the innate and adaptive immune systems. Given that older adults are a heterogeneous group, we sought to test whether categorical measures of clinical Frailty Syndrome, as measured by the Fried Frailty Phenotype, may be better indicators of weaker immunity (e.g., disease susceptibility or poorer vaccine response) than chronological age alone. Cytomegalovirus (CMV) is a common latent persistent herpes virus infection associated with CD8 T cell expansion and decreased T cell diversity, and has been associated with frailty. This study explores the associations of age, CMV and other immune parameters by frailty category. This case control study included 129 persons from the Arizona Center on Aging Frailty Cohort, 62 non-frail (45 CMV+), 67 pre-frail or frail (42 CMV+). Eligibility criteria

included > age 55, MMSE >23 (cognitively intact), and able to walk 30 feet (could use assistive device). Initial measures included: Fried frailty criteria (METS/week, fatigue, grip strength, walking speed and weight loss), and CMV status. Preliminary findings suggest that for sub-group analysis of those who were CMV+ there was significantly increased total number of monocytes (p=0.0083), decreased Hemoglobin (p= 0.0063), decreased hematocrit (p= 0.0160), and IL6 was elevated (p=0.0674) when comparing CMV+ pre-frail/frails to non-frail CMV+ controls. Significance for all relationships was lost when not controlling for CMV. All groups analyzed were of comparable age range, and medians; the pre-frail/frail cohort had a median age (73yrs) vs non-frail (71yrs) p= 0.1554. Validity of each immune measure was obtained using Nonparametric Mann-Whitney-U-test. Multivariate polychotomous regression will be performed to investigate the independent effects of each immune variable in predicting frailty category. Odd Ratios (OR), sensitivities and specificities will be calculated for variables shown to have an independent effect on frailty category. Overall, our preliminary findings highlight the associations between CMV, immune function and frailty status, including associations with gait, physical performance and total physical activity variables, and should provide basis for further detailed exploration of cellular mechanisms of frailty.

Proliferative Senescence & Telomeres

80. Telomerase Activity and Telomere Length in Daphnia

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Often studied for their role in cellular aging and senescence, telomeres are oligonucleotide repeats that flank the ends of linear chromosomes. An enzyme named telomerase functions to impart telomeric repeats to the ends of chromosomes and is essential for maintaining the length of the telomeres. Due to the end replication problem, which arises due to DNA polymerase only functioning in the 5' to 3' direction, each cellular replication event results in a progressive shortening of the telomeres in telomerase negative mammalian somatic cells. In mammals, somatic cell proliferation is high in embryonic stages but very low to none as the organism reaches adulthood. Studies examining the role of telomeres in cellular aging led to the development of the telomere hypothesis of cellular aging which states that telomeres serve as a mitotic clock in telomerase negative cells and when telomeres have been eroded, cellular senescence and death occur. In our present study, we examined telomerase activity, processivity and telomere length of Daphnia. Daphnia are microcrustaceans that inhabit bodies of freshwater around the world and are an established ecotoxological model system. They are also an emerging model system in the study of aging and longevity. Our study examines two closely related ecotypes named Daphnia pulex and Daphnia pulicaria. D. pulex inhabits transitory bodies of water and live on average 25-30 days while D. pulicaria inhabits stratified lakes and live 60-65 days. We determined the telomeric repeat to be TTAGG, which is identical to insects and other crustaceans and in accordance with the repeat length we found the telomerase assay produces a 5-nucleotide periodicity. Comparing the two ecotypes, we show that there is no age dependent decline in telomerase activity, processivity, or telomere length in the short lived D. pulex; however, in the long lived D. pulicaria, there is an age dependent decline in telomerase activity, processivity, and telomere length. We provide the first study examining telomeres and telomerase in Daphnia and our results indicate that a mechanism other than telomere erosion is leading to the short life span of D. pulex.

81. Correlating cellular proliferation with age, size, and longevity

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The long-term objective of this research is to better understand the biochemical, genetic and physiological mechanisms of aging and age related changes in the domestic dog, Canis familiaris, in comparison to the changes known in human aging. With the American population's life span nearly doubling in the last century, it is economically and socially vital to understand the biological mechanisms allowing for healthy old age. The domestic dog is a valuable candidate for investigation of the cellular senescence theory of aging because access to canine pedigree and medical information is readily available; investigation of its natural variation in life expectancy provides a unique study of the genetic components affecting aging. Our hypothesis is that fibroblasts extracted from smaller dogs, who have the longest lifespan within the species, will proliferate at a slower pace than fibroblasts extracted from larger dogs, who have a shorter lifespan. Further, cellular respiration and energy production will quantitatively reflect these differences. The hypothesis is tested by collection of skin biopsies from various dog breeds: 3 small breeds (<20lbs.), 3 medium breeds (30-60 lbs.), and 3 large breeds (> 80 lbs.). Primary fibroblasts are isolated from skin biopsies and cultured under standard conditions. Proliferation rates are measured daily, and cellular respiration is measured at determined optimum passages and concentrations. We found that fibroblasts from smaller dog breeds grew at a significantly increased rate over those isolated from larger dog breeds. However, the senescence rate for the smaller breeds' cells was much delayed when compared to the larger breeds' fibroblasts. Cells were measured for base respiration rates at passage 3 on the Seahorse Biosciences XFp extracellular flux analyzer. Results were compared among various breeds, grouped by size. The cellular respiration and proliferation rates were further aligned to known longevity parameters.

82. Tissue accumulation of dormant senescence-prone cells (DSPC) memorizes individual life history of genotoxicity and increases the risk of accelerated aging

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Cellular senescence is one of the outcomes as a response to DNA damage and accumulation of senescent cells is considered as a likely cause of aging and thus various age-related diseases in mammals. Exposure of mammalian organism to severe genotoxic stress, such for example as chemo and radiation therapy, is expected to result in accelerated aging. Surprisingly, however, mice that received high doses (7-13 Gy) of total body irradiation (TBI) and rescued from radiation-induced lethality by bone marrow transplantation showed neither massive accumulation of senescent cells in their tissues nor accelerated acquisition of bone fide aging phenotype. However, close to 100% of mesenchymal cells from organs of mice that received 11 or more Gy of TBI rapidly converted into senescence, exhibiting full senescent phenotype, including SA-beta-Gal staining and SASP acquisition upon plating in culture indicating that they persisted as

dormant senescence-prone cells (DSPC) in tissues. The conversion of DSPC to fully senescent cells was p53 dependent and preceded with DSPC's entry to S-phase and activation of DNA damage response (DDR), which was not activated in these cells in vivo following TBI. After irradiation, DSPCs remain in tissues during the entire life of irradiated mice and can be detected when they are forced to proliferate. Switching of TBI to a high fat diet accelerates development of aging phenotype as reflected by rapid increase in their frailty index presumably by massive conversion of DSPC into senescence associated with their cell cycle entry during lipogenesis. Potential implications of these findings to biodosimetry of genotoxicity and to prophylaxis of accelerated aging in people subjected to genotoxic stresses did not escape our attention.

83. Association of Leukocyte Telomere Length with plasma Homocysteine in Singapore Chinese population

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Telomere Length (TL), a major determinant of biological age, is considered to be one of the most promising emerging indicators for risk of age-related diseases. In cardiovascular diseases (CVD) particularly, current biomarkers are still inefficient to assess and monitor CVD risk. Although the significance of TL has been increasingly appreciated in age-related disease risk indication, epidemiological basis is largely controversial and human studies are surprisingly relatively limited. Furthermore, there is a paucity of data from different ethnic backgrounds including Asian populations. In this study, 100 samples from the Singapore Chinese Health Study (SCHS) cohort were tapped on to explore the association of leukocyte telomere length (LTL) with CVD risk factors. The cohort recruited Chinese individuals between the age of 47 to 74 years and demographic data was collected through face-to-face interviews. Blood was collected at the time of recruitment and CVD biomarkers were measured. LTL was measured using the Southern Blot method, which is the gold standard for TL measurement. LTL was found to be inversely associated with homocysteine (p of trend = 0.014) and with serum urate at borderline significance (p of trend = 0.056) after adjustment for age, gender, smoking status, education and dialect. Although the other CVD risk factors such as low-density lipoprotein and nutrients such as folate showed the expected direction of association with LTL, they did not reach statistical significance. This significant and specific association of LTL with homocysteine in overt CVD free population from the SCHS cohort indicates the role of LTL in CVD risk development. Evidence from this study will therefore assist in validation of TL as an early complimentary biomarker for CVD risk and may also indicate possible nutritional intervention strategies targeted towards optimal telomere maintenance in CVD development.

Environment, Exercise and Obesity

84. Intraspecific diversity in genetically-mediated lifespan extension under reduced temperature

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Environmental change and the perception of environmental change induce plasticity in lifespan. Lower temperatures have been shown to increase lifespan in a range of species, a phenomenon once thought to be due to a decrease in the "rate of living" but increasingly understood to be controlled by genetic mechanisms. Recent work showed that the lifespan of a Brachionus monogonont rotifer was significantly extended by exposure to low temperature early in life and implicated environmental sensing by transient receptor proteins (TRP) in the response. Rotifers are a valuable animal model system for aging, as they have a distinctive aging phenotype and a median lifespan of approximately 14 days, have not undergone the extensive genome reduction of other invertebrate model systems and thus share more genes in common with humans, and provide increased evolutionary breadth in studies of aging as basal metazoans. We investigated the conservation of the lifespan change response to decreased temperature in 11 closely related isolates from the Brachionus species complex. Growth at 16 °C instead of 21 °C increased maximum lifespan by 27% to 110% and changed median lifespan by -6% to 100%, depending on the isolate. Isolates with the same basal lifespan at 21 °C had significantly different responses to growth at 16 °C. Differences between isolates in their response to cooler temperature were not correlated with differences we previously observed in their response to caloric restriction, suggesting the responses to these two environmental changes are mediated via different mechanisms. We found that an isolate that responded to growth at 16 °C with lifespan extension had increased resistance to heat, oxidative, and salinity stresses at 21 °C when grown for the first 4 days of life at 16 °C, while no resistance was conferred to an isolate that did not respond to growth at 16 °C with lifespan extension. We compared lifespan and stress response in these two isolates after exposure to chemical agonists of TRPM8 and TRPA1 and conducted comparative qPCR to determine which receptors and pathways are involved in low-temperature induced lifespan plasticity. Our work argues against lifespan extension via a simple thermodynamic slowing of metabolism at cooler temperatures and for an active sensing of the thermal environment and a coordinated genetic response that changes aging.

85. Treadmill exercise attenuates aging-related bradykinesia: potential involvement of increased nigral GFR-α1 expression and dopamine tissue content

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Given the burgeoning increase in our elderly population, lifestyle strategies that mitigate aging-related impairments are essential. Bradykinesia, a cardinal symptom of Parkinson's disease (PD), also affects up to 30% of the elderly population. Exercise may improve locomotor deficits in PD models and patients, but the neuroanatomical and molecular basis for these effects have not been pinpointed to striatal dopamine (DA) recovery. In PD, striatal DA loss exceeds 80% at onset of bradykinesia, but in aging, striatal DA loss has not ever been reported to exceed 50%. However, in aging and PD alike, the onset of bradykinesia may be associated with 50% DA loss in the substantia nigra (SN). Here, we hypothesize that an established treadmill exercise regimen could attenuate aging-related bradykinesia (ARB) in conjunction with increased DA and the glial cell line-derived neurotrophic factor (GDNF) receptor, GDNF family receptor-alpha 1 (GFR-α1) in the SN. The rationale for this hypothesis is based on observations that striatal infusion of GDNF in aging models increases locomotor activity and DA in the SN, but not striatum. Exercise may also increase GDNF expression, and exogenous GDNF may increase GFR-α1 expression in the SN. We have also reported that GFR-α1 decreases only in the SN in aging, and replenishing the quantity of GFR-α1 lost due to aging

increases locomotor activity in combination with increased DA and tyrosine hydroxylase (TH) expression in SN, but not striatum, in aged rats. Using our treadmill exercise regimen, we assessed the impact of short- and long-term exercise on ARB and GDNF signaling in aged rats. Our results demonstrate that two rounds of our exercise regimen increased GFR-α1 expression and DA tissue content in SN of aged rats: a result that reflects the previously reported effect of exogenous GDNF. Notably, a repeated regimen of long-term exercise followed by an equal amount of rest eventually attenuated ARB when compared to non-exercise rats. These studies may be applicable in PD models, in that reduction of age-related loss of DA in the SN may be an important mechanism of reducing bradykinesia. Finally, our work may suggest that a therapeutic strategy that reduces ARB and increases DA synthesis in the SN may be a sufficient means to target bradykinesia, particularly in those who may be physically unable or unwilling to exercise.

86. Effect of altered day length on measures of healthspan in male C57BL/6 mice

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Animals perceive and respond to photoperiod cues that stimulate both behavioral and physiological responses. Photoperiod, specifically light, entrains the central circadian clock which influences these responses. Metabolic processes follow a diurnal pattern driven in part by a similar diurnal pattern of hormone release ultimately under the control of the circadian clock. Since the passage of chronological time is intrinsically linked to the number of circadian cycles, the extent to which an animal's perception of passing time, and subsequent cycles of the circadian clock, affects lifespan is not known. In this study, we sought to decouple chronological time and perceived time by either contracting or expanding perceived time by 10% relative to the 24-hr day. We hypothesize mice randomized to the contracted day will experience more circadian cycles (accompanied by a greater number of metabolic and hormonal cycles), will perceive time as passing more quickly, and as a result, will senesce faster than mice randomized to the 24-hr day. Conversely, mice randomized to the expanded day will experience fewer circadian cycles, will perceive time as passing more slowly, and will senesce at a slower rate than mice randomized to the 24-hr day. Male C57BL/6 mice (n=80 per group, age=12 weeks) were randomly assigned to one of three groups: 22-, 24-, or 26-hr day. Body weights were measured weekly starting at age 12 weeks, and healthspan was evaluated using grip strength tests and total distance traveled during 4 min in an open field (locomotion) starting at age 73 weeks. To date, survival is 71, 87, and 84% for the 22-, 24-, and 26-hr groups, respectively. At ages 73, 85, and 98 weeks, body weights exhibited the following trend: 22-hr group > 24-hr group > 26-hr group, but the differences were not significant ($p=0.05$). At age 73 weeks, the maximum grip strength trend of 24-hr group > 26-hr group > 22-hr group was not significant ($p=0.09$). At age 85 weeks, the maximum grip strength trend 26-hr group > 24-hr group > 22-hr group was significant ($p<0.001$). However, at age 98 weeks, the maximum grip strength trends among groups returned to that observed at 73 weeks ($p=0.44$) but absolute values had declined over time ($p<0.001$). At age 76 weeks, locomotion was highest for the 24-hr group ($p=0.02$) but lowest at age 101 weeks for the 24-hr group ($p<0.01$). This longevity study is ongoing. The current data suggest perceived time imparts an effect on grip strength and locomotion.

87. Effects of exercise and antioxidant supplementation on oxidative stress in brain regions

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Aging is associated with a decline in motor and cognitive function, which has been related to an increase in molecular oxidative damage and in a pro-oxidizing redox state disrupting redox signaling. Due to the high oxygen consumption, high levels of polyunsaturated fatty acids and low antioxidant defenses, brain tissue is particularly vulnerable to changes in oxidative stress. A large number of health conscious individuals often combine exercise with vitamin supplementation, anticipating an additive effect maximizing their performance. However, recent studies have indicated a potential antagonistic action of the antioxidants on the beneficial effects of exercise. While our preliminary behavioral outcomes suggest that the combination of both interventions increased benefits in some aspects of motor and cognitive function, it is important to establish whether it is associated with a decrease in oxidative burden. Separate groups of young (4 months) and old (20 months) male C57BL/6J mice were assigned to one treatment group: Sedentary/control diet (SedCon), Sedentary/antioxidant-rich diet (α-tocopherol (128 IU/kg /d) and ascorbate (189 mg/kg/d) (SedEC); Exercise/control diet (ExCon); Exercise/antioxidant-rich diet (ExEC). After 16 weeks on the treatment, brains were dissected and prepared for measurement of glutathione, and catalase activity. The outcomes of the treatments seemed to be dependent on age and the brain region studied. Catalase activity seemed to be decreased only in cortex and hippocampus, and increased in midbrain. In young mice, catalase activity was decreased in exercised mice in cerebellum and hippocampus, and in SedEC mice in cortex. In old mice, all treatments seemed to increase activity in the cerebellum, while only SedEC increased activity in the cortex and SedEC and ExEC increased it in the hippocampus. In old mice, GSSG levels seemed to be reduced in the hippocampus of all treated mice, in the cerebellum of the ExEC mice, and in the cortex of the SedEC and ExCon mice when compared to controls. Our data indicate that each treatment have differential effects that are dependent on the age of the mice as well as the brain regions being studied. There does not seem to be any additive effects of exercise and antioxidant supplementation on these two measures of oxidative stress. Further investigations of oxidative stress status will be required to determine the true nature of the interaction between exercise and antioxidants.

Biomarkers & Models

88. Autofluorescence In Tissues of Aging *Drosophila melanogaster*

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Age pigment, or lipofuscin, has been shown to accumulate in aging *Drosophila melanogaster* based on electron microscopy and quantification of autofluorescent material extracted from fly tissues. The objective of the current study was to measure the intensity of autofluorescence quantitatively in the cells of fly tissues in situ, across a range of excitation and emission wavelengths, and document any changes associated with aging. The hypothesis tested was that autofluorescence at wavelengths characteristic of

lipofuscin would increase during aging, as has been observed in vitro. Freshly dissected fly tissues were imaged in phosphate-buffered saline using confocal laser scanning microscopy, with excitation wavelengths of 405, 458, 477, 488, 514, 563 and 641 nm, and spectral detection of emissions from 430-750 nm; 14-25 μm wire spacers were placed between the slide and coverslip to minimize tissue damage. Results will be presented for the brain, anterior Malpighian tubules, posterior midgut, hindgut, flight muscles and trachea.

89. Systems Biology of Human Aging - Network Model 2015

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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 most 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 the molecular to the whole-body level. Effects due to externalities, lifestyle, environment, and proposed interventions are highlighted around the margins of the network.

90. The Skeptical Researcher's Metabolic and Behavioral Measurement Toolbox

John Lighton, Ph.D.
President and chief scientist, Sable Systems International

Aging induces a panoply of diverse cardiopulmonary, vascular, neuromuscular, metabolic and behavioral changes to the intact, functioning organism, whether human or model-animal. Reductionistic, in vitro approaches have greatly advanced our understanding of the etiology of aging. However, in many cases the effects of specific therapeutic interventions (e.g. mTOR inhibitors) or experimental genetic changes (e.g. knockouts, CRISPR manipulations) have unpredictable effects in vivo. Because they may have multiple effects on complex, interacting

physiological systems, they must be tested on the whole organism. Traditional whole-organism metabolic and behavioral monitoring systems provide low bandwidth data streams characterized by low sampling frequency. In the areas of metabolism, activity and intake measurement and analysis, the greater the bandwidth of the system, the more valuable is the information that can be mined from real-world data streams. I suggest that the optimal bandwidth is 100-1000x greater than typical systems allow, particularly in the metabolic arena. Using actual data from a Promethion high throughput metabolic and behavioral phenotyping system, I demonstrate several data extraction modalities of potential interest to researchers of aging. These include novel indices of ambulatory activity, energy cost of activity, and whole-animal water balance in mice. I close with an example of a whole-organism ROS tolerance assay in Drosophila.

Denham Harman, M.D., Ph.D.

February 14, 1916 – November 25, 2014

In Memoriam

Dr. Denham Harman was the driving force behind the founding of the American Aging Association (AGE) and is credited with formulating the Free Radical Theory of Aging. He incorporated AGE in Omaha, Nebraska in 1970 and served as its first president. He served on the faculty of the University of Nebraska Medical Center for 52 years before stepping down in 2010.
Inspired by his passion, dedication, generosity and humility, we dedicate this meeting to his memory.