Friday, Pre-Meeting: June 3, 2011

LATE ONSET INTERVENTION AGAINST AGING: TOOLS, APPROACHES, IMPACT

Chair: Aubrey de Grey

8:00 am – 8:50 am	**Pre-Conference Registration and Continental Breakfast**
8:50 am – 10:00 am	**SESSION I** **THE BIOTECHNOLOGICAL PROMISE OF LATE-ONSET INTERVENTIONS IN AGING**
8:50 am – 9:00 am	1. Aubrey de Grey, SENS Foundation **Postponing Aging Starting in Middle Age: Risky Research, Rampant Rewards**
9:00 am – 9:30 am	2. Walter Funk, BioTime **The Potential and Limitations of Personalized Medicine for Aging**
9:30 am – 10:00 am	3. Anthony Atala, Wake Forest University **The Promise of Regenerative Medicine for Aging**
10:00 am – 10:30 am	***BREAK***
10:30 am – 12:00 pm	**SESSION II** **STATUS AND PROSPECTS FOR SLOWING AGING STARTING LATE IN LIFE**
10:30 am – 11:00 am	4. Michael Rae, SENS Foundation **Late-Onset Calorie Restrictions in Rodents and Humans**
11:00 am – 11:30 am	5. David Sharp, University of Texas Health Sciences Center San Antonio **Rapamycin: A Late-Onset Calorie Restriction Mimetic?**
11:30 am – 12:00 pm	6. David Harrison, Jackson Labs **Late Versus Early Onset Intervention Studies in Animal Models**
12:00 pm –1:00 pm	***LUNCH BREAK (on your own)***
1:00 pm – 3:00 pm	**SESSION III** **PROGRESS TOWARDS REVERSING AGING STARTING LATE IN LIFE**
1:00 pm – 1:30 pm	7. Jacques Mathieu, Rice University **Pre-Empting Atherosclerosis by Eliminating Stored Oxysterols**
1:30 pm – 2:00 pm	8. Megan Smithey, University of Arizona **Simultaneous Repair of Both Major Components of Immunosenescence**

2:00 pm – 2:30 pm	9. Zhenyu Ju, Hangzhou Normal University **Dissecting the Role of Telomere Dysfunction in Hematopoietic Failure in Telomerase Deficient Mice**
2:30 pm – 3:00 pm	10. Jan Vijg, Albert Einstein College of Medicine **Do Mice Accumulate Epimutations in the Brain During Adulthood?**
3:00 pm – 3:30 pm	***BREAK***
3:30 pm – 5:30 pm	**SESSION IV** **SOCIETAL IMPACT OF LATE-ONSET INTERVENTIONS STUDIES IN AGING**
3:30 pm – 4:00 pm	11. Natalia Gavrilova and Leonid Gavrilov, University of Chicago **How Much Would Late-Onset Interventions in Aging Affect Demographics?**
4:00 pm – 4:15 pm	12. Svetlana Ukraintseva, Duke University **Feasibility of Intervention Against Aging with Currently Approved Drugs: Relevance of Age of Onset and Genetic Background**
4:15 pm – 4:30 pm	13. Anatoli Yashin, Duke University **Genes and Compression of Mortality**
4:30 pm – 5:00 pm	14. Aubrey de Grey, SENS Foundation **Impact of Biomedical Compression or Postponement of Morbidity: Relevance of Age of Onset**
5:00 pm – 5:30 pm	PANEL DISCUSSION **How Can Research on Late-Onset Interventions in Aging be More Prioritized?**

PRE-CONFERENCE ADJOURNS

6:30 pm – 8:00 pm	**AAA Conference Registration** **AAA WELCOME RECEPTION**

Saturday, June 4, 2011

MECHANISMS OF AGING: EMERGING CONCEPTS

Chair: Holly Brown-Borg
University of North Dakota

7:30 am **Continental Breakfast**

8:30 am – 8:40 am **Welcome and Opening Remarks**
Holly Brown-Borg, University of North Dakota
President American Aging Association

8:40 am – 9:40 am ***Keynote Address:***

Norman Sharpless
University of North Carolina-Chapel Hill

The Amazing INK4a/ARF Locus and Human Aging

9:45 am – 12:15 pm **Session 1**
MICRORNA AND AGING
Chair: Heidi Scrable, Mayo Clinic, Rochester, MN

9:45 am – 10:15 am 15. Frank Slack, Yale University
MicroRNAs and Aging in C. elegans

10:15 am – 10:45 am 16. Izumi Horikawa, National Cancer Institute
The P53-MicroRNA Connection: Clues From Cellular Senescence to Organismal Aging

10:45 am – 11:15 am ***BREAK***

11:15 am – 11:45 am 17. Scott Hammond, University of North Carolina
MicroRNAs in Development and Disease

11:45 am – 12:15 pm 18. Heidi Scrable, Mayo Clinic, Rochester
Epigenetic Modifiers in Stem Cells

12:15 pm –1:45 pm ***Vincent Cristofalo Memorial Rising Star Award and Lecture***

Matt Kaeberlein
University of Washington

Molecular Mechanisms Linking Diet, Disease and Aging

Sponsored by:
The American Federation for Aging Research (AFAR)
In collaboration with
The Glenn Foundation for Medical Research
Margaret F. Cristofalo
The Gerontological Society of America

1:45 pm – 3:45 pm	**Session 2** **COMPARATIVE BIOLOGY OF AGING** *Chair: Shelley Buffenstein*
1:45pm – 2:15 pm	19. Shelley Buffenstein **Lessons on Longevity from the Longest Living Rodent**
2:15 pm – 2:45 pm	20. Mary Ann Ottinger, University of Maryland **Role of Endocrine Hormones in Vertebrate Aging**
2:45 pm – 3:15 pm	21. Woody Wright, University Texas Southwestern Medical Center at Dallas **Comparative Biology of Telomeres and Their Role in Species Longevity**
3:15 pm – 3:45 pm	22. Zoltan Ungvari, University of Oklahoma Health Sciences Center **Animal Models, Cardiovascular Function and Aging**
3:45 pm – 4:15 pm	***BREAK***
4:15 pm - 5:15 pm	***Submitted Papers Sessions 3A and 3B are Dual Sessions*** **SUBMITTED PAPERS SESSION 3A** *Chair:*
4:15 pm – 4:30 pm	23. Graham Pawelec, University of Tuebingen **Road-Mapping European Aging Research: The Next Decade of EAR**
4:30 pm – 4:45 pm	24. Anshu Agrawal, University of California Irvine **Mechanisms Underlying the Altered Dendritic Cell Function in Aged Humans**
4:45 pm – 5:00 pm	25. Anatoliy Yashin, Duke Univeristy **Polygenic Influence on Life Span: Insights for Mechanisms of Aging and Disease Development**
5:00 pm – 5:15 pm	26. Karl Rodriguez, University of Texas Health Science Center San Antonio **When Compared to the Mouse Proteasome the Naked Mole-Rat Proteasome Shows Increased Activity and Resistance to Inhibition**
4:15 pm – 5:15 pm	**SUBMITTED PAPERS SESSION 3B** *Chair: Christy Carter, University of Florida*
4:15 pm – 4:30 pm	27. Christy Carter, University of Florida **Usefulness of Preclinical Models for Assessing the Efficacy of Late-Life Interventions for Functional Decline**
4:30 pm – 4:45 pm	28. Lauren Sloane, University of Texas Health Science Center San Antonio **Developing Healthspan Assays in C57BL/6 Mice**
4:45 pm – 5:00 pm	29. Dudley Lamming, Massachusetts Institute of Technology **Depletion of mTOR and mLST8 Uncouples Longevity From Rapamycin-Induced Changes in Glucose Homeostasis**

5:00 pm – 5:15 pm	30. Thomas Register, Wake Forest Baptist Health Medical Center **Cardiovascular Function and Aging in a Non-Human Primate Model**
5:30 pm – 7:00 pm	**Poster Session 1** **Competition for Glenn Award and Nicolai Prize**
5:30 pm – 7:00 pm	**COCKTAIL RECEPTION**
7:00 pm – 8:30 pm	**Special Student Session: Data Blitz** ***Sponsored by*** ***The American Aging Association*** ***and The Gerontological Society of America***
7:00 pm – 10:00 pm	**AGE BOARD OF DIRECTORS DINNER MEETING**

Sunday, June 5, 2011

7:30 am	**Continental Breakfast**
8:30 am – 10:30 am	**Session 4** **STRESS RESISTANCE IN AGING & DISEASE** *Chair: Valter Longo, University of Southern California* ***SPONSORED BY*** ***THE ELLISON MEDICAL FOUNDATION***
8:30 am – 9:00 am	31. Valter Longo, University of Southern California **Differential Cellular Protection and Sensitization in Cancer Treatment**
9:00 am – 9:30 am	32. Keith Blackwell, Harvard Medical School **Mechanisms Through Which TORC1 Signaling and Rapamycin Influence *C. elegans* Longevity**
9:30 am – 10:00 am	33. James Mitchell, Harvard School of Public Health **Dietary Amino Acid Restriction, Stress Resistance and Longevity**
10:00 am – 10:30 am	34. Rafael de Cabo, National Institute on Aging **Stress Resistance by NRF2: Its Role in Calorie Restriction and Aging**
10:30 am – 11:00 am	***BREAK***
11:00 am – 12:30 pm	**Session 5** **TRANSLATIONAL AGING RESEARCH (Arthur Balin Session)** *Chair: James Kirkland* **SPONSORED BY**
11:00 am – 11:30 pm	35. Nathan LeBrasseur, Mayo Clinic, Rochester **Muscling Into Translational Research: From Mighty Mice to Men**
11:30 am – 12:00 pm	36. Jeremy Walston, Johns Hopkins University **Biology of Frailty and Late Life Decline**
12:00 pm – 12:30 pm	37. James Kirkland, Mayo Clinic, Rochester **Low Hanging Fruit: Early Opportunities to Translate Advances from the Basic Biology of Aging into Clinical Applications**
12:30 pm – 1:00 pm	**AMERICAN AGING ASSOCIATION AWARDS LUNCHEON** **(ALL ATTENDEES ARE INVITED TO ATTEND)** **Awards Presentations:** **Distinguished Achievement Award** **Denham Harman Award**

1:00 pm – 2:00 pm **PLENARY SESSION: DENHAM HARMAN AWARD LECTURE**

Sessions 6A and 6B are Dual Session

2:00 pm – 5:00 pm **Session 6A**

NATHAN SHOCK CENTER SYMPOSIUM

Sponsored by
The National Institute on Aging
Nathan Shock Centers of Excellence

Chair: Felipe Sierra, PhD, Director, Division of Aging Biology, National Institute on Aging

2:00 pm –2:30 pm 38. Carolos J. Orihuela, University of Texas Health Sciences Center
Inflammaging and Cellular Senescence Prime the Lungs for Bacterial Pneumonia

2:30 pm – 3:00 pm 39. Dana L. Miller, University of Washington
Effects of Hydrogen Sulfide on Protein Homeostasis and Aging

3:00 pm – 3:30 pm ***BREAK***

3:30 pm – 4:00 pm 40**.** Gary A. Churchill, The Jackson Laboratory
New Mouse Resources for Genetic Research in Aging

4:00 pm – 4:30 pm 41**.** Scott Pletcher, University of Michigan
Neurosensory Modulation of Physiology and Aging in Drosophila

4:30 pm – 5:00 pm 42**.** Derek M. Huffman, Albert Einstein College of Medicine
IGF-1 Action in the Brain on Insulin Sensitivity and Body Fat Distribution with Aging

2:00 pm – 5:00 pm **Session 6B**

TRANSATLANTIC SYMPOSIUM

Chair: Richard Faragher

BSRA Korenchevsky Award Presentation

2:00 pm – 2:30 pm 43. Elaine Emmerson, University of Manchester
Estrogen Receptor Mediated Effects on Wound Healing in the Elderly

2:30 pm – 3:00 pm 44. Angela Sheerin, University of Brighton
Is Lifespan in a long-Lived Mutant Mouse Strain Limited by Cell Senescence?

3:00 pm – 3:30 pm ***BREAK***

3:30 pm – 4:00 pm 45. Deborah Dunn-Walters, King College London
The B Cell Repertoire in Ageing

4:00 pm – 4:30 pm	46. Gregg Scutt, University of Brighton **Ageing in a Defined Neuronal Population**
4:30 pm – 5:00 pm	47. Jill Saffrey, Open University **Ageing of the Mammalian Gastrointestinal System**
5:00 pm – 5:50 pm	**PLENARY SESSION: SPECIAL LECTURE**

Thomas Perls, Boston University
The Increasing Genetic Contribution to Exceptional Longevity with Increasing Age Beyond 100 Years

Sponsored by
The Glenn Foundation

6:00 pm – 6:30 pm	AAA Business Meeting – **Open to all members**
6:30 pm – 8:00 pm	**POSTER SESSION II** **Competition for Glenn Award and Nicolai Prize**
6:30 pm – 8:00 pm	**COCKTAIL RECEPTION**

Monday, June 6, 2011

7:00 am – 8:00 am	**Continental Breakfast**
8:00 am – 10:00 am	***Sessions 7A and 7B are Dual Session***
8:00 am – 10:00 am	**SESSION 7A: THE ENVIRONMENT AND AGING** *Chairs: J. Christopher Corton and Robert MacPhail, U.S. Environmental Protection Agency*
8:00 am – 8:30 am	48. J. Christopher Corton, U.S. Environmental Protection Agency **Characterization of the Impact of Life Stage on Gene-Chemical Interactions in the Liver**
8:30 am – 9:00 am	49. Staci Bilbo, Duke University **Implications of Early-Life Experience for Cognitive Function Throughout the Lifespan**
9:00 am – 9:30 am	50. John Hong, National Institutes of Environmental Health Sciences **Inflammation-Mediated Degeneration of Dopaminergic Neurons: Mechanisms, Interventions and Relevance to Parkinson's Disease**
9:30 am – 10:00 am	51. Robert MacPhail, U.S. Environmental Protection Agency **Aging and the Environment: Importance of Variability Issues in Understanding Risk**
10:00 am -10:30 am	***BREAK***
8:00 am-10:00 am	**SESSION 7B: CARDIOVASCULAR AGING** *Chair: Julie Mattison, National Institute on Aging*
8:00 am – 8:30 am	52. Carol A. Shively, Wake Forest University **Psychosocial Stress, Disease and Aging in Female Nonhuman Primates**
8:30 am – 9:00 am	53. Anna Csiszar, University of Oklahoma Health Sciences Center **Vascular Oxidative Stress in Aging: A Homeostatic Failure Due to Dysregulation of Nrf2-Mediated Antioxidant Response**
9:00 am – 9:30 am	54. Julie Mattison, National Institute on Aging **Resveratrol: Protecting the Heart and Vessels of Mice and Monkeys**
9:30 am – 10:00 am	55. Ed Lakatta, National Institute on Aging **The Stress of Aging Viewed from the Arterial Wall**

10:30 am – 12:00 pm	**SESSION 8: AGE Pioneers: A TRIBUTE TO JIM JOSEPH AND MARK A. SMITH** *Chair: Don Ingram, Pennington Biomedical Research Center and Barbara Shukitt-Hale, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University*
10:30 am – 11:00 am	56. Donald Ingram, PhD, Pennington Biomedical Research Center **Jim Joseph: Davy Crockett at the Frontier of Neurogenerontology**
11:00 am – 11:30 am	57. Barbara Shukitt-Hale, PhD, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University **Food For Thought: What Jim Joseph Taught Me About Aging and Nutrition**
11:30 am – 12:00 pm	58. Gemma Casadesus, PhD, Case Western Reserve University **The Journey from Normal Aging to Alzheimer's Disease: Lessons Learned from my Mentor Jim A. Joseph and my Husband Mark A. Smith**
12:00 pm – 12:30 pm	59. George Perry, PhD, The University of Texas at San Antonio **Chrysler Newport's and Triangles: Mark Smith's Legacy**
12:30 pm – 1:00 pm	**AWARDS CEREMONY (TRAINEES)**
1:00 pm	**MEETING ADJOURNS**

AGE PROGRAM SPEAKER ABSTRACTS

Abstract Number corresponds to speaker presentation number in the program schedule
(P) Denotes Presenter (G) Denotes Post-doctoral Candidate for Glenn Award
(N) Denotes Pre-Doctoral Candidate for Nicolai Award

1. Postponing Aging Starting in Middle Age: Risky Research, Rampant Rewards

Aubrey de Grey (P)
SENS Foundation

Biomedical gerontology, despite being highlighted over 25 years ago in the name of this society's international counterpart, remains a relative backwater within research on the biology of aging, largely because of pessimism that our current knowledge about how aging works is sufficient to permit the rational design of interventions to postpone aging. However, this is all relative: when compared to the situation even as little as a decade ago, intervention-focused biogerontology is finally burgeoning. What, then, is the next step? This one-day symposium seeks to highlight what an increasing number of researchers see as the key remaining "blind spot" in the research priorities of the biogerontology community: the failure to devote significant effort to developing interventions that can be effective even when begun in middle age. The perception persists that until we can achieve much more than is currently possible with early-onset or germline interventions, it is premature even to consider late-onset ones. This is powerfully challenged by the rapidity of progress in regenerative medicine, which can in principle be applied to aging by periodically repairing the accumulating molecular and cellular damage of aging and thus postponing ill-health. I will introduce the symposium by summarising the promise of this approach.

2. The Potential and Limitations of Personalized Medicine for Aging

Walter Funk (P)
BioTime

Personalized medicine is a catch-all phrase that covers topics as diverse as sensitivities to drugs, genetic disease and risk factors, and cellular therapeutics. Certain "personalized" practises such as transplantation or genetic testing for heriditable disease are well established medical treatments whose costs and efficacy are well described. Much of the recent excitement on personalized applications focus on cellular therpeutics and predictive genetics, both of which are in their relative infancies in terms of medical applications. I'll review two examples of these classes; cellular therapeutics vs orthopedic surgery for knee injury and genetic testing for ApoE in Alzheimer's disease. The issues of cost and therapeutic benefit will increasingly drive development and commercialization decisions for these personalized medical technologies.

3. Regenerative Medicine: Current Concepts and its Promise for Aging

Anthony Atala, M.D. (P)
Wake Forest University, Winston-Salem, NC

Patients with diseased or injured organs may be treated with transplanted organs. There is a severe shortage of donor organs which is worsening yearly due to the aging population. Regenerative medicine and tissue engineering apply the principles of cell transplantation, material sciences, and bioengineering to construct biological substitutes that may restore and maintain normal function in diseased and injured tissues. Stem cells may offer a potentially limitless source of cells for tissue engineering applications and are opening new options for therapy. Recent advances that have occurred in regenerative medicine will be reviewed and applications of these new technologies that may offer novel therapies for patients with end-stage tissue and organ failure will be described.

4. Late-onset Calorie Restriction: Findings and Prospects from Mouse to Man

Michael Rae (P)
SENS Foundation

Calorie restriction without malnutrition (CR) remains the best-established and most well-characterized environmental intervention for the retardation of biological aging. The unresolved issues of the range of efficacy, mechanisms, and human translatability of the "anti-aging" effects of CR are central issues in to individual practice and the development of "Calorie restriction mimetics" to harness the mechanisms CR pharmacologically for aging humans without requiring strict dietary adherence. These issues are particularly salient in the case of persons who are already entering the initial stages of age-related physical decline, where the medical and societal imperative is greatest and the evidence is sparsest. This presentation will address these questions based on animal and human CR studies, as well as the relevance and

interpretation of the epidemiological findings surrounding anthropometry and mortality risk.

5. Rapamycin as a Prolongevity and Healthspan Intervention

Zelton Dave Sharp (P), Paul Hasty, Barbara Christy, Carolina Livi, Ana Garcia, Randy Strong, Elizabeth Fernandez, Martin A Javors, Arlan Richardson

Barshop Institute for Longevity and Aging Studies, The University of Texas Health Science Center at San Antonio, San Antonio, TX

Mounting evidence indicates that the target of the rapamycin (TOR) is an important regulator of aging in unicellular organisms and metazoans. Results in Ames dwarf mice indicated that mammalian TOR complex 1 (mTORC1) is down regulated in liver and skeletal muscle. Diet restriction and growth factor restriction has been shown to increase lifespan in several animal systems. Recent results indicate a circadian periodicity in the response of mTORC1 to diet restriction of mice. These data suggested that restriction of either of these major stimuli (nutrient/energy state and/or growth factors), which are integrated by cell autonomous mTORC1 signaling, extend mammalian life span via its down regulation. This led to the proposal that long-term treatment with rapamycin, which, together with its intracellular receptor FKBP12, allosterically restricts mTORC1 function, would mimic nutrient and/or growth factor restriction for life span extension in mammals. The Intervention Testing Program (ITP) directed by Nancy Nadon at the NIA and site directors (Randy Strong, Barshop Institute, UTHSC San Antonio, Rich Miller, University of Michigan, and Dave Harrison, The Jackson Labs) rigorously tested this idea. In pilot experiments to establish a dose for long-term studies, we discovered stability issues of rapamycin in food preparations. To overcome this obstacle, Randy Strong developed a novel microencapsulation formulation of rapamycin that is stable in food, and which delivers the drug to the intestinal tract, resulting in excellent blood levels. The first cohort of genetically heterogeneous mice to receive encapsulated rapa-containing diet was 20 months of age (60 in human years) at the time they started the drug. Results from this study showed that a long-term intervention with rapamycin starting late in life significantly extended both median and maximum life span of both genders. A second cohort of mice started encapsulated rapa diet beginning at 9 months of age. Again, both genders demonstrated significant extension of median and maximum life span with additional evidence of improved healthspan. Ongoing studies of rapa-based interventions in various mouse models of age-related diseases will be discussed.

Supported by an RC2 Grand Opportunity grant (AG036613) from the NIH (ZDS, RS, and PH) and by a grant from the Glenn Foundation (ZDS).

6. Modeling Declining Physical Function and Late-Life Intervention Strategies

Christy Carter (P)

Department of Aging and Geriatric Research, University of Florida, Gainesville, FL

Age-related changes in body composition have important clinical implications given that the loss of muscle mass, gains in fat mass, and the consequent negative impact on muscle strength may be independently associated with declining performance as well as increased risk for disability and mortality in older persons. In some experimental settings, behavioral interventions, such a moderate caloric restriction and physical exercise have proven beneficial against age-associated changes in body composition and physical performance in targeted populations; however, very little is known regarding what benefit these interventions might have when initiated late in life. This is an extremely important empirical question given that these behavioral interventions are not appropriate for all older adults. Therefore, we describe several late-life interventions studies from our laboratories which directly assess the relationship between physical function, sarcopenia, and body composition. We begin with providing a conceptual framework for the ever-changing definition of sarcopenia and a rationale for the use of an appropriate rodent model of this condition which emphasizes the importance of functional outcomes in determining the efficacy of various interventions. Finally, we provide our perspective regarding the implications of this body of work and future areas of research that may also contribute to the ultimate goal of maintaining functional independence in older individuals through extending their healthspan.

7. Strategies for the Mitigation of Oxysterol-Induced Cytotoxicity

Jacques Mathieu (P) and Pedro Alvarez

Rice University, Houston, TX

7-ketocholesterol (7KC) is a cytotoxic oxysterol that plays a role in many age-related degenerative diseases. 7KC formation and accumulation may occur in the lysosome, hindering enzymatic transformations which may reduce its toxicity, and increasing the chance for lysosomal membrane permeabilization. We assayed the potential to

mitigate 7KC cytotoxicity and enhance cell viability by transiently transfecting human fibroblasts to overexpress several 7KC-active enzymes. One of our engineered constructs, a lysosomally-targeted cholesterol oxidase that lacked isomerization activity, significantly increased cell viability at concentrations up to 50 μM 7KC relative to controls. Additionally, both overexpression of the lysosomal membrane protein LAMP1 and treatment with 0.9% hydroxypropyl β-cyclodextrin attenuated 7KC-mediated cell death. The overexpression of cholesterol oxidase, sterol 27-hydroxylase, and 11β-hydroxysteroid dehydrogenase, which were localized to the cytoplasm, mitochondria, and endoplasmic reticulum respectively, resulted in either decreased cell viability or no significant change from controls. These results indicate that the lysosome should be the target for treatment of oxysterol-mediated toxicity, and support the utility of using microbial enzymes for therapeutic benefit.

8. Rejuvenation Strategies to Rebalance the Aging T-cell Repertoire

Megan J. Smithey (P)
Department of Immunobiology, College of Medicine,University of Arizona, Tucson, AZ

Immunosenescence is a poorly characterized hallmark of aging believed to underlie the increased susceptibility of aged individuals to new and emerging pathogens, and poor vaccine responses. These problems may be compounded in individuals with lifelong persistent viral infections, particularly with the Herpesvirus family members CMV and HSV. In both mouse and man, repeated interactions between these reactivating viruses and antiviral T cells leads to memory T-cell inflation (MI) with increasing accumulation of these cells over the lifespan. It is hypothesized that MI carries a price for the immune system: competition between memory and naïve T-cells for survival signals may impair the maintenance of a diverse naïve T-cell pool, consequently leaving the individual at a disadvantage when exposed to a new pathogen. We have directly tested this using a mouse model of lifelong persistent infection with either mCMV or HSV-1. Young mice were infected with either virus, and then monitored for MI until age 20 months before challenge with *Listeria monocytogenes*. Subsets of each cohort underwent therapeutic interventions including: (1) lifelong treatment with antiviral Famvir (HSV); or late-life treatment with (2) partial lymphocyte depletion, (3) Kepivance/KGF to increase thymic output, or (4) young naïve T-cell infusion in attempts to "rebalance" the naïve T-cell repertoire. Our results suggest that although lifelong persistent viral infection does not further impair the reduced bacterial clearance or magnitude of the anti-*Listeria* response in old mice, it alters the effector cytokine profiles of *Listeria*-specific T cells. Treatment with Famvir or KGF modestly restored the appropriate effector cytokine profiles. Young T-cells infused into aged recipients showed a "young" cytokine profile, indicating the aged environment can support their effector development. These data suggest that such interventions may improve the quality of immune responses in aged individuals, even in the presence of MI to lifelong persistent viral infections.

9. Dissecting the Role of Telomere Dysfunction in Hematopoietic Failure in Telomerase Deficient Mice

Zhenyu Ju (P)
Institute of Aging Research, Hangzhou Normal University College of Medicine, Hangzhou, China

A functional decline of adult stem cells could contribute to the aging process and impaired organ homeostasis and functionality. The mechanisms of stem cell aging are still poorly understood. Telomere dysfunction represent one of the molecular mechanisms limiting adult stem cell function by triggering both cell intrinsic checkpoints and cell extrinsic alterations. Telomerase deficient mouse model provides an invaluable tool to genetically dissect the in vivo consequences of telomere dysfunction on adult stem cell aging, hematopoiesis and tumour formation. We found that deletion of DNA damage checkpoints induced by telomere dysfunction can rejuvenate the aging stem cells and improve organ function in 3rd generation telomerase knockout mice (G3Terc$^{-/-}$). However, the engrafted wild-type HSCs in G3Terc$^{-/-}$ mice showed abnormal hematopoiesis, which was associated with environmental defect induced by telomere dysfunctional in a age dependent manner. This age-dependent telomere dysfunction environmental defects including both bone marrow stromal cells and altered systemic environmental factors, which is responsible for the skewed hematopoiesis in telomere dysfunctional mice. By using a genetically modified mouse model, we tested the hypothesis that whether HSC transplantation could slow down the telomere-driven aging in the setting of ameliorate environmental defects. We found that wild-type HSC engrafted in G3Terc$^{-/-}$ knockout mouse showed impaired hematopoiesis, whereas HSC engrafted in Exo-1$^{-/-}$, G3Terc$^{-/-}$ double knockout mouse showed normal hematopoiesis.

Further analysis showed an increased survival in Exo-1$^{-/-}$, G3Terc$^{-/-}$ double knockout mouse after wild-type HSC transplantation compared to those untreated mice. These data indicate that tranplantation of wild-type HSC could rescue the survival of telomere dysfunctional mice in the setting of ameliorated environmental defects.

10. Single-Cell Epigenomics in Aging

Jan Vijg, Ph.D. (P)
Albert Einstein College of Medicine

Stochastic alterations in the genome or epigenome have been implicated as a cause of aging. Especially epigenetic alterations now appear to be widespread and have been identified as a major causal factor in cancer through the inactivation of tumor suppressor genes by hypermethylation. Epigenetic information is typically collected from millions of cells, thereby precluding a more precise understanding of cell-to-cell variability and the pathogenic history of epimutations. To access putative cell-to-cell variation in the epigenome during aging we developed a procedure to analyze DNA methylation patterns in single cells or nuclei down to the basepair level using bisulfite conversion, whole genome amplification and massively parallel sequencing. This procedure made it possible, for the first time, to directly measure the rate of epimutations in organs and tissues during aging. Results will be presented for mouse liver and brain.

11. How Much Would Late-Onset Interventions in Aging Affect Demographics?

Natalia S. Gavrilova (P) and Leonid A. Gavrilova (P)
Center on Aging, NORC at the University of Chicago, 1155 East 60th Street, Chicago, Illinois, 60637, USA
Email: gavrilova@longevity-science.org

A common objection against starting a large-scale biomedical war on aging is the fear of catastrophic population consequences (overpopulation). This fear is only exacerbated by the fact that no detailed demographic projections for radical life extension scenario have been conducted so far. In this study we explore different demographic scenarios and make population projections in order to clarify what could be the demographic consequences of a successful biomedical war on aging. We specifically explored the effects of late-onset anti-aging interventions, which start at age 60 years. A general conclusion of this study is that population changes are surprisingly slow in their response to a dramatic life extension. For example, we applied the cohort-component method of population projections to 2005 Swedish population for several scenarios of life extension and a fertility schedule observed in 2005. Even for very long 100-year projection horizon, with the most radical life extension scenario (assuming rejuvenation after age 60), the total population increases by 22% only. Moreover, if some members of society reject to use new anti-aging technologies for some religious or any other reasons (inconvenience, non-compliance, fear of side effects, costs, etc.), then the total population size may even decrease over time. Thus, even in the case of the most radical life extension scenario, population growth could be relatively slow and may not necessarily lead to overpopulation. Therefore, the real concerns should be placed not on the threat of catastrophic population consequences (overpopulation), but rather on such potential obstacles to a success of biomedical war on aging, as scientific, organizational, and financial limitations.

12. Genes That Jointly Increase Longevity: What are Their Functions and Relevance to Interventions in Aging?

Svetlana V. Ukraintseva (P), Deqing Wu, Konstantin G. Arbeev, Anatoliy I. Yashin
Duke University, Durham, NC, E-mail: svo@duke.edu

Recently we have shown that a substantial portion of genetic influence on life span can be explained by joint genetic effects involving large numbers of common SNP alleles, each with small and/or low significant effect (Yashin et al. 2010). Using various statistical approaches to selecting individual pro-longevity alleles from data on genome-wide genotyping of 550K SNPs in Framingham Study participants we identified a core set of 27 SNP alleles that manifest both substantial and significant additive influence on life span, regardless of statistical method used for their selection. This suggests potentially key roles of respective SNPs for achieving longevity. The fact that majority of these SNPs (74%) are located within genes, compared to only 40% SNPs in the original 550K set, also indicates functional significance of the selected SNPs. We therefore performed extensive search of literature and online information data bases to investigate functional properties of genetic variants from the core set of 27 SNPs. We found that genes closest to these SNPs are mainly involved in cell proliferation, senescence, apoptosis, cell adhesion, and CNS information processing, which processes have relevance to both known aging pathways and common disease development, especially to cancer and brain disorders. Relevance of the identified

genes as potential targets for interventions in aging is discussed.

13. Genes and Compression of Mortality

Anatoliy I. Yashin (P), Deqing Wu, Konstantin G. Arbeev, Eric Stallard, Kenneth C. Land, Svetlana V. Ukraintseva
Duke University, Durham, NC

The compression of mortality or the process of rectangularization of survival curve has been observed in populations of developed countries in the first half of the last century. The improvements in environmental and living conditions as well as advances in health care and medical treatment are considered responsible for such trends. It turns out than dependence of survival curves on the number of longevity alleles contained in persons' genomes shows similar pattern. Using set of longevity alleles selected in genome wide association studies of the Framingham Heart Study (FHS) data we investigated possible mechanisms which could be involved in forming observed differences in age patterns of human mortality and survival. The analyses of available genetic data using modern concepts and biodemographic models of aging and longevity bring us to conclusion that differences in the number of longevity genes affect dynamics of aging related changes in resistance to stresses. We confirm this conclusion by testing differences in average age trajectories of physiological indices evaluated for sub-cohorts of the original FHS cohort having different genetic background. Respective sub-cohorts also show substantial differences in incidence rates associated with major human diseases and mortality by cause. Observed similarities in patterns of survival changes in response to radically different factors (over time in case of mortality compression, and over genetic dose in case of differences in genetic background) indicate the presence of important systemic biological mechanisms involved in life span regulation. Although these mechanisms could be different for different stimuli, their functional roles could be similar.

14. Impact of Biomedical Compression of Morbidity: Relevance of Age of Onset

Aubrey de Grey (P)
SENS Foundation

Since the term "compression of morbidity" was coined by Fries over 30 years ago, it has enjoyed immense prominence as a stated goal of biomedical gerontology. However, both its feasibility and its desirability are highly dependent on details of how it is achieved that are often neglected - a shortcoming that may have greatly diminished biogerontologists' success in attracting appropriate levels of funding, especially public funding, to their work. It may be highly valuable to distinguish various flavours of compression of morbidity and to compare and contrast them, in order to focus both research and advocacy more productively. Specifically, we must form realistic views as to how much a given amount of postponement of the onset of morbidity will also postpone the end of morbidity (i.e. death); how much the rate at which more and more postponement of morbidity is achieved impacts the demographics, i.e. what proportion of the population are in terminal decline at any given time; and most importantly of all, how much the economics of postponing aging dare affected by the dependence of age of initiation of a given intervention on its effectiveness. In this talk I will explain why these details of the "compression of morbidity" concept make such a big difference to the potential of our work to benefit humanity.

15. MicroRNAs and Aging in *C. elegans*

Frank Slack (P)
Yale University, New Haven, CT

Aging is under genetic control in *C. elegans* but the mechanisms of lifespanregulation are not completely known. MicroRNAs (miRNAs) regulate various aspects of development and metabolism and one miRNA has been previously implicated in lifespan. We showed that multiple miRNAs change expression in *C. elegans* aging, including novel miRNAs, and that mutations in several of the most up-regulated miRNAs lead to lifespan defects. Some act to promote normal lifespan and stress resistance while others inhibit these phenomena. We found that these miRNAs genetically interact with genes in the DNA damage checkpoint response pathway and in the insulin signaling pathway. Our findings reveal that miRNAs both positively and negatively influence lifespan. Since several miRNAs up-regulated during aging regulate genes in conserved pathways of aging and thereby influence lifespan in *C. elegans*, we propose that miRNAs may play important roles in stress response and aging of more complex organisms.

16. The p53-MicroRNA Connection: Clues from Cellular Senescence to Organismal Aging

Izumi Horikawa (P)
Laboratory of Human Carcinogenesis, National Cancer Institute

p53 regulates DNA repair, apoptosis, cellular senescence and many other cellular functions,

which may influence aging processes. Cellular senescence, or aging at the cellular level, may directly affect organ functions and homeostasis, and therefore aging in humans at the organismal level. Cellular senescence in stem cell populations in the elderly may result in impaired tissue regeneration and organ dysfunction. A growing number of microRNAs (miRNAs) have been identified to regulate p53 or to be regulated by p53. Investigation of these p53-related miRNAs provides evidence that the p53-miRNA connection plays key roles at the crossroad of cellular senescence, stem cell biology and human aging. For example, miR-34a, a miRNA transcriptionally activated by p53, is an endogenous regulator of replicative senescence, is down-regulated during reprogramming from somatic to stem cells, and targets SIRT1, a human homolog of yeast longevity protein Sir2. MiR-145, a miRNA post-transcriptionally activated by p53, also induces cellular senescence when overexpressed and is diminished with stem cell phenotypes. High-throughput profiling of *in vivo* miRNA expression during physiological aging (e.g., using CD8+ T lymphocyte model) identifies a further set of miRNAs to be investigated.

17. MicroRNA's in Development and Disease

Scott M. Hammond (P)
University of North Carolina

MicroRNAs (miRNAs) are short, non-coding RNAs that post-transcriptionally regulate gene expression. Over 900 miRNA genes have been identified in the human genome. We have undertaken the study of miRNA function in mammals. Using a custom microarray platform, we investigated miRNA expression patterns in mammalian development and in cancer (Thomson et al. 2004). In early development, several families of miRNA are highly expressed. This includes the oncogenic cluster miR-17-92 (He et al. 2005). Regulation of this cluster occurs largely at the level of transcription, with E2F and Myc playing a critical role (Woods et al. 2007). A second group of miRNA are not expressed in early development, but are induced strongly during mid-gestation. This includes tissue restricted miRNAs, and also widely expressed miRNAs in the Let-7 and miR-125 families. These same miRNAs are often reduced in expression in cancer. We have investigated the regulation of these miRNAs in development and in cancer (Thomson et al. 2006). Let-7 biogenesis is regulated post-transcriptionally at distinct steps. A major component of this regulation is via the RNA binding protein Lin28 (Newman et al. 2008). This protein blocks maturation of Let-7, and promotes uridylation of precursor species, leading to degradation. We have expanded on this observation. We find that Lin28 blocks Let-7 maturation in the absence of uridylation. Blockade occurs at the Drosha and Dicer steps, depending on Let-7 family member. Let-7 precursor uridylation does occur, but by two distinct mechanisms. Lin28 promotes uridylation of Let-7 precursors in embryonic cells, while a Lin28 independent mechanism leads to uridylation in myriad cell types. We have developed strategies to profile precursor sequences. We find extensive uridylation of all miRNA families in a Lin28 independent manner. We have partially mapped turnover pathways for uridylated miRNA precursors. This data paints a complex picture of miRNA regulation and turnover in mammalian cells.

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18. Epigenetic Modifiers in Stem Cells

Heidi Scrable (P)
Kogod Center on Aging, Mayo Clinic, Rochester, MN

We have discovered that p53 activity early in embryogenesis controls the level of four enzymes responsible for establishing the nascent epigenome, including DNA methyltransferases 1, 3a, and 3b and Myst2/HBO1, a major histone acetyltransferase (HAT). Although changes in epigenetic modifiers, such as DNMTs and HATs at such early stages of development would be predicted to alter the expression of many genes and could be responsible for phenotypic consequences

on many traits, we have chosen to focus on frailty as a major target. Frailty is a major clinical entity in geriatric medicine and an outcome of old age that is thought to affect virtually all people at the end of their lives. Despite the importance of this syndrome, however, there is a dearth of information about what constitutes frailty at the level of the gene, transcript, or protein.

19. Successful Aging and Sustained Good Health in the Naked Mole-Rat

Rochelle Buffenstein (P), Yael Edrey, Mario Pinto, Kaitlyn Lewis, Kelly Grimes
Barshop Institute and Department of Physiology, Univeristy of Texas Health Science Center at San Antonio, San Antonio, TX

Animals that have evolved exceptional capabilities, such as extraordinary longevity may reveal pertinent and potentially critical insights into biomedical research that are not readily apparent in standard laboratory animals. Naked mole-rats (*Heterocephalus glaber*; NMRs) are the longest-living rodents known exhibiting a maximum lifespan of ~30 years which is 5 times longer than expected on the basis of body size. These highly social mouse-sized rodents are naturally found in subterranean burrows in the arid and semiarid regions of the horn of Africa, and are commonly used in behavioral, neurological and ecophysiological research. Very old animals (>28 years), like humans, show signs of age-associated pathologies including sarcopenia, osteoarthritis and cardiac disease, however they remain cancer-free and show no signs of neurodegeneration despite measureable levels of both soluble and insoluble beta amyloid. Indeed, for at least 75% of their long-lives NMRs maintain normal activity, body composition, reproductive and physiological functions with no obvious age-related gradual increases in mortality rate. Surprisingly, even at an early age naked mole-rats in captivity appear to have unexceptional defences against oxidative stress and exhibit high levels of oxidative damage to proteins, lipids and DNA, yet nevertheless are able to abrogate the translation of this damage into age-related diseases and the concomitant decline in healthspan. Elucidating these mechanisms will provide useful information for enhancing human life- and healthspan and as such the naked mole-rat is an important "super model" for aging research and resistance to chronic age-associated diseases.

20. Role of Endocrine Hormones in Vertebrate Aging

Mary Ann Ottinger (P)
University of Maryland, College Park, MD

For both males and females, healthy aging is associated with many hormone related functions that go beyond reproduction, including receptor response and metabolic syndrome, circadian rhythms, immune response; energy, feeling of well being, and muscle structure and function. Extensive studies have characterized age-related changes in circulating hormones, physiological impacts leading to reproductive senescence and changes in metabolic endocrine function. Interesting similarities have been observed in overall patterns of change in reproductive hormones across vertebrates, including declining ovarian function, reduced hypothalamic response, and accompanying altered circulating gonadotropins. Female rodents experience a marked period of constant estrus during aging; whereas other mammals have more transient periods of unopposed estrogen due to loss of ovulation and subsequent progesterone production by the ovary. Not surprisingly, rhesus macaque females follow a similar age-related decline in ovarian function, experiencing the peri-menopausal transition and accompanying changes in circulating hormones to those in human females. In human females, the controversy surrounding hormone replacement therapy has made the issue of hormones and aging more complex. Short lived birds show a similar age-related progression in loss of ovarian function and ultimately ovarian collapse; whereas long lived birds, such as terns show little evidence of ovarian failure or changing circulating hormones. In contrast vertebrate males, including primates have a gradual age-related reproductive decline, evidenced by declining circulating androgens and in many cases, rising circulating estrogens. As aging progresses, other hormonal changes accompany altered physiological and behavioral responses. In the avian male, age-related loss in neuroendocrine and behavioral components of reproduction are restored in senescent males with exogenous steroids, providing evidence of retained neuroplasticity. These and other data will be discussed in the context comparative biology of aging and insights provided across vertebrate models.

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21. Comparative Biology of Telomeres and Their Role in Species Longevity

Woodring E. Wright(P)[1], Nuno Gomes[1], Oliver Ryder[2], Marlys Houck[2], Suellen Charter[2], and Jerry W. Shay[1]
[1]University of Texas Southwestern Medical Center, Dallas, TX and [2]Arnold and Mabel Beckman Center for Conservation Research, Escondido, CA

Humans have short telomeres, repress telomerase in most somatic cells, and use replicative aging as a brake against cancer progression while inbred laboratory rodents have extremely long telomeres, express telomerase in most dividing somatic tissues, and do not count cell divisions. We examined telomeres/telomerase in cultured cells from >60 mammalian species in order to place different uses of telomeres in a broad mammalian context. Phylogeny based statistical analysis (PLGS) was used to reconstruct ancestral states. The ancestral mammalian phenotype of short telomeres and repressed telomerase suggests the initial adaptation to the increased mutational load of homeothermy was the repression of telomerase and the consequent adoption of replicative aging. Telomere length inversely correlated with lifespan, while telomerase expression co-evolved with body size. Multiple independent times smaller, shorter-lived species changed to having long telomeres and expressing telomerase. Trade-offs involving reducing the energetic/cellular costs of specific oxidative protection mechanisms (needed to protect short telomeres in the absence of telomerase) could explain this abandonment of replicative aging. These observations provide a conceptual framework for understanding different uses of telomeres in mammals, support a role for short telomeres in allowing longer lifespans to evolve, demonstrate the need to include telomere length in the analysis of comparative studies of oxidative protection in the biology of aging, and identify which mammals can be used as appropriate model organisms for the study of the role of telomeres in human cancer and aging.

22. Animal Models, Cardiovascular Function and Aging

Zoltan Ungvari (P)
University of Oklahoma Health Sciences Center, Oklahoma City, OK

23. Road-Mapping European Aging Research: The Next Decade of EAR

Graham Pawelec (P)
Center for Medical Research, University of Tübingen Medical School, Tuebingen, Germany

The European Commission (EC) has provided dedicated funding for ageing research networks and collaborations over the last two decades. In 2009, a road-mapping program was initiated as a consultation exercise in biogerontology, in parallel with a similar program in the societal aspects of ageing research. Here, I will briefly summarize the nature of the former consultation and its outcome. The program, designated "WhyWeAge" (WWA) was coordinated by Olivier Toussaint, University of Namur, Belgium. Eleven separate workshops were organized during 2010, covering what was considered the most important research areas as follows: 1. Biomarkers of ageing and longevity; 2. Telomere biology, DNA damage, mitochondrial biology and senescence; 3. Oxidative stress, protein damage and protein maintenance; 4. Systems biology; 5. Immunosenescence and Inflammation; 6. Metabolism; 7. Nuclear receptors; 8. Vascular ageing; 9. Muscle weakness, sarcopenia and physical exercise; 10. Skin age-related modifications, elastic tissues; and skin biotechnology; 11 Clinical Biogerontological studies. Nutrition, psychosocial and other issues were recognized as important areas but were being covered by the other program. Each 2-day workshop was limited to the participation of 10 invited experts with 10 free registrations for interested scientists. At the end, each group distilled their conclusions into a brief report, which was presented at a congress of representatives of all 11 workshops in May 2010. A finalized set of recommendations was presented to the EC as guidance from "front-line" scientists regarding their ideas of the most important topics for research over the next decade at a summit conference in Brussels in December 2010. In this short talk, I will briefly summarize these conclusions and illustrate the potential for collaborative projects also with scientists outside Europe.

Supported by EC 7FP project no. 200970, "WhyWeAge—A road map for European ageing research", see http://ec.europa.eu/research/health/medical-research/human-development-and-ageing/projects/whyweage_en.html

24. Mechanisms Underlying the Altered Dendritic Cell Function in Aged Humans

Sangeetha Ulagaoozhian, Jia-Ning Cao, Sudhanshu Agrawal, Sudhir Gupta, Anshu Agrawal (P)
University of California Irvine, Irvine, CA

Dendritic cells (DC) are the major antigen presenting cells of the body that are critical for generation of immunity and maintenance of

tolerance. Advancing age has a profound affect on dendritic cell functions. DCs from aged display a higher basal level of activation and secrete pro-inflammatory cytokines even without activation leading to chronic inflammation. This also compromises the capacity of aged DCs to maintain peripheral self tolerance contributing further to inflammation. In contrast to self antigens, DCs from aged subjects are impaired in their ability to mount effective immune responses against foreign antigens such as influenza virus. There is reduced Interferon secretion as well as reduced capacity to prime T cell responses. The mechanisms underlying the altered DC function in aged humans are not well understood. We have previously shown that functions of NFkB and PI3Kinase signaling pathways are altered in aged DCs. Our more recent studies with Affymatrix gene analysis of DCs from aged and young subjects suggests that processes such as antigen presentation, ubiquitination and transcription are also significantly affected with age. Studies using chromatin-immunoprecipitation (CHIP-IP) with histone antibodies (H3K4, H3K9) suggest chromatin remodeling may also be a key player in altering the response of DCs with age. We found that there may be increased association of IFN-I and IFN-III promoters with suppressor histone, H3K9 in aged DCs. Age-associated alterations at the genetic as well as chromatin remodeling may therefore be responsible for the changes in the function of DCs from aged subjects.

25. Polygenic Influence on Life Span: Insights for Mechanisms of Aging and Disease Development

Anatoliy Yashin (P), Deqing Wu, Konstantin Arbeev, Svetlana Ukraintseva
Duke University, Durham, NC

Recent genome wide association studies (GWAS) of life span and other complex traits have shown that these traits can be affected by a large number of common SNP alleles each having small individual effect and little predictive value. Such genetic variants are often excluded from further analyses either of their connection with other phenotypic traits, or their roles in molecular biological regulation of such traits. In this paper we show that despite small individual effects of such variants their joint influence on life span can be substantial and highly statistically significant. We evaluate such influence using an index characterizing additive genetic component of life span (AGC index) using data on 550K SNPs available for the Framingham Heart Study (FHS) participants. To construct this index we use six allele selection procedures based on six different statistical models describing potential link between SNP genetic variant and life span. The overlapping set of genetic variants is used for calculating the AGC index as well as its surrogate measure counting the number of longevity variants contained in person's genome (NGV index) and evaluating joint influence of respective genetic variants on life span. The predictive power of selected genetic variants is tested using data on the offspring FHS cohort. For this purposes genetic variants selected in the original FHS cohort are used for calculating AGC and NGV indices and testing their influence on life spans of individuals from the offspring FHS cohort. We show that selected genetic variants are likely to influence organisms' resistance to stresses. We show that these variants jointly influence incidence of chronic aging related diseases, mortality rates by cause, the age trajectories of physiological indices, and that they also play important roles in molecular pathways and metabolic networks involved in regulation of human aging and disease development.

26. When Compared to the Mouse Proteasome the Naked Mole-Rat Proteasome Shows Increased Activity and Resistance to Inhibition

Karl Rodriguez (P) and Rochelle Buffenstein
University of Texas Health Science Center San Antonio, San Antonio, TX

The proteasome, the essential protease of the ubiquitin-proteasome pathway (UPP), is responsible for the controlled cleavage of short- and long-lived proteins. This multi-protein complex degrades misfolded or oxidatively damaged proteins and serves as quality control service for the cell. However, if protein degradation does not match de novo synthesis the proteasome may contribute to the metabolic slowdown observed in aging tissue. The naked-mole rat (NMR; Bathyergidae; Heterocephalus glaber), the longest-lived rodent on record, has a maximum lifespan of 32 years. Older mole-rats maintain robust proteasome activity when compared to old mice. NMRs also showed significantly lower levels of ubiquitinylated proteins than mice suggesting less damaged or misfolded proteins. We have shown using sub-fractionated (microsomal, nuclear and cytosolic) NMR liver lysates two-fold greater proteasome activity in both chymotrypsin-like (ChT-L) and trypsin-like (T-L) active centers when compared to age-typed mouse samples. In addition, cytosolic fractions from NMRs require 10 times the amount

of proteasome inhibitor (both MG132 and Adh(VS)) than do mouse fractions in order to completely ablate ChT-L and T-L activities. Analyses with native gels show subtle differences in the distribution of proteasome subassemblies between NMRs and mice, that may explain the observed 'inhibition resistance' and/or increase in activity. Characterization of the NMR proteasome could lead to important insights on how the cells in these animals handle increased stress and protein damage to maintain a longer health in their tissues and ultimately a longer life.

27. Usefulness of Preclinical Models for Assessing the Efficacy of Late-Life Interventions for Functional Decline

Christy Carter (P)[1], Drake Morgan[1], Christiaan Leeuwenburgh[2], Todd Manini[2], Thomas Foster[3], Leanne Groban[4], Philip Scarpace[5], Emanuele Marzetti[6]

[1]Department of Psychiatry, University of Florida; [2]Department of Aging and Geriatric Research, University of Florida; [3]Department of Neuroscience, University of Florida; [4]Department of Geriatrics and Gerontology, Wake Forest University; [5]Department of Pharmacology and Therapeutics, University of Florida; [6]Department of Orthopedics and Traumatology, Catholic University

Caloric restriction and physical exercise have proven beneficial against age-associated changes in body composition and declining physical performance; however, little is known regarding the functional benefit these interventions might have when initiated late in life. Pharmacological mimetics of diet and exercise may create synergy when partnered with these behavioral approaches and may be most effective in vulnerable aged populations (obese, frail, arthritic, etc) which are most challenged to engage in these activities. We have focused on the use of the angiotensin converting enzyme inhibitor (ACEi) enalapril as a late-life intervention designed to mitigate sarcopenia and changes in body composition. Beyond its impact on hemodynamic functioning, enalapril has been shown to modulate an amalgam of biological processes inherent to overall metabolic function. In addition observational studies in humans and converging evidence from our laboratory suggest that the use of ACEi's attenuate age-related increases in whole-body adiposity and physical performance decline, in the absence of any sizeable muscle hypertrophic effect. Indeed, these data lend credence to the hypothesis that it is not necessary to optimize the quantity of muscle that is preserved with an intervention but that it is the quality of muscle which determines functionality. This benefit is not isolated to skeletal muscle in that enalapril treatment mitigates dysfunction in other organ systems including kidney and heart. Furthermore, similar effects of ACEis on body composition in various strains of rats and mice, across different ages, and under normal and high-fat feeding scenarios has been observed. Therefore, preclinical intervention models, especially those for which function is a primary outcome, represent a critical translational link for the more rapid translation of treatments to the clinical arena insofar as they may serve as a tool for the relatively rapid systematic assessment of traditional and non-traditional interventions initiated late in life.

28. Developing Healthspan Assays in C57BL/6 Mice

Lauren Sloane (P), Vanessa Soto, Michael Walsh, Keith Maslin,Quixia Dai, Merry Lindsey, Holly Van Remmen, Arlan Richardson, Steven Austad

Barshop Institute University of Texas Health Science Center San Antonio, San Antonio, TX

Measures of healthspan offer a more unified index of overall aging than measuring lifespan or longevity alone, as healthspan includes assays on a range of functional systems within the individual. The objectives of this study were to identify robust and reproducible functional measures of healthspan, and to determine the inter-relationship of these measures. The study uses 16 different assays (e.g., metabolic rate, gait analysis, nerve conduction & cardiac function) to evaluate overall healthspan in C57BL/6 male mice of 4 different ages (4, 20, 28, and 32 months of age). Many of these assays show significant consistent declines with age. By identifying age-related changes in these functional patterns we can begin to develop biomarkers of aging that will help both research scientists and clinicians identify relevant patterns of aging. For example, geriatric frailty involves the inter-relationship of multiple variables—involuntary weight loss, exhaustion, lack of spontaneous activity, slow walking speed and reduced grip strength. By measuring these variables in aging rodents we can develop models that explore the physiology and etiology of such complex phenomena and provide critical insights into the mechanisms associated with age-related decline.

29. Depletion of mTOR and mLST8 uncouples longevity from rapamycin-induced changes in glucose homeostasis

Dudley Lamming[1], Lan Ye[2], Pekka Katajisto[1], Marcus Goncalves[2], Maki Saitoh[1], Deanna

Stevens[1], James Davis[2], Adam Salmon[3], Arlan Richardson[3], Rexford Ahima[2], David Guertin[1], David Sabatini[1], Joseph Baur[2]
[1]Whitehead Institute; [2]University of Pennsylvania School of Medicine; [3]University of Texas Health Science Center at San Antonio, San Antonio, TX

Rapamycin, an inhibitor of mechanistic target of rapamycin complex 1 (mTORC1), extends the lifespan of yeast, flies, and mice through mechanisms that remain unclear. Improved glucose homeostasis is considered a key feature of interventions that extend life in mammals, including caloric restriction, which has been proposed to work via mTORC1 and thus may share a common mechanism with rapamycin. However, we find that mice chronically treated with rapamycin have substantially impaired glucose tolerance and are insulin resistant. We demonstrate that chronic treatment with rapamycin disrupts a second mTOR complex, mTORC2, in vitro and in vivo, and that this complex is required for the suppression of hepatic gluconeogenesis. Further, disruption of glucose homeostasis is separable from mTOR-mediated lifespan extension, as female mice heterozygous for both mTOR and mLST8 exhibit decreased mTORC1 activity and extended lifespan, but have normal glucose tolerance and insulin sensitivity. These results indicate that attenuation of mTORC1 signaling is sufficient to extend lifespan independently from changes in glucose homeostasis, and moreover, that rapamycin extends lifespan despite impairing insulin action. Our data further reveal that mTORC2 disruption is an important mediator of rapamycin's effects in vivo.

30. Cardiovascular Function and Aging in a Non-human Primate Model

Thomas Register (P), Dalane Kitzman, Leanne Groban1, Craig Hamilton, Greg Hundley, Jay Kaplan, Carol Shively, Kevin High, John Stehle, Thomas Clarkson, David Herrington, Matt Jorgenson, J Carr
Wake Forest University School of Medicine, Winston-Salem, NC

Non-human primates provide excellent models for the study of inter-relationships between complex phenotypes and age related diseases such as cardiovascular disease. The Vervet Research Colony (VRC) is a population of African green monkeys (Chlorocebus aethiops sabaeus) with a current census of over 400 individuals ranging in age from infant to over 25 years of age. Among the nonhuman primates (NHPs), the vervet has become a major biomedical research model, and proven useful for the creation of widely-used cell lines, vaccine testing, and the investigation of behavior, body composition, physical function, and cardio-metabolic phenotypes. The Vervet Genome Sequencing Project (VGSP) of the National Human Genome Research Institute (NHGRI) will sequence genomes of numerous unrelated vervets, leading to the development of most comprehensive single nucleotide polymorphism (SNP) resource available for any NHP, enabling the scientific community to conduct genetic studies of age-related phenotypes with a power and precision which will profoundly shape scientific knowledge in the future.

In the present work, we assessed cardiovascular characteristics in young adult (8-10 years, comparable to ~30 year old people) and older (age 21-26 years, comparable to 65-80 year old people) female vervets which had consumed a low fat "chow" based diet over their lifetime. Cardiac phenotypes assessed by quantitative echocardiography and cardiac magnetic resonance imaging (CMRI) revealed that three out of five of the aged (and none of the young adult) VRC females had significant left ventricular hypertrophy (LVH), one of these showed signs of aortic insufficiency, one showed tricuspid regurgitation, another displayed a sinus arrhythmia on EKG. These results demonstrate that the vervets developed age-related cardiopathologies despite consuming a healthy low fat diet. Comprehensive assessment of cardiovascular and other phenotypes combined with well-defined life histories and genetic information will provide important insights into the genetic and environmental determinants relevant to cardiovascular aging.

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31. Differential Cellular Protection and Sensitization in Cancer Treatment

Valter Longo (P)
Univeristy of Southern California, Los Angeles, CA

The dietary recommendation for cancer patients receiving chemotherapy, as described by the American Cancer Society, is to increase calorie and protein intake. Yet, in simple organisms, mice and humans, fasting induces a wide range of changes that promote cellular protection, which would be difficult to achieve even with pharmacological interventions. In mammals, the protective effect of fasting is mediated, in part, by an over 50%

reduction in glucose and insulin-like growth factor 1 (IGF-I) levels. Because proto-oncogenes function as key negative regulators of the protective changes induced by fasting, cells expressing oncogenes, and therefore the great majority of cancer cells, are predicted to not respond to the protective signals generated by fasting, promoting the differential protection (Differential Stress Resistance) of normal and cancer cells from a variety of chemotherapy drugs. Preliminary reports indicate that fasting for up to 5 days followed by a normal diet, can also protect patients against chemotherapy without causing chronic weight loss or other detrimental side effects. By contrast, the long-term 20 to 40% restriction in calorie intake (dietary restriction, DR), whose effects on cancer progression have been studied extensively for many decades, requires weeks–months to be effective, causes much more modest changes in glucose and/or IGF-I levels, and promotes chronic weight loss in both rodents and humans, which prevent its clinical applications. I will discuss our basic as well as clinical studies on fasting, cellular protection and chemotherapy. Although additional pre-clinical and clinical studies are necessary, fasting has the potential to be translated into effective clinical interventions for the protection of patients and the improvement of therapeutic index.

32. Mechanisms Through which TORC1 Signaling and Rapamycin Influence *C. elegans* Longevity

Stacey Robida-Stubbs, Kira Glover-Cutter, Prashant Raghavan, Masaki Mizunuma, Theresa Operaña, T. Keith Blackwell (P)
Joslin Diabetes Center, Harvard Stem Cell Institute, and Department of Pathology, Harvard Medical School, Boston, MA

The TOR (target of rapamycin) kinase is central to growth regulation, and influences aging in diverse species. In the context of the TORC1 complex, TOR promotes protein synthesis in response to nutrient and growth signals. Less is known about the related complex TORC2. TORC1 signaling has been implicated in effects of dietary restriction on longevity, and inhibition of TOR through genetics or treatment with rapamycin, a clinically used TORC1 inhibitor, extends lifespan from yeast to mice. It is a central challenge in the aging and growth signaling fields to understand how TOR influences longevity and resistance to stresses.

Previous work in *C. elegans* indicated that reduction of TOR activity extends lifespan independently of DAF-16/FOXO, suggesting that TOR and insulin/IGF-1 signaling (IIS) influence longevity through independent mechanisms. Here we show that TORC1 inhibition and rapamycin increase longevity and stress resistance by acting through the SKN-1/Nrf and DAF-16/FOXO transcription factors. Adulthood knockdown of individual TORC1 complex genes provides longevity that requires both *skn-1* and *daf-16*. In contrast, Rapamycin confers *skn-1*-dependent, *daf-16*-independent longevity. This appears to involve inhibition of both TORC1 and TORC2, because TORC2 inhibition confers longevity that does not require *daf-16*. Longevity deriving from TORC1 inhibition is independent of the germline longevity pathway, which we show also inhibits SKN-1 as well as DAF-16. Importantly, TORC1 inhibition and rapamycin each direct SKN-1 and DAF-16 to induce transcription of protective genes. Our results indicate that the IIS, TOR, and germline pathways each influence aging by acting through distinct mechanisms to inhibit SKN-1 and DAF-16. The data have important implications for understanding effects of TOR-based therapies, and relationships between nutrient availability, growth regulation, and aging.

33. Activation of the amino acid starvation response in stress resistance and longevity

Jay Mitchell (P)
Department of Genetics & Complex Diseases, Harvard School of Public Health, Boston, MA

Dietary restriction (DR) extends lifespan and increases stress resistance in most organisms tested. In mammals, the underlying nutritional triggers and genetic requirements of these benefits remain elusive. Previously we reported that short-term DR as well as and fasting increase resistance to the acute stress of renal ischemia reperfusion injury, a phenomenon we call dietary preconditioning. Here we examined the nutritional and genetic basis of dietary preconditioning. We found that total protein deprivation for 6-14 days in the absence of reduced calorie intake led to increased resistance to renal ischemia reperfusion injury. Deprivation of individual essential amino acids including tryptophan, methionine and leucine, as well as pharmacological activation of the amino acid starvation response with halofuginone, also improved outcome following ischemic kidney injury. The role of amino acid sensing in stress resistance was tested using mice deficient in a key mediator of the amino acid starvation response, Gcn2. Gcn2 is activated by uncharged tRNAs and can thus in principle detect deficiency in any single amino acid. Consistent with a role for amino acid sensing in modulation of stress resistance, the benefits of tryptophan

deficiency and halofuginone were lost in Gcn2 knockout mice. Together, these data point to amino acid sensing as a key modulator of acute stress resistance by dietary preconditioning with implications for practical applications in the clinic and our mechanistic understanding of extended longevity by amino acid restriction.

34. Stress Resistance by NRF2: Its Role in Calorie Restriction and Aging

Rafael de Cabo (P)
National Institute on Aging, Baltimore, MD

Caloric restriction (CR) is the most potent intervention known to both protect against carcinogenesis and extend lifespan in laboratory animals. Acting through the antioxidant response element, a variety of anti-carcinogens and CR mimetics induce and activate the NF-E2-related factor 2 (Nrf2) pathway. Nrf2 is a transcription factor that serves as cellular sensor of oxidative and electrophilic stress generated from endogenous reactions and exogenous agents, and is one of the main regulators of intracellular redox balance. Nrf2 offers a promising target for anti-carcinogenesis and anti-aging interventions. We used Nrf2-disrupted mice to examine its role on the biological effects of CR. Here we show that Nrf2 is responsible for most of the anti-carcinogenic effects of CR, but is dispensable for increased insulin sensitivity and lifespan extension. Nrf2 deficient mice developed tumors more readily in response to carcinogen exposure than did wild-type mice, and CR was ineffective in suppressing tumors in the Nrf2-deficient mice. Further, we investigated the role of Nrf2 on cellular immortalization and lifespan of murine embryonic fibroblasts (MEFs). Genetic deletion of Nrf2 leads to MEFs immortalization due to an early loss of p53-dependent gene expression. When compared with their Wt counterparts, immortalized Nrf2-/- MEFs exhibited decreased growth rates and impaired expression of NQO1. SirT1 levels were also significantly reduced in Nrf2-/- MEFs and these cells exhibited shorter lifespan. Our results underscore the importance of Nrf2 in cellular and organismal aging processes.

35. Muscling into Translational Research: From Mighty Mice to Men

Nathan K. LeBrasseur, PT, PhD (P)
Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester, MN

The loss of skeletal muscle is one of the most dramatic changes in the human body consequent to advancing age. An excessive reduction in skeletal muscle mass is now commonly referred to as *sarcopenia*. Sarcopenia has emerged as a critical mediator of age-related changes in muscle performance, functional status and metabolic homeostasis. In recent years, we have focused on quantifying these outcomes in mice and humans to foster translational, bench-to-bedside, research. The purpose of this seminar is to discuss the application of these methods in studies of mouse models of aging and age-related diseases, and clinical trials of older individuals. Specifically, their utility in assessing the therapeutic potential of myostatin inhibition for improving muscle mass, physical function and whole-body metabolism will be presented.

36. Biology of Frailty and Late Life Decline

Jeremy Walston (P)
Johns Hopkins University, Baltimore, MD

37. Low Hanging Fruit: Early Opportunities to Translate Advances from the Basic Biology of Aging into Clinical Application

James L. Kirkland (P)
Mayo Clinic, Rochester, MN

The aging field has moved from a descriptive/discovery phase through delineation of mechanisms into developing interventions that enhance survival. Whether these interventions enhance healthspan needs to be determined. Some of them are close to being ready for clinical application. Translation is an iterative process requiring partnership between basic and clinical investigators. Attention to regulatory steps and intellectual property protection are necessary for commercialization. Effects of interventions ideally need to be detectable within a short time frame using clear, validated, clinically-relevant outcomes that are acceptable to the FDA in subjects who are already symptomatic. Parallel outcomes that reliably capture age-related changes across major physiological and functional domains need to be devised for the experimental animal models used in preclinical studies. An important issue is whether long term interventions are practical in humans. Lifestyle interventions are particularly challenging. For example, it is unlikely that lifelong caloric restriction would be widely adopted, particularly in the face of an obesity epidemic. Interventions will need to have few or no side effects to be acceptable for use in younger, asymptomatic individuals with the expectation of a much later impact on survival. Interventions that can be initiated in later life and that are potentially effective in subjects already afflicted by frailty or multiple age-related conditions are more likely to succeed. Perhaps

interventions effective over the long term in experimental animals (e.g., caloric restriction, sirtuin agonists, or IGF-1, mTOR, or protein aggregation inhibitors) might have beneficial short term effects in humans. Indeed, short term caloric restriction favorably influences predisposition to age-related diseases and possibly, tolerability of chemotherapy in humans. Studies of interventions effective in increasing lifespan in animals can also be "piggybacked" onto studies for other indications in humans (e.g., cardiovascular or metabolic disease). Frailty is a particularly attractive indication. "Frailty" denotes the most vulnerable subset of older individuals. It becomes evident over time as increased vulnerability to physiological stress, with reduced ability to maintain or regain homeostasis after a destabilizing event, such as an infection, injury, or surgery. Clinicians have developed scales to diagnose frailty. These include combinations of weakness, fatigue, weight loss, decreased physical activity, impaired motor performance, immobility, imbalance, social withdrawal, cognitive dysfunction, altered body composition, and vulnerability to physiological stress. Subjects meeting clinical frailty criteria generally have increased inflammatory biomarkers, impaired glucose metabolism, increased clotting, falls, disability, hospitalization, and mortality. Mechanisms of frailty appear to be related to those involved in lifespan. Because frailty is associated with high mortality, interventional studies to ameliorate it are more likely to receive IRB approval than studies in healthy subjects. Frailty-related clinical trial outcomes that might be impacted by interventions that extend lifespan or reduce dysfunction associated with aging include: chemotherapy side effects, chemotherapy therapeutic index, recovery after elective surgery (hip, prostate), success of bone marrow transplantation, surgical wound healing, or hospital readmission. Where interventions based on longevity outcomes in experimental animals have short term clinical indications, such as sirtuin agonists in treating diabetes in obese individuals, effects on lifespan might eventually become apparent in post-marketing analyses. We need to take the scientific, training, and organizational steps to orchestrate efforts to translate findings into clinical application. Training and career-long funding mechanisms for geriatricians in basic science research and for basic scientists in geriatric issues are needed. While we must find the resources to do so, we need to continue, indeed increase funding for descriptive/high throughput/discovery and mechanistic basic aging research using cell and experimental animal model systems, and not shift funds from these essential areas into supporting translation. We need to expand the overall funding envelope for aging research, particularly since we could be poised to delay age-related chronic diseases, frailty, and disabilities as a group.

38. Inflammaging and Cellular Senescence Prime the Lungs for Bacterial Pneumonia

Pooja Shivshankar[1], Angela Rodriguez Boyd[1], Claude Jourdan Le Saux[2], I-Tien Yeh[3], and Carlos J. Orihuela(P)[1]

[1]Department of Microbiology and Immunology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas, 78229 USA. [2]Department of Medicine, Division of Cardiology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas, 78229 USA. [3]Department of Pathology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX

Cellular senescence is an age-associated phenomenon that promotes tumor invasiveness due to the secretion of pro-inflammatory cytokines, proteases, and growth factors. Herein we show that cellular senescence also increases susceptibility to bacterial pneumonia caused by *Streptococcus pneumoniae*, the leading cause of infectious death in the elderly. Aged mice had increased lung inflammation as determined by cytokine analysis and histopathology of lung sections. Using BrdU/PI labeling and immunoblotting for p16, pRb, and mH2A we found that aged humans and mice have increased levels of these senescence markers and a greater number of cells in G0/G1 and G2/M phase in the lungs versus young counterparts, respectively. *In vitro* we determined that senescent lung cells expressed elevated levels of the pneumococcal ligands Keratin 10 (K10), Laminin Receptor (LR), and Platelet activating factor receptor (PAFr) and were up to 5-fold more permissive for bacterial adhesion. Aged mice also had increased levels of these ligands and were highly susceptible to pneumococcal challenge in a bacterial adhesin-dependent manner. Exposure of normal cells to conditioned media from senescent lung cells increased PAFr levels and their permissiveness for bacterial attachment. Finally, genotoxic stress induced by bleomycin and oxidative stress enhanced susceptibility of young mice to pneumonia and was positively correlated with enhanced p16, inflammation, and LR and PAFr levels. These findings suggest that cellular senescence primes the lungs for bacterial adhesion and helps to explain the increased incidence of community-acquired pneumonia in the elderly.

This study is the first to report a second negative consequence for SASP.

39. Effects of Hydrogen Sulfide on Protein Homeostasis and Aging

Dana L. Miller (P)
University of Washington, Seattle, WA

Hydrogen sulfide (H_2S) increases lifespan and thermotolerance in *C. elegans*, and can protect against the effects of hypoxia and ischemia/reperfusion injury in mammals. We have found that H_2S also improves the ability to maintain protein homeostasis. We are investigating the effects of adaptation to H_2S on different aspects of protein metabolism. We have found that global translation rate is not decreased upon adaptation to H_2S. In addition, our data suggests that two conserved longevity factors, HIF-1 and SKN-1, coordinate a broad transcriptional response to H_2S that results in the up-regulation of many gene products involved in protein turnover. Interestingly, we have recently found that *hif-1* expression in neurons is sufficient to *C. elegans* to survive exposure to H_2S, suggesting the possibility that the effects of H_2S are centrally regulated. Together, our data support a model in which H_2S influences longevity assurance mechanisms that mediate protein homeostasis.

40. Genetic Resource Populations for the Laboratory Mouse

Gary A. Churchill (P)
The Jackson Laboratory, Bar Harbor, ME

Genome-wide association studies in human populations have raised the bar for genetic mapping and analysis of Mendelian and complex traits. However, the genetic structure of human populations, uncontrolled environmental variables, and limitations on experimental interventions and phenotyping present significant barriers to investigations of biological processes in humans. If we could design an ideal model system for genetic studies, what properties would it have? High genetic diversity is desirable to enable the broadest possible scope of discovery. High mapping resolution is needed to identify causal genes with confidence and precision. Absence of population structure and rare alleles combined with full genomic sequences of ancestral haplotypes will substantially improve power and reduce required sample sizes. A good model system does not need to have a natural population structure but the evolutionary origin and context in which segregating variants arose should be understood. The ability to reproduce genotypes leads us to consider inbred models, but natural heterozygosity is also desirable. In depth phenotyping tools, a high-density genotyping platform, and methods to work with transgenic constructs are essential. An experimental system should allow for both discovery and validation. I will describe how the Collaborative Cross and Diversity Outbred mouse populations together fulfill these criteria and provide an ideal system for genetic analysis in a mammalian model organism.

41. Neurosensory Modulation of Physiology and Aging in Drosophila

Scott Pletcher (P)
University of Michigan, Ann Arbor, MI

Evidence from work in the nematode, *Caenorhabditis elegans*, and from our work in the fruit fly, *Drosophila melanogaster*, has established that aging is strongly modulated by sensory systems and that this modulation is evolutionarily conserved. Ablation of specific sensory neurons in the worm increases lifespan, as do mutations in genes required for sensory signal transduction. We have shown that exposure of *Drosophila* to food-based odorants is sufficient to reduce lifespan and limit the beneficial effects of dietary restriction. Here I will discuss new efforts that address the specific sensory stimuli and neural circuits that transduce sensory information that is relevant for aging and other health-related phenotypes in flies. I will describe new olfactory and gustatory manipulations that influence the lifespan of the fly, and I will describe our understanding of the molecular mechanisms that underlie a novel diet- and sensory-related phenotype, DR-induced sleep fragmentation.

42. Unraveling the IGF-1 Paradox of Aging: New Strategies for 'Humanizing' the IGF-1 Axis in Rodents

Derek M. Huffman, PhD (P)
Department of Medicine, Division of Endocrinology, Institute for Aging Research and The Einstein Nathan Shock Center of Excellence in the Biology of Aging, Albert Einstein College of Medicine, Bronx, NY

Evidence from across species consistently show that diminished growth hormone/ insulin–like growth factor-1 (GH/IGF-1) signaling extends lifespan. Likewise, in humans, low IGF-1 in the periphery is associated with reduced risk for most cancers and we have shown that a functional mutation in the IGF-1 receptor, linked to dampened IGF-1 signaling, is enriched in centenarians. In contrast, low peripheral IGF-1 levels in humans are paradoxically linked with increased risk for

numerous other diseases, including cardiovascular disease, type 2 diabetes and cognitive decline, suggesting that findings from model systems may not be entirely relevant to humans. To further address the complexity of this axis, we have recently uncovered novel actions of IGF-1 signaling when targeted to the brain with potentially beneficial implications for human aging. Indeed, when we raise central IGF-1 levels either acutely or chronically in young and old male Sprague-Dawley rats, we observe an improvement in peripheral insulin sensitivity and a reduction in visceral fat mass. We are now working toward identifying the important site(s) of IGF-1 action in mediating these effects as well as its universality in other models. In summary, taking into account its distinct sites of action and consequences, we believe that IGF-1 signaling should be viewed as a balance between its central and peripheral effects in order to make our understanding of this axis more relevant to human aging. Furthermore, our current working model proposes that strategies designed to shift the balance of IGF-1 action from peripheral to central will maximize the *'good'* effects of IGF-1 while avoiding its *'bad'* effects on cancer risk in the periphery to promote healthy aging and longevity.

43. Estrogen receptor-mediated manipulation of wound healing in the elderly

Elaine Emmerson (P), Laura Campbell, Matthew Hardman

The University of Manchester, Manchester, United Kingdom

Human skin undergoes structural changes with age, becoming thinner, fragile and more susceptible to trauma. Cutaneous wounds take much longer to heal with advancing age. Rapid expansion of the elderly population has lead to increased incidence of non-healing wounds, associated with substantial cost to the NHS and pronounced patient morbidity. While chronological age is a risk factor for delayed healing our recent work implicates estrogen decline, rather than intrinsic ageing per se, as a critical regulator of delayed healing in elderly subjects. With increasing age systemic estrogen levels fall, in both males and females, and the rate of healing declines. Estrogen replacement (HRT) can reverse this delay, but unfortunately long term HRT increases breast cancer risk, such that steroidal estrogen is now listed as a carcinogen. Our recent work has begun to functionally dissect the role of estrogen signalling during repair at the molecular, cellular and physiological levels. Our data have shown estrogen to be a pleiotropic regulator of wound healing, influencing multiple wound cell types. Subsequent studies, combining pharmacological manipulation and genetic ablation, have revealed novel diametrically opposed roles for the two estrogen receptor isoforms, ERα and ERß, during healing. We have exploited this to demonstrate the in vivo therapeutic potential of compounds with receptor selective agonistic/antagonistic activity. We are currently translating this research into human studies with the ultimate aim of developing targeted therapeutics for the treatment of delayed acute and chronic wounds in the elderly.

44. Is Lifespan in a Long-Lived Mutant Mouse Strain limited by Cell Senescence?

Dr Angela Sheerin(P)[1]; Professor Richard Faragher[1]; Professor Dame Linda Partridge[2]; Professor David Kipling[3]; Professor Dominic Withers[4].

[1]Centre for Biomedical & Health Science Research (CBHSR), School of Pharmacy & Biomolecular Sciences, Huxley Building, University of Brighton, Brighton, East Sussex; [2]Centre for Research on Ageing, Department of Biology, University College London, London; [3]Department of Pathology, School of Medicine, Cardiff University, Cardiff ; [4]Centre for Diabetes and Endocrinology, University College London, Rayne, Institute, London, UK.

The primary goal of ageing research is to promote a healthy and independent old age. The mechanisms controlling the rate of aging in mammals remain poorly understood but there is strong evidence that the insulin/insulin-like growth factor signalling pathway (IIS) is a key regulator of lifespan in many different species. The purpose of this research is to determine whether mutations in the IIS extend healthy mouse lifespan by reducing the rate of accumulation of senescent cells. The cell senescence hypothesis of ageing proposes that the progressive accumulation of senescent cells contributes to, but does not exclusively cause, the ageing of tissue in which they reside. Despite the intense interest in how mutations in IIS affect lifespan no studies have as yet been undertaken to determine the frequency of senescent cells in key tissues.

In vitro analysis for markers of cellular senescence, using the senescence associated β galactosidase (SA-β gal) assay and $p16^{INK4a}$, was investigated in frozen sections of lung, liver and kidney tissue for both wild type and $Irs1^{-/-}$mutants (80-90 versus 700days old). Using co-localisation of 53BP and telomere (TIF localisation assay), as another marker of senescence, analysis was carried out using frozen sections of lung tissue from wild type

and *Irs1*$^{-/-}$mutants at 700days old. Preliminary data emerging from these studies shows interesting differences between wild type and *Irs1*$^{-/-}$mutants between 80-90 versus 700 days old.

We can provisionally conclude that the data is suggestive that senescent cells accumulate at a reduced rate in three different tissues from long-lived animals compared to their controls. This suggests that the long-lived animals act through a mechanism that reduces the number of senescent cells.

45. B Cell Repertoire in Ageing

Deborah K. Dunn-Walters (P)
Peter Gorer Department of Immunobiology, King's College London School of Medicine, London, UK.

Older people are particularly susceptible to morbidity and mortality from infectious diseases, especially from pulmonary diseases such as pneumococcal pneumonia. The pneumococcal polysaccharide vaccine (PPV23) has a reduced efficacy in older adults but the reasons for this are not well understood. B cell repertoire in the old is reduced, and this is correlated with frailty. An investigation of the molecular and antibody responses to winter vaccination was undertaken to determine whether a reduced B cell repertoire diversity in old age could be the cause of impaired humoral responses. B cell CDR3 repertoire was assessed in an isotype-specific manner by spectratyping of all samples, and by high throughput sequencing in a limited number of samples, alongside ELISA measurements of pneumococcal-specific serum antibody. Serum IgM and IgA pneumococcal responses are significantly impaired in older people, with no difference in IgG levels. B cell spectratype analysis showed a clear change in the repertoire at day 7 after vaccination, with a return to the baseline levels at day 28. The changes at day 7 reflected expansion of *IGH* sequences that have smaller, more hydrophilic, CDR3 regions and these changes are reduced in older people. The old are also more likely to have spectratypes indicating a reduced diversity at day 0 and day 28. In conclusion, IgA and IgM responses are significantly impaired in the elderly pneumococcal response and are likely key mediators of protection. Hydrophobicity and/or small size of the *IGH* CDR3 appear to be important in these responses. The *IGHM* spectratype analysis seems to be the most promising in terms of its predictive ability for vaccine responses, although there are likely many factors other than a diminished B cell repertoire that are also functionally relevant in the senescent humoral immune response.

This work was supported by Research into Ageing

46. Age Related Changes to Ca^{2+} - K_{Ca} Signalling Within Neurones

Gregg Scutt (P)
University of Brighton, Brighton, East Sussex, UK

The central nervous system (CNS) of the common pond snail *Lymnaea stagnalis* is a useful model for studying the effects of age on the biophysical properties of identified neurones. Contained within the cerebral ganglia of the CNS are a pair of electrically coupled, serotonergic neurones, which modulate the feeding circuitry of the animal, and are involved in learning and memory. As the CGCs age they undergo characteristic age-related changes, similar to those observed in mammalian hippocampal neurones. These include an increase in the duration of the slow afterhyperpolarisation (sAHP), and a reduction in firing frequency. We have evidence to suggest that an increased Ca^{2+}-activated K^+ current (K_{Ca}) may be responsible for these changes. Further to this, we have recently found that the strength (f_{adapt}) and speed (τ_{adapt}) of Spike Frequency Adaptation (SFA), a process usually controlled by K_{Ca}, is also altered with advancing age (during a 5 s current injection at 10, 20 and 30 nA: f_{adapt} 71.5, 74.9, 75.9% vs 78.3, 82.4, 83.3% and τ_{adapt} 556, 752, 805 ms vs 659, 878, 923 ms, young and old respectively, $p<0.05$). Interestingly, the age-related changes to SFA and the post current injection sAHP can be reversed by blockers of Ca^{2+} release from internal stores. This suggests that during ageing, there is an increased contribution to K_{Ca} activation from Ca^{2+} originating from internal stores. An estimation of the time constant for Ca^{2+} decay (τ_{Ca}) following artificial depolarisation can be made from the ratio $\tau_{adapt}/(1-f_{adapt})$. We find this to increase significantly with age suggesting either an age-related change in Ca^{2+} buffering, or a prolonged increase in intracellular Ca^{2+} from internal or external sources (during a 5 s nA current injection at 10, 20 and 30: τ_{Ca} 1979, 3086, 3454 ms vs 3887, 5383, 5678 ms, in young and old respectively, $p<0.05$).

47. Aging of the Mammalian Gastrointestinal System

M.Jill Saffrey PhD (P)
Department of Life Sciences, Open University, Milton Keynes, UK

Gastrointestinal (GI) dysfunction is a common and distressing cause of morbidity in the aging

population. Constipation and incontinence involve dysfunction of the large bowel, while efficient nutrient absorption and appetite may be affected by changes in the stomach and small intestine. Understanding the causes of physiological changes in the GI system requires analysis of cellular and molecular changes in the aging gut. Such analysis is challenging, however, because of the complex mixed cellular composition of the system, which includes smooth muscle, different types of epithelial, interstitial and connective tissue cells as well as neurons and glia. The intestine has a highly complex intrinsic nervous system, the enteric nervous system, which contains some 20 different types of neuron. Subpopulations of these neurons are vulnerable to age-related degenerative changes and significant numbers of enteric neurons are lost both in aged animals and humans. The nature of enteric neuronal damage and mechanisms of neuronal loss have not been fully characterised. Importantly, the functional correlates of neuronal changes and the interrelationships of cellular and molecular changes between neurons and their effector cells have also not been studied in detail. We are adopting a multidisciplinary approach using the mouse to investigate the nature of the cellular and molecular changes in the neuromuscular system and the mucosal epithelium of the aging GI system and analysing how these changes impact upon specific physiological functions.

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48. Characterization of the Impact of Life Stage on Gene - Chemical Interactions in the Liver

J. Chris Corton (P)
Integrated Systems Toxicology Division, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC

Differences in responses to environmental chemicals and drugs between life stages are likely due in part to differences in the expression of xenobiotic metabolizing enzymes and transporters (XMETs). We have carried out a comprehensive analysis of the mRNA expression of XMETs through life stages in mice and humans. Using full-genome arrays, the mRNA expression of all XMETs and their regulatory proteins was examined during fetal (gestation day (GD) 19), neonatal (postnatal day (PND) 7), prepubescent (PND32), middle age (12 months), and old age (18 and 24 months) in the male C57BL/6J (C57) mouse liver and compared to young adults (2-6 months). Fetal and neonatal life stages exhibited dramatic differences in XME mRNA expression compared to the relatively minor effects of old age. The number of XMETs that differed from adults was 597, 467, 77, 5, 42, and 93 for GD19, PND7, PND32, 12 months, 18 months and 24 months, respectively. At all life stages except PND32, under-expressed genes outnumbered over-expressed genes. The altered XMETs included those in all of the major metabolic and transport phases including introduction of reactive or polar groups (Phase I), conjugation (Phase II) and excretion (Phase III). In the fetus and neonate, parallel increases in expression were noted in the dioxin receptor, Nrf2 components and their regulated genes while nuclear receptors and regulated genes were generally down-regulated. Suppression of male-specific XMETs was observed at early (GD19, PND7) and to a lesser extent, later life stages (18 and 24 months). To determine if aged humans exhibit changes in XMET expression, we examined gene expression profiles from young (21-45 years) and old (69+ years) men and women. Five to seven livers per age group were profiled. Compared to mice, there were relatively few consistent changes in gene expression in the livers from aged humans compared to younger humans. We identified 370 genes that were altered between young and old men and 1163 genes that were altered between young and old women. Top canonical pathways were identified using Ingenuity® Pathway Analysis. The protein ubiquitination pathway is affected in both older men and women. We found that age caused minimal numbers of changes in the gene expression of XMETs (8 in males and 33 in females between young and old). Most of these changes were in the expression of Phase III genes. The expression of solute carriers increased with age in men while the majority decreased with age in women. These studies indicate that the livers from aging humans exhibit a number of changes in XMEs that may lead to differences in the metabolism of xenobiotics. We also identified chemicals that may be differentially metabolized at different life stages due to the changes in the XMETs. Chemicals were identified using the Comparative Toxicogenomics Database (CTD) in which gene-chemical interactions of interest can be identified and grouped into different functional classes. In summary, our analysis revealed dramatic differences in the expression of the XMETs through different life stages in the mouse with more subtle differences in older humans. We hope to use the XMET expression patterns and known gene-chemical interactions to predict life stage-specific responses to environmental

chemicals and drugs and to test these predictions in relevant in vitro models. (This is an abstract of a proposed presentation and does not reflect US EPA policy.)

49. Implications of Early-Life Experience for Cognitive Function Throughout the Lifespan

Staci D. Bilbo (P)
Duke University, Department of Psychology & Neuroscience, Durham, NC

There is significant individual variability in cognitive decline during aging, suggesting the existence of "vulnerability factors" for eventual deficits. Neuroinflammation may be one such factor; increased glial reactivity is a common outcome of aging, which in turn is associated with numerous neurodegenerative conditions. Early-life infection leads to cognitive impairment in conjunction with an inflammatory challenge in young adulthood, which led us to explore whether it might also accelerate the cognitive decline associated with aging. Rats were treated on postnatal day 4 with PBS or live *E. coli*, and then tested for learning & memory at 2 or 16 month of age, using fear conditioning and a spatial water maze task. Neonatally-infected rats exhibited memory impairments in both fear-conditioning and in the water maze, but only at 16 month. Neonatally-infected rats also exhibited greater aging-induced increases in glial markers (CD11b and MHC II on microglia, and GFAP on astrocytes) within the hippocampus, but not in amygdala or parietal cortex compared to controls. Taken together, these data suggest that early-life infection leads to less successful cognitive aging, which may be linked to changes in glial reactivity within the hippocampus.

50. Role of Inflammation in the Pathogenesis of Neurodegenerative Disease: Models, Mechanisms, and Therapeutic Interventions

Jau-Shyong Hong (P)
Neuropharmacology Sections, Laboratory of Toxicology and Pharmacology, National Institutes of Environmental Health Sciences/National Institute of Health, Research Triangle Park, NC

The theme of this talk is first to describe newly created rodent Parkinson's disease (PD) models that mimic the progressive disease development in PD patients. With these models, we have elucidated the novel mechanisms of microglial activation that lead to inflammation-mediated neurodegeneration. These insights have then led us to develop innovative anti-inflammatory therapy for PD

1. Creation of inflammation-mediated rodent PD models.

In an effort to evaluate the role of inflammation in the pathogenesis of PD, we first developed new PD animal models and primary midbrain neuron-glia cultures. The salient features of these models are: a) prominent inflammation in the process of producing neuronal death; b) **delayed, progressive, and selective** nature of dopaminergic (DA) neuronal death, both *in vivo* and *in vitro*. These models were the first to mimic the delayed and progressive nature of the disease symptoms in PD patients. These unique features are absent from existing PD models, such as 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), because this toxin kill DA neurons rapidly after injections.

2. Mechanism of inflammation-mediated degeneration: role of microglia

We have demonstrated that microglia are the major player in mediating the inflammation related-neurodegeneration of DA neurons, triggered by a variety of environmental toxins. These findings are critical to the novel concept that microglia do not merely serve a passive role as scavengers. Instead, microglia play an active role in the pathogenesis of PD and other neurodegenerative diseases. We have identified a series of pro-inflammatory factors released from activated microglia, which mediate toxin-induced neuronal damage. Moreover, we have elucidated the molecular mechanisms underlying the regulation of expression for these pro-inflammatory genes.

3. Development of novel anti-inflammatory therapy for PD

Information generated from the above studies has provided insights for us to develop a series of novel anti-inflammatory and neuroprotective agents. Unique from the conventional strategies for developing anti-inflammatory drugs, which often target a specific pro-inflammatory factor, our approach is to prevent the over-production of the majority of pro-inflammatory factors through the inhibition of the over-activation of microglia, which would prevent the subsequent inflammatory process. Furthermore, we have discovered several small molecules, including peptides and alkaloids, which are both anti-inflammatory and neuroprotective at femtomolar concentrations. In addition to their potential therapeutic benefits, the discovery of femtomolar-acting peptides also offers valuable insight to the potential physiological mechanisms governing microglial activation and DA neuron survival in the substantia nigra.

51. Aging and the Environment: Importance of Variability Issues in Understanding Risk

Robert C. MacPhail (P)
Toxicity Assessment Division, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC

Of the many features of aging that could enhance susceptibility to environmental stressors, including toxic chemicals, the role of variability is arguably the least understood. This conclusion is surprising, since increased variability is a widely accepted feature of old age. Increased variability could complicate the demonstration of enhanced susceptibility, by reducing statistical power, as well as the estimation of toxic risk due to old age. A number of studies have shown increased variability in baseline function with advancing age, which may indeed be prodromal of neurodegenerative disease. Considerably fewer investigations have focused on age-related variability in response to toxicant exposures. Results will be described of studies on the effects of the volatile organic solvent toluene on the behavior of adolescent, young-adult, middle-age and senescent male Brown Norway rats. Under baseline conditions, behavior (motor activity) generally decreased with age, while variability increased both between- and within-subjects. Toluene (1000 mg/kg) increased motor activity in direct proportion with age; variability in toluene's effect also increased with age. Creating distributions of response for each age group, based on z-scores, allowed quantitative estimation of the increase in susceptibility to toluene in senescent rats. The results suggest that increased variability in toxic response due to old age can greatly amplify risk estimates.

This is an abstract of a proposed presentation and does not reflect US EPA policy.

52. Psychosocial Stress, Disease, and Aging in Female Nonhuman Primates

Carol A. Shively, Ph.D. (P)
Wake Forest University, Winston-Salem, NC

Normal human aging is accompanied by progressive brain tissue loss, cognitive and physical decline, and increasing morbidity and mortality. However, several factors are thought to influence the rate of aging. Obesity – particularly visceral obesity, depression, and CHD are accompanied by brain tissue loss. All three share high rates of comorbidity, and increase rates of morbidity and mortality. In contrast, physical activity is associated with greater brain volume and reduced obesity, depression and CHD. We have studied factors which influence risk of obesity, depression and CHD risk in socially housed female cynomolgus monkeys consuming a Western diet. When housed in groups social status hierarchies form. Socially subordinate monkeys appear behaviorally and physiologically to be stressed relative to their dominant counterparts. Subordinates deposit more fat in the viscera, develop more extensive coronary artery atherosclerosis, the pathological process underlying CHD, and have higher rates of depression than their dominant counterparts. Subordinates and depressed monkeys have lower activity levels, and lower activity levels are associated with more coronary artery atherosclerosis. Depressed monkeys have twice the coronary artery atherosclerosis of subordinates, and smaller hippocampi. Thus, in female primates, psychosocial stress promotes visceral obesity, depression and coronary artery atherosclerosis, an array of pathologies typical of Western society, some of which exacerbate each other. Psychosocial stress is an important contributor to morbidity, mortality, and perhaps brain aging, in later life.

53. Vascular Oxidative Stress in Aging: A Homeostatic Failure Due to Dysregulation of Nrf2-Mediated Antioxidant Response

Anna Csiszar (P)
University of Oklahoma Health Sciences Center, Oklahoma City, OK

The oxidative stress theory of aging postulates that increased production of ROS induces a variety of macromolecular oxidative modifications, and accumulation of such oxidative damage is a primary causal factor in the aging process. Although the exact role of increased production of ROS in regulation of lifespan is controversial, there is a consensus that oxidative stress contributes to the development of age-associated diseases. There is strong evidence that oxidative stress develops with age in the arterial system both in humans and laboratory animals. Increased production of ROS in the aged vasculature results in endothelial dysfunction and promote the development of atherosclerotic vascular diseases (including myocardial infarction, stroke, vascular dementias), which are responsible for the majority of age-related increases in morbidity and mortality in the Western world.

NF-E2–Related Factor-2 (Nrf2) is a transcription factor, which is activated by reactive oxygen species (ROS) in the vasculature of young animals

leading to the up-regulation of numerous ROS detoxifying and antioxidant genes. In young organisms this homeostatic response serves to attenuate vascular oxidative stress and limit the damage caused by the increased production of ROS. We assessed aging-induced changes in ROS production in arteries of F344xBN rats and correlated these changes with Nrf2 expression and activity and expression of Nrf2-driven antioxidant enzymes. Using cultured arteries isolated from young and aged rats we also determined whether aging impairs the ability of vascular cells to mount an effective antioxidant response in response to oxidative stressors (H_2O_2 treatment, model hyperglycemia) by inducting Nrf2-regulated ROS detoxification systems. We found that in aging vessels increased production of ROS fails to activate Nrf2 resulting in increased blood vessels sensitivity to the deleterious effects of ROS.

54. Resveratrol: Protecting the Heart and Vessels of Mice and Monkeys

Julie Mattison (P)
National Institute on Aging, Baltimore, MD

Resveratrol, a naturally occurring polyphenol found in grapes and other plant species, is reported to have diverse health benefits particularly in obesity, cardiovascular disease, diabetes, cancer, and survival. A chronic high fat diet alters central and peripheral circulating signals which can act on the arterial wall and predispose it to arterial dysfunction and thus, cardiovascular stress. Studies in mice fed a high calorie diet supplemented with resveratrol showed overall improvement on many markers of health. Pathology scoring of the cardiovascular tissue demonstrated beneficial effects on measures of fatty lesions, vacuolization, degeneration, and inflammation. Resveratrol with either a standard diet or a high calorie diet in mice improved vascular function as measured by aortic vasodilation response. To further evaluate the effect of resveratrol in an animal more closely related to humans, rhesus monkeys were supplemented while on a high fat and sugar diet for two years. On measures of pulse wave velocity, an indirect assessment of vascular stiffness, resveratrol monkeys were significantly improved compared to placebo controls. Additionally, resveratrol protected endothelial integrity and prevented macrophage infiltration, foam cell formation, and fat or calcium deposits in the thoracic aorta. Resveratrol-fed monkeys also had a reversal of the transcription pathways, re-setting the groupings of arterial genes from a high fat diet into functional pathways of a healthy diet. Thus, it is possible that resveratrol can help maintain arterial health in spite of an unhealthy diet but more study is needed to determine if these effects will translate to human health benefits.

55. The Stress of Aging Viewed from the Arterial Wall

Edward G. Lakatta (P)
National Institutes of Health, National Institute on Aging Intramural Research Program, Gerontology Research Center, Baltimore MD 21224

Arterial diseases, e.g. atherosclerosis and hypertension, are rampant in our society and increase exponentially with advancing age. Progressive changes occur throughout life in the structure and function of central arteries in numerous species. These changes include diffuse intimal and medial thickening, and enhanced stiffening (1). Since predominantly systolic hypertension is prevalent in epidemic proportion among older persons (1); it is reasonable to hypothesize that specific mechanisms that underlie alterations in the arterial substrate that accompany "aging" may be intimately linked to the age-associated exponential increase in predominantly systolic hypertension (1). Indeed, recent studies show pulse wave velocity, an index of arterial stiffness, is an independent predictor of the future increases in SBP and of incident hypertension (2).

Age-associated remodeling of the aortic wall of both animals and humans involves a proinflammatory profile of arterial cell and matrix properties (3). This profile features increased production of angiotensin II (Ang II) and downstream Ang II/AT_1 receptor signaling molecules, e.g., matrix metalloproteases (MMPs), calpain-1 and monocyte chemoattractant protein (MCP-1), transforming growth factor β1 (TGF-β1) NFκb, TNFα, iNOS, and VCAM. Activation of calpain-1, MMPs, TGF-β, and NADPH oxidase within the arterial wall is increased, and nitric oxide bioavailability is reduced (1,2,4). Both invasive and proliferative capacities of early passage vascular smooth muscle cells (VSMC) isolated from the aged arterial wall are increased, and are linked to augmented Ang II signaling. This age-associated arterial proinflammatory secretory profile within the grossly appearing arterial wall and related structural/functional remodeling, is reproduced in young rats by chronic infusion of Ang-II (4).

A recent comprehensive quantitative proteomic study in young (8 months) and old (30 months) rats has discovered additional molecules linked to Ang-

II signaling related VSMC proliferation and invasion (5). Transcription and translation of one such novel protein, the Milk Fat Globule protein Epidermal growth factor 8 (MFG-E8), substantially increase with aging in several mammalian species, including humans (5). MFG-E8 increases BrdU incorporation and also elevates levels of VSMC cell cycle accelerators, p-ERK1/2, CDK4, PCNA, and also increases the invasive capacity in VSMC from young rat aorta to levels isolated in VSMC from untreated old rat aortae. Both invasion and proliferation effects of MFG-E8 are substantially reduced by neutralizing antibodies against the cellular integrin receptors of MFG-E8, αvβ3 or αvβ5. Conversely, siRNA knock-down of MFG-E8 in VSMC from aortae of old rats reduces MCP-1 and invasion capacity, and these effects are also inhibited by the MCP-1 receptor blocker vCCI (5). siRNA knockdown of MFG-E8 also reduces levels of PCNA and CDK4 expression, raises levels of cell cycle decelerators ATM, p53, and p21, and facilitates cell entry into a growth-arrested state (senescence). MFG-E8 colocalizes with both angiotensin II and MCP-1 within VSMCs of the thickened aged aortic wall. Chronic infusion of Ang II into young rats increases aortic MFG-E8, MCP-1 and PCNA, an index of cellular proliferation, to levels in untreated old rats. Exposure of early passage VSMCs from young aortae to elevated angiotensin II markedly increases their MFG-E8 level, and invasive capacity (5), to levels observed in untreated VSMCs from old rats. Thus the age-associated increase in MFG-E8 is a novel pivotal relay element within the angiotensin II/MCP-1/PERK, CDK4 VSMC invasion and proliferation signaling cascades.

A megacept emerges with the realization that in arteries of younger animals, in response to experimental induction of hypertension or early atherosclerosis, parts of this proinflammatory profile within the arterial wall that have been studied to date are strikingly similar to the profile that occurs with advancing age (1). Thus, ‘‘aging’’-associated arterial changes and those associated with hypertension (and early atherosclerosis) are fundamentally intertwined at the cellular and molecular levels (1). In humans, other well-known risk factors (e.g., altered lipid metabolism, smoking, and lack of exercise) likely interact with this arterial substrate that has been altered during aging, and that renders the aging artery a “fertile soil” that facilitates the initiation and progression of these arterial diseases (1). Lifestyle and pharmacologic interventions have already proved to be effective in preventing or ameliorating hypertension associated with aging. The cellular/molecular proinflammatory mechanisms that underlie arterial aging are novel putative candidates to be targeted by interventions aimed at attenuating arterial aging, and thus attenuating the major risk factor for hypertension.

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56. Jim Joseph: Davy Crockett at the Frontier of Neurogerontology

Donald Ingram, (P)
Pennington Biomedical Research Center, Baton Rouge, LA

This lecture will highlight the major scientific contributions of James A. Joseph. Through a career spanning over 30 years, Jim was truly a pioneer in the neurobiology of aging and nutritional neuroscience. He served in a variety of posts at the U.S. National Institute on Aging, the U.S. Armed Forces Radiobiological Laboratory, the U.S. Department of Agriculture, and also in the pharmaceutical industry at American Cynamid.

Over this period Jim made seminal findings in several areas including (1) relating age-related changes in dopaminergic and cholinergic systems to motor dysfunction; (2) also linking these changes to alterations in neuronal membrane composition; (3) identifying potential detrimental effects of heavy particles on motor and cognitive function that might affect the deep space travel of astronauts; and (4) most notably, describing beneficial effects on brain aging and behavioral function of diets enriched in fruits and vegetables with high antioxidant activity. The latter work was conducted while he served as Chief of the Neuroscience Laboratory of the USDA/Tufts University Human Nutrition Research Center on Aging. While his important contributions were many, this collection of work over the last decade has stimulated major growth in research on botanicals and brain aging and represents a lasting and fitting legacy.

57. Food for Thought: What Jim Joseph Taught Me about Aging and Nutrition

Barbara Shukitt-Hale (P)
USDA, HNRC on Aging Tufts University, Boston, MA

Motor and cognitive behavioral deficits occur in senescence, and in cases of severe deficits, hospitalization and/or custodial care would be a likely outcome. Unless some way is found to reduce these age-related decrements in neuronal function, health care costs will continue to rise exponentially. Thus, it is extremely important to explore methods to retard or reverse age-related neuronal deficits, as well as their subsequent, behavioral manifestations, in order to increase healthy aging. In this regard, for the last 15 years, the laboratory of Jim Joseph has studied how consumption of fruits and vegetables high in antioxidant and anti-inflammatory activity, such as blueberries, blackberries, and strawberries, can prevent and even reverse the occurrence of the neurochemical and behavioral changes that occur in aging. We have shown that berry extracts are able to reverse several parameters of brain aging as well as age-related motor and cognitive deficits when fed to rats from 19-21 months of age. The polyphenolic compounds found in berry fruits may exert their beneficial effects either through their ability to lower oxidative stress and inflammation, or directly by altering the signaling involved in neuronal communication, calcium buffering ability, neuroprotective stress shock proteins, plasticity, and stress signaling pathways. These interventions, in turn, may exert protection against age-related deficits in cognitive and motor function.

58. The Journey from Normal Aging to Alzheimer's Disease: Lessons Learned from my Mentor Jim A. Joseph and my Husband Mark A. Smith

Gemma Casadesus(P)[1], Jaewon Chang[1], Agnes Rimaldo[2], Barbara Shukitt-Hale[4], Mark A. Smith[3], and James A. Joseph[4]
[1] Department of Neurosciences, Case Western Reserve University, Cleveland OH; [2] USDA-ARS, Natural Products Utilization Research, University, MS; [3] Department of Pathology, Case Western Reserve University, Cleveland OH; [4]USDA-ARS, Human Nutrition Research Center on Aging, Tufts University, Boston, MA

Scientific dogmas and F.A.D.S. are hard to break. Only courageous investigators that are willing to sacrifice the commodities of following the pack to stay true to science are able to transcend scientific stagnation and bring progress forward. I was blessed to have a Ph.D. mentor, Jim Joseph, and a husband and collaborator, Mark Smith, that were true examples of such character. With ruthless honesty and a wit and charisma that few of us are lucky to have, Jim and Mark have enlightened the way we view neuronal aging, antioxidant/oxidative stress research, and AD pathology. My work sits between these their two pedestals – The study of age-related pathological events that drive benign aging to become AD. Infected by the same "Dogma/fad science questioning bug", here I present data demonstrating that pterostilbene, a little known resveratrol analog, is substantially more potent than resveratrol when given at diet achievable doses, at modulating age-related pathological signaling and AD pathology in the SAMP8 mouse, a mouse model of accelerated aging with many pathological markers of AD. Importantly, this is driven independently of Sirt-1 activation, via PPAR alpha activation.

59. Mark A. Smith: Homeostatic Balance in Aging and Alzheimer Disease

Xiongwei Zhu[1] and George Perry (P)[2]
[1]Department of Pathology, Case Western Reserve University, Cleveland, OH; [2] College of Sciences, The University of Texas at San Antonio, San Antonio, TX

Mark entered into the free radical aging scene, and in less than 20 years placed oxidative stress as a foundation of disease pathogenesis. Building on the pillars of Earl Stadman, Robert Floyd, Bruce Ames, Bill Markesbery, Flint Beal and James Joseph, Mark established that every macromolecule class and every antioxidant response was altered in Alzheimer disease (AD). Beginning with the finding of glycation modification of the lesions of AD he soon found

that oxidative abnormalities pre-dated lesions and were both the earliest change of AD and dependent on mitochondria turnover abnormalities. Instead of marking the course to late AD, oxidative abnormalities distinguish people in midlife. Instead of simply accepting these elegant results as part of the spectrum of disease Mark worked tirelessly to integrate them into the physiology of aging. The American Association for Aging played a major role in his thinking. AAA influenced his development of the cell cycle, signal transduction, mitochondria, metal, gonadotropin and two hit hypotheses of AD, ideas continuing to influence the field. His work had a deep respect for the biological features that controlled disease, and of course, aging and could be the most amenable to therapeutic intervention. But even more, those that knew him were influenced by his great generosity to friends and colleagues.

Poster Session I

Saturday, June 4, 2011

60	**Misfolding Diseases May Result From The Collapse of Physicochemically Vulnerable Pathways** *Prajwal Ciryam (P, N), Gian Tartaglia, Richard Morimoto, Christopher Dobson, Michele Vendruscolo*
61	**Advanced Paternal Age: Age-related Changes in AP Endonuclease I Abundance in Spermatogenic Cells** *Jamila Momand (P, N), Rebecca Garcia, Kim Hildreth, Kristine Vogel, Christi Walter*
62	**Rapamycin and Dietary Restriction: Do They Share a Common Mechanism in Lifespan Extension?** *Wilson Fok (P, N), Yiqiang Zhang, Adam Salmon, Arunabh Bhattacharya, Carolina Livi, William Wood, Yongqing Zhang, Kevin Becker, Walter Ward, Arlan Richardson, Viviana Perez*
63	**Protein Oxidation and Proteostasis in Cultured Cells. Does Oxygen Tension Matter?** *Viviana Perez (P), Mina Konigsberg, Asish Chaudhuri, Arlan Richardson, Holly Van Remmen*
64	**Chemical Analysis of Aging Tissue in Drosophila Melanogaster** *Elizabeth Ostler (P), Aamira Iqbal, Matthew Piper, Declan Naughton, Linda Partridge, Richard Faragher*
65	**Longitudinal Dynamics of Physiological Indices in Relation to Risks of Diseases: Analysis Using the Stochastic Process Model** *Konstantin Arbeev (P), Svetlana Ukraintseva, Igor Akushevich, Alexander Kulminski Liubov Arbeeva, Irina Culminskaya, Deqing Wu, Anatoliy Yashin*
66	**Insights in Genetic Origin of Health and Lifespan in Humans in the Light of Aging** *Alexander Kulminski (P) and Irina Culminskaya*
67	**Antagonistic Association of the APOE Polymorphism with the Ages at Onset of CVD and Cancer Influences Human Lifespan** *Alexander Kulminski (P), Irina Culminskaya, Svetlana Ukraintseva, Konstantin Arbeev, Liubov Arbeeva, Igor Akushevich, Kenneth Land, Anatoli Yashin*
68	**Declining Autophagy in Aging Non-transgenic and 3xTg-AD Mouse Mitochondrial Neurons** *Aaron Barnett (P, N) and Gregory Brewer*
69	**Extension of Yeast Replicative Lifespan through Nuclear tRNA Sequestration** *Joe Delaney (P)*
70	**A Role for the HDAC-Mef2 Pathway in Neuromuscular Aging** *Michael Walsh (P) and Holly Van Remmen*
71	**Oxidizing Brain Neurons as We Age: NADH, Redox and Glutathione Levels Change Before ROS Damage in Alzheimer and Control Mice** *Gregory Brewer (P) and Debolina Ghosh*
72	**The importance of Caspase-2 in Age-Dependent Osteoporosis** *Danielle Victor (P), Difernando Vanegas, Ramaswamy Sharma, Meenakshi Tiwari, Marisa Lopez-Cruzan, Kathleen Woodruff, Sherry Abboud-Werner, Brian Herman*
73	**Aging as the Last Epigenetic Stage of Development: How Reversible is the Histone Code with Age and Alzheimer's Disease?** *Michael Walker (P) and Gregory Brewer*
74	**Novel Olfactory Acuity Assay in Measuring Olfactory Perceptiveness in Mice** *Samantha Rendon, Kathleen Fischer, Steven Austad*
75	**Mitochondria-Targeted Overexpression of MsrA: Is this Beneficial or Detrimental to Health- and Life-Span?** *Adam Salmon (P, G), Holly Van Remmen, Arunabh Bhattacharya, Yuhong Liu, JennaLynn Styskal, Daniel Pulliam, Rod Levine, Arlan Richardson*
76	**Lipid Peroxidation Potential and Chain Length Contribute to Diverse Longevities of *C. elegans* Near-Isogenic Mutants** *Robert Shmookler Reis(P), Lulu Xu, Hoonyong Lee, Minho Chae, John Thaden, Puneet Bharill, Cagdas Tazearslan, Eric Siegel, Ramani Alla, Piotr Zimniak, Srinivas Ayyadevara*

77	**Genes, GO Where I Send Thee; How Shall I Send Thee? A Novel Analytic Method for Microarray Data with Functional Categories** *Robert Shmookler Reis (P), Taewon Lee, Robert Delongchamp*
78	**Enhanced Leptin Sensitivity Leads to Obesity Resistance and Healthy Aging In A PKA-Disrupted Mouse Model** *Linda Enns (P, G), Hannah Richards, Ken Bible, Warren Ladiges*
79	**Impact of Calorie Restriction (CR) on Cellular Aspects of Metabolic Decline in Aging Rhesus Skeletal Muscle.** *Rozalyn Anderson (P)*
80	**A Novel Conserved Mechanism of Extended Longevity in a Mouse Model of Mitochondrial Dysfunction** *Daniel Pulliam (P, N), Deepa Sathyaseelan, Yuhong Liu, Holly Van Remmen*
81	**Longevity and Proteome Stability in Marine Bivalves** *Stephen Treaster (P, N), Keith Maslin, Iain Ridgeway, Asish Chaudhuri, Steven Austad*
82	**The *C. elegans* Ribosome: An Aging Factor and a Model for Human Ribosomopathy** *George Sutphin (P, G)*
83	**Life Span in Wild-Type and p66Shc-/- Mice Fed 5% or 40% Calorie Restricted Diets** *Roger McDonald, Dianna Tran, Kevork Hagopian, Gino Cortopassi, Kent Lloyd, Steve Griffey*
84	**Aspirin Extends Caenorhabditis Elegans Lifespan and Ameliorates Aging-Associated Functional Decline** *Puneet Bharill (P, N), Srinivas Ayyadevara, Abhijit Dandapat, Magomed Khaidakov, Changping Hu, Robert Shmookler Reis, Jawahar Mehta*
85	**Differential Expression of DNA Methylation Enzymes in the Ames Dwarf Mouse** *Vanessa Armstrong (P, N), Sharlene Rakoczy, Holly Brown-Borg*
86	**XIAP Regulation by Asparagine Deamidation and PIMT** *L Solorzano (P, G), B Powell, J Lowenson, J Enama, S Clarke, O Fearnhead, E Alnemri*
87	**Monogonont Rotifers as a New Model System: A Survey of Aging-Related Genes** *Kristin Gribble and David Mark Welch*
88	**Systems Biology of Human Aging 2011 Network Model** *John Furber (P)*
89	**An Observational Assessment of Aging in Laboratory Rats: Strain Comparison** *Pamela Phillips(P), Kimberly Jarema, David Kurtz, Christopher Gordon, Robert MacPhail*
90	**Increased Median Life Span and Overall Longevity in FAT10ko Mice: A Novel Model Linking Adiposity, Inflammation and Aging** *Allon Canaan(P, G), Jason Defuria, Eddie Perelman, Hui-Young Lee, Varman Samuel, Martin Obin, Sherman Weissman*
91	**C57BL/6 Life Span Study: Muscle Function and Dysfunction** *T. G. Graber (P, N), J-H Kim, R. W. Grange, L. K. Mcloon, L. V. Thompson*
92	**Comparative Biology with New Invertebrate Models to Test Theories of Aging and Senescence** *Terry Snell (P) and Allison Fields*
93	**Ovariectomy and Dietary Restriction Show Different Storage Levels but Similar Feeding Rates & Life Extensions in Grasshoppers** *John Hatle (P), Michelle Drewry, J. Williams, Alycia Macdonald, James Kellenberger, Ephraim Viray*
94	**Ultraviolet Exposure Extends Lifespan in *Brachionus manjavacas (Rotifera)* Through Hormesis** *Jarrett Smith (P), Allison Fields, Terry Snell*
95	**Postreproductive Life Span - How Could it Evolve** *Josh Mitteldorf(P) and Charles Goodnight*

Poster Session I Continued:

Saturday, June 4, 2011

96	**Daphnia: A Model System for Understanding Genome-Environment Interactions in Aging** *Jeffry Dudycha (P), Rekha Patel, Sean Place*
97	***p16-LUC*: An *in vivo* Reporter Allele for Cancer and Aging** *Christin E. Burd (P), Kelly S. Clark, Jessica A. Sorrentino , Norman E. Sharpless*
98	**Transcriptional and Translational Changes Across Species; Profiling of Skeletal Muscle During Aging** *Evi M Mercken (P, G), Bethany A Carboneau, Julie A Mattison and Rafael de Cabo*
99	**Association Between Serum Adiponectin and Ferritin Level in Apparently Healthy Women** *Jee Yon Lee (P)*
100	**High- Normal Fasting Plasma Glucose Decreased Testosterone in Non-Diabetic Middle Aged Men** *Jinyoung Shin(P), Byoungjin Park, Eunki Park, HyeRee Lee*
101	**Age-Related Loss of Fenestrations Impairs Hepatic Uptake of The Water Soluble Substrate Acetaminophen** *Sarah Mitchell (P, G), Aniko Huizer-Pajkos, Victoria Cogger, David Le Couteur, Andrew McLachlan, Rafael de Cabo, Brett Jones, Sarah Hilmer*
102	**Age-Related Changes in T Cell Subtypes in African Green Monkeys** *John Stehle (P), Maria Blevins, Kevin High*
103	**Aging Exacerbates Oxidative Stress in Disused Type II Muscles** *Chiao-nan (Joyce) Chen (P) and LaDora Thompson*
104	**Highest Audible Pitch and Memtrax Image Recognition Correlate with Age and Each Other in a Kronos-MMT Reliability Study** *Rolf Martin(P), Susan Kaib, Terrence Rose, S. Mitchell Harman, Peter Bayley, J. Wesson Ashford*
105	**A Double-Blind, Placebo-Controlled Trial of PCBN-21 for Age-Associated Memory Impairment** *Rolf Martin (P)*

106	**Dietary Restriction and Surgical Removal of Adipose Tissue Reduce Age-Dependent Intolerance to Inflammatory Stress** *Marlene Starr (P,N), B Evers, Hiroshi Saito*
107	**TLR2-Induced Signaling Defects In Aged Alveolar Macrophages Occur Downstream to IRAK-1 Following Infection with *S. pneumoniae*** *Angela Boyd (P,N), Pooja Shivshankar, Perla Rodriguez, Carlos Orihuela*
108	**Impaired CD8+ T Cell Priming in Aged Mice is Due to Poor CD8 Dendritic Cell Responses?** *Gang Li (P, G)*
109	**Long-Term Rapamycin Differentially Affects Immunity in Young and Old Mice** *Adriana Benavides (P, N), Elizabeth Fernandez, Z. Dave Sharp, Randy Strong, Ellen Kraig*
110	**A Molecular Mechanism for TNF-α-Mediated Down-Regulation of Mouse B Cell Responses in Aging** *Bonnie B Blomberg (P), Maria Romero, Alain Diaz, Ana Marie Landin, Richard L Riley, Daniela Frasca*
111	**Autocrine TNF-α Down-Regulates Aged Human B Cell Function** *Daniela Frasca (P), Alain Diaz, Maria Romero, Bonnie B Blomberg.*
112	**Effect of Resveratrol on Proinflammatory Profile of a Non-Human Primate Model of Obesity** *Jimenez-Gomez Y (P, G), Martin-Montalvo A, Mattison J, de Cabo R.*
113	**In Silico Evidence Supports a Role for DNA Methylation in Sirt1-Mediated Effects of Dietary Restriction** *Laura Ions (P), Luisa Wakeling, Dianne Ford*
114	**Serum 25-Hydroxyvitamin D and DHEA-S Level are Associated with Depression in Community-Dwelling Older Women** *Hyun mee Kim (P), Jee Yon Lee, Chung Ji Youn, Duk Chul Lee*
115	**Muscle Iron Accumulation in Old Rats is Associated with High Levels of Oxidative Stress and Impaired Recovery from Atroph** *Jinze Xu (P), Judy Hwang, Hazel Lee, Stephanie Wohlgemuth, Mitchell Knutson, Andrew Judge, Esther Dupont-Versteegden, Emanuele Marzetti, Christiaan Leeuwenburgh*
116	**Calorie Restriction Suppresses the Occurrence and Retards the Growth of Ethyl-nitrosourea-Induced Glioma in Rats** *Megan Mahlke, Lisa Cortez, Celeste Webb, Melanie Ortiz, Yiqiang Zhang, Shuko Lee, Gene Hubbard, Yuji Ikeno (P)*
117	**Anti-Inflammatory Effects of Walnut-Associated Fatty Acids in BV-2 Microglia** *Amanda Carey(P, G), Derek Fisher, Donna Bielinski, Barbara Shukitt-Hale*
118	**The Influence of Dietary Lipid Composition on Life Span in Calorie Restricted Mice** *Jon Ramsey (P), Kevork Hagopian, Dianna Tran, Jose Villalba, Placido Navas, Guillermo Lopez Lluch, Roger McDonald*
119	**Energy Metabolism Pathways in p66Shc-/- Mice** *Kevork Hagopian (P), Gino Cortopassi, Roger McDonald, Jon Ramsey*
120	**Effects of Phenylalanine Supplementation on Age-related Hearing Loss** *Shinichi Someya and Christiaan Leeuwenburgh*
121	**2011 Blueberry Health Study Report: Memory Scores Continue To Improve; Power Milestones Bring Personalized Research Closer** *Rolf Martin(P), Diana Burns, David Doiron, Kurt Gerstmann, Kathy Hull, Amy Kokesh, Bruce Kristal, Alec Pruchnicki, Howard Raphaelson, Barrie Sachs, Roseanne Schnoll, Joseph Vogelman, Anthony Wetherell*
122	**Delaying Aging with Available Interventions May Lead to Large Economic as well as Quality of Life Benefits** *Rolf Martin(P) and Gregory Brewer*

Poster Session II Continued:

Sunday, June 5, 2011

123	**Timing of High Fat Diet Onset has Differential Effects on Health and Survival in Mice** *Robin K. Minor (P,G) and Rafael de Cabo*
124	**NQO1 Overexpression Increases Antioxidant Protection and Glucose Homeostasis In Mice** *Alejandro Martín-Montalvo (P,G), Yolanda Jimenez-Gomez, Rajib Paul, Kavitha Sankavaram, Theresa Ward, Andrew Levette and Rafael de Cabo*
125	**Activation of GPR30 Attenuates Diastolic Dysfunction and LV Remodeling in Oophorectomized mREN2.Lewis Rats** *Hao Wang (P), Jewell Jessup, Sarah Lindsey, Marina Lin, Clarissa Chagas, Leanne Groban*
126	**IGF-1 Deficiency Impairs Vascular Oxidative Stress Resistance in Mice** *Lora Bailey-Downs(P), Matthew Mitschelen, Peter Toth, Danuta Sosnowska, Jim Henthorn, Akos Koller, William Sonntag, Zoltan Ungvari, Anna Csiszar*
127	**Vascular Stiffness is Independently Associated with Central Adiposity Rather Than with Chronological Age in Nonhuman Primate** *Kylie Kavanagh (P), Ashley Wylie, William Strawn, Jason Lazar, Stephen Kritchevsky, Jay Kaplan*
128	**Circulatory Diseases in the U.S. Elderly in the Linked National Long Term Care Survey-Medicare Database** *Igor Akushevich (P), Julia Kravchenko, Svetlana Ukraintseva, Konstantin Arbeev, Anatoli Yashin*
129	**High Oxidative Stress and Detectable Levels of Aβ in the Naked Mole-Rat Brain** *Yael Edrey(P, N), Martha Hanes, Mario Pinto, James Mele, Rochelle Buffenstein*
130	**A Potential Role for Redox Sensitive Signaling-Dependent Demyelination of Motor Neurons** *Ryan Hamilton (P,G), Michael Walsh, Arunabh Bhattacharya, Yun Shi, Holly VanRemmen*
131	**Role of Neuronal mTOR in Aging** *Stacy Hussong (P,G) and Veronica Galvan*
132	**Signaling through mTOR and the Heat Shock Response in Cognitive Aging and Neurodegeneration** *Veronica Galvan (P), Jonathan Halloran, Natalia Podlutskaya, Anson Pierce*
133	**Humor Usage in Geriatric** *Gurprit Lamba (P), Vijay Aswani, James Ellison*
134	**Role of Thioredoxin 1 and 2 in Cancer and Aging** *Lisa Cortez, Celeste Webb, Megan Mahlke, Melanie Ortiz, Arunabh Bhattacharya, Yiqiang Zhang, Adam Salmon, Wenbo Qi, Yuhong Liu, Shuko Lee, Holly Van Remmen, Arlan Richardson, Gene Hubbard, Yuji Ikeno (P)*
135	**Effect of Hypoxia on Bioenergetics of Myoblasts Precursor Cells (MPC) Isolated from Young and Old Mice** *Mina Konigsberg (P), Viviana Pérez, Carmen Rios, Sukkyoo Lee, Yun Shi, Holly Van Remmen*
136	**Enhanced Stress Resistance of Fibroblasts from Long-Lived Birds** *James Harper (P), Joseph Williams, Richard Miller*
137	**Stress Resistance and Lifespan: The Bare Necessities** *Kaitlyn Lewis (P,N), James Mele, Rochelle Buffenstein*
138	**The Differential Impact of Calcineurin Isoforms in the Early Unfolded Protein Response** *Yanan Chen (P)*
139	**Mild Oxidative Stress Increases Oxidative Stress Tolerance Through NRF2 Regulated Proteasome Expression in Flies and Worms** *Andrew Pickering (P,N), Derek Sieburth, John Tower, Kelvin Davies*
140	**Insulin Resistance can be Regulated by the Protein Oxidation Repair Enzyme Methionine Sulfoxide Reductase A (MsrA)** *JennaLynn Styskal (P,N)*

141	**Heated Hydrotherapy Induces the Heat Shock Response in Nonhuman Primates and Improves Cardiometabolic Health** *Kylie Kavanagh (P), David Flynn, Kurt Jenkins, Matthew Davis, Janice Wagner*
142	**Skeletal Muscle HSP70 is Affected by Western Diet and not by Chronological Age in Nonhuman Primates** *Kylie Kavanagh (P), Ashley Wylie, Tara Chavanne, Lawrence Rudel, Stephen Kritchevsky, Jay Kaplan*
143	**Environment and Aging: Life-Stage Susceptibility to Toxicants** *Kimberly Jarema(P), Pamela Phillips, Robert MacPhail*
144	**Negative Regulation of STAT3-mediated Cellular Respiration by SirT1** *Susan Krzysik-Walker, Michel Bernier, Alejandro Martin-Montalvo, Morten Scheibye-Knudsen, Shaoming Song, and Rafael de Cabo*
145	**How Well Do Sleep and Activity Behaviours Predict Lifespan in Drosophila?** *Sofocles Koudounas, Edward Green, David Clancy(P)*
146	**Predictors of Sleep Disturbances in Seniors: A Population-based Study** *Joseph Finkelstein (P) and Eunme Cha*
147	**Serum Carcinoembryonic Antigen Levels Associated with Arterial Stiffness in Female Korean Non-Smokers?** *Ji Won Lee (P), Ki-Deok Park, Duk-Chul Lee, Soo Jin Jung, Ji Youn Chung, Jeing Ah Hong, Jeeyon Lee*
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150	**Long-Lived SURF1 Knockout Mice with Decreased Mitochondrial Complex IV Activity Exhibit Increased Insulin Sensitivity** *Deepa Sathyaseelan (P), Daniel Pulliam, Adam Salmon, Lauren Sloane, Holly Van Remmen*
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153	**Nectarine Extract Reduces Oxidative Damage and Promotes Longevity in Drosophila melanogaster** *Xiaoping Sun (P), Olga Boyd, Peter Weng, Thomas Alberico, Mara Laslo, Bradley Kern, Sige Zou*
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155	**Timing of Antioxidant Intervention During Life Determines Functional Outcome** *Nathalie Sumien (P), Uzoma Ikonne, Theresa, Carbonaro, Jacques D. Nguyen, Kiran Chaudhari, and Michael J. Forster*

KEYNOTE ADDRESS

The Amazing INK4a/ARF Locus and Human Aging

Norman Sharpless
University of North Carolina-Chapel Hill

VINCENT CRISTOFALO MEMORIAL RISING STAR AWARD AND LECTURE

Molecular Mechanisms Linking Diet, Disease and Aging

Matt Kaeberlein
University of Washington, Seattle, WA

Aging is controlled by a complex interaction between environmental and genetic factors. The best-characterized environmental modulator of longevity is dietary restriction, defined as a reduction in nutrient availability in the absence of malnutrition. In addition, several genetic pathways have been implicated in aging across evolutionarily divergent species. These include sirtuins, the target of rapamycin (TOR) kinase, insulin/IGF-1-like signaling, and the hypoxic response. Here I will describe our ongoing studies to define the mechanistic basis for the interaction between these genetic and environmental factors as they pertain to aging. I will also describe efforts to understand the molecular processes that determine how genotype influences the respone to dietary restriction.

DENHAM HARMAN AWARD LECTURE

Modulation of the Innate Immune System in Aging as a Target for Increasing Endogenous Repair and Synaptic Plasticity

Paula C. Bickford, PhD
University of South Florida, Tampa, FL

Changes in innate immune function are early and critical events in brain aging. Importantly, neurodegenerative diseases emerge and are exacerbated within this context. Functional changes in microglial phenotype can lead to dysregulation of pro-inflammatory molecules that initiates neuronal dysfunction and reduced synaptic plasticity in the aged brain. Microglia in young and non-diseased brain are constantly surveying the environment for "danger" signals. In this so-called "resting" phenotype, microglia are not static, but rather quite active. Neurons produce signaling molecules e.g. CX3CL1 (fractalkine) that turn off production of iNOS, IL1β, TNFα and IL-6 among other pro-inflammatory molecules. With aging, we and others have demonstrated that there is a decrease in the production of these "calming" signals, which leads to a disrupted regulation of microglia in the brain. Additional changes with age in the peripheral immune system also influence the brain as serum proteins and peripheral immune cells communicate with the brain, and may also contribute to the disruption of "resting" microglial phenotype. These extrinsic factors have been shown to have a negative impact on the brain and the neurogenic niche. Over the years we have examined a number of ways to modulate the innate immune system including stem cells, nutraceutical supplements and chemokines.

SPECIAL LECTURE

The Increasing Genetic Contribution to Exceptional Longevity With Increasing Age Beyond 100 years

Thomas Perls
Boston University, Boston, MA

AGE PROGRAM POSTER ABSTRACTS

Abstract Number corresponds to speaker presentation number in the program schedule
(P) Denotes Presenter; (G) Denotes Post-doctoral Candidate for Glenn Award;
(N) Denotes Pre-Doctoral Candidate for Nicolai Award

60. Misfolding Diseases May Result From The Collapse of Physicochemically Vulnerable Pathways

Prajwal Ciryam (P, N)[1], Gian Tartaglia[2], Richard Morimoto[3], Christopher Dobson[1], Michele Vendruscolo[1]

[1]Department of Chemistry, University of Cambridge, Cambridge, UK [2]Centre for Genomic Regulation; [3]Dept of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, IL

A fundamental requirement for life is that proteins remain soluble in the cell. It has been observed that the solubility requirement of proteins forms an "edge" in which maximum expression level anti-correlates with aggregation propensity. When protein homeostasis fails to maintain solubility, toxic protein aggregates can form, leading to a number of age-related disorders, including Alzheimer's, Parkinson's, Huntington's, and other diseases. The mechanism by which aggregation causes disease has not yet been fully established. However, it is thought that misfolded proteins — like amyloid beta in Alzheimer's and a-synuclein in Parkinson's — gain toxic functions that cause cellular damage. One important way this may occur is by disrupting protein homeostasis, which may then lead to a further burden of misfolded species. Recent evidence suggests that such disruption can cause widespread misfolding in the cell. Using a bioinformatics approach, we show that the biochemical pathways associated with neurodegenerative diseases are highly enriched in poorly soluble proteins, which makes them particularly susceptible to collapse. The loss of function of proteins in these pathways may underlie misfolding diseases. In support of this possibility, we find that proteins with greater functional importance have more stringent solubility requirements. This suggests that there is selection against misfolding-induced loss of function. These results imply that toxic aggregates trigger a misfolding cascade in physiochemically vulnerable pathways, ultimately leading to cellular dysfunction and death.

61. Advanced Paternal Age: Age-related Changes in AP Endonuclease I Abundance in Spermatogenic Cells

Jamila Momand (P, N), Rebecca Garcia, Kim Hildreth, Kristine Vogel, Christi Walter

The University of Texas Health Science Center at San Antonio, San Antonio, TX

A 30% increase in the number of older fathers during the last three decades, has made the paternal age effect an increasingly important topic in aging reproductive health. Using lacI transgenic C57Bl/6 male mice, in conjunction with mice harboring inactivated alleles of various base excision repair genes, we have determined that normal base excision repair activity is essential in maintaining a low mutant frequency in spermatogenic cells. Comparisons of base excision repair activity in nuclear extracts prepared from spermatogenic cells obtained from young, middle-aged and old mice revealed that repair activity is reduced by 50% in extracts prepared from old mice. This decreased base excision repair activity appears to be mediated by reduced abundance of a key base excision repair protein, AP endonuclease 1 (APEN1). Our objective is to delineate the mechanisms mediating reduced base excision repair in spermatogenic cells with increasing age by identifying the molecular changes that result in reduced APEN1 abundance and activity. TRP53 has been shown to play a role in regulating APEN1 expression and APEN1 expression was reduced by 40% in pachytene spermatocytes obtained from p53 null mice relative to wild-type animals. However, northern blot analysis revealed that the abundance and integrity of the Apex1 transcript remains constant with increasing age. This suggests that the age-related change in APEN1 abundance is regulated at the translational or post-translational level. Spermatogenic cells isolated from Apex1 heterozygous mice, also carrying the lacI mutation reporter, demonstrated an accelerated increase in germ cell mutagenesis. Conversely, transgenic mice that over-express APEN1 were protected from the age-related increase in mutant frequency. Combined, these results indicate a strong relationship between APEN1 abundance and germline mutagenesis and that TRP53 may be a regulator of APEN1 abundance in spermatogenic cells.

62. Rapamycin and Dietary Restriction: Do They Share a Common Mechanism in Lifespan Extension?

Wilson Fok (P, N)[1], Yiqiang Zhang[1], Adam Salmon[1], Arunabh Bhattacharya[1], Carolina Livi[1], William Wood[2], Yongqing Zhang[2], Kevin Becker[2], Walter Ward[1], Arlan Richardson[1], Viviana Perez[1]

[1]University of Texas Health Science Center at San Antonio, San Antonio, TX; [2]National Institute on Aging, Baltimore MD

Rapamycin (rapa) is a specific inhibitor of the target of rapamycin, which acts as a key regulatory nexus in the responses of eukaryote cells to nutrients, growth factors, and cellular energy status. Rapamycin has been found to extend lifespan in yeast, *C. elegans*, Drosophila, and recently mice. Rapa has been hypothesized as a dietary restriction (DR) mimetic and act through similar mechanisms. To test this hypothesis, we used male C57BL6 mice fed ad libitum (AL), DR fed 60% of AL diet, and AL supplemented with encapsulated rapa (14 ppm), the rapa concentration used in the studies showing rapa increased the lifespan of mice. We compared the similarities or differences in genes expression and signaling pathways associated with DR or rapa interventions for six months, starting at 2 months of age.

We observed no differences in body composition, with exception in fat mass, in which only DR treatment had a significant decrease in fat mass. The mTOR signaling pathway was determined by the phosphorylated levels of the downstream target S6. Our data showed significantly reduced phosphorylation of S6 (60%) by DR and Rapa which indicates that both treatment affected mTOR activity similarly. Also, both DR and rapa treatment increased levels of autophagy when measured by the ratio of LC3II/LC3I. From our microarray analysis of the liver, we observed that DR has a more dramatic effect on genes than rapa. Over 2000 genes were significantly changed with DR compared to 1400 genes in rapa. Of these gene changes, about 600 genes were shared by DR and Rapa. We have recently begun to validate some of of these gene changes observed in the microarray but our preliminary data indicates that although DR and rapa may have similar effects on mTOR activity and autophagy, there are still major differences at the gene and pathway level.

63. Protein Oxidation and Proteostasis in Cultured Cells. Does Oxygen Tension Matter?

Viviana Perez (P)[1], Mina Konigsberg[2], Asish Chaudhuri[3], Arlan Richardson[1], Holly Van Remmen[1]

[1]C&SB/Barshop Institute for Longevity and Aging Studies; [2]Barshop Institute for Longevity and Aging Studies; [3]Biochemistry/ Barshop Institute for Longevity and Aging Studies, University of Texas at San Antonio, San Antonio, Texas

Protein oxidation is an important contributor to the progressive loss of functional cellular processes that lead to aging. Oxidation of specific amino acids induces changes in structure and function, resulting in protein misfolding and aggregation that cause cellular disorders and age-related diseases. In order to study the relation between oxidative stress and aging, cell cultures have been widely used. However, traditionally they were performed at ambient oxygen tension (21%), which is significantly higher than physiological levels (2 - 5%). It has been shown that cells grown under low O2 tension (3%) display lower levels of oxidative damage to DNA and proteins, and a decrease in the formation of oxidized/cross-linked protein aggregates (lipofusin). Existing data suggest that 21% oxygen tension is itself an oxidative stress for cells; thus the question remains to as whether the higher levels of "basal" oxidation observed in "old" cells are indeed real, or whether they are an artefactual effect of high oxygen levels under the cell culture conditions utilized. It is possible that what is really being measured is the capability of the cells to respond and repair the oxidative damage induced by 21% oxygen. In order to address this question, primary myoblast obtained from young and old mice were cultured either at 21% or 3% pO2. After 2 weeks in culture, cells were harvested and the cytosolic (soluble) and insoluble fraction were analyzed. Our data indicate that cells cultured at 21% pO2 showed more protein aggregates, these findings suggest that oxygen tension might play an important role in protein oxidation and/or protein homeostasis.

This work was supported by Ellison Medical Foundation AG-NS-0705-10/PEREZ. MK is a Fulbright and a CONACyT (147827) scholar

64. Chemical Analysis of Aging Tissue in Drosophila Melanogaster

Elizabeth Ostler (P)[1], Aamira Iqbal[1], Matthew Piper[2], Declan Naughton[3], Linda Partridge[2], Richard Faragher[1]

[1]University of Brighton, Brighton, UK; [2]University College, London, UK; [3]University of Kingston, Kingston upon Thames, UK

There is growing evidence that aging may be caused by the accumulation of highly-diverse, thermodynamically-stable chemical species occurring as a side effect of normal metabolic

processes. Under ordinary circumstances repair or removal of such "stable damage" is energetically unfavoured. However, under conditions where resources are scarce (eg during dietary restriction) broad spectrum detoxification processes, capable of "recycling" the affected macromolecules, are upregulated. Equally, under reduced environmental temperatures, poikilotherms may accumulate damage more slowly, due to reduced rates of non-enzymic reactions. It is postulated that interventions which extend lifespan may therefore do so by invoking these recycling processes. This extension of the oxidative damage theory of ageing is known as the "Green Theory" of aging. Environmental and genetic interventions were applied to examine their effects on age-related changes in the chemical composition of Drosophila melanogaster. Novel analytical methods were developed and validated to investigate the accumulation, with age, of damaged compounds in flies. The results obtained [1] are consistent with the predictions of the Green Theory of aging, and were later independently confirmed.[2] To test further the predictions made by Green Theory, proton magnetic resonance and mass spectrometric as well as fluorimetric analyses of *Drosophila melanogaster* were undertaken in the presence and absence of interventions which extend lifespan.Cohorts of Drosophila with extended lifespans showed a reduced rate of accumulation of signals consistent with damage during ageing compared to wild-type cohorts cultured under normal conditions. Spectrometric analysis also revealed distinct age-associated qualitative changes. These spectral data are now being deconvoluted in order to identify the compounds responsible. This work represents the first use of a range of analytical techniques to characterise and quantify compounds associated with, and possibly causing, different rates of aging in Drosophila melanogaster.

1. Iqbal, A. et al. (2009) AGE 31, 343-351.
2. Jacobson J. et al. (2010) Aging Cell 9, 466-477.

65. Longitudinal Dynamics of Physiological Indices in Relation to Risks of Diseases: Analysis Using the Stochastic Process Model

Konstantin Arbeev (P), Svetlana Ukraintseva, Igor Akushevich, Alexander Kulminski Liubov Arbeeva, Irina Culminskaya, Deqing Wu, Anatoliy Yashin
Duke University, Durham, NC

Data on individual age trajectories of physiological indices collected in longitudinal studies of aging, health, and longevity provide information on dynamic characteristics of such indices, which may be associated with morbidity and mortality risks. In our earlier studies we showed that dynamic characteristics of such indices at middle and old ages are related to risks of death and onset of diseases at later ages in participants of the original cohort of the Framingham Heart Study (FHS). Respective aging-related mechanisms linking the dynamics of age trajectories of physiological indices and mortality/morbidity risks can be analyzed using the stochastic process model (SPM) of aging. In this study, we applied the SPM to analyze relationship between the risk of onset of aging-related diseases (cancer, cardiovascular diseases, or diabetes) and longitudinal measurements of six physiological indices (body mass index, diastolic blood pressure, hematocrit, pulse pressure, pulse rate, and serum cholesterol) in participants of the FHS (original cohort). We show how different components of the aging process, such as the decline in resistance to stresses and adaptive capacity, can be evaluated from age trajectories of these indices and data on onset of diseases, and how they can contribute to an increase in the risk of onset of diseases with age. We calculated respective characteristics in females and males and evaluated their contribution to the observed sex-specific patterns of incidence rates. The results indicate the presence of substantial gender difference in the aging-related decline in stress resistance and adaptive capacity, as well as in accumulation of allostatic load, which contributes to the difference in the shape of the sex-specific patterns of incidence rates of aging-related diseases. The determinants of such differences (which may have genetic or non-genetic origin) deserve further studies.

66. Insights in Genetic Origin of Health and Lifespan in Humans in the Light of Aging

Alexander Kulminski (P) and Irina Culminskaya
Duke University, Durham, NC

Despite notable progress of the candidate gene and genome-wide association studies, understanding the role of genes contributing to human health and longevity is still very limited. Targeting the fundamental process of biological aging could be a promising alternative to currently prevailing genetic strategies in health and aging research. We focus on participants of the original and offspring cohorts of the Framingham Heart Study to gain insights in genetic predisposition to health and longevity by focusing on systemic processes in aging organisms. We show that lifespan and multiple aging-related traits can be controlled by

the same networks of allelic variants. This finding suggests that such phenotypes can be regulated by a common systemic mechanism likely associated with biological aging. Single nucleotide polymorphisms (SNPs) in these networks exhibit correlated patterns even so such SNPs can be on non-homologous chromosomes. Empirical Kaplan-Meier estimates show that carriers of the minor-risk alleles of these SNPs live significantly shorter lives (life expectancy [LE]=79.0 years, 95% confidence interval [CI]=78.0-80.1 years) than their major allele homozygous age-peers (LE=87.7 years, CI=87.3-88.1 years). The minor allele carriers are at highly significant risk of premature death (log rank=9.6×10-63). Survival curves for them resemble those for the major allele homozygotes except virtually parallel shift to the left suggesting faster aging rate for the risk allele carriers. Individuals carrying the risk alleles experience cardiovascular diseases and cancers up to about seven years earlier in life than the non-risk allele age-peers. This result strengthens the conclusion on premature aging of the risk allele carriers. Genes for these SNPs tend to be organized in networks associated with fundamental processes in an aging organism related to cell communication, transcriptional regulation, development, homeostasis/immunity, and stress response. The results document challenging role of gene networks in regulating health and lifespan in humans.

67. Antagonistic Association of the APOE Polymorphism with the Ages at Onset of CVD and Cancer Influences Human Lifespan

Alexander Kulminski (P), Irina Culminskaya, Svetlana Ukraintseva, Konstantin Arbeev, Liubov Arbeeva, Igor Akushevich, Kenneth Land, Anatoli Yashin
Duke University, Durham, NC

Progress in unraveling the genetic origins of healthy aging is tempered, in part, by a lack of replication of effects, which is often considered a signature of false positive findings. We convincingly demonstrate that the lack of genetic effects on an aging-related trait can be due to trade-offs in the gene action. We focus on the well-studied apolipoproetin E (APOE) e2/3/4 polymorphism and on lifespan and ages at onset of cardiovascular diseases (CVD) and cancer, using data on 3,924 participants of the Framingham Heart Study Offspring cohort. Kaplan-Meier estimates show that the e4 allele carriers live shorter lives than the non-e4 allele carriers (log rank=0.016). The adverse effect was attributed to the poor survival of the e4 homozygotes, whereas the effect of the common e3/4 genotype was insignificant. The e3/4 genotype, however, was antagonistically associated with onsets of those diseases predisposing to an earlier onset of CVD and a later onset of cancer compared to the non-e4 allele genotypes. This trade-off explains the lack of a significant effect of the e3/4 genotype on survival; adjustment for it in the Cox regression model makes the detrimental effect of the e4 allele highly significant (p=0.002). This trade-off is likely caused by the lipid-metabolism-related (for CVD) and non-related (for cancer) mechanisms. An evolutionary rationale suggests that genetic trade-offs should not be an exception in studies of aging-related traits. Deeper insights into biological mechanisms mediating gene action are critical for understanding the genetic regulation of a healthy lifespan and for personalizing medical care.

68. Declining Autophagy in Aging Non-transgenic and 3xTg-AD Mouse Mitochondrial Neurons

Aaron Barnett (P, N) and Gregory Brewer
Southern Illinois University School of Medicine, Springfield, IL

The processes of autophagy and mitochondrial ROS damage are integral parts of aging. As autophagy becomes defective, mutated molecules and organelles accumulate in the cell, interfering with normal processes, a theme in neurodegenerative disease pathology. Normal or age-induced ROS damage to mitochondria render them less efficient and in need of repair. Damaged mitochondria are typically removed through autophagy, recycling their components, to allow the production of new, more efficient mitochondria. Since neurons and other post-mitotic cells cannot dilute their cargo of defective mitochondria by going through the cell cycle, they must rely more heavily on autophagy to clear the cell of these components. Since autophagy impacts neurodegenerative pathology, the reversal or prevention of neurodegeneration through the regulation of autophagy is a worthy target. We hypothesize that stimulation of autophagy will reduce age-related and neurodegenerative pathology. If the process of autophagy is not functioning properly, damaged mitochondria will accumulate, having a negative impact on the energy producing capabilities of the cell, especially under stress. Damaged mitochondria also produce ROS at a higher rate than more efficient mitochondria, enforcing a downward spiral of more ROS damage throughout the cell. We are testing the effects that age has on the process of autophagy

and accumulation of defective mitochondria in mouse neurons in a controlled culture environment using fluorescent microscopy. Lysosome counts/cell increased until middle age, then declined in old age. Both mitochondrial and cytosolic cytochrome C increased up to middle-age then plateaued in non-Tg neurons. However, these measures were higher in 3xTg-AD neurons, suggesting compensation for damage with increased numbers of mitochondria. These steady-state measures need to be informed by measures of the rate of autophagy. The combination of more mitochondria and less lysosomes suggests that autophagy declines with age, especially in this 3xTg-AD mouse neuron model.

69. Extension of Yeast Replicative Lifespan through Nuclear tRNA Sequestration

Joe Delaney (P)
University of Washington, Seattle, WA

A wide range of proteins have been implicated in the life extending properties of dietary restriction (DR). Tor, S6 kinase, ribosomal proteins, and reduction of translation all function downstream of DR. However there are many forms of dietary restriction and each one may follow an independent pathway of lifespan extension. One intriguing possibility is that nutrient deprivation causes an increase in nuclear tRNA and restricts the cytoplasm of mature tRNA for use in translation. Transport of tRNA in yeast is regulated by the Ran dependent exportin Los1p and a high copy suppressor of los1Δ, Sol1. Deletions of Los1 and Sol1 result in heightened accumulation of tRNA in the nucleus and an extension of replicative lifespan in yeast. Lifespan extension occurred in a Sir2 independent manner and was also independent of the yeast specific extrachromosomal ribosomal DNA circle aging pathway. Remarkably, nuclear accumulation of tRNA occurs without any loss of translation as measured by growth rate assays, suggesting nuclear tRNA acts as a signaling event rather than a strategy to separate tRNAs from ribosomes in the cytoplasm. Exploration of the regulation of tRNA maturation will better define how the cell's response to nutrient starvation is translated to a longer lifespan.

70. A Role for the HDAC-Mef2 Pathway in Neuromuscular Aging

Michael Walsh (P) and Holly Van Remmen
University of Texas Health Science Center at San Antonio, San Antonio, TX

Motor neurons form a highly specialized synapse with skeletal muscle known as the neuromuscular junction, and degeneration of this neuromuscular interaction has been implicated in disease and aging. The myocyte enhancer factor 2 (Mef2) transcription factor is partially responsible for maintaining the neuromuscular junction. Recently, a role for histone deacetylases (HDAC) in the regulation of the Mef2 pathway has been established. HDAC4 and HDAC5 are expressed in skeletal muscle and interact with the Mef2 transcriptional complex. HDAC4 is concentrated at the neuromuscular junction, and during denervation, HDAC4 enters the nucleus and associates with Mef2, inhibiting its transcriptional activity.

Our laboratory and others have reported extensive neuromuscular changes with age. The goal of this study is to determine the role of the HDAC-Mef2 pathway in neuromuscular aging. We have found increased protein levels of HDAC4 in the skeletal muscle of old mice when compared to young mice. Using sciatic nerve crush, which mimics age-related neuromuscular degeneration, we have found that the HDAC inhibitor sodium butyrate (NaBu) protects against neuromuscular degeneration. Seven days post-nerve crush, the gastrocnemius in the control group lost 25% of its mass, while the NaBu-treated group lost only 11% of its mass. This HDAC inhibition prevents the induction of the muscle-specific E3 ubiquitin ligase, atrogin-1, which has been implicated in neuromuscular disease. In the superoxide dismutase 1 knockout mouse (Sod1-/-), a model of accelerated age-related muscle atrophy, Mef-2 is not induced seven days post-nerve crush, as in the control group. We have observed reductions in the sciatic nerve conduction velocities in old wild type and young Sod1-/- mice compared to young controls, and predict that NaBu treatment will protect against this functional decline of the peripheral nervous system. In conclusion, the HDAC-Mef2 pathway likely contributes to age-related neuromuscular degeneration.

71. Oxidizing Brain Neurons as We Age: NADH, Redox and Glutathione Levels Change Before ROS Damage in Alzheimer and Control Mice

Gregory Brewer (P)[1] and Debolina Ghosh[2]
[1]Southern Illinois University School of Medicine, Springfield, IL; [2]Calcutta University, India

The brain depends on redox electrons from NADH to produce ATP. Since oxyradical (ROS) damage is prominent in aging and Alzheimer's disease (AD), we wanted to know whether an oxidative redox shift is up- or downstream of ROS

production in a mouse model of AD and whether this preceded cognitive decline. We used non-transgenic (Tg) and the 3xTg-AD mouse model of AD which displays cognitive deficits beginning at 4 months. Hippocampal neurons were isolated across the age-span and cultured in a common culture environment to control for possible hormonal and vascular differences. The redox state of individual neurons was measured as NADH and FAD intrinsic fluorescence. ROS production was measured in single cells simultaneously with anti-oxidant reducing power as glutathione. We found non-Tg neurons increase NADH levels until middle age, followed by a decline in old age, while the 3xTg-AD neurons failed to generate as much NADH, which declined further in old age. The redox state followed a similar pattern with age, but continuously declined in the AD neurons. However with stress from 2 months on, the NADH regenerating capacity was significantly lower in 3xTg-AD than non-Tg neurons, both of which declined in old age. The rate of ROS production was low at 2 and 4 months for both genotypes then increased until middle age, accompanied by an increase in glutathione. However, after middle age, glutathione levels decreased, even lower in 3xTg-AD neurons. Surprisingly, macromolecular ROS damage was not detected with age or genotype. The present data suggest that a more oxidized redox state and a lower antioxidant glutathione defense can occur without ROS damage in both old age and precedes the onset of cognitive deficits in the 3xTg-AD model. Moreover, deficits in NADH regenerating capacity and glutathione synthesis in old age may be more seminal than macromolecular damage.

72. The importance of caspase-2 in age-dependent osteoporosis

Danielle Victor (P)[1], Difernando Vanegas[1], Ramaswamy Sharma[1], Meenakshi Tiwari[1], Marisa Lopez-Cruzan[1], Kathleen Woodruff[2], Sherry Abboud-Werner[2], Brian Herman[1]
[1]University of Texas Health Science Center at San Antonio, Department of Cell and Biol, [2]University of Texas Health Science Center at San Antonio, Department of Pathology

Normal bone homeostasis is maintained by balancing osteoclast-mediated bone resorption with osteoblast-induced bone formation. If this delicate balance is chronically tipped toward excess bone resorption, osteoporosis occurs. Age-related osteoporosis in men is of particular importance since mortality following fracture is higher in men than in women. We have previously shown that old male mice deficient in caspase-2, a cysteine protease involved in apoptosis, exhibit severe age-related osteoporosis. Specifically, micro-computed tomography (μCT) and dual energy x-ray absorptiometry (DEXA) analyses of long bones from old Caspase-2-/- mice demonstrated decreased bone mineral density (BMD). Furthermore, immunoblots show increased cleaved caspase-2 in old male mice compared to young. In order to assess how local bone factors are affected by the loss of caspase-2 in aging male mice, we compared levels of factors promoting osteoclast differentiation (CSF-1) and osteoblast differentiation (BMP-2) between old Caspase-2-/- and wild-type (WT) mice. Using ELISA, we found elevated CSF-1 levels (>2-fold) in whole bone lysates from old Caspase-2-/- mice compared to WT mice. Additionally, immunoblots show decreased BMP-2 levels in old Caspase-2-/- mice compared to WT mice. These results suggest that despite the increased osteoclastogenesis influenced by high CSF-1 levels, factors promoting osteoblastogenesis, such as BMP-2, are not upregulated to compensate for increased bone resorption in old Caspase-2-/- mice. Additionally, caspase-2 can act as a sensor for oxidative stress and therefore, we have also found via immunocytochemistry that osteoclasts express caspase-2 when treated with mitochondrial oxidative stressors such as rotenone and antimycin A. However, caspase-2 is not expressed in osteoclasts treated with a general oxidative stressor, H2O2. Elucidating the function of caspase-2 in bone under normal conditions and during age-dependent osteoporosis, as well as by characterizing factors involved in maintaining osteoblast-osteoclast interactions, will lead to the identification of molecular targets that may result in new and valuable anti-osteoporosis therapies.

73. Aging as the Last Epigenetic Stage of Development: How Reversible is the Histone Code with Age and Alzheimer's Disease?

Michael Walker (P) and Gregory Brewer
Southern Illinois University School of Medicine, Springfield, IL

Aging is the leading epidemiological factor contributing to the onset of Alzheimer disease (AD). A metabolic shift to an oxidized redox state is associated with aging and possibly AD. This shift is postulated to affect transcription factors which enforce epigenetic controls (EORS theory of aging). In our culture model, neurons from aged rats and mice maintain increased sensitivity to various stressors despite being grown in the same environmental conditions as young neurons. This led us to hypothesize that an imposed epigenetic

switch controls aging. Since aging and AD affect neurons within the hippocampus and cortex, determination of the epigenetic state of these cells is essential to understanding the relationship between histone modification and aging as well as the etiology of AD. Acetylation and methylation of histone lysine residues are important epigenetic modifications associated with chromatin modulation of gene transcription. We cultured hippocampal/cortical neurons from non-transgenic (non-Tg) and the 3xTg-AD mouse model across the age-span to compare neuron-intrinsic age-related and AD-model changes that might enforce some of the cognitive and pathological manifestations of aging and AD. Neurons were analyzed by single-cell immunofluorescence of acetylation and methylation levels of histones H3 and H4. Here we show in non-Tg neurons that only acetylation of H4 declines with age. But 3xTg-AD neurons have significantly higher levels of acetylation in H3 and H4 that increases across the lifespan. Histone acetylation and methylation can be rapidly changed by inhibition of histone deacetylases and methyltransferases. In agreement with higher H3K9 methylation repressing gene expression, qPCR indicates that BDNF gene expression is lower in 3xTg-AD mice and can be reversed by inhibition of a specific methyl transferase. These data show that the epigenetic states of non-Tg and 3xTg-AD mice are profoundly different and reversible beginning at 4 months when the first memory deficits are observed in this model.

74. Novel Olfactory Acuity Assay in Measuring Olfactory Perceptiveness in Mice

Samantha Rendon, Kathleen Fischer, Steven Austad

University of Texas Health Science Center at San Antonio, San Antonio, Texas

Olfactory acuity in mice is a sensory feature that not only has relevance to a mouse's survival in the wild, but as a consequence is likely informative about overall health status. Therefore, an assay utilizing serial dilutions of urine to assess variations in olfactory acuity should provide meaningful information about how a mouse's sense of smell changes as it ages. Surprisingly, age-related changes in olfactory acuity have not been previously assessed in mice.

This blinded, biologically relevant assay compares the length of time a mouse spends sniffing near a diluted sample of urine versus a distilled water control. Scents are presented from most dilute to most concentrated to identify the lowest concentration a mouse of a given age is able to detect. Mice have the ability to differentiate between urine and water at a dilution of 1:1,000; however, they usually cannot distinguish urine from water at 1:10,000 (Rendón, unpublished data). We describe a method that uses urine concentrations between 0.001 and 0.0001. Sampling in this range allows us to pinpoint and compare discriminatory ability and to more accurately assess the influence of factors, such as age, or drugs, on rodent olfactory acuity.

75. Mitochondria-Targeted Overexpression of MsrA: Is this Beneficial or Detrimental to Health- and Life-Span?

Adam Salmon (P, G)[1], Holly Van Remmen[1], Arunabh Bhattacharya[1], Yuhong Liu[1], JennaLynn Styskal[1], Daniel Pulliam[1], Rod Levine[2], Arlan Richardson[1]

[1]Barshop Institute, University of Texas Health Science Center at San Antonio, San Antionio, TX; [2]NIH/NHLBI

Mitochondria are the primary source of oxidative stress in eukaryotic cells because superoxide radicals are generated as a byproduct of the synthesis of ATP. Mitochondrial proteins are constant targets of oxidation due to their proximity to this reaction; oxidation reduces protein function, thereby leading to decreased mitochondrial efficiency and exacerbation of oxidative stress. To protect mitochondria, oxidized proteins are removed or repaired with the repair of oxidized proteins regulated primarily by methionine sulfoxide reductases (Msr). Ubiquitously expressed MsrA is the primary Msr isoform in mammals, however only ~25% of total cellular content of MsrA is localized to mitochondria. We generated mice with mitochondria-targeted overexpression of MsrA (TgMito_MsraMTS) to test the hypothesis that increased mitochondrial protein repair/reduced mitochondrial protein oxidation can extend mammalian health- and life-span. Compared to wild-type mice (WT), TgMito_MsraMTS mice express MsrA at levels >10-fold higher and with ~99% of all cellular MsrA localized to the mitochondria. Young and adult TgMito_MsraMTS mice are similar to WT in body mass, tissue masses, body composition, and glucose homeostasis. Contrary to our prediction, mitochondria from TgMito_MsrAMTS mice produce significantly more oxidative stress than WT. In mitochondria isolated from skeletal muscle and brain from TgMito_MsraMTS mice, and in fibroblasts, production of hydrogen peroxide and superoxide are 2- to 5-fold higher than WT.

Paradoxically, ATP synthesis, membrane potential, and mitochondrial respiration were unchanged in TgMito_MsrAMTS mitochondria, showing that mitochondrial efficiency is preserved despite high levels of oxidative stress. We are currently evaluating oxidation of the mitochondrial proteome to determine whether mitochondrial MsrA maintains protein homeostasis even under high levels of peroxide and superoxide generation. In addition, our preliminary lifespan analysis (through 28 months) shows that the lifespan of TgMito_MsrAMTS mice is no shorter than WT. Further exploration is needed, but these mice may represent a significant argument against the mitochondrial theory of aging.

76. Lipid Peroxidation Potential and Chain Length Contribute to Diverse Longevities of *C. elegans* Near-Isogenic Mutants

Robert Shmookler Reis(P)[1], Lulu Xu[2], Hoonyong Lee[2], Minho Chae[3], John Thaden[2], Puneet Bharill[4], Cagdas Tazearslan[5], Eric Siegel[6], Ramani Alla[2], Piotr Zimniak[7], Srinivas Ayyadevara[2]

[1]Central AR Veterans Healthcare System; University of Arkansas Medical School; [2]Department of Geriatrics, University of Arkansas for Medical Sciences; [3]National Center for Toxicologic Research, Jefferson, Arkansas; [4]Biochem & Molecular Biololgy Department, University of Arkansas for Medical Sciences, Little Rock, Arkansas; [5]Department of Genetics, Albert Einstein College of Medicine, Bronx, New York; [6]Department of Biostatistics, University of Arkansas for Medical Science Little Rock, Arkansas; [7]Department of Pharmacology, University of Arkansas for Medical Science, Little Rock, Arkansas

Many lifespan-modulating genes are involved in either generation of oxidative substrates and end-products, or their detoxification and removal. Among such metabolites, only lipoperoxides have the ability to produce free-radical chain reactions which inflict widespread damage. For this study, we created a near-isogenic panel of *C. elegans* mutants and reassessed their lifespans in a uniform genetic background. This panel provides a useful tool for the initial discovery of parameters that correlate with longevity, across a tenfold range of median adult lifespans. Fatty-acid profiles were determined by GC-MS, for N2DRM (wild-type) adults and 9 longevity mutants. Short-chain fatty acids increased, while long-chain lipids decreased with lifespan. Similarly, monounsaturated fatty acids (MUFAs) increased over 1.5-fold, whereas polyunsaturated fatty acids (PUFAs) declined 50%. As a result, increasing lifespan was accompanied by declines in average lipid chain length ($R=-0.87$, $P<0.002$; rank-order $Rs=0.49$, $P<0.003$), and susceptibility to lipoperoxidation ($R=-0.85$, $P<0.002$; rank-order $Rs=0.39$, $P<0.02$), especially in the longest-lived mutants affecting the insulinlike-signaling pathway. This suggested a functional model in which longevity benefits from a reduction in lipid-peroxidation substrates, offset by a coordinate rise in MUFAs and a decline in fatty-acid chain length, both serving to maintain membrane fluidity. This model was tested by disrupting the underlying steps in lipid biosynthesis, using RNAi knockdown to deplete transcripts of genes involved in fatty-acid metabolism. As predicted, reduced expression of genes encoding desaturases (especially fat-4, required to form the most desaturated PUFAs) and elongases (elo-1 and elo-2) extended the lifespan of wild-type adults. These functional interventions imply that wild-type worms also benefit from the "strategies" employed by the longest-lived mutants, with respect to fatty-acid abundance and expression of lipid-biosynthesis genes. Furthermore, nearly all knockdowns produced concordant effects on hydrogen-peroxide-stress survival, which can trigger lipoperoxide chain reactions – consistent with membrane resistance to lipoperoxidation contributing to the determination of lifespan.

77. Genes, GO Where I Send Thee; How Shall I Send Thee? A Novel Analytic Method for Microarray Data with Functional Categories.

Robert Shmookler Reis (P)[1], Taewon Lee[2], Robert Delongchamp[3]

[1]University of Arkansas Medical School; Central AR Veterans Healthcare System, Little Rock, Arkansas; [2]Korea University, Jochiwon, Chungnan, 339-700, Korea; [3]University of Arkansas for Medical Sciences, Little Rock, Arkansas

High-throughput transcript assays generate copious data. Current practice is to estimate by-gene variance across microarrays to define genes with "significantly differential expression" between groups, such that the false-discovery rate (FDR) remains below some arbitrary threshold such as 0.05. These lists often differ between laboratories or experiments, probably reflecting high false-positive and false-negative rates. To reduce the impact of errors and favor robust conclusions, meta-analysis is commonly performed wherein functional-annotation (e.g., Gene Ontology or GO) terms are associated with genes. Investigators then ask whether their "hit list" is significantly enriched for any category (GO-term) relative to all genes defined for that genome. This provides a partial solution to the problem, but discards most

microarray information, and inflates the significance of GO categories by ignoring correlations in expression pattern among class members. We developed an alternative meta-analysis procedure in which p-values are combined within functional categories, to assess overall inter-group significance and correct for within-category correlations. Using this procedure to reanalyze a comparison of two extremely long-lived age-1 mutants to a moderately long-lived allele [Aging Cell 8:706–725, 2009], the number of significant terms distinguishing these mutants increased from 15 to 85. Terms with =0.1% FDR now include (*,GO term previously significant; ^,trait confirmed independently): membrane*^, transmembrane receptors/transport*, adult lifespan determination^, zinc-ion binding*, iron-ion binding*, calcium-ion binding*, growth^, reproduction^, larval development^, protein binding, cytochrome-C oxidase, lipid storage^, transcription factors*, DNA binding*, mitochondrial inner membrane, sensory perception of chemical stimuli^, locomotion^, metabolism*^, morphogenesis^, intracellular signaling^, oxidation-reduction*^, TGF-ß signaling^, tRNA modification, ATP binding*, FAD binding, heme binding, collagen^, protein/amino-acid methylation, voltage-gated K+ channel, ion transport, protein kinase*^, receptor-mediated endocytosis, queuine-tRNA-ribosyltransferase, peptide receptor, vulval development^, glutamate receptor, cytoskeleton, dauer entry^, alcohol dehydrogenase, acyl-coA dehydrogenase, carbohydrate metabolism^, and aconitase^. Thus, our novel procedure for functional-annotation analysis improves both sensitivity and specificity of selection for altered categories.

78. Enhanced Leptin Sensitivity Leads to Obesity Resistance and Healthy Aging In A PKA-Disrupted Mouse Model

Linda Enns (P, G), Hannah Richards, Ken Bible, Warren Ladiges
University of Washington, Seattle, WA

Protein kinase A (PKA) plays a major role in the genetic regulation of obesity and energy balance. Mice lacking the Cß subunit of PKA are resistant to diet-induced obesity. We have found that while these mice had similar levels of both activity and food intake compared to WT, they showed 6% higher metabolic rates (VO2)(P<0.01).Although their metabolism was enhanced, serum leptin levels were over 3 times lower than WT (P=0.026).Young Cß null mice were found to have similar resting metabolic rates compared to WT, but when injected twice daily with leptin (4 µg/g) for a week, showed 11% higher VO2 (P<0.0005). Current aging studies have shown that mutants are also resistant to age-related obesity. By 12 months of age, WT females displayed body fat percentages of 28% compared to 24% for Cß null mice (P=0.016). By 24 months of age, 43% (20/43) of an aging WT cohort had body weights corresponding to almost 40% body fat; none of the Cß null mice had achieved this body weight. Livers of WT mice at this age were 15 % larger than mutants (P=0.028). Healthy aging was especially noticeable in the hearts of 24 month-old Cß null mice. At this age, WT mice had enlarged hearts, 20% heavier than mutants (P=0.024). Echocardiography and doppler imaging showed diastolic dysfunction in the WT mice, attenuated in the mutants, whose hearts had significantly higher Ea/Aa ratios, MPI, and fractional shortening and lower ventricular circulation times and aorta/left atrium ratios. Mutants at 12 months of age showed serum leptin levels almost half that of WT (P=0.05), and indirect calorimetry showed metabolic rates to be on average 7% higher in the mutants (P<0.05). We conclude that leptin sensitivity in in the Cß null mouse leads to enhanced metabolic rates, and resistance to age-induced obesity and related pathologies.

79. Impact of Calorie Restriction (CR) on Cellular Aspects of Metabolic Decline in Aging Rhesus Skeletal Muscle.

Rozalyn Anderson (P)
University of Wisconsin-Madison, Madison WI

In skeletal muscle, mitochondrial integrity is a key component of healthy tissue. Mitochondrial dysfunction has been associated with aging, diabetes and metabolic syndrome. We have identified a shift in skeletal muscle fiber type distribution in rhesus monkeys that is coincident with the early stages of sarcopenia. Age negatively impacts the activity of the mitochondrial electron transport system and the intracellular distribution of the most active mitochondria. Autofluorescent detection using multiphoton imaging indicates that levels of both NADH and FAD+ metabolic cofactors decline with age; and fluorescent decay lifetime analysis indicates that the cellular milieu is altered. A change in lipid distribution that is consistent with mitochondrial insufficiency is observed. Microarray analysis points to altered lipid metabolism as a possible mechanism by which CR maintains skeletal muscle mass. These data indicate that innate mechanisms of cellular adaptation contribute to aging, and suggest CR

specifically activates these metabolic pathways to promote longevity.

80. A Novel Conserved Mechanism of Extended Longevity in a Mouse Model of Mitochondrial Dysfunction

Daniel Pulliam (P, N), Deepa Sathyaseelan, Yuhong Liu, Holly Van Remmen
University of Texas Health Science Center at San Antonio and Barshop Institute for Aging, San Antonio, TX

Dysfunctional mitochondria can lead to disruptions in ATP production and increase the reactive oxygen species related to aging. Previously it was thought that dysfunctional mitochondria would lead to a decrease in lifespan, however mutations or knockdowns resulting in a decrease in the activity of the mitochondrial electron transport chain have been shown to increase longevity in C. elegans, drosophila and recently mice. However, in C. elegans, disruption of the mitochondrial unfolded protein response (mtUPR) reverses this lifespan extension. The mtUPR is a retrograde signaling response resulting in an increase in mitochondrial specific chaperones and proteases aimed at refolding misfolded proteins and degrading damaged proteins.

Here we explore a novel conserved mechanism of longevity in the mitochondrial complex IV deficient Surf1 knockout mouse. Knockout of Surf1, which codes for a complex IV assembly factor, results in a 50-80% decline in complex IV activity and a 20% increase in median lifespan. This dysfunction results in tissue specific differences in membrane potential, ATP production and decreased oxygen consumption in isolated fibroblasts. Interestingly, these fibroblasts are more resistant to the superoxide generator paraquat and the protein unfolding effects of heat shock. Here we show that these mice have an increase in mitochondrial specific chaperones (HSP60) and proteases (CLPP) that have been linked to mtUPR providing a possible mechanism for the increased resistance to cellular stresses. Taken together, the Surf1 knockout mouse could have enhanced longevity due to a novel mechanism conserved from invertebrates to mammals. This project is supported by a grant from the Ellison Medical Foundation.

81. Longevity and Proteome Stability in Marine Bivalves

Stephen Treaster (P, N)[1], Keith Maslin[1], Iain Ridgeway[2], Asish Chaudhuri[1], Steven Austad[1]
[1]University of Texas Health Science Center; University of Texas Health Science Center; [2]Bangor University, Bangor Whales, UK

Marine bivalves provide a unique and powerful model system for comparative studies of aging. The different species encompass a wide range of lifespans, from one to over five hundred years as determined by counting growth rings in the shell. Many informative species are available commercially at minimal costs. This combination eliminates the waiting involved in traditional aging models and gives researchers easy access to an exceptional range of validated ages.Using this model system, we are investigating the relationship between proteome stability and lifespan. Specifically, we are testing for markers of oxidative and unfolding damage to proteins in basal and under stressed conditions. Novel fluorescent based assays were utilized, capable of directly binding to the damaged structures of the protein. The fluorescein-5-thiosemicarbazide (FTC) probe binds to carbonyls, which are biomarkers of oxidative damage to proteins. 6-iodoacetamidofluorescein (6-IAF) binds to free thiols, a measurement of cysteine oxidation. The apolar BisANS probe binds to hydrophobic regions of proteins exposed as they unfold. To date, we find that under temperature, urea, and TBHP stress, protein unfolding decreases with increasing lifespan. This corresponds with the proteome stability theory. The theory also predicts that longer-lived species should exhibit reduced oxidative damage markers. Surprisingly, carbonyl levels in the soluble fraction increased with lifespan, yet did not increase in insoluble, aggregated proteins. This suggests that despite the presence of carbonyls in the soluble fraction, these proteins may not be functionally compromised. Furthermore, long-lived species exhibited higher free thiol levels in the nuclear and ribosomal fractions, indicating reduced cysteine oxidation. However, free thiol levels were lower in the mitochondrial fraction. This could be due to selection against oxidation prone cysteines in the mitochondrial genome, as the longer-lived species would benefit from more stable residues in their place.

82. The *C. elegans* Ribosome: An Aging Factor and a Model for Human Ribosomopathy

George Sutphin (P, G)
University of Washington, Seattle, WA

Ribosomopathy is a class of human disease resulting from impaired ribosome biogenesis. Ribosomopathies typically occurs as a result of

mutations in genes encoding components of the ribosome or ribosome processing factors, and include cases of Schwachman-Diamond syndrome, Diamond-Blackfan Anemia, X-linked dyskeratosis congenita, cartilage hair hypoplasia, Treacher Collins syndrome, and 5q syndrome. These diseases share a recurring array of clinical features, including anemia, craniofacial defects, elevated cancer risk, and short stature. Pathology is thought to result from ribosome insufficiency through activation of p53 and subsequent cell cycle arrest and/or apoptosis in effected tissues. In support of this hypothesis, ribosomal proteins (RPs) have been shown to influence p53 activity through two separate mechanisms. First, RPs interact with MDM2/HDM2 to regulate p53 ubiquitination and degradation. Second, several RPs have been shown to directly interact with p53 mRNA to regulate translation. In Caenorhabditis elegans, we find that knockdown of RPs leads to elevated occurrence of protruding vulva (Pvl) and ruptured vulva (Rup), which we collectively refer to as vulva integrity defects (Vid). The increase in Vid in response to RP deficiency is prevented by inactivation of the C. elegans p53 functional ortholog, CEP-1. This parallel regulation of p53/CEP-1 by RPs suggests that Vid in C. elegans may represent a model for human ribosomopathies. In addition to the role of RPs in ribosome-specific disease, RPs and mRNA translation have been implicated in aging. In worms, reduced expression of genes encoding proteins of both the large and small ribosomal subunits extends lifespan. Here we show that the lifespan extension and the increased Vid in response to RP knockdown are highly dependent on both age and temperature, and on the presence of CEP-1. We also explore the mechanistic role of CEP-1 mediated apoptosis and cell cycle arrest in both processes.

83. Life Span in Wild-Type and p66Shc-/- Mice Fed 5% or 40% Calorie Restricted Diets.

Roger McDonald[1], Dianna Tran[2], Kevork Hagopian[2], Gino Cortopassi[2], Kent Lloyd[3], Steve Griffey[4]

[1]University of California, Department of Nutrition; [2]University of California, Molecular Biosciences; [3]University of California, Anatomy/Physiology & Cell Biology; [4]University of California, Comparative Pathology Laboratory

A previous investigation reported that knocking out the gene for the p66Shc protein increases mean and maximal life span in mice. While several investigations have evaluated possible mechanisms for the life extension, the life span study has not been replicated. We evaluated the life span characteristics in the identical strain of mice used in the original investigation. To this end, 100 p66Shc knockouts (KO) and 100 wild-type (WT) mice were fed ad libitum a chow diet until 4 months of age. Then, each strain was divided into two groups, 50 animals receiving daily 95% of ad libitum food intake and 50 animals receiving daily 60% of ad libitum intake for the remainder of their life span. Body weights were collected weekly on all mice. Mice were allowed to live out their natural life span or were euthanized when the investigators determined that death was imminent. Mean and maximal life span of the KO and WT mice fed 95% ad libitum did not differ significantly, although body weights were significantly less in the KO animals. With approximately 40% of the 60% -fed mice alive at the time of this submission, the KO vs. WT animals have a median life span that appears significantly greater, 1078 vs. 1002, respectively. The casue of death and common age-related histopathology did not differ between the KO and WT mice fed 95% or 60% ad libitum. While life span is not increased in KO compared to WT mice fed a 5% CR diet, there is evidence for life span increases in the KO vs. WT mice when consuming a 40% CR diet. The results of this study suggest that that decreases in Shc level may make animals especially receptive to life span extension with CR

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84. Aspirin Extends Caenorhabditis Elegans Lifespan and Ameliorates Aging-Associated Functional Decline

Puneet Bharill (P, N)[1], Srinivas Ayyadevara[2], Abhijit Dandapat[2], Magomed Khaidakov[3], Changping Hu[3], Robert Shmookler Reis[4], Jawahar Mehta[3]

[1]University of Arkansas; [2]Central Arkansas Veterans Healthcare Center; [3]University of Arkansas, Department of Medicine; [4]University of Arkansas, Department of Geriatrics, Biochemistry, Little Rock, Arkansas

Inflammation and oxidative stress are associated with aging. Acetylsalicylic acid (aspirin) is a widely used anti-inflammatory drug which is also used for prevention of myocardial infarction and stroke. Both of these conditions are associated with oxidative stress and inflammation, and may be considered partial surrogates of aging. In this study, we examined aspirin's ability to modulate lifespan of Caenorhabditis elegans. We observed that both aspirin and its salicylic acid moiety, each at a non-toxic concentration, significantly extended the lifespan of *C. elegans* by 20-25% (each $P<0.01$). Both agents also attenuated the toxicity of

5-mM hydrogen peroxide for *C. elegans*. The lifespan-enhancing effect of aspirin requires a functional FOXO transcription factor, DAF-16, since no life extension was observed in *C. elegans* carrying an inactivating mutation in the daf-16 gene. We next studied the effect of aspirin on age-associated declines in motility and pharyngeal pumping, reliable indices of physiological decline in aging *C. elegans*. In keeping with the extension of lifespan, aspirin significantly prevented or delayed the age-associated declines in motility and pharyngeal pumping. Furthermore, aspirin treatment reduced the formation of protein aggregates, a robust molecular correlate of *C.elegans* aging, inversely related to longevity. Aggregate formation was significantly reduced by aspirin in older worms, but did not affect the lower levels seen in young worms. Although other mechanisms are possible, the most parsimonious explanation for all of the above data is that aspirin delays the aging process itself, as reflected by diverse biomarkers of physiological and molecular aging.

85. Differential Expression of DNA Methylation Enzymes in the Ames Dwarf Mouse

Vanessa Armstrong (P, N), Sharlene Rakoczy, Holly Brown-Borg
University of North Dakota, Grand Forks, ND

Methylation reactions are important to establish and maintain the epigenetic methylation tags on DNA and histone molecules that are critical to the development and life-long function of an organism. DNA and histone methylation are altered in aging, thus one aspect of increased pathologies associated with aging could be a gradual process resulting from epigenetic dysregulation. Ames dwarf mice are growth hormone deficient, live 50-68% longer than their wild type counterparts, exhibit increased liver methionine enzyme activities, reduced SAM:SAH ratios and differential methylation patterns without increased disease pathology. We hypothesized that the mechanisms maintaining and creating methylation patterns differ between dwarf and wild type mice. The main enzymes responsible for the overall methylation patterns of DNA, DNA methyltransferase 1 (Dnmt1), and DNA methyltransferases 3a and 3b (Dnmt3a and Dnmt3b), were examined in a cross-sectional study at three different age groups (3, 12 and 24 months). Dnmt1, Dnmt3a and Dnmt3b mRNA expression levels were increased in dwarf mice when compared to wild type mice ($p<0.003$). In addition, 3 and 12 month old dwarf mice exhibited a greater abundance of Dnmt3a protein levels (>100%) when compared to their wild type counterparts. These differences suggest that maintenance and de novo methylation of DNA in the dwarf mouse may play an important role in the lifespan extension of growth hormone mutant mice.

86. XIAP Regulation by Asparagine Deamidation and PIMT

L Solorzano (P, G)[1], B Powell[2], J Lowenson[3], J Enama[2], S Clarke[3], O Fearnhead[4], E Alnemri[1]
[1]Thomas Jefferson University, Philadelphia, PA; [2]US Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD; [3]University of California; [4]National University of Ireland

X-linked IAP (XIAP) is a major member of the Inhibitor of Apoptosis Proteins (IAP). It interacts and strongly inhibits pro-apoptotic proteases called caspases. Then XIAP down-regulation by proteasome degradation or fragmentation by cytosolic HtrA2/Omi is often a requisite during apoptosis commitment. On parallel XIAP expression is up-regulated in different types of cancer while down-regulated during aging. In the present work we aimed to further the current understanding of post-translational regulation of XIAP expression. Analysis by two-dimensional electrophoresis evidenced ubiquitous post-translational modifications of XIAP. However the pattern of post-translational modifications changed on parallel to XIAP down-regulation during synchronized apoptosis of human cell lines induced by nocodazole. Analysis by mass spectrometry indicated that XIAP contains asparagine deamidation in the linker regions. These asparagines sites were found to be highly conserved during evolution and similarly located in other IAP family members. Anti-aging protein-L-isoaspartyl-methyl-transferase (PIMT) and mutations of deamidated sites altered the two-dimensional pattern and confirmed the nature of XIAP post-translational modifications. Mutations of deamidated sites and changes in culture media pH were found to regulate XIAP expression. However the direction or degree of regulation depended on the number of modified asparagines rather than the position along XIAP. Likewise PIMT chemical inhibition, stable down-regulation by shRNAi or deficiency in mouse embryos significantly regulated XIAP expression. Furthermore, XIAP anti-apoptotic activity reflected protein expression changes induced by asparagine deamidation. Therefore we propose that asparagine deamidation and PIMT regulates the expression and anti-apoptotic activity of XIAP.

87. Monogonont rotifers as a new model system: a survey of aging-related genes

Kristin Gribble and David Mark Welch
Marine Biological Laboratory, Woods Hole, MA

Rotifers are a valuable new invertebrate model to investigate the cellular, molecular, and genetic processes of senescence, homeostasis, and tissue repair. Among the most experimentally tractable basal triploblast animals, rotifers provide evolutionary breadth to comparative studies of aging. They appear not to have undergone the extensive genome reduction of many traditional model species, and thus may share with humans many aging-related genes absent in D. melanogaster and C. elegans. Rotifers have many additional features making them attractive as a study system for aging, including alternating sexual and asexual reproduction, allowing asexual propagation of clonal cultures and comparison of how reproductive mode influences lifespan in the same genetic background; haploid males, allowing direct expression of alleles and simplified crosses; highly stable diapausing eggs; and nearly a century of aging-related research combined with growing genomic and transcriptomic tools. Finally, the monogonont rotifer genus Brachionus contains many well-studied, closely related isolates that differ in their response to stimuli that influence aging, such as temperature and dietary restriction, facilitating genetic dissection of the aging process. Here we present a survey of next-generation transcriptomic and genomic libraries of three Brachionus species for homologs of more than 100 genes known to be involved in aging in humans and other model systems, including genes in insulin-activated, mitogen-activated, stress-activated, DNA damage response, FOXO, Sirtuin, TOR, PI-3K and AMPK pathways; and report the initial results of differential gene expression between isolates Brachionus in which dietary restriction has different impacts on longevity.

88. Systems Biology of Human Aging 2011 Network Model

John Furber
Legendary Pharmaceuticals, Gainesville, FL

The many observable signs of human senescence have been hypothesized by various researchers to result from several primary causes. Close 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 diagram is presented to aid in conceptualizing the many processes and interactions among them, including 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
Several researchers have proposed to adapt the network model's contents into an interactive website with links to references and background materials. A symposium to promote this development was held at Arizona State University, December 2008; abstracts are at http://legendarypharma.com/meetings/2008ASU_SysBioAging/aging.html
http://circas.asu.edu/symposia/aging/ .
A second symposium was held 8-9 Dec 2009 at the National Institute on Aging in Baltimore, Maryland. This network model includes both intracellular and extracellular processes. It ranges in scale from the molecular to the whole-body level. Important pathways include:

- Extracellular proteins become damaged. Altered environmental niches for cells contribute to transdifferentiation, arrested cell division, cell death, cancer, stem cell depletion, tissue wasting, neurodegeneration, and organ malfunction.
- Lysosomes accumulate reactive, crosslinked lipofuscin.
- Mitochondrial DNA mutates.
- Lamin-A spice-variant, progerin, accumulates in nucleus, impairing cell division.
- Nuclear envelope pore proteins become oxidized.
- Nuclear mutations, telomere shortening, chromosome breaks, chromatin alterations and epigenetic DNA adducts change gene expression.
- Oxidized aggregates in cytoplasm become crosslinked.
- Increased redox poise alters signaling and enzyme activities, and erodes telomeres.
- Inflammatory cascades, promoted by damaged molecules and sick cells, further damage tissues.
- Neuroendocrine and immune systems degrade.
- ER stress: Misfolded proteins accumulate in ER.

89. An Observational Assessment of Aging in Laboratory Rats: Strain Comparison

Pamela Phillips(P)[1], Kimberly Jarema[1], David Kurtz[2], Christopher Gordon[1], Robert MacPhail[1]
[1]US Environmental Protection Agency, [2]Experimental Pathology Laboratories, Research Triangle Park, NC

The growth of the aging population highlights the need for laboratory animal models to (1) efficiently monitor the health of aging research colonies, and (2) aid in unraveling the mechanisms of susceptibility to toxic chemicals and disease. An observational assessment was developed that provides rank scores of appearance, posture, mobility, and muscle tone on a 5-point scale. A score of 1 indicates no impairment while a score of 5 indicates severe impairment. The assessment of a single rat can be completed in about one minute. The assessment was applied to male Brown Norway (BN) rats between the ages of 12 and 36 months (n=32), and to male Long Evans (LE) rats between the ages of 5 and 23 months (N=15). BN rats are a popular strain in aging research, while LEs are popular in neuroscience but have not been used as extensively in aging research. In BN rats, aging-related signs of impairment did not appear before 18 months. Assessment scores for each of the measures subsequently increased with age. Variability in scores also increased with age. In contrast, scores for male LE rats showed clear impairment at 5 months of age. LE impairment scores increased to a maximum that was reached at 17 months, an age at which there was no evidence of impairment in BN rats. Further analysis indicated the age at which half-maximum impairment was reached was 5 months in LEs compared to 24 months in BNs. The observational assessment provides an efficient means to chart aging in rats and can detect substantial strain differences in the aging trajectory.

This abstract does not necessarily reflect USEPA policy

90. Increased Median Life Span and Overall Longevity in FAT10ko Mice: A Novel Model Linking Adiposity, Inflammation and Aging

Allon Canaan(P, G)[1], Jason Defuria[2], Eddie Perelman[3], Hui-Young Lee[1], Varman Samuel[1], Martin Obin[2], Sherman Weissman[1]
[1]Yale University School of Medicine, New Haven, CT; [2]Tufts University; [3]Ben Gurion University, Israel

The HLA-F adjacent transcript (FAT10/UBD) encodes a protein with duplicated ubiquitin-like sequences which conjugates to target proteins. FAT10 expression is highly induced by inflammatory mediators (TNF-α, INF-γ) and FAT10ko mice demonstrate increased susceptibility toward LPS - implicating FAT10 involvement in inflammatory responses. Surprisingly, FAT10ko mice exhibited delayed onset of aging biomarkers (grey hair, sarcopenia, decreased activity) and improved metabolic profile. FAT10ko mice show an overall reduction of adipose tissues and a dramatic decrease of perigonadal (~visceral) adipose mass. Visceral adipose tissue decrease is positively associated with life span in mammals, and emerging evidence implicates reduction in fat mass as a mechanism underlying the anti-aging effect of caloric restriction. To study the physiologic role of FAT10, we examined features of adipose tissue and metabolic homeostasis, inflammation and aging. In contrast to WT mice, FAT10ko mice maintain a lower weight as they age. Leaner body composition was manifested as early as 10 weeks in FAT10ko mice by necropsy and MRI (45% less adipose tissue). They have a higher respiratory exchange ratio and are metabolically more active as demonstrated by caloric intake and energy expenditure. Insulin and glucose tolerance tests revealed significantly enhanced glucose tolerance and increased insulin sensitivity. RNA-Sequencing analysis identified FAT10 as the third most highly up-regulated gene in intra-abdominal adipose tissue of obese, insulin resistant WT mice. Obesity-associated up-regulation of the FAT10 gene occurred selectively in adipocytes during 12 weeks of high fat diet (>100-fold increase by QPCR), coinciding with increasing adiposity, adipose tissue TNF-α expression and macrophage infiltration. Mortality analysis of FAT10ko and WT was performed by the Kaplan-Meir method. FAT10ko mice demonstrated an increase in total (~20%) and median life span (~19%). These data were statistically significant by Wilcoxon, Mantel-Heanszel and Tarone-Ware tests ($P<0.0001$). These observations implicate FAT10 as a modulator of metabolic and inflammatory stresses and aging.

91. C57BL/6 Life Span Study: Muscle Function and Dysfunction

T. G. Graber (P, N), J-H Kim, R. W. Grange, L. K. Mcloon, L. V. Thompson
University of Minnesota, Medical School, Minneapolis, MN

Loss of muscle power by the elderly correlates with reduction in functional ability, loss of independence and may contribute to the onset of frailty. The primary purpose of this study is to investigate whether age-induced changes in functional performance (power=force x velocity) are associated with changes in key velocity regulating proteins (e.g., myosin light chains--MLC). A secondary goal is to examine whether there is an age-associated decline in motor function and contractility, and to establish Rota-rod and inverted grip tests as biomarkers of frailty and as predictors of protein expression for documenting mouse healthspan. We are evaluating five age

groups of C57BL/6 mice: juvenile (2 months), young (6 months), adult (12-20 months), old (24 months) and elderly (28 -30 months). Rota-rod (measuring overall motor function) and the inverted grip test (measuring strength) describe function at the organism level. Whole skeletal muscle contractility (e.g., force, force-frequency and force-velocity/ power) using in vitro methodology portrays tissue level function. Electrophoresis (with mass spectrometry identification) and Western immunoblots provide identification of skeletal muscle proteomic profiles. We found a significant reduction in performance on both the Rota-rod and grip tests between the young and old groups. There is a trend of decreases in peak twitch (Pt) and maximal isometric force (P0), a shift to the right in the force frequency curve and a decrease in maximal power in the old and elderly groups. Proteomic data show age-specific expression levels including a significantly lowered expression of MLC3f starting with the old group. Overall, the data support the concept of an age-related decline in performance markers mirroring a decrease in muscle contractility, and altered expression of proteins that control velocity. In addition, this work implies that the Rota-rod and grip tests may ultimately prove to be valid predictors of frailty in mouse models.

92. Comparative Biology with New Invertebrate Models to Test Theories of Aging and Senescence

Terry Snell (P) and Allison Fields
Georgia Institute of Technology, Atlanta, GA

Comparative biology has a central role in aging studies and new invertebrate models of senescence can be especially informative. Animals in the phylum Rotifera have an interesting life cycle, including sexual and asexual reproduction, two kinds of diapause, adult cells that are all post-mitotic, and minimal inflammatory response. There is a growing appreciation of epigenetic phenomena in aging through the accumulation of post-translationally modified proteins. Some epigenetic modifications are developmentally programmed like DNA methylation, but others are stochastic like ROS damage and crosslinking. Oxidative damage compromising proteome stability can be retarded by slowing the rate at which metabolism creates ROS or by removal of ROS by scavenging antioxidants. If true, exposure to certain antioxidants should reduce rates of senescence and extend lifespan. A complex network of antioxidants has evolved to manage oxidative damage and we screened 16 antioxidants from various categories of this network, including vitamins, thiols, peptides, enzymes, and plant derivatives, for their ability to extend rotifer lifespan. Rotifer exposure to 20-100 μM concentrations of five antioxidants (indole 3-propionic acid, melatonin, ß-carotene, EUK-8, and pyrroloquinoline quinone) significantly extended lifespan from 14-44%. Life extension was achieved by extending the reproductive and post-reproductive age classes, whereas the pre-reproductive age classes were unaffected. Concentrations of superoxide in rotifer mitochondria were quantified with MitoSOX, a selective fluorescent probe. As rotifers aged from birth to the mean lifespan of 8 days, superoxide concentrations in mitochondria fell 6-fold. Superoxides did not accumulate during 25 years of diapause and rotifers hatching from diapause eggs had 2.4X less superoxide in their mitochondria than those hatching from non-diapausing eggs. Thus, diapause may reduce the superoxide load with which rotifer hatchlings begin their life. It is plausible that the protective mechanisms that enable diapause and defend cells from oxidative damage could also extend life in non-diapausing animals.

93. Ovariectomy and Dietary Restriction Show Different Storage Levels but Similar Feeding Rates & Life Extensions in Grasshoppers

John Hatle (P)[1], Michelle Drewry[1,2], J. Williams[1], Alycia Macdonald, James Kellenberger[1]; Ephraim Viray[1]
[1]University of North Florida; [2]Jacksonville University, Jacksonville, FL

Dietary restriction (DR) and reduced reproduction each extend life span in females of most animals, but their relationship is unclear. Typically, DR reduces fecundity. This leads to the question of whether the two treatments are extending life span in similar ways. Here, we address whether life extensions via DR and OVX act through similar mechanisms in lubber grasshoppers. First, we tested whether the life extensions due to DR and OVX were additive. Treatments were fully fed and sham-operated (ShamFD), fully fed and ovariectomized (OVX FD), diet restricted and sham-operated (ShamDR), and OVX DR. Median life spans were: ShamFD=245 d, OVX FD=285 d, ShamDR=286 d, and OVX DR=322 d. Proportional hazards models revealed an effect of DR (P=0.001) and an effect of OVX (P=0.003). There was no interaction of DR and OVX (P=0.439), implying an additive relationship. The ShamDR group consumed 70% of that eaten by the ad libitum group, while OVX FD consumed 64%

of ad libitum, and OVX DR consumed 44% of ad libitum. Hence, the OVX females naturally reduced their feeding. These same females were bled every ~30 d. As seen previously, vitellogenin (the egg yolk-precursor protein) levels were ~5-fold higher in OVX females, but were not affected by DR. Total anti-oxidant activity levels (per ug protein) in the hemolymph were significantly reduced by OVX ($P=0.017$), but were not affected by DR ($P=0.347$). In a follow up experiment, ingestion rates of sham and OVX females were matched. Ovariectomy doubled hemolymph volume and fat body mass (indexes of storage in insects; $P<0.0001$), but DR did not affect storage ($P=0.178$). These data suggest that DR and OVX produce very different physiological responses, despite similar life extensions and feeding rates. The two treatments may extend life span via different mechanisms.

94. Ultraviolet Exposure Extends Lifespan in *Brachionus manjavacas (Rotifera)* Through Hormesis

Jarrett Smith (P), Allison Fields, Terry Snell
School of Biology, Georgia Tech, Atlanta, GA

Interest in hormesis has recently increased in aging research. Hormesis occurs when an organism mobilizes cellular defenses in response to low doses of a stressor and receives protection from processes like oxidation that cause cellular damage and senescence. A common result of a hormetic response is extended lifespan and increased reproduction. Hormesis has been demonstrated in C.elegans and Drosophila in response to numerous stressors including caloric restriction, heat shock and osmotic shock. The phylum Rotifera is comprised of over 2000 species, and as Lophotrochozoans, are only distantly related to the Ecdysozoans C. elegans and Drosophila. Rotifers have a life cycle that provides interesting opportunities for testing theories of aging, including asexual and sexual reproduction, eutelic development, haplo-diploid sex determination, and diapause. Furthermore, life table analysis can be easily performed and many stressors can be tested for hormesis in just a few weeks. We investigated whether brief exposures to UV light were able to induce hormesis in the rotifer Brachionus manjavacas. After screening various exposure intensities and durations, we found the most effective conditions producing hormesis to be four minutes of UV exposure (2.6 mJ cm-2 of 312 nm) at the beginning of the rotifer lifespan (mean lifespan 8 days). These exposures caused a significant life extension of up to 36%, with a small effects on reproduction. UV exposure of 8 minutes caused 70% of rotifers to exhibit a prematurely aged phenotype in middle age, including a decreased length/width ratio, slower swimming, cessation of reproduction, and increased opacity of the pseudocoelom. Rotifer life extension through hormesis also was achieved by exposure to hyposomotic stress and high temperature.

95. Postreproductive Life Span - How Could it Evolve

Josh Mitteldorf(P)[1] and Charles Goodnight[2]
[1]University of Arizona, Tucson AZ; [2]University of Vermont, Burlington, VT

Menopause in females was once regarded as a phenomenon unique to social mammals that nurture their young. But in recent years, it has been discovered that post-reproductive life span (PRLS) is ubiquitous in nature: Post-reproductive populations of many bird and mammal species, fish, even worms and budding yeast have been identified in nature. This phenomenon cannot be explained with kin selection. All three of the widely-accepted evolutionary theories of senescence predict that PRLS should be absent. We propose that the function of PRLS is ecological: it helps to stabilize population dynamics, preventing local extinctions. In times of famine or epidemic, vulnerability of the youngest and smallest individuals poses a special danger to the population. Reproduction can be effectively blocked, and the population risks extinction. If a senescent, post-reproductive sub-population is the most vulnerable age class, they can serve as a buffer, absorbing the threat, protecting the youth, helping to promote survival through the crisis. With an individual-based computational model derived from the classic Lotka-Volterra equations, we show that (1) an age class of small, immature and vulnerable individuals de-stabilizes the population dynamic, and (2) a post-reproductive senescent class that is even more vulnerable than the very young can stabilize the dynamic, and decrease the frequency of extinctions. In a meta-population version, the model evolves both senescence and post-reproductive life span.

96. Daphnia: A Model System for Understanding Genome-Environment Interactions in Aging.

Jeffry Dudycha (P), Rekha Patel, Sean Place
University of South Carolina, Columbia, SC

Daphnia are small freshwater crustaceans that have long been a model system in ecology and population genetics. Recently, the entire genome of

Daphnia pulex was sequenced and shown to be a closer match to humans than are other arthropods, including ~1000 human homologs not found in Drosophila. Daphnia have a life cycle that incorporates both asexual and sexual phases. Thus, natural populations can harbor large amounts of genetic variation, but researchers can produce many genetically identical individuals in the lab easily. We have begun developing this as a model system for understanding the biology of aging, focusing in particular on the clone whose genome was sequenced (TCO) and natural populations that have been selected for rapid and slow aging. We show two-fold genetic variation in median lifespan (30 – 60 days) between different populations of Daphnia. In addition, we see moderate extension of lifespan in the TCO clone in response to resveratrol, diminished responses to heat shock in old Daphnia of long-lived genotypes, and age-dependence in the capacity to regenerate excised antennae. We have also conducted molecular evolutionary analyses of genes involved in aging-related pathways (e.g., Sirtuin homologs), and document positive selection on key regions of these genes associated with the divergence of short- and long-lived Daphnia.

97. *p16-LUC*: an *in vivo* reporter allele for cancer and aging

Christin E. Burd (P)[1,3], Kelly S. Clark[1,3], Jessica A. Sorrentino[3,4] and Norman E. Sharpless[1,2,3]
Department of [1]Genetics, [2]Medicine, [4]The Curriculum in Toxicology and [3]The Lineberger Comprehensive Cancer Center, University of North Carolina School of Medicine, Chapel Hill, NC

The $p16^{INK4a}$ tumor suppressor is upregulated and serves to limit proliferation in response to cellular stressors and oncogenic events. Induction of $p16^{INK4a}$ is highly dynamic during tumorigenesis and aging, ranging from undetectable in unstressed cells to $>10^5$ mRNA copies per cell when maximally activated. This property, led to the hypothesis that $p16^{INK4a}$ could be an excellent marker of tumorigenesis and aging. Toward that end, we developed a novel knock-in mouse model, *p16-LUC*, wherein the endogenous $p16^{INK4a}$ promoter drives single-copy expression of firefly luciferase. Using homologous recombination in ES cells, the open reading frame of firefly luciferase was inserted in-frame at the transcriptional start site of $p16^{INK4a}$, preserving the structure and regulation of other genes ($p19^{ARF}$ and $p15^{INK4b}$) encoded by the *Cdkn2a/b* locus. Murine embryo fibroblasts (MEFs) derived from *p16-LUC* animals demonstrated marked ($>10^5$) upregulation of luciferase activity with culture, paralleling that observed for the endogenous allele. To test the ability of this allele to serve as a biomarker of oncogenic stress, we crossed *p16-LUC* mice to genetically engineered mouse models (GEMMs) of human cancer. In these animals, potent induction of luciferase activity was apparent in tumors driven by a wide variety of oncogenes. Moreover, weeks before visual or palpable tumors were detected, *p16-LUC* expression localized to future tumor sites. To detect endogenous *p16-LUC* expression, we crossed this allele to an immune competent, hairless mouse strain (SKH1-E). In these animals, endogenous luciferase activity was readily detectable and increased following exposure to ionizing radiation. Together, our findings show that the *p16-LUC* allele has potential to advance the study of *de novo* tumor formation and aging by providing a mechanism to visualize the dynamics of these processes *in vivo*.

98. Transcriptional and translational changes across species; profiling of skeletal muscle during aging

Evi M Mercken (P, G), Bethany A Carboneau, Julie A Mattison and Rafael de Cabo
Laboratory of Experimental Gerontology, National Institute on Aging, National Institutes of Health, Baltimore, MD

Aging is associated with a loss of muscle mass, strength and function, a condition known as sarcopenia. These age-associated changes have a devastating effect on quality of life in the elderly and the healthcare related expenses are enormous. At the present, there are no effective therapies to treat this condition. While sarcopenia occurs in all species, the impact of aging on muscle wasting may be different across species. As such models of sarcopenia and any effective therapy gained from understanding these models may not be translatable to humans. Therefore, the purpose of this study is to examine the effects of aging on global mRNA expression profiles across species. For this purpose, we carried out high density oligonucleotide expression profiling in muscle from young, middle-aged and old male, mice, rats and female squirrel monkeys. There is a significant age-related decrease in muscle mass, which was observed in rodents and non-human primates. However, gene expression patterns of aging on skeletal muscle in rodents did not resemble the age-related alterations in this species of monkeys. In rodents most alterations in gene expression were found between young and old, while in the monkeys most changes were observed between the young and middle-aged monkey cohorts. These results clearly demonstrate that the impact and

progression of aging on muscle wasting in rodents induces gene-expression profiles that do not overlap those of non-human primates. In future studies we will assess the effect of aging on skeletal muscle in rhesus monkeys as well as in humans.

99. Association Between Serum Adiponectin and Ferritin Level in Apparently Healthy Women

Jee Yon Lee (P)
Sevrance Hospital, Seoul, South Korea

Aim: Serum adiponectin and ferritin are associated with insulin resistance (IR), oxidative stress and metabolic syndrome. But bilateral relationship between serum ferritin and adiponectin in apparently healthy woman has been unknown. The aim of the study was to evaluate the association between serum adiponectin and ferritin level in apparently healthy women. Subjects and methods: We evaluated a total of 111 subjects aged 32–68 years old. Clinical and laboratory measurements, including serum levels of their lipid profiles, high-sensitivity C-reactive protein (hs-CRP), fasting glucose levels, fasting insulin levels adiponectin, ferritin levels and total antioxidant status (TAS) were measured. Results: Serum adiponectin levels were negatively correlated with body mass index (BMI), triglyceride, homeostatic model assessment of insulin resistance (HOMA-IR), ferritin and positively correlated with HDL cholesterol levels. Serum ferritin levels were positively correlated with age, BMI, HOMA-IR, TAS and negatively correlated with adiponectin levels. By step-wise multiple regression analysis, serum ferritin, triglyceride were found to be independent factors associated with serum adiponectin, and serum adiponectin, TAS independently affected serum ferritin levels. Conclusions: Our findings suggest that the relationship between serum ferritin and adiponectin levels might play an important role in glucose metabolism, IR and oxidative stress. Further studies on the causality between adiponectin and ferritin are warranted.

100. High- Normal Fasting Plasma Glucose Decreased Testosterone in Non-Diabetic Middle Aged Men

Jinyoung Shin(P)[1], Byoungjin Park[1], Eunki Park[2], HyeRee Lee[1]
[1]Gangnam Severance Hospital, Yonsei University College of medicine; [2]Department of Urology, Yonsei Joy Clinic

Background: It was known that testosterone is related with metabolic syndrome, cardiovascular disease as well as body and mood, consciousness, sexual function and bone metabolism in men. Testosterone level was decreased in diabetes patients. When it was recently reported that high-normal fasting glucose level is a risk factor of cardiovascular disease, we investigated the association between fasting plasma glucose and endogenous sex hormone (serum total testosterone, sex hormone binding globulin, estradiol and the ratio of testosterone/estradiol) in middle-aged non-diabetes men. Methods: This study included 388 men aged more than 40 years old who visited the health promotion center of a university hospital from May 2007 to August 2008. Anthropometric measurement, medical history and lifestyle information, such as smoking and alcohol ingestion habits were taken. Fasting plasma glucose, testosterone, sex hormone binding globulin and estradiol were measured and the ratio of testosterone/estradiol was calculated. We assessed the correlation analysis and multiple linear regression analysis, which divided by quartile of fasting plasma glucose in non-diabetes men. Results: Total testosterone was inversely related with fasting plasma glucose ($r=-0.198$, $P<0.01$). After adjustment for age, body mass index, non high-density lipoprotein cholesterol, smoking and alcohol ingestions, testosterone was independently associated with fasting glucose in non-diabetic population ($ß=-0.082$, $P<0.01$). Especially, at the high glucose level (above 88mg/dL), testosterone level was decreased (mean testosterone 20.64±1.1, 17.47±0.6, $P<0.005$, respectively). Sex hormone binding globulin, estradiol and testosterone/estradiol were correlated with fasting plasma glucose, but not statistically significant. Conclusion: Total testosterone was independently associated with fasting plasma glucose in non-diabetic middle aged men. High-normal fasting glucose was associated with the decrease of the total testosterone. Further research is needed to search the glycemic target value for the preventing a decrease of testosterone in clinical and public health settings.

101. Age-Related Loss of Fenestrations Impairs Hepatic Uptake of The Water Soluble Substrate Acetaminophen

Sarah Mitchell (P, G)[1,2], Aniko Huizer-Pajkos[3], Victoria Cogger[1], David Le Couteur[1,4], Andrew McLachlan[1,4], Rafael de Cabo[2], Brett Jones[3], Sarah Hilmer[1,3]
[1]University of Sydney, Sydney, New South Whales, Australia; [2]National Institute on Aging; [3]Royal North Shore Hospital, Sydney Australia; [4]Concord Hospital

Objective

Age-related pseudocapillarisation of the hepatic sinusoids, which includes thickening and defenestration of the sinusoidal endothelium, impedes the transfer of small lipoproteins from the sinusoidal blood to the hepatocytes and may also affect the disposition of medications. This study aimed to investigate the effect of age-related pseudocapillarisation of the sinusoidal endothelium on the hepatic disposition of acetaminophen in rats.

Methods
The multiple indicator dilution technique in the isolated perfused rat liver was used to determine the hepatic disposition of tracer amounts of 14C-acetaminophen and reference markers (Evans blue, 3H-sucrose) in young (n=11) and old (n=12) male Fischer 344 rats.

Results
Scanning electron microscopy confirmed reduced fenestrations in the sinusoidal endothelium of old rats compared to young. The recovery of acetaminophen after a single pass through the liver was significantly increased (0.64±0.04, old; 0.59±0.05, young; $p<0.05$) and the apparent volume of distribution of acetaminophen as a fraction of the sucrose volume was significantly reduced (1.95±0.17 old vs. 2.51±0.26 young $p<0.005$) in old rats compared to young. The permeability surface-area (PS) product for the transfer of acetaminophen across the sinusoidal endothelium was also reduced in the old rats (0.034±0.006 ml/s/g,old; 0.048±0.014 ml/s/g,young; $p<0.005$).

Conclusion
Age-related pseudocapillarisation of the liver sinusoid was associated with increased recovery, decreased volume of distribution and decreased PS product of acetaminophen following a single pass through the isolated perfused liver. This is consistent with exclusion of acetaminophen from the space of Disse in old age and has implications for the pharmacokinetics and toxicity of acetaminophen in old age.

102. Age-Related Changes in T Cell Subtypes in African Green Monkeys

John Stehle (P), Maria Blevins, Kevin High
Wake Forest University Baptist Medical Center, Winston-Salem, NC

Background: Nonhuman primate models for aging research provide rapid translation to humans. Using a colony of African Green Monkeys (AGMs) at the Wake Forest University (WFU) Primate Center, we assessed T cell profiles for age-related changes. Design: Animals in paired housing were evaluated in a simple cross sectional study design (baseline tests, no interventions). T cell subsets (CD4+, CD8+, CD8+bright, CD8+dim, and CD28+) were evaluated using flow cytometry in four middle aged (9-12 years) and four old aged (19-26 years) animals in peripheral blood mononuclear cell (PBMNC) and splenocyte fractions. Results: Age-related CD28 dropout on murine, human and rhesus monkey CD8+ cells were not present in AGMs. Splenocytes show no change in the CD28+ expression on CD8+ T cells with age while CD28 expression actually increased with age in the PBMNCs (30% in middle-aged vs. 42.7% CD8+/CD28+ cells in old age ($p=0.05$)). Further, the percent of CD8+bright cells decreased with age in PBMNCs (middle: 45.08%; old: 27.95%; $p=0.05$); splenocytes displayed no change in this fraction. CD8+ dim cells showed a non-significant increase with age in the PBMNCs (middle: 29.48%, old: 36.13%) and splenocytes (middle: 21.38%, old: 30.63%, $p=0.0767$). The percent of T cells demonstrating CD4 expression fell by 45% in the PBMNC fraction (middle: 6.43%; old: 3.58%, $p=0.06$), while splenocytes show a highly significant reduction of 50% in the CD4+ cells (middle: 10.35%; old: 5.28%, $p=0.005$). Conclusion: CD28 dropout on CD8+ T cells observed in other animal models may not serve as a marker of immune senescence in AGMs. An age-related decline in the percent of T cells expressing CD4 may be a more consistent indicator if our findings are confirmed in larger studies and correlate with important outcomes such as vaccine responses.

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103. Aging Exacerbates Oxidative Stress in Disused Type II Muscles

Chiao-nan (Joyce) Chen (P)[1] and LaDora Thompson[2]
[1]Chang Gung University, Taiwan; [2]University of Minnesota, Minneapolis, MN

Background: One mechanism underlying skeletal muscle dysfunction with aging and disuse is oxidative stress. While most studies focused on type I muscles, it is unknown whether aging influences the disuse-related increase of oxidative stress in type II muscles that are affected most by the aging process. In this study we hypothesized that (a) the adaptation of antioxidants to muscle disuse is age-dependent and (b) the accumulation

of oxidized protein is greater in disused muscles of aged animals.

Methods: Fischer 344 rats aged 13 months old (adult, n = 40) and 26 months old (old, n = 40) were randomized into 4 groups: normal weight-bearing, 3, 7, and 14 days of hindlimb unloading (HU3, HU7 and HU14). Tibialis anterior muscles were used for analysis. Activities/content of antioxidants Mn SOD, Cu-Zn SOD, catalase, glutathione peroxidase (GPX), and glutathione (GSH) were measured spectrophotometrically. Oxyblot was used to detect the accumulation of oxidized proteins. The data were analyzed statistically using 2-way-ANOVA and Tukey-Kramer Multiple Comparison Test.

Results: Mn SOD, Cu-Zn SOD and GPX adapted with disuse age-dependently, and catalase and GSH adapted with disuse age-independently. Specifically, activities of Mn SOD and Cu-Zn SOD did not change with disuse in adult muscles, but Mn SOD activity increased and Cu-Zn SOD activity dropped dramatically in muscles of old HU14. GPX activity decreased in muscles of adult HU3 while the activity was unchanged in aged muscles with disuse. The level of oxidized proteins changed with disuse age-independently with the greatest level in muscles of HU3.

Conclusion: The dramatic decrease of Cu-Zn SOD activity in aged muscles with disuse may result in greater superoxide levels and protein damage. The age-independent change of oxidized proteins in muscles with disuse suggests the efficiency of damaged protein removal is similar between adult and old animals.

104. Highest Audible Pitch and Memtrax Image Recognition Correlate with Age and Each Other in a Kronos-MMT Reliability Study

Rolf Martin(P)[1], Susan Kaib[2,3], Terrence Rose[1], S. Mitchell Harman[2,4], Peter Bayley[5], J. Wesson Ashford[6]

[1]MMT Corporation, Sherman, CT; [2]Kronos Science Laboratory, Phoenix Arizona; [3]University of Arizona, Phoenix, AZ, [4]Kronos Longevity Research Institute, Phoenix, AZ; [5]Department of Veteran's Affairs, Palo Alto, CA; [6]Stanford / VA Aging Clinical Research Center, VA Palo Alto Health Care System

BACKGROUND: The Kronos Science Laboratory, MemTrax, and Blueberry Study (BBS/MMT) groups compared their offline and online cognitive performance measurements by determining test-retest reliability, within-person standard deviations and associations with chronological age for each measurement.

METHODS: Offline and online measurement sessions, 30 minutes in length, were completed each weekday during two-week evaluation periods for each study cohort. Measurements completed by the 12 participants were highest audible pitch (HAP), reaction time and movement speed, vibrotactile sensation, visual accommodation (VA), MemTrax continuous image recognition memory, BBS face recognition memory, and a delayed pattern-match to sample measurement from the ANAM test battery developed by the Department of Defense. To minimize order effects, measurements were rotated each day according to a multiple, intersecting Latin square design. Measurement pages and detailed methods are posted for review and comment at: Blueberrystudy.com/KronosIRBapp

RESULTS: For the offline H-SCAN (Hochschild, R., Corona del Mar, CA) VA and HAP measurements, online face recognition and MemTrax measurements, and online HAP measurement, test-retest reliability (correlation, r) and within-person standard deviations (SD) were significant ($r > 0.7$ and $SD < 20\%$). Correlations with age were: HAP offline, -0.610; HAP online, -0.787; MemTrax (beta), 0.610; face recognition (reaction time), -0.27; VA, -0.646. MemTrax and face recognition correlated more closely with HAP than chronological age (e.g., MemTrax-age, 0.610; MemTrax-HAP, 0.767) suggesting that HAP may represent biological rather than chronological age. Correlations between offline and online HAP measurements were 0.927, MemTrax and face recognition, 0.752, and visual accommodation and online HAP, 0.712.

CONCLUSIONS: MemTrax, VA and HAP appear to be useful measures of age-associated cognitive change. The high correlations obtained indicate that relatively precise hearing and visual image memory measurements can be obtained with online testing, and strength of testing can be increased with repeated measurements.

105. A Double-Blind, Placebo-Controlled Trial of PCBN-21 for Age-Associated Memory Impairment

Rolf Martin

MMT Corporation, Sherman, CT

During initial analysis, this randomized, double-blind, placebo-controlled, parallel group study of two doses of propriety formulation PCBN-21

(1400 and 2800 mg/dy) failed to improve delayed memory scores on the Rey Auditory Verbal Learning Test (RAVLT). The treatments appeared safe at each dose and may have proven effective if the study included longer treatment periods, additional participants, strict control of seasonal effects, more repeated measurements, and/or more carefully balanced treatment and placebo groups based on multivariate matching prior to randomization. During post hoc analysis based on effect sizes and standard deviations actually obtained, the study proved to be underpowered. A re-analysis was then performed after data cleaning to remove (i) 'noisy' individual data sets with high, within-person standard deviations, (ii) outliers identified by multivariate analysis, and (iii) interference from subgroups that may have contributed to statistical power collapse. Data cleaning conducted with Microsoft Excel and Umetrics' SIMCA software (Kinnelon, NJ) resulted in identification of a relatively large subgroup within which significant short-term memory score improvements occurred following consumption over 3 months of low or high doses of PCBN-21. Significant memory score seasonal variation ($p = 0.004$) was also corrected by score transformation. Cleaning and seasonal adjustment permitted identification of a subgroup within which short-term memory scores improved 15.6% more in the combined low and high dose treatment groups compared to the placebo group ($n=59$, $p=0.027$). Memory retention half life also decreased significantly in the treatment group ($p=0.044$) suggesting that this nutrient combination may facilitate both encoding and decoding mechanisms. Conclusion: PCBN-21 may provide significant benefits to individuals within the identified subgroup. Because multiple tests of significance were applied to the cleaned data, this conclusion must be confirmed by follow-up investigations before final conclusions are reached.

106. Dietary Restriction and Surgical Removal of Adipose Tissue Reduce Age-Dependent Intolerance to Inflammatory Stress

Marlene Starr (P,N), B Evers, Hiroshi Saito
University of Kentucky, Lexington, KY

Aging is associated with stress intolerance and an impaired ability to regulate excessive inflammation. Dietary restriction (DR), a well-established and extensively-studied area of research, is known to extend the life-span of multiple laboratory species, including mice, and has been proven to delay or prevent the onset of many age-associated pathophysiologies. Surgical removal of visceral adipose tissue (VAT) in rodents has also been shown to have beneficial effects with respect to longevity and insulin action. We recently found that VAT is one of the major tissue sources which express pro-inflammatory and pro-coagulant factors such as interleukin (IL)-6, plasminogen activator inhibitor (PAI)-1 and -2, tissue factor (TF), and thrombospondin (Thbs)-1 in an age-dependent fashion during acute inflammatory stress. In the present study, we tested the hypothesis that loss of fat mass in middle-aged (12-months) and aged (22-24-months) mice would remove the major source of many pro-inflammatory and pro-coagulant factors and thus, improve age-associated sensitivity to acute inflammatory stress. To reduce fat mass, two methods were utilized: short-term DR and surgical removal of VAT. Short-term DR in middle-aged mice effectively reduced body weight and fat mass, and resulted in significantly lower levels of circulating cytokines (IL-6 and IL-1ß) and adipose-tissue derived pro-inflammatory (IL-6) and pro-coagulant (TF, PAI-1, PAI-2, Thbs-1) factors during lipopolysaccharide (LPS)-induced inflammatory stress. Conversely, in aged mice, short-term DR was ineffective and did not improve the pathophysiology of age-related acute inflammation. Surgical VAT removal improved survival by 20-40% in aged mice after injection with a lethal dose of LPS. Reduction in the quantity of VAT through DR shows promise as a therapeutic intervention to reduce susceptibility to inflammatory stress in middle-age, however, in old age the beneficial effects of short-term DR are lost. Therefore, complete or partial surgical removal of adipose tissue depots may provide some benefit.

107. TLR2-Induced Signaling Defects In Aged Alveolar Macrophages Occur Downstream to IRAK-1 Following Infection with *S. pneumoniae*

Angela Boyd (P,N), Pooja Shivshankar, Perla Rodriguez, Carlos Orihuela
The University of Texas Health Science Center at San Antonio, San Antonio, TX

Streptococcus pneumoniae (Spn) is a leading cause of infectious death for those > 65 years of age. Alveolar macrophages (AMs) are the first immune cell to sense an invading pathogen and coordinate the inflammatory response within the lungs. Studies have suggested that macrophages exhibit age-related deficiencies in Toll-like receptor (TLR) function; however, the molecular mechanisms altered with age remain poorly understood. In this study we examined TLR expression and signaling in young, mature and aged BALB/c AMs following infection with Spn to identify the molecular

mechanisms contributing to macrophage immunosenescence. Using a murine lung infection model, we demonstrate that resistance to pneumococcal pneumonia declines with age, as does the ability of AMs to respond to Gram-positive cell wall (GPCW) and ethanol-fixed Spn (ethSpn) with a pro-inflammatory cytokine response. AMs from TLR2 knock-out mice were unable to respond to GPCW or ethSpn, confirming that recognition of Spn is TLR2-dependent. Studies using flow cytometry determined that TLR2 surface expression was significantly increased ($P = 0.02$) on aged AMs suggesting that intracellular signaling defects occur following TLR engagement. Following infection of AMs with Spn, we observed a significant age-related decline in TLR2-induced phosphorylation of p65 NFκB and p38 MAPK by quantitative immunoblot, both of which are critically involved in cytokine transcription and translation, respectively. To identify potential defects in the common pathway from TLR2 engagement to p65 NFkB and p38 MAPK activation, we examined IRAK-1 phosphorylation. Importantly, IRAK-1 phosphorylation did not differ with age indicating alterations occur downstream of IRAK-1. These data are the first to identify that TLR2-dependent recognition of Spn by aged AMs is impaired and that TLR2-induced signaling defects occur downstream to IRAK-1 phosphorylation. Future studies are undergoing to examine signaling downstream of IRAK-1 and whether antiaging therapies such as Rapamycin or Caloric Restriction can counter age-related TLR dysfunction.

108. Impaired CD8+ T cell priming in aged mice is due to poor CD8 dendritic cell responses?

Gang Li (P, G)
University of Arizona, Tucson, AZ

Previously, we have observed that the increased susceptibility to intracellular pathogens (e.g. Listeria monocytogenes (LM) and West Nile virus in old mice correlates with a limited and incompletely differentiated CD8+ T cell response. However the underlying basis for these defects remains incompletely understood. In this study, we have asked whether differences in the priming environment contribute to reduced CD8+ T cell expansion in old mice. Prior to LM-OVA infection, OVA specific OTI CD8+ T cells from adult mice were transferred into adult (2-3 months) and old (>18 months) C57BL/6 mice and enumerated at 7 days post-infection. We found that adult OTI CD8+ T cells expanded significantly better in adult mice. By transferring OT-1 cells into adult and old recipients on different days post-infection, we observed that differences in CD8+ T cell priming can be traced to the critical period between days 3 and 5 post infection. While the total number of CD11c+ DCs in the spleen did not change following LM infection, adult mice contained significantly more CD8a+ DCs between days 3 and 5 post infection. To determine if impaired CD8+ T cell priming in old mice can be restored by increasing numbers of DCs, Flt3L was administered to old mice prior to infection. At 7 days post-infection, OT-1 numbers were again enumerated and were found to be increased ~2-fold in mice that received Flt3L treatment. Altogether, this data suggests that reduced numbers of CD8-alpha DCs in response to infection is at least partly responsible for poor CD8+ T cell expansion in old mice. Mechanistic reasons for this age-related difference in DC numbers are under investigation.

109. Long-term Rapamycin Differentially Affects Immunity in Young and Old Mice

Adriana Benavides (P, N), Elizabeth Fernandez, Z. Dave Sharp, Randy Strong, Ellen Kraig
University of Texas Health Science Center at San Antonio, San Antonio, TX

It is suggested that rapamycin might be efficacious in slowing the aging process. However, the potential use of this immunosuppressive drug in elderly individuals must be considered cautiously due to their immunosenescent state and increased susceptibility to autoimmune and infectious diseases. Because of this, it is important to understand how rapamycin affects immunity as individuals age. To investigate, young and old mice were fed a control or rapamycin diet and then immunized 3 times with the protein antigen, Aa Omp34/alum. Rapamycin was shown to repress humoral responses in young mice whereas responses in old mice were negligible. To assess whether rapamycin's effects were due to TREGS, mice were pre-treated with αCD25 or an isotype control antibody prior to immunization. Treatment had no effect, and FACS analysis of splenocytes revealed no increase in the TREG population in rapamycin-fed mice, suggesting that rapamycin was not eliciting its suppression through TREGS. Furthermore, mice were challenged with another protein antigen, TAChRα/CFA. The repression observed with Omp34/alum was not seen in the young, suggesting that a strong immunogen can overcome rapamycin's inhibition. However, in old mice, rapamycin did suppress the response but only in females. T-cell responses to p146-162, the

immunodominant epitope of TAChRα, was modestly affected by rapamycin in the young. Moreover, when TAChRα transgenic mice were immunized with p146-162, T-cell tolerance was maintained in rapamycin-fed mice. Because it was demonstrated that rapamycin increased efficacy of viral and DC-based vaccines, I hypothesized responses to organisms could be differentially affected. Mice were challenged with viable Aa. Antibody responses to Aa was higher in older rapamycin-fed mice. No equivalent increase was seen in younger animals. The conclusions from my studies to-date suggest that long-term vs. short-term rapamycin may have different immune consequences and that the effects might be very different in older individuals.

110. A Molecular Mechanism for TNF-α-Mediated Down-Regulation of mouse B Cell Responses in Aging

Bonnie B Blomberg (P), Maria Romero, Alain Diaz, Ana Marie Landin, Richard L Riley, Daniela Frasca
Department of Microbiology and Immunology, University of Miami Miller School of Medicine, Miami FL

Aging in humans has been shown to be associated with a low-grade chronic pro-inflammatory status referred to as "inflammaging". In the present study, we have shown that old (greater than 20 months) unimmunized BALB/c mice also have increased inflammatory serum cytokines (i.e. TNF-α). We have previously shown that autonomous B cell function is impaired in aged mice. Here we investigated whether B cells can contribute to inflammaging by secreting pro-inflammatory cytokines, such as TNF-α, and whether adding a pro-inflammatory cytokine can impair B cell function in responding to stimuli such as LPS. Our hypothesis is that in aging there is a feedback mechanism of inflammatory cytokines that act in an autocrine fashion on B cells, lowering the expression of activation-induced cytidine deaminase (AID), crucial for class switch recombination (CSR). Our results show that unstimulated (*ex vivo*) B cells from old mice make significantly more TNF-α mRNA and protein than B cells from young mice. Pre-incubation of B cells with additional TNF-α before stimulation with LPS decreases both young and old B cell responses. B cell function was restored by adding anti-TNF-α antibody before LPS in young and more significantly in old cultured B cells. To address a molecular mechanism, we found that pre-incubation of B cells with TNF-α, before LPS stimulation, induces tristetraprolin, a physiological regulator of mRNA stability of the transcription factor E47, crucial for CSR. These results reveal new molecular mechanisms which likely contribute to reduced antibody responses in aging and potentially other conditions of increased chronic inflammation.

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111. Autocrine TNF-α Down-Regulates Aged Human B Cell Function

Daniela Frasca (P), Alain Diaz, Maria Romero, Bonnie B Blomberg
Department of Microbiology and Immunology, University of Miami Miller School of Medicine, Miami FL

Aging is characterized by increased plasma levels of pro-inflammatory cytokines such as TNF-a which can have deleterious effects as they are implicated in the pathogenesis of several disabling diseases of the elderly. We hypothesize that this pro-inflammatory status of the elderly, known as "inflammaging", is partially contributed to by B cells and more importantly that the autocrine production of B cell TNF-a impairs the capacity of the individual to make protective antibodies and to respond to vaccination. Our data indicate that aged human B lymphocytes produce increased TNF-a and that this negatively impacts their function, including the ability to up-regulate activation-induced cytidine deaminase (AID) and undergo class switch recombination (CSR) which are critical for optimal effector function of the antibodies produced in response to exogenous antigens and vaccines. We show that the levels of AID in *in vitro* mitogen-stimulated B cells is negatively correlated with the levels of TNF-a in unstimulated B cells and that most old B cells have lower levels of AID and higher levels of TNF-a before stimulation. TNF-a is also negatively correlated with AID induced in a specific response, such as the influenza vaccine response, elicited in cultures of vaccine-stimulated B cells 28 days after vaccination. These studies will have immediate impact on crucial development of effective vaccines and especially for the elderly as well as treatment of autoimmune disorders (e.g. anti-TNF-a treatments of Rheumatoid Arthritis and Intestinal Bowel Disease).

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112. Effect of Resveratrol on Proinflammatory Profile of a Non-Human Primate Model of Obesity

Jimenez-Gomez Y (P, G), Martin-Montalvo A, Mattison J, de Cabo R.
Laboratory of Experimental Gerontology, National Institute on Aging, National Institutes of Health, 251 Bayview Blvd, Baltimore, MD

The number of overweight individuals worldwide has increased dramatically due, in part, to consumption of high-fat diets. Obesity is recognized as a chronic low-grade systemic inflammation that is associated with an increase risk for type 2 diabetes mellitus and cardiovascular disease. Lifestyle changes including healthier diet and exercise should be a primary intervention to attenuate the development of obesity and its associated diseases. Resveratrol, a naturally occurring polyphenol that is found in the skins of grapes and nuts, has been shown to exert an anti-inflammatory effect on a number of biological systems. The present study focuses on establishing the effect of resveratrol on the pro-inflammatory profile caused by a high-fat, high-sugar diet (HFS diet) in adipose tissue and blood from non-human primates. For this purpose, 24 male non-human primates (*Rhesus Macaques*) were randomly assigned to one of three diets for two years: Standard healthy control (SD; 13% kcal fat and < 5% kcal sucrose by weight); HFS (42% kcal fat and 27% kcal sucrose by weight) and HFS+resveratrol diets, supplemented with 40 mg/twice daily (12 months timepoint) followed by 240 mg/twice daily (24 months timepoint). Plasma pro-inflammatory cytokine concentrations (IL1beta, TNFalpha, IL6) at 0, 12 and 24 months, and IkappaBalpha protein levels, an inhibitor of NF-kappaB´s inflammatory response, in omental adipose tissue at 24 months were determined by ELISA and Western Blot, respectively. We found that the HFS+Resv diet induced a significant increase in IkappaBalpha protein levels as compared to the HFS diet ($P = 0.008$) in omental adipose tissue from *Rhesus* monkeys at 24 months of dietary intervention. However, comparisons of the effect of time, diet, and the interaction both factors (time and diet) for the concentrations of IL1beta, TNFalpha and IL6, the only significant effect was time on study for IL1beta at 24 months ($P< 0.001$). All other comparisons showed no effect. Our results reveal a potential anti-inflammatory role of chronic administration of resveratrol in omental adipose tissue of non-human primates fed a diet specifically formulated to produce obesity and, thus, a chronic low-grade inflammation. However, we did not observe any significant differences in plasma levels of pro-inflammatory cytokines IL1beta, TNFalpha and IL6 when we compared the three diets. This may be explained, at least in part, by the overall healthy characteristics of our sample population.

113. In Silico Evidence Supports a Role for DNA Methylation in Sirt1-Mediated Effects of Dietary Restriction

Laura Ions (P), Luisa Wakeling, Dianne Ford
Institute for Cell and Molecular Biosciences, Newcastle University, UK

The histone deacetylase Sirt1/Sir2 is implicated in increasing lifespan in response to dietary restriction (DR) across diverse species. In silico analysis indicated that overlaps between mouse gene lists compiled from published studies identifying: i) loci that bind Sirt1, ii) transcripts showing changes in expression after DR, and iii) loci showing altered methylation in older compared with younger intestinal tissue, were greater than expected by chance. This analysis indicates an association between genes that respond to DR, genes that show altered methylation with ageing and genes that bind Sirt1, consistent with our hypothesis that responses to DR include epigenetic effects, and specifically effects on DNA methylation, resulting from Sirt1 activity.

Ten genes fell into the "three way" overlap, compared with an expected number of 4 (representation factor 2.5; $P=0.0079$). Methylation levels of 8 of these genes were measured by pyrosequencing using a human intestinal Caco-2 cell line model in which SIRT1 expression was increased (overexpression from a transgene) or reduced (siRNA). For 6 of the 8 genes (CDC7, EIF5, IRX3, KLF3, PCTY1A, PTPRG, SLC39A4 and TBX3) at least one CpG site assayed showed a statistically significant effect of SIRT1 expression levels on methylation. As examples, comparing individual CpG sites under conditions of SIRT1 overexpression versus SIRT1 knockdown respectively: $1.78 \pm 0.32\%$ versus $5.61 \pm 0.89\%$ for EIF5; $8.00 \pm 1.38\%$ versus $5.00 \pm 0.41\%$ for PTPRG; $90.11 \pm 2.12\%$ versus $71.89 \pm 2.92\%$ for TBX3; mean ± SEM ($n = 9$); $P < 0.05$ by Kruskal Wallis then Dunn's post test.

The findings support our hypothesis that some beneficial effects of DR may be through reversal by Sirt1 of ageing-associated DNA methylation changes. Establishing the genome-wide effects of Sirt1 manipulation on gene methylation may support further these ideas and identify specific genes with a role in the beneficial response to DR.

114. Serum 25-Hydroxyvitamin D and DHEA-S Level are Associated with Depression in Community-Dwelling Older Women

Hyun mee Kim (P)1, Jee Yon Lee2, Chung Ji Youn2, Duk Chul Lee2

1Department of Family Medicine, Miz-medi Hospital , Seoul, Korea; 2Department of Family Medicine, Yonsei University, College of Medicine, Seoul, South Korea

Background: Even though some previous studies suggested that vitamin D deficiency may contribute to depressive disorder, the association between vitamin D deficiency and depression has been controversial. Furthermore, only few studies been have done in the general population. Dehydroepiandrosterone-sulfate (DHEA-S) is most abundant steroid hormone secreted by adrenal cortex, known to have diverse role in human including stabilizing mood. Aim of this study was to investigate the association between serum 25-hydroxyvitmin D and DHEA-S and depression. Methods: This study conducted as a part of the Yonsei Aging Study (YAS). YAS was designed as a survey to investigate the factors related to depression, cognitive function and physical performance in community dwelling old people in Korea. A total of 136 women aged older than 60 years. Blood sample was obtained in October and November in 2008. Depressive symptoms were self-reported by Geriatric depression scale-15(GDS-15). Depressive disorder is defined as a GDS-15 score of 7 or higher. Cognitive abilities were assessed using the Mini-Mental State Examination (MMSE). Additionally, cardiometabolic risk factors, DHEA-S and 25-(OH) vitamin D level and physical performance index (by gait speed, chair stand test, and tandem standing test) were measured. Results: The prevalence of depression was vitamin D deficiency is 46.3% in this study population. Mean values of 25(OH) vitamin D and DHEA-S (18.47±7.10 ng/ml, 52.98±31.96 μg/dl respectively)in women with depression were lower than those in normal women (22.01±8.09 ng/ml, 44.58±32.54 μg/dl respectively; $p<0.02$, $p=0.12$ repsectively). The prevalence of vitamin deficiency (25-OH vitamin D <10ng/ml) and vitamin insufficiency (25-OH vitamin D <20ng/ml) were 6.62 % and 46.32% respectively. Correlation analysis showed GDS scores associated with 25-(OH) vitamin D level ($r=-0.26$, $p<0.005$), physical performance index ($r=-0.22$, $p<0.01$), and DHEA-S level (-0.20, $p<0.05$). Conclusion: This study suggested that 25-hydroxy vitamin D and DHEA-S levels are the independently associated with depression in apparently healthy community-dwelling older women.

115. Muscle Iron Accumulation in Old Rats is Associated with High Levels of Oxidative Stress and Impaired Recovery from Atrophy

Jinze Xu (P)[1], Judy Hwang[1], Hazel Lee[1], Stephanie Wohlgemuth[1], Mitchell Knutson[1], Andrew Judge[1], Esther Dupont-Versteegden[2], Emanuele Marzetti[1], Christiaan Leeuwenburgh[1]

[1]University of Florida, Gainesville, FL; [2]University of Kentucky, Lexington, KY

In the present study, we investigated the effects of 7 and 14 days of re-loading following 2-week muscle unweighting (hindlimb suspension, HS) on non-heme iron levels and oxidative damage in the gastrocnemius muscle of young (6 months) and old (32 months) male Fischer 344 x Brown Norway rats. We show that aged rats were associated with lower muscle mass, higher levels of total non-heme iron and oxidative damage in skeletal muscle in comparison with young rats. Protein levels of cellular iron transporter, such as divalent metal transport-1 (DMT1), transferrin receptor-1 (TfR1), Zip14, and ferroportin (FPN), and their mRNA abundance were determined. The extremely low expression of FPN in skeletal muscle might lead to inefficient iron export in the presence of iron overload and play a critical role in age-related iron accumulation in skeletal muscle. HS was associated with higher protein levels of DMT1 and Zip14 in aged, but not young muscle, suggesting that DMT1- and Zip14-mediated iron uptake during HS may be at least partly responsible for non-heme iron overload in the aged atrophying muscle. Moreover, oxidative stress was much greater in the muscles of the older animals measured as 4-hydroxy-2-nonenal (HNE)-modified proteins and 8-oxo-7,8-dihydroguanosine levels. These markers remained fairly constant with either HS or re-loading in young rats. In old rats, HNE-modified proteins and 8-oxo-7,8-dihydroguanosine levels were markedly higher in HS and were lower after 7 days of recovery. However, no difference was observed following 14 days of recovery between control and re-loading animals. In conclusion, advanced age is associated with disruption of muscle iron metabolism which is further perturbed by disuse and persists over a longer time-period.

116. Calorie Restriction Suppresses the Occurrence and Retards the Growth of Ethyl-nitrosourea-Induced Glioma in Rats

Megan Mahlke1, Lisa Cortez[1], Celeste Webb[1], Melanie Ortiz[1], Yiqiang Zhang[1], Shuko Lee[2], Gene Hubbard[1], Yuji Ikeno (P)[1]

[1]Barshop Institute/University Texas Health Science Center at San Antonio, San Antonio, TX; [2]Audie Murphy VA Hospital, San Antonio, TX

The anti-tumor action of calorie restriction (CR) and the possible underlying mechanisms on tumor growth were investigated using ethylnitrosourea (ENU)-induced glioma in rat. ENU was given transplacentally at gestational day 15. The brain from 4-, 6-, and 8-month-old rats fed either ad libitum (AL) or calorie restricted diets (40% restriction of total calories compared to AL rats) were studied. Tumor burden was assessed by comparing the size and number of gliomas present in the brain. Immunohistochemical analysis was used to detect the lipid peroxidation products [4-hydroxy-2-nonenal (HNE), malondialdehyde (MDA), and acrolein] and nitrotyrosine to document oxidative stress, levels of glycated end products, cell proliferation activity (PCNA), and cell death (ssDNA) associated with the development of gliomas. The results showed that the number of gliomas did not change with age in the AL groups; however, the average size of the gliomas was significantly larger in the 8-month-old group compared to that of the younger groups. Immunopositive areas for HNE, MDA, acrolein, nitrotyrosine, and glycated end products increased with the growth of gliomas. The CR group showed both reduced number and size of gliomas, less accumulation of oxidative damage, and less glycated end products compared to the AL group. Furthermore, the CR group showed less PCNA positive and more ssDNA positive cells. Interestingly, we also discovered that the anti-tumor effects of CR were associated with less accumulation of hypoxia inducible factor-1α (HIF1α) levels and a reduction in the mammalian target of rapamycin (mTOR) signaling. Our results are very exciting because they could not only demonstrate the anti-tumor effects of CR on oligodendroglioma, but also indicate the possible underlying mechanisms, i.e., anti-tumor effects of CR could be mediated by the changes in redox-sensitive and/or nutrient sensing signaling pathways.

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117. Anti-Inflammatory Effects of Walnut-Associated Fatty Acids in BV-2 Microglia

Amanda Carey(P, G)[1], Derek Fisher[1], Donna Bielinski[2], Barbara Shukitt-Hale[1]
[1]United States Department of Agriculture; [2]Tufts University, Boston, MA

Walnuts are a rich source of essential fatty acids, including the polyunsaturated fatty acids (PUFAs) alpha-linolenic acid (ALA) and linoleic acid (LA). Essential fatty acids have been shown to modulate a number of cellular processes in the brain, including the activation state of microglia. Microglial activation can result in the generation of cytotoxic intermediates and is associated with a variety of age-related and neurodegenerative conditions. In vitro, microglial activation can be induced with the bacterial cell wall component lipopolysaccharide (LPS). Previously, we demonstrated that walnut extract dose-dependently decreased LPS-induced nitrite production and TNFα release, and the anti-inflammatory effect of walnut extract may be modulated via internalization of the LPS receptor, toll-like receptor 4 (TLR4). The present study focused on the effects of PUFAs on LPS-induced microglial activation of inflammatory markers in vitro. Cells were treated with fatty acids before overnight exposure to LPS, which increased production of pro-inflammatory mediators such as nitric oxide and pro-inflammatory cytokines. The results suggest that pretreatment with LA or ALA was similarly protective against LPS-induced NO release, iNOS release, COX-2 expression and TNFα. It is well known that ALA has anti-inflammatory properties; however in this model, it appeared that both ALA and LA reduced LPS-generated increases in inflammatory indices. Additionally, microglia were also treated with a combination of LA and ALA at the concentration found in our methanolic walnut extract. The combination of LA and ALA did not produce a synergistic antagonism of the LPS-induced activation of the inflammatory markers. We further investigated if PUFAs induced internalization of TLR4 and if the anti-inflammatory effects of PUFAs were dependent on functional activation of phospholipase D2 (PLD2). These studies add to research from our laboratory investigating the anti-inflammatory effects of walnuts in microglia and could lead to nutritional interventions in the prevention and treatment of neurodegeneration.

118. The Influence of Dietary Lipid Composition on Life Span in Calorie Restricted Mice

Jon Ramsey (P)[1], Kevork Hagopian[1], Dianna Tran[1], Jose Villalba[2], Placido Navas[3], Guillermo Lopez Lluch[3], Roger McDonald[1]
[1]University of California – Davis, Davis, CA;
[2]Universidad de Cordoba, Cordoba, Argentina;
[3]Universidad Pablo de Olavide, Seville, Spain

Calorie restriction (CR), without malnutrition, has been shown to increase life span in many species of animals. However, the mechanism responsible for the retardation of aging with CR is still not entirely known. It has been proposed that a decrease in membrane unsaturated fatty acids, especially n-3 fatty acids, may play a central role in life span extension with CR. To test this theory, membrane fatty acid composition was manipulated in CR mice by feeding diets that differed in lipid composition. At 4 months of age, male C57BL/6 mice were divided into four groups (control and 3 CR groups) and fed AIN93M diets at 95% (control) or 60% (CR) of ad libitum intake. The primary dietary fats for the 3 CR groups were soybean oil (same as control), lard or fish oil. Life span and end of life pathology were determined for each group of mice. As expected, the fish oil group had increased levels of n-3 fatty acids ($P<0.05$) in mitochondrial phospholipids when compared to the other groups of mice. Despite this increase in phospholipid n-3 fatty acids, median life span was increased by 21% ($P<0.05$) in the CR fish compared to control group. Median life span was also increased in both the CR lard and CR soy oil groups when compared to the control group ($P<0.05$). These results indicate that a decrease in membrane n-3 fatty acids is not required for life span extensions with CR. Nonetheless, dietary lipid composition can influence life span in CR animals since median life span was increased in the CR lard group compared to either the CR fish or CR soy groups. Thus, it is important to consider dietary lipid source when designing a diet to optimize life extension with CR.

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119. Energy Metabolism Pathways in p66Shc-/- Mice.

Kevork Hagopian (P), Gino Cortopassi, Roger McDonald,Jon Ramsey
University of California

It has been previously reported that p66Shc-/- (KO) mice are long-lived, insulin-sensitive and resistant to weight gain on high fat diet. However, recent studies indicate that lifespan extension in the KO mice may only occur under calorie restriction (CR) conditions. This suggests that p66Shc may influence life span through changes in energy metabolism. The purpose of the present study was to determine the influence of Shc proteins on the activities of enzymes of key metabolic pathways. The activities of enzymes of glycolysis, ß-oxidation and ketone catabolism, as well as pyruvate dehydrogenase complex (PDH) were measured in skeletal muscles from wild-type (WT) and KO mice, under fed and fasted conditions. In the glycolytic pathway, the activities of hexokinase (HK), phosphofructokinase-1 (PFK-1) and pyruvate kinase (PK) were decreased significantly in the fasted KO compared to WT mice. On the other hand, the activities of the ß-oxidation enzymes acyl-CoA dehydrogenase, hydroxyacyl-CoA dehydrogenase and ketoacyl-CoA thiolase all increased ($P < 0.05$) in skeletal muscle from fasted KO compared to WT mice. Also, the ketone metabolism enzymes acetoacetyl-CoA thiolase and ß-hydroxybutyrate dehydrogenase were increased in fasted KO compared to wild-type mice ($P < 0.05$). The results of our studies suggest that Shc proteins play an important role in energy metabolism. In particular, a decrease in Shc proteins leads to an increased capacity for ß-oxidation, ketone body metabolism, amino acid catabolism and gluconeogenesis under fasting conditions. Decreases in Shc proteins may play an important role in transitioning the animal from a fed to fasted state and may help animals adapt to sustained periods of CR.

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120. Effects of Phenylalanine Supplementation on Age-related Hearing Loss

Shinichi Someya and Christiaan Leeuwenburgh
University of Florida, Department of Aging and Geriatric Research, Gainesville, FL

Age-related hearing loss (AHL) is a common feature of mammalian aging and is the most common sensory disorder in the elderly population. It is well established that the amino acids L-phenylalanine (Phe) and L-tyrosine (Tyr) function in the brain as precursors for the neurotransmitter dopamine. Phe can be converted into Tyr, which in turn is converted into L-DOPA and eventually to dopamine. A large number of studies describe the beneficial effects of the dopamine precursor L-DOPA on Parkinson's disease. L-DOPA administration has also been shown to extend the mean lifespan of mice by 50%, showing a link between dopamine and aging. In this study, we found that supplementation with the dopamine precursor Phe delayed the onset of AHL in C57BL/6 mice. Auditory Brainstem Response (ABR) hearing tests revealed that 15-month-old mice fed control diet (control diet mice) displayed severe hearing loss at low, middle, and high frequencies, while age-matched mice fed a diet supplemented with Phe (Phe diet mice) displayed

normal hearing or slight hearing loss at these frequencies. In agreement with the hearing test results, middle-aged Phe diet mice displayed only minor loss of neurons and hair cells in the cochlea, while age-matched control diet mice displayed severe loss of neurons and hair cells. Cell counting also revealed that the mean neuron survival in basal cochlear turns from middle-aged Phe diet mice was significantly higher than that of age-matched control diet mice. These results suggest that Phe supplementation may protect cochlear neurons by preventing the decline in dopamine levels in the inner ear.

121. 2011 Blueberry Health Study Report: Memory Scores Continue To Improve; Power Milestones Bring Personalized Research Closer

Rolf Martin(P)[1], Diana Burns[2], David Doiron[2], Kurt Gerstmann[1], Kathy Hull[3], Amy Kokesh[1], Bruce Kristal[4], Alec Pruchnicki[5], Howard Raphaelson[2], Barrie Sachs[6], Roseanne Schnoll[7], Joseph Vogelman[8], Anthony Wetherell[9]

[1]MMT Corporation, Sherman CT; [2]Mansfield Senior Center, Mansfield, CT; [3]New Fairfield Senior Center, New Fairfield, CT; [4]Brigham & Women's Hospital, Boston, MA; [5]St. Luke's Roosevelt Hospital, New York, NY; [6]H.R. Herbs, Teas and Gifts, Sherman, CT; [7]Brooklyn College of the City University of New York, Brooklyn, NY; [8]Orentreich Foundation for the Advancement of Science, Cold Spring, NY; [9]Defence Science & Technology Laboratory, UK (retired)

BACKGROUND: To investigate effects of daily blueberry consumption on memory, volunteers have consumed one half to two cups daily of wild Maine blueberries since 2002. Goals of this study are to (i) determine if blueberries delay long-term memory decline, (ii) measure how long benefits last and rule out the possibility that harmful effects may occur after several years of blueberry consumption, and (iii) develop low cost, precise measurements enabling individuals to measure memory changes as small as 1% per year at 95% confidence. This allows them to monitor their personal responses to medicines and other interventions and conduct personalized research to support effective personalized medicine.
METHODS: Measurement pages are posted at Blueberrystudy.com to enable high-precision measurement from the comfort of each person's home, including a new hearing page that enables this study to determine if hearing is improved by blueberries as well as other antioxidant supplements.
RESULTS: Approximately four of every five study participants obtain memory scores that improve steadily over time. In no case has long-term improvement been followed by significant decline that might be linked to delayed negative effects. Two participants have this past year set precision milestones that to our knowledge have not been previously achieved. In one case, scores on noun, verb and name recognition tests improved for four consecutive years with each year increasing at 95% confidence compared to the previous year. After cumulative improvements of more than ten percent, another participant measured a further annual increase of 0.56% at 95% confidence (p=0.007), exceeding by a comfortable margin the previous minimum statistically significant annual change of 0.75% (p=0.030).
CONCLUSION: In the past we have calculated statistical power for each participant and predicted that individuals could measure 1% performance changes one year to the next at 95% confidence. The most recent data indicate the accuracy of these predictions.

122. Delaying Aging with Available Interventions May Lead to Large Economic as well as Quality of Life Benefits

Rolf Martin(P)[1] and Gregory Brewer[2]

[1]MMT Corporation, Sherman, CT; [2]Southern Illinois University School of Medicine, Springfield, IL

BACKGROUND: Because economic consequences of longevity-related research are important to policy makers and private donors who influence funding for such research, we analyzed Centers for Disease Control (CDC) Mortality Multiple Cause data files for 2003-2007 to identify trends related to future medical expenses. The scenario for rapid, essentially instantaneous decline in aging rate that we model is based on development of a medicine and/or nutrient combination that re-activates misfolded enzymes, as B.N. Ames has proposed for acetyl-L-carnitine, persistent activation of 600+ NRF2 protective genes, telomerase induction, and 30% extension of remaining life expectancy as observed after rapamycin administration in the NIA Intervention Testing Program.
METHODS: Data sets for 2003-2007 were examined using Turbo Pascal software that was validated by comparing our tallies with figures published elsewhere by the CDC. The software accepts different cost estimates so can accommodate different cost assumptions.
RESULTS: If average aging rate is delayed ten years so that for example biological age of individuals aged 70-79 becomes equivalent to those aged 60 to 69, then the percentage of individuals who enter nursing homes, assuming other factors remain unchanged, is projected to

decrease by 32% for circulatory/cardiovascular diseases, 38% for flu and pneumonia, 28% for chronic obstructive pulmonary disease, 20% for diabetes, 45% for Alzheimer's disease and 9% for malignant neoplasms. Projected annual savings range from $14 to $60 billion dollars depending on assumptions.
LIMITATIONS: This analysis models healthspan-shortening effects of divorce, cancer, circulatory diseases, diabetes and Alzheimer's disease but does not take into account one-time historic events such as the Great Depression and World War II.
CONCLUSIONS: If physiologically younger seniors have significantly reduced medical expenses, then financial effects of delayed aging and reduced morbidity may be large. Research funding at 1% of projected medical expenses may yield disproportionate benefits if support targets morbidities with most favorable cost-benefit impacts.

123 Timing of High Fat Diet Onset has Differential Effects on Health and Survival in Mice

Robin K. Minor (P, G) and Rafael de Cabo
Laboratory of Experimental Gerontology, National Institute on Aging, National Institutes of Health, Baltimore, MD

As overweight and obesity rates continue to rise in the US and abroad, so too do the rates of overweight and obesity among increasingly younger age groups. In fact, recent results from the 2007–2008 NHANES indicate nearly 17% of children and adolescents aged 2–19 years are obese. Obese children often become obese adults who are at increased risk for many chronic conditions such as diabetes, cardiovascular disease, and certain cancers. While high fat diet treatment successfully produces rodent models of obesity, whether obese mice will present with increased mortality from the aforementioned conditions is unclear. Here we tested the effects of long-term treatment with high fat diets on health and longevity in C57Bl/6J mice started at different ages. Four diet groups were compared: standard diet (SD) controls, either started at 27 weeks of age (SD-Y) or 56 weeks of age (SD-O), and high fat diet (HFD) treated mice, started at 27 wks (HFD-Y) or 56 wks (HFD-O). Mean lifespan in the HFD-Y mice was 89 wks while HFD-O mice lived to 95 wks on average. Maximum lifespan was less affected by age of HFD onset, with the longest-lived 5% of the HFD-Y cohort living to 135 ± 1.2 wks and the HFD-O mice surviving to 137 ± 2.2 wks. We also present data on major pathologies evident at necropsy in the groups. These findings suggest that, like humans, mice also respond to early-onset high fat diet exposure with greater risk for obesity, obesity-related disease, and increased mortality.

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124. NQO1 Overexpression Increases Antioxidant Protection and Glucose Homeostasis In Mice

Alejandro Martín-Montalvo (P, G), Yolanda Jimenez-Gomez, Rajib Paul, Kavitha Sankavaram, Theresa Ward, Andrew Levette and Rafael de Cabo
Aging, Metabolism and Nutrition Unit, Laboratory of Experimental Gerontology, National Institute on Aging, National Institutes of Health, Baltimore, MD, USA.

One of the most important benefits of Calorie Restriction (CR) is an improvement of the plasma membrane redox system. CR enhances the activities and content of antioxidant compounds known to decline with age. Previous studies from our laboratory have shown that the levels of expression and activity of NADH coenzyme Q oxidoreductase 1 (NQO1) are increased in rodent plasma membranes under long-term CR. This overexpression promotes quenching of free radicals and other oxidizing compounds, and it contributes to the regulation of cellular redox homeostasis, particularly the NAD/NADH ratio. We present here the characterization of NQO1 overexpression in transgenic mice produced in our laboratory. These mice harbor the rat orthologue of NQO1 which was cloned into a pRC/CMV-rDTD vector. The construction was stably incorporated into the genome and the gene expression was controlled by a promoter associated with human cytomegalovirus and the appropriate sequences for polyadenylation. NQO1 overexpression resulted in lower body weight gain under high fat feeding conditions (HF) and resulted in a myriad of beneficial physiological effects. Particularly interesting was an increase in oxygen consumption, which might explain the decreased weight gain under HF. In addition, overexpression of NQO1 prevented the onset of insulin insensitivity and it protected against protein oxidative damage. Furthermore, NQO1 KI mice showed an increase in the NAD/NADH ratio as well as ATP levels, but these increases were not associated with increased mitochondrial content. These metabolic changes in NQO1 KI mice lead to a decrease of inflammatory markers, improvements in insulin sensitivity decreased protein oxidation damage in HF and increased oxidative stress tolerance in an acute oxidative treatment of the superoxide producer diquat in a standard diet.

These findings support that NQO1 overexpression, as seen in CR, has long-term benefits and prevents the deleterious effects of HF in mice.

Our future research will determine the particular pathways and proteins that are involved in the induced benefits of NQO1 overexpression, the mechanisms underlying glucose homeostasis improvements and the long-term effects of NQO1 overexpression during an ongoing longevity study. We predict that NQO1 overexpression will lead to healthspan and lifespan benefits.

125. Activation of GPR30 Attenuates Diastolic Dysfunction and LV Remodeling in Oophorectomized mREN2.Lewis Rats

Hao Wang (P), Jewell Jessup, Sarah Lindsey, Marina Lin, Clarissa Chagas, Leanne Groban
Wake Forest University School of Medicine, Winston-Salem, NC

The incidence of diastolic dysfunction increases in women after menopause but the mechanisms involved are not completely understood. GPR30 is a novel estrogen receptor expressed in various tissues including the heart. Studies have shown that activation of GPR30 by its agonist G1, improves contractile function and reduces infarct size in isolated rat and mouse hearts subjected to ischemia/reperfusion injury and attenuates diastolic dysfunction in a salt-sensitive rat model of hypertension. We hypothesized that the activation of GPR30 by G1 protects against the development of diastolic dysfunction and cardiac hypertrophy in oophorectomized mRen2.Lewis rats, an established model that emulates the cardiac phenotype of the postmenopausal woman. Surgical bilateral oophorectomy (OVX) was performed in female mRen2.Lewis rats at 4 weeks of age, and G1 (50 or 100 μg/kg/day) was given subcutaneously via minipump starting at 13 weeks of age for 2 weeks. Both doses of G1 significantly improved lusitropic function and structure, independent of changes in blood pressure. Compared to vehicle-treated OVX rats, G1 reduced the tissue Doppler-derived index of left ventricular filling pressure (E/e'), left ventricle mass, wall thickness, and the biomarkers of hypertrophy, ANF and BNP mRNA levels. Using cultured H9c2 cardiomyocytes, in vitro studies further showed that 1) G1 inhibited angiotensin (ANG) II-induced hypertrophy, evidenced by reductions in cell size, and ANF and BNP gene expression; 2) the antagonist of GPR30, G15, inhibited estrogen's protective effects on ANG II-induced hypertrophy; and 3) G1 induced phosphorylation of Erk and Akt. These data demonstrate the protective role of GPR30 in a model of diastolic dysfunction and cardiac hypertrophy, and provide insight into the underlying mechanisms.

126. IGF-1 Deficiency Impairs Vascular Oxidative Stress Resistance in Mice

Lora Bailey-Downs(P)[1], Matthew Mitschelen[1], Peter Toth[2], Danuta Sosnowska[1], Jim Henthorn[1], Akos Koller[2], William Sonntag[1], Zoltan Ungvari[1], Anna Csiszar[1]
[1]Reynolds Oklahoma Center on Aging, Department of Geriatric Medicine, Oklahoma City, OK; [2]Department of Physiology, New York Medical College, Valhalla, NY

Age-related dysfunction of Nrf2-driven pathways impairs cellular redox homeostasis, exacerbating age-related cellular oxidative stress and increasing sensitivity of aged vessels to oxidative stress-induced cellular damage. Circulating levels of IGF-1 substantially decline during aging, which significantly increase the risk for cardiovascular diseases in humans. To test the hypothesis that isolated, adult-onset IGF-1 deficiency impairs Nrf2-driven pathways in the vasculature, we utilized a novel mouse model with a liver-specific adenoviral knockdown of the IGF-1 gene using Cre-lox technology (Igf1f/f +MUP-iCre-AAV8), which manifests a significant decrease (~50%) in circulating IGF-1 levels. In the aorta of IGF-1 deficient mice there was a trend for decreased expression of Nrf2 and the Nrf2 targets genes GCLC, NQO1 and HMOX1. In cultured aorta segments of IGF-1 deficient mice treated with oxidative stressors (high glucose, oxidized LDL and H_2O_2) induction of Nrf2-driven genes was significantly attenuated as compared to control vessels, which was associated with an exacerbation of endothelial dysfunction, increased oxidative stress and apoptosis, mimicking the aging phenotype. In conclusion, IGF-1 deficiency is associated with dysregulation of Nrf2-dependent antioxidant responses in the vasculature, which likely promotes adverse vascular phenotypic alterations in pathophysiological conditions associated with oxidative stress (i.e. diabetes mellitus, hypertension) and results in accelerated vascular impairments in aging.

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127. Vascular Stiffness is Independently Associated with Central Adiposity Rather Than with Chronological Age in Nonhuman Primates

Kylie Kavanagh (P)[1], Ashley Wylie[1], William Strawn[1], Jason Lazar[2], Stephen Kritchevsky[1], Jay Kaplan[1]

[1]Wake Forest University School of Medicine, Winston-Salem, NC; [2]SUNY Downstate Medical Center, Brooklyn, NY

Vascular stiffness is presumed to result from age-related changes in the arterial wall; however, atherosclerosis develops concurrently and therefore it is not known what proportion of vascular stiffening is related to age effects alone. In the study presented here, pulse wave velocity (PWV) was measured in female vervet monkeys (*Chlorocebus aethiops*) that were maintained under controlled conditions which included a lifetime exposure to a low fat, low cholesterol diet which is known to prevent atherosclerosis development in this monkey model. Brachial-femoral PWV, plasma lipids, systolic blood pressure (SBP) and adiposity were measured in mid-life to aged female vervet monkeys (n=131, 9-24 years of age with maximal lifespan ≈ 25). In addition, a composite metabolic health score was developed by ranking monkeys 1 through 4 based on waist, triglycerides, HDL cholesterol, and SBP to mimic metabolic syndrome health risk. Average PWV was 7.14 (SD 1.9) m/s, which is comparable to measures in older people. PWV was moderately associated with age ($R=0.20$, $p=0.02$) but more strongly associated with waist circumference ($R=0.33$, $p<0.001$) and systolic blood pressure ([SBP] $R=0.30$, $p=0.001$). Using a multiple regression model, only waist circumference predicted PWV and accounted for 7% of PWV variance ($p=0.002$). When PWV was examined by quartile, waist circumference and SBP differed significantly among levels of vascular stiffness ($p=0.008$ and 0.02 respectively). A linear increase in PWV was seen with increasing metabolic risk score (6.29, 6.92, 7.38, and 8.63 m/s), which remained significant with adjustment for age ($p=0.005$) but not after addition of waist circumference ($p=0.30$) suggesting a primary role for central obesity in metabolic disease and associated vascular stiffness. No relationships were seen with plasma lipids. In conclusion, this primate model demonstrates that vascular stiffening is dependent on central adiposity and related health consequences rather than chronological age, and occurs in the absence of atherosclerosis.

128. Circulatory Diseases in the U.S. Elderly in the Linked National Long Term Care Survey-Medicare Database

Igor Akushevich (P)[1], Julia Kravchenko[2], Svetlana Ukraintseva[3], Konstantin Arbeev[3], Anatoli Yashin[3]
[1]Duke University; [2]Duke Cancer Institute, Duke University; [3]Center for Population Health and Aging, Duke University, Durham, NC

Heart disease and stroke cause about 50% of deaths at ages 65+ in the U.S., thus evaluation of trends of their incidence, prevalence, disability and co-morbidities is crucial for medical services, costs planning, and insurance expenditures. However, no nationally representative data could provide with required information. We evaluated whether Medicare data linked to the National Long Term Care Survey (NLTCS) for 1991- 2004 could provide with the information valid at the national level. Incidences of circulatory diseases declined by 14%-24% per 5-year interval (trend was significant). Incidence rates of stroke and heart failure were twice higher in severely disabled individuals and increased with age (15%-25% per 5-year). Acute coronary heart disease (ACHD) tended to have stable rates at all ages, non-dependent on disability level. Persons with high comorbidity index had higher rates of all circulatory diseases (10%-20% per the Charlson index unit). The obtained age patterns were in agreement with those reported in literature for all studied diseases. Stability of reconstructed incidence rates was confirmed by sensitivity analysis (including the effects of alternative definitions of disease onset, latent censoring, study design, etc.). Thus, the national age-specific incidence patterns of circulatory diseases can be adequately evaluated from the Medicare-NLTCS data and used in studying the effects of diagnostic, screening and therapeutic innovations and medical cost projections. Detected maxima at incidence curve and, especially, monotonic decline (detected for ACHD) with age do not agree with hypothesis that geriatric disease risks correlate with accumulation of adverse health events (genetic mutations, deterioration of vascular system, immunosenescence, etc.). To explain this phenomenon, the effect of selection (when frail individuals do not survive to advanced ages), possible under-registration of diagnoses at advanced ages (cannot be proved by available data), and the effects of basal, ontogenetic, and time-dependent components on individual aging could be suggested.

129. High Oxidative Stress and Detectable Levels of Aβ in the Naked Mole-Rat Brain

Yael Edrey(P, N), Martha Hanes, Mario Pinto, James Mele, Rochelle Buffenstein
Barshop Institute and Physiology Department, University of Texas Health Science Center San Antonio, San Antonio, TX

Age is the greatest risk factor to Sporadic Alzheimer's disease (AD)- the most common form of dementia. AD has been widely studied for over a

century yet the mechanisms that drive its development remain unknown. Laboratory rodents (mice and rats) do not naturally develop this disease and this has been attributed to both their short lifespan and to a different Aβ amino acid sequence than that of humans. Genetically manipulated mice carrying human genes are therefore widely used as biomedical models to study AD. These transgenic mice recapitulate the more rare form of familial AD, however a well-characterized model of sporadic AD has yet to be developed. Naked mole-rats (NMRs; Heterocephalus glaber) are the longest-living rodents known (maximum lifespan 32 years) and show prolonged good health for at least 70% of their long lives. These subterranean rodents, having evolved in a hypoxic habitat devoid of sunlight are naturally vitamin D deficient and in captivity incur high levels of oxidative damage evident even at a young age. Both these traits have been associated with Alzheimer's disease (AD) in humans. We hypothesize that NMRs, in keeping with their long lifespan and physiological traits considered high risk for the development of AD, will manifest in the symptoms characteristic of sporadic AD in old age. We sequenced the Aβ peptide and to our surprise, found that it was more similar to the human peptide than to that of its mouse counterpart. NMR brains had detectable amounts of both the soluble and insoluble fractions of Aβ even at a young age and showed significantly higher levels than young adult humans or even transgenic mice carrying human Aβ. Taken together, these results suggest that the longest-living rodents may prove a valuable natural model with which to study sporadic AD.

130. A Potential Role for Redox Sensitive Signaling-Dependent Demyelination of Motor Neurons

Ryan Hamilton (P,G), Michael Walsh, Arunabh Bhattacharya, Yun Shi, Holly VanRemmen
University of Texas Health Science Center at San Antonio, San Antonio, TX

Increasing evidence suggests that oxidative stress plays an inhibitory role in the proper myelination of motor neurons in the PNS that is associated with a loss in muscle and fiber size. We have previously shown that mice lacking Sod1 have an accelerated muscle atrophy phenotype and high levels of oxidative stress. Furthermore, Sod 1 null mice also show a reduction in nerve conduction velocity, suggesting increased demyelination. In support of this is a significant decrease in neuronal conduction velocity of the SOD -/- mice at all ages compared to the controls. We hypothesized that oxidative stress plays a role in demyelination through domain specific redox sensitive signaling of stress activated protein kinases (SAPKs) that inhibits myelination and that demyelination exacerbates the muscle atrophy phenotype as observed in the SOD1 knockout mice. To test this hypothesis, 1) Schwann cell cultures were treated with H_2O_2 to determine signaling, myelin protein synthesis and degradation and 2) SOD +/+, SOD-/- mice at 6, 10, 15 and 27 months of age were used to determine muscle loss, changes in signaling and myelination. H_2O_2 increased SAPKs ERK-1,2 and JNK-1,2 phosphorylation dose-dependently which are known to inhibit myelination. In addition, there was a concomitant decrease in the myelinating signal AKT phosphorylation. Accompanying these observed decreases in signaling was a decrease in myelin protein zero and an increase in MCP-1, a known mediator of macrophage-dependent myelin proteolysis. In spinal cords isolated from the Sod 1 null mice we found an increase in ERK-1,2 phosphorylation and JNK-1,2 phosphorylation at all ages compared to control suggesting an inhibitory pathway to myelination. Further, there was a concomitant decrease in AKT phosphorylation suggesting a further reduction in myelination. Altogether, these data suggest a link between oxidative stress and muscle atrophy that may be partly explained through a demyelination phenotype of motor neurons.

131. Role of Neuronal mTOR in Aging

Stacy Hussong (P,G) and Veronica Galvan
University of Texas Health Science Center at San Antonio, San Antonio, TX

Reduction of target of rapamycin (TOR) signaling has been shown to extend lifespan in invertebrates as well as in adult mice. In other genetic models of longevity, neuronal-specific manipulations are sufficient to extend lifespan.

To determine whether reduction of neuronal mammalian TOR (mTOR) signaling in adult mice is sufficient to extend lifespan and improve healthspan, we will decrease or eliminate expression of mTOR or of key downstream effectors in the mTOR pathway in neurons of adult mice. Conditional knock-out mice for mTOR, regulatory associated protein of mTOR (Raptor) as well as S6K1, a major downstream effector of mTOR were crossed with mice that inducibly express Cre recombinase in neurons. This experimental system makes it possible to study the role of neuronal mTOR signaling without the complicating effects from altered development.

An in vitro system was developed as a reference for the PCR and Western blot-based quantitative analyses that will enable us to determine the penetrance of the inducible, Cre-mediated excision of floxed alleles of genes in the mTOR pathway. This system involves the use of mouse embryonic fibroblasts isolated from heterozygous and homozygous conditional knock-out mice and adenoviral delivery of Cre recombinase. We will report on the generation of our calibration system and on the generation of animals homozygous for the different conditional knock-out alleles and heterozygous for doxycycline-inducible neuronal-specific Cre, that will be either left uninduced or treated with doxycycline after completion of developmental maturation at 2 months of age in experiments that will determine if decreased mTOR signaling in the mouse nervous system is sufficient to extend lifespan and improve healthspan.

132. Signaling Through mTOR and the Heat Shock Response in cognitive Aging and Neurodegeneration

Veronica Galvan (P), Jonathan Halloran, Natalia Podlutskaya, Anson Pierce
University of Texas Health Science Center at San Antonio, San Antonio, TX

The target of rapamycin (TOR) pathway is a major signaling hub that integrates nutrient/growth factor availability with cell metabolism. Reduced activity of TOR extends invertebrate lifespan, and, in mice, pharmacologic reduction of mTOR signaling during adulthood prolongs life, possibly by retarding aging. Consistent with this idea, we recently showed that systemic, long-term treatment with rapamycin prevents Alzheimer's (AD)-like deficits and reduces Aß accumulation in a mouse model of the disease. Moreover, long-term inhibition of the mTOR pathway improved cognitive function and reduced anxiety as well as depressive-like behavior in wild-type mice. To determine the mechanisms by which long-term inhibition of mTOR modulates cognitive function we performed proteomic and gene expression studies. The chaperone/heat shock response (HSR) family was overrepresented among the proteins upregulated in rapamycin-treated brains. In agreement with the upregulation of heat shock proteins (HSP), activity of heat shock factor 1 was increased. The small chaperone alpha-B crystallin (hsp16), which is already augmented in brains of untreated AD mice, showed the highest level increase as a result of rapamycin treatment, suggesting that long-term inhibition of mTOR enables alpha-B crystallin to further accumulate and possibly contribute to the amelioration of AD-like deficits in mice. Our results suggest that maintenance of proteostasis may have a key role in sustaining cognitive function and preventing affective dysfunction during aging and in AD-like neurodegeneration. Low-level, long-term inhibition of the mTOR pathway may thus act by boosting the activation of the chaperone network as a response to the accumulation of aggregated or damaged proteins during aging, and in AD. Our data support the hypothesis that inhibition of mTOR delays aging, and suggest that rapamycin, already used in clinical settings, may be a potentially effective therapeutic agent for the treatment of a continuum of age-associated cognitive dysfunctions, depressive-like disorders, and AD.

133. Humor Usage in Geriatrics

Gurprit Lamba (P)[1], Vijay Aswani[2], James Ellison[3]
[1]St Elizabeth's Medical Center; [2]Marshfield Clinic;
[3]McLean and Harvard Medical School

Background
The potential of humor to enhance and promote therapeutic relationships in healthcare is being recognized but clinical data is limited.

Purpose
The purpose of this study is to review humor in the scientific and medical literature and explore various angles from its evolution to its usage as a clinical tool in medicine.

Method
A literature search was conducted in PubMed using MeSH terms 'Laughter Therapyin Elderly' and 'Humor and Laughter'. Additional articles from the references cited in relevant publications were also reviewed. These were critically analyzed to review the use of humor in care of the elderly.

Results
This poster provides an overview of the field of humor highlighting what is known and not known about humor in aging in medicine. This information potentially serves and contributes to highlight the development of formal training in the use of humor in medicine.

Conclusions
Based on the review we argue that knowledge of humor should be imparted to healthcare providers as a therapeutic tool in a holistic approach to care of the elderly in the field of medicine.

134. Role of Thioredoxin 1 and 2 in Cancer and Aging

Lisa Cortez[1], Celeste Webb[1], Megan Mahlke[1], Melanie Ortiz[1], Arunabh Bhattacharya[1], Yiqiang Zhang[1], Adam Salmon[1], Wenbo Qi[1], Yuhong Liu[1], Shuko Lee[2], Holly Van Remmen[1], Arlan Richardson[1], Gene Hubbard[1], Yuji Ikeno (P)[1]
[1]Barshop Institute, University of Texas Health Science Center at San Antonio; [2]Audie Murphy VA Hospital, San Antonio, TX

Our laboratory has conducted the first detailed study on the effect of overexpressing or down-regulating thioredoxin 1 (Trx1: cytosol) or thioredoxin 2 (Trx2: mitochondria) on aging. Interestingly, we found that the Trx2Tg mice showed a significant extension of lifespan (25.6%) compared to wild-type mice, although we observed a little increase in survival of the Trx1Tg mice. The extension of lifespan of Trx2Tg mice was correlated to less reactive oxygen species (ROS) production from mitochondria and less oxidative stress. These data indicate that overexpressing Trx in the mitochondria may be more important than in the cytosol on aging because mitochondria are a major source of ROS. When we tested the effects of reduced levels of Trx in cytosol or mitochondria on aging, we surprisingly observed the reversed effects, i.e., a significant increase in survival of the Trx1KO mice (12.6%) compared to wild-type mice, while the Trx2KO mice showed little effects on lifespan. The extension of lifespan of Trx1 KO mice was associated with less cancer compared to wild-type mice at 22-24 months of age. These data indicate that reduced cancer in the Trx1KO mice could be one of the contributing factors of extended lifespan. Our data are exciting in that we show 1) overexpressing Trx in the mitochondria increases lifespan, but overexpressing Trx in the cytosol has little effect on lifespan, which is similar to the results of mCAT mice; and 2) down-regulating Trx in the cytosol increases lifespan and reduces cancer, but down-regulating Trx in mitochondria has no effect on lifespan or cancer. These paradoxical, but intriguing results could indicate that the Trx2Tg and Trx1KO mice attenuate aging through different mechanisms, e.g., protection of mitochondria against oxidative stress and reduced age-related pathology, e.g., cancer.

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135. Effect of Hypoxia on Bioenergetics of Myoblasts Precursor Cells (MPC) Isolated from Young and Old Mice

Mina Konigsberg (P)[1,2], Viviana Pérez[3], Carmen Rios[1], Sukkyoo Lee[1], Yun Shi[1], Holly Van Remmen[3]
[1]Barshop Institute, University of Texas Health Science Center at San Antonio, San Antonio, TX; [2]Universidad Autonoma Metropolitana, Mexico City, Mexico; [3]Cellular and Structural Biology Department, Barshop Institute, University of Texas Health Science Center at San Antonio, San Antonio, TX

The progressive loss of muscle mass during human aging produces a decline in strength and function, which results in the deterioration of the quality of life and has also been associated with a variety of illnesses. To study skeletal muscle atrophy, in vitro studies with isolated myoblasts precursor cells (MPC) have been performed. However, most of the in vitro cell cultures have been performed at ambient oxygen tension (21% O_2), despite the fact that physiological oxygen pressure at the organ level is much lower, and varies from tissue to tissue (2-5%). Previous studies modifying pO_2 in tissue cultures have shown an effect on cell growth and differentiation as well as the establishment of cellular senescence, although the biochemical and molecular mechanisms leading to these outcomes are still poorly understood. Therefore it is important to study the variation in pO_2 and its effect on cell metabolism and response to toxins while studying cellular deterioration associated to aging. The aim of this study was to determine the effect of pO2 on MPC mitochondrial respiratory capacity and bioenergetics after an acute oxidative insult in relation to age. MPC isolated from young (3-4 m) and old (29-30 m) C57BL/6 mice, were grown under hypoxia (3% O_2) or normoxia (21% O_2) for two weeks. Cells were treated with an acute oxidative insult of 100 μM H2O2 for 19 h. Mitochondrial bioenergetics such as oxygen consumption, glycolysis and fatty acid oxidation, were measured using the Seahorse Bio-science XF24 extracellular flux analyzer. Our results show a difference in the reserve capacity between the MPC isolated from old and young mice after an oxidative insult, as well as a shift to a more glycolytic metabolism in the old mice MPC.

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136. Enhanced Stress Resistance of Fibroblasts from Long-lived Birds

James Harper (P)[1], Joseph Williams[2], Richard Miller[1]
[1]University of Michigan, Ann Arbor, MI; [2]Ohio State University, Columbus, OH

Evolutionary senescence theory postulates that aging results from the declining force of natural selection with increasing chronological age. A goal of comparative studies in the biology of aging is to identify genetic and biochemical mechanism(s) driving species-specific differences in the aging process that are the end product of life history tradeoffs. We hypothesized that cells from long-lived bird species are more resistant to stress agents than are cells from short-lived species, and that cells from birds are more resistant to stress than are cells from relatively short-lived mammals of similar size. Using primary fibroblast cultures from 35 species of free-living birds we found that cell lines from longer-lived species were resistant to death caused by cadmium ($R_2 = 0.27$, $p = 0.002$), paraquat ($R_2 = 0.13$, $p = 0.03$), hydrogen peroxide ($R_2 = 0.09$, $p = 0.07$), and methyl methanesulfonate ($R_2 = 0.13$, $p = 0.03$), as well as to the metabolic inhibition seen in low glucose medium ($R_2 = 0.37$, $p < 0.01$). They did not differ in their resistance to UV radiation, or to thapsigargin or tunicamycin, inducers of the unfolded protein response. These results were largely consistent even after accounting for the influence of body mass and phylogeny. In addition, avian fibroblasts were significantly more resistant than rodent fibroblasts to each of the tested stressors. These results support the idea that cellular resistance to injury may be an important contributor to the evolution of slow aging and long life span among bird species, and may contribute to the relatively long life span of birds as compared with rodents of the same body size. It remains to be seen which molecular mechanisms are responsible for this differential stress resistance, both among, and within, taxa.

137. Stress Resistance and Lifespan: The Bare Necessities

Kaitlyn Lewis (P,N)[1], James Mele[1], Rochelle Buffenstein[2]
[1]University of Texas Health Science Center at San Antonio; [2]Barshop Institute, University of Texas Health Science Center at San Antonio, San Antonio, TX

The transcription factor Nrf2 (nuclear factor-erythroid 2-related factor-2) is present in all tissues and regulates the expression of over 200 cytoprotective genes playing key roles in protection against cancer, toxins, inflammation and neurodegeneration. New data suggest that Nrf2 also may be important in aging and longevity; however, the mechanism behind this is yet to be elucidated. Basal Nrf2 levels are regulated by Keap1 (Kelch-like ECH-associated protein 1) mediated proteasomal degradation. Under stressful conditions, cysteine modifications inactivate Keap1, thus reducing degeneration, increasing levels of free Nrf2 and increasing Nrf2 half-life. Free Nrf2 translocates into the nucleus where it binds to the ARE (antioxidant response element) to activate the transcription of an array of downstream antioxidant and detoxicant enzymes and molecules. We show that there is a positive correlation with Nrf2 signaling activity and species longevity in several similarly-sized rodent species with a range in maximum lifespan (MLSP) from 3 to 32 years. The naked mole-rat, with an extraordinary MLSP of 32 years, has significantly higher levels of in vivo Nrf2-signaling activity compared to shorter-lived rodent species. This enhanced cytoprotective signaling may contribute to its profound longevity, minimal decline in healthspan, marked resistance to toxins and cancer-free existence. The key to upregulated Nrf2 signaling activity appears to be the dramatically decreased levels of Keap1 in the naked mole-rat and concomitant increased levels of free Nrf2. Naked mole-rats are both physiologically and biochemically more resistant to in vivo challenges with toxins than are shorter-lived rodents, showing smaller changes in body temperature, less irreversible cysteine damage and more rapid Nrf2 signaling. Similarly, both dwarf mice and caloric-restricted mice show evidence of an upregulated and sensitive Nrf2 signaling pathway. Enhanced Nrf2-signaling may be a conserved mechanism facilitating extended healthspan and prolonged lifespan.

138. The Differential Impact of Calcineurin Isoforms in the Early Unfolded Protein Response

Yanan Chen (P)
University of Texas Health Science Center at San Antonio

A crucial function of the Endoplasmic Reticulum (ER) is to insure correct protein folding and processing. ER stress leads to an accumulation of unfolded proteins, which in turn, activates a series of adaptive responses that attempt to restore ER homeostasis and are collectively called the Unfolding Protein Response (UPR). The most immediate response is the attenuation of mRNA translation, which is initiated by autophosphorylation of PKR-like ER kinase (PERK). This is followed by phosphorylation of the eukaryotic initiation factor-2α (eIF2α), which attenuates protein synthesis. Recently our lab reported that calcineurin-Aα (CN) strengthened the early UPR by binding to PERK and enhancing its autophosphorylation (Bollo et al PLoSOne 5 (8): e11925). Given the existence of two isoforms of CN-A (α and ß) in astrocytes, we hypothesized a

differential regulation of the early UPR. First, we treated astrocytes with thapsigargin, a Ca2+-ATPase inhibitor that is known to induce ER stress. We found that CN-Aα levels were rapidly increased while the levels of CN-Aß decreased. Second, RNAi knockdown of CN-Aα impaired the UPR while CN-Aß RNAi treatment did not. Third, the binding affinity of CN-Aß to PERK was unaffected while CN-Aα binding to PERK was increased. Finally, astrocytes deficient in CN-Aß exhibited a significantly higher level of cell death and eIF2α phosphorylation at rest. Oxygen and Glucose Deprivation (OGD) treatment did not further increase cell death or eIF2α phosphorylation in CN-Aß -/- cells, but did so in both CN-Aα -/- and wild-type siblings. Taken together, we suggest that CN-Aß is bound to the PERK monomer at rest and is responsible for maintaining ER homeostasis. During stress CN-Aα competes off CN-Aß and helps to initiate the early UPR. This is a new function for an old phosphatase that remarkably, increases autophosphosphorylation of PERK, thereby enhancing early cell survival during stress.

139. Mild Oxidative Stress Increases Oxidative Stress Tolerance Through NRF2 Regulated Proteasome Expression in Flies and Worms

Andrew Pickering (P,N), Derek Sieburth, John Tower, Kelvin Davies
University of Southern California, Los Angeles, CA

The Antioxidant Response Element (ARE) transcription factor NRF2 (CNC-C, SKN-1) has, in *Drosophila melanogaster* and *Caenorhabditis elegans*, been reported to play an important role in determining life-span. Additionally several long lived mutants have been generated by knockout or knock-down of NRF2 inhibitors. We observe an increase in tolerance to oxidative stress, following mild oxidative pre-treatment in flies, worms and murine epithelial cells. This hormesis occurs through a conserved pathway that involves increased 20S proteasome synthesis (as well as immunoproteasome in mice) and that is regulated by NRF2. Because of the connection between NRF2 and lifespan, and our ability to trigger NRF2 linked stress-adaptation through mild oxidative stress, we speculate a potential role for mild oxidative stress in longevity.

140. Insulin Resistance can be Regulated by the Protein Oxidation Repair Enzyme Methionine Sulfoxide Reductase A (MsrA)

JennaLynn Styskal (P,N)
University of Texas Health Science Center at San Antonio, San Antonio, TX

Aging is associated with the accumulation of oxidative damage which can regulate the development of age-related diseases. A significant source of oxidative stress is adipose tissue, which when accumulated has been shown to alter redox signaling and increase oxidative damage to cellular macromolecules. Proteins oxidation is particularly detrimental because oxidation can alter conformation which leads to reduced function and accumulation. MsrA plays an important role in the regulation of protein oxidation by reducing methionine sulfoxide residues to non-oxidized methionine, acting as an antioxidant specifically on proteins. We have found that MsrA has a significant effect on the development of obesity-induced insulin resistance. High fat fed WT mice show significant reduction in response to insulin tolerance tests, and reduced insulin signaling in skeletal muscle, adipose tissue and liver. Mice that lack MsrA (MsrA-/-) mice fed a high fat diet show a significant reduction in insulin response beyond that of WT mice, whereas high fat fed mice that overexpress MsrA (MsrATg) mice show significant improvement. These results suggest that oxidative damage, specifically to proteins, may play an important role in obesity-induced insulin resistance. To address how protein oxidation may cause insulin resistance, we have utilized ex-vivo studies using skeletal muscle and adipose tissue to test the effect of MsrA on oxidative stress-induced insulin resistance. Interestingly, MsrA seems to have a significant effect on the development of insulin resistance in this system. We show that tissues from MsrA-/- mice are sensitive to oxidative stress, whereas those from MsrATg mice are protected in this ex vivo system. These findings suggest that levels of protein oxidation can be correlated with the degree of insulin resistance in this system. Future studies will focus on functionality of the insulin signaling proteins isolated from these models as well as their oxidation status.

141. Heated Hydrotherapy Induces the Heat Shock Response in Nonhuman Primates and Improves Cardiometabolic Health

Kylie Kavanagh (P), David Flynn, Kurt Jenkins, Matthew Davis, Janice Wagner
Wake Forest University School of Medicine, Winston-Salem, NC

The heat shock response (HSR) and heat shock proteins (HSPs) decrease with age. The HSR results in transcription of HSPs 70 and 90. HSP70 is lower in obesity and diabetes, and HSP90 increases vasorelaxation through facilitating

endothelial nitric oxide synthase. Cardiovascular disease and reduced glycemic control are common comorbidities of aging and should benefit from induction of the HSR. We aimed first to investigate whether induction of the HSR using heated hydrotherapy (HT) successfully increases HSPs 70 and 90 tissue levels in primates. Four adult monkeys were submerged to neck level in a heated whirlpool with continuous rectal temperature monitoring, and maintained between 39 and 41°C for 30 minutes. Muscle biopsies were taken prior to, immediately following, and 1 and 4 hours post-HT in 4 monkeys. HT significantly induced gene expression of HSP70 nine-fold ($p<0.01$), and HSP90 two-fold ($p<0.05$) with elevations persisting to 4 hours. We then conducted a pilot clinical trial in aged primates (average age 17 years) to determine the vascular and glycemic effects of repeated HSR induction. Four monkeys underwent twice weekly HT for 5 weeks and 3 control [CTL] monkeys had normal body temperature maintained under sedation. Muscle biopsies, blood pressure (BP) measurements, and glucose tolerance tests were performed one week before and one week after the trial. In the HT monkeys, tissue levels were variable but showed a trend towards elevated HSP70 protein even 5 days after the final HT. Systolic BP was reduced by > 40mmHg and diastolic BP by >20mmHg ($p=0.06$ for both) in HT monkeys with a notable trend towards reduced heart rate ($p=0.11$). Glucose tolerance worsened in CTL, but HT monkeys were stable with signs of improved insulin secretion. We conclude that HT in aged primates induces the HSR and shows promise for the treatment of hypertension and insulin resistance.

142. Skeletal Muscle HSP70 is Affected by Western Diet and not by Chronological Age in Nonhuman Primates

Kylie Kavanagh (P), Ashley Wylie, Tara Chavanne, Lawrence Rudel, Stephen Kritchevsky, Jay Kaplan
Wake Forest University School of Medicine, Winston-Salem, NC

Tissue heat shock protein (HSP)70 decreases with both normal aging and insulin resistance and/or hyperglycemia. As aging is often associated with coincident insulin resistance and higher blood glucose levels, we aimed to understand how these factors relate to muscle HSP70 levels in an age-diverse colony of vervet monkeys (*Chlorocebus aethiops*). We correlated glycemic endpoints, plasma lipids, adiposity, with muscle HSP70 in 284 adult monkeys (3-24 years, lifespan ≈ 26) who were fed a low fat/cholesterol laboratory chow diet. Aging monkeys showed increased adiposity (BMI and waist circumference) and less healthy lipid profiles (increased triglycerides and low-density-lipoprotein cholesterol). We found there was no association of HSP70 with age ($r=0.04$, $p=0.53$). Modest positive associations were found only with glucose ($r=0.12$, $p=0.04$), triglycerides ($r=0.13$, $p=0.03$) and very-low-density lipoprotein cholesterol ($r=0.15$, $p=0.008$). Muscle HSP70 was not different in a subset of diabetic monkeys, ($n=10$, $p=0.50$) when compared to the non-diabetic colony monkeys. Results were also not tissue-specific to muscle, as when liver was compared in younger (n=5;9-12 years) and older (n=5; 20-26 years) colony monkeys, no differences were found ($p=0.37$). In contrast, a subset of adult vervet monkeys (n=9; 5-13 years) that had consumed a high fat, cholesterol containing diet for 6 years, showed a striking negative association between muscle HSP70 and glucose ($r=-0.84$, $p=0.004$) and adiposity (waist circumference $r=-0.69$, $p=0.04$). These monkeys had normal glucose values but were insulin resistant as compared to the chow fed colony monkeys (HOMA mean 5.8[SE=1.5] vs. 3.33[SE=0.26]). The conclusions from this study demonstrate that a low fat/cholesterol diet may preserve tissue HSP70 with aging and that dietary lipid or cholesterol may be an important determinant for HSP70 expression in response to changes in insulin sensitivity. Finally, studies that examine HSP proteins should utilize an appropriate nutritional context that is relevant to people, to allow experimental findings to be translational.

143. Environment and Aging: Life-Stage Susceptibility to Toxicants

Kimberly Jarema(P), Pamela Phillips, Robert MacPhail
US Environmental Protection Agency

Understanding the role of aging in susceptibility to environmental chemicals becomes increasingly important as the aging population swells. As part of a larger program on environmental pollution and life-stage susceptibility, these experiments determined the effects of toluene, an organic solvent, and the carbamate pesticide methomyl on the behavior of adolescent, young-adult, middle-age and senescent male Brown Norway rats. Motor activity was tested at weekly intervals in photocell devices that recorded both horizontal and vertical activity during 30-min sessions. Toluene (corn-oil vehicle, 300-1000 mg/kg) was administered p.o., 30 min before testing. Methomyl (deionized water, 0.5- 2.75 mg/kg) was given orally 15-min before a

test session. All compounds were administered orally to each rat in a mixed order. Following vehicle, horizontal and vertical activity was highest in young adults and lower in the middle-age, senescent and adolescent rats. Toluene produced dose-related increases in horizontal activity that increased proportionately with age. Methomyl produced dose-related decreases in both horizontal and vertical activity that also increased with age. An additional, longitudinal, study on the effects of another carbamate, carbaryl (17 mg/kg), showed decreases in both horizontal and vertical activity that became amplified at 20 months of age and beyond. These results indicate aging increases susceptibility to toluene, methomyl and carbaryl in Brown Norway rats; they also emphasize the importance of evaluating toxicant effects at multiple life stages in order to make informed inferences regarding susceptibility and risk.

This is an abstract of a proposed presentation and does not reflect US EPA policy)

144. Negative Regulation of STAT3-mediated Cellular Respiration by SirT1

Susan Krzysik-Walker, Michel Bernier, Alejandro Martin-Montalvo, Morten Scheibye-Knudsen, Shaoming Song, and Rafael de Cabo
Laboratory of Experimental Gerontology, National Institute on Aging, National Institutes of Health, Baltimore, MD

In mammals, the transcriptional activity of signal transducer and activator of transcription 3 (STAT3) is regulated by the deacetylase SirT1. However, whether the newly described non-genomic actions of STAT3 toward mitochondrial oxidative phosphorylation are dependent on SirT1 is unclear. In this study, SirT1 gene knockout MEF cells were used to delineate the role of SirT1 in the regulation of STAT3 mitochondrial function. Here, we show that STAT3 mRNA and protein levels and the accumulation of serine phosphorylated STAT3 in mitochondria were increased significantly in SirT1-KO cells as compared to wild-type MEFs. Various mitochondrial bioenergetic parameters, such as the oxygen consumption rate in cell cultures, enzyme activities of the electron transport chain complexes in isolated mitochondria, and production of ATP and lactate, indicated that SirT1-KO cells exhibited higher mitochondrial respiration as compared to wild-type MEFs. Two independent approaches, including ectopic expression of SirT1 and siRNA-mediated knockdown of STAT3, led to reduction in intracellular ATP levels and increased lactate production in SirT1-KO cells that were approaching those of wild-type controls. Comparison of profiles of phospho-antibody array data indicated that the deletion of SirT1 was accompanied by constitutive activation of the pro-inflammatory NF-κB pathway, which is key for STAT3 induction and increased cellular respiration in SirT1-KO cells. Thus, SirT1 appears to be a functional regulator of NF-κB-dependent STAT3 expression that induces mitochondrial biogenesis. These results have implications for understanding the interplay between STAT3 and SirT1 in pro-inflammatory conditions.

145. How Well Do Sleep and Activity Behaviours Predict Lifespan in Drosophila?

Sofocles Koudounas[1], Edward Green[2], David Clancy(P)[1]
[1]Lancaster University; [2]University of Leicester, UK

There are currently no reliable biomarkers of ageing in any animal. A biomarker should indicate physiological age, that is, the amount of an animal's total lifespan it has lived and, therefore, the amount of time it has remaining. Some potential biomarkers cannot be validated as such because their measurement involves harm or death of the animal, so that its ultimate lifespan cannot be determined. A non-destructive biomarker would allow us to test molecular markers potentially involved directly in the ageing process, to monitor the effectiveness of therapeutic interventions to delay ageing, and provide a useful measure of general health of the organism. In the model organism Drosophila, various behavioural phenotypes change directionally with age but we do not know whether they predict lifespan. Here we measure activity and sleep parameters in 64 wildtype male flies over the course of their natural lives to see whether lifespan is predicted by any absolute measures or changes over time. Indices of sleep fragmentation were the best predictors of lifespan. However considerable variation between individuals and within individuals over time rendered even the best predictor, decline in longest sleep duration, slightly less accurate and less precise than chronological age at predicting 50% lifespan. A caveat is that populations must be healthy (early mortality must be low) for chronological age to reflect physiological age.

146. Predictors of Sleep Disturbances in Seniors: A Population-based Study

Joseph Finkelstein (P) and Eunme Cha
Johns Hopkins University School of Medicine, Baltimore, MD

Background: Seniors often suffer from sleep disturbances caused by age-related health

deterioration. In this study we assessed the prevalence of sleep disturbances in different age groups and predictors of sleep disturbances in seniors using population-based data.
Methods: Based on the National Health Interview Survey (NHIS) for year 2007, seniors (>=65) with sleep disturbances were included in the study sample. Sleep disturbances were defined by the following questions: "During the past 12 months, have you regularly had insomnia or trouble sleeping" and "Have you regularly had excessively sleepiness during the day?" Subjects who answered "Yes" to these questions were included, and those who "Refused" or answered "Not ascertained" or "Don't know" were excluded from the analyses. The logistic regression was performed controlling for gender, age, poverty income ratio (PIR), race, marital status and education.
Results: The Sample Adult File from the year 2007 consisted of 23,393 subjects. The age stratified prevalence of sleep disturbances was: 18.3% (19-39), 22.7% (40-54), 25.0% (55-64), and 28.0% (>=65). From the youngest to the oldest age group, an increasing trend of sleep disturbances was observed. Among >=65 age group, sex (female, OR 1.3, p=0.007), low income status (PIR<1, OR 1.5, p=0.006), and presence of any co-morbidities (OR 4.1, p<.0001) strongly predicted the sleep disturbances. Hispanics, African Americans, and Asians were less likely to report sleep disturbances than Whites, but only African Americans (OR 0.7, p=0.009) showed statistical significance. Compared to seniors who completed 12+ years education, those with <12 years of education showed slightly higher risk (OR 1.2, p=0.09) for sleep disturbances, but with no significance.
Conclusions: In this study, we identified predictors of sleep disturbances in seniors who are 65 or older. Female sex, income level, and other co-morbidities were strongly associated with self-report of sleep disturbances. Further investigation can be focused on those predictors.

147. Serum Carcinoembryonic Antigen Levels Associated with Arterial Stiffness in Female Korean Non-Smokers?

Ji Won Lee (P)[1], Ki-Deok Park[2], Duk-Chul Lee[3], Soo Jin Jung[3], Ji Youn Chung[3], Jeing Ah Hong[3], Jeeyon Lee[3]
[1]Yonsei University College of Medicine Severance Hospital; [2]Department of Rehabilitation Medicine, Gachon University of Medicine; [3]Department of Family Medicine, Severance Hospital, Yonsei University

Objective—Carcinoembryonic antigen (CEA), a serological malignant tumors, may show a modest increase under some nonmalignant conditions, such as ageing and cigarette smoking. We marker of have investigated whether serum CEA levels are associated with arterial stiffness in female Korean non-smokers.
Methods and Results—Cross-sectional data from 124 female Korean non-smokers who underwent general health screening were analyzed. The interquartile of cutoff values of serum CEA levels were 0.58, 1.07, and 1.39 ng/mL. Intima media thickness and pulse wave velocity were associated with increased serum CEA levels. Multiple regression analysis adjusted for age, menopause status, alcohol intake, exercise, body mass index, serum lipid and glucose profiles, and C-reactive protein the CEA levels were independently associated with intima media thickness.
Conclusions—Serum CEA was associated with arterial stiffness independently of atherogenic risk factors and markers of inflammation. Our data suggest that a slight elevation of CEA may not be an innocuous observation from the viewpoint of early atherosclerosis.

148. Adiponectin in Women with Polycystic Ovary Syndrome

Hyun-Young Shin(P), Duk-Chul Lee, JeeYon Lee, Ji Youn Chung, Jeong Ah Hong, Ji-Won Lee
Department of Family Medicine, Severance Hospital, College of Medicine, Seoul, South Korea

Background Though adiponectin has been associated with insulin resistance and cardiovascular risk factors, the relationship between adiponectin and polycystic ovary syndrome(PCOS) remains controversial. The aim of this study was to compare adiponectin level in women with PCOS and without PCOS, and to investigate the relationship between adiponectin level and metabolic variables.
Methods 60 women with PCOS were enrolled along with a control group of 80 healthy women, matched for age and body mass index(BMI). We measured hormonal and metabolic parameters, as well as the plasma adiponectin concentrations of each participant. We estimated the insulin sensitivity according to the quantitative insulin sensitivity check index(QUICKI).
Results The PCOS group displayed significantly lower levels of adiponectin (P<0.001) after adjustment for age, BMI, mean blood pressure, fasting glucose, fasting insulin, triglyceride (TG), high-density lipoprotein(HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, estradiol (E2), luteinizing hormone/follicle-stimulating hormone (LH/FSH), total testosterone and sex-hormone binding globulin (SHBG) levels. Adiponectin levels were positively correlated with

QUICKI in the PCOS group (P<0.001) and the control group (P=0.03). Following step-wise multiple regression analysis, however, adiponectin levels were positively correlated with QUICKI in the control group only (P=0.03). In addition, adiponectin levels were found to be independently associated with HDL-cholesterol levels (P<0.001) and BMI (P=0.02) in the PCOS group and independently associated with HDL- cholesterol (P=0.02) in the control group.
Conclusion Lower adiponectin levels in the PCOS group, when compared to the non-PCOS group, were observed and adiponectin is associated with both lipid metabolism and obesity, which, in turn, is related to insulin resistance in PCOS. Further studies are needed to clarify the mechanism of adiponectin in PCOS.

149. Serum Adiponectin Level is Independently Associated with Physical Performance in Community Dwelling Old Women

Duk Chul Lee, Ji Youn Chung, Jee Yon Lee (P), Hyun Mee Kim
Department of Family Medicine, Yonsei University, College of Medicine, Seoul, South Korea

Adiponectin is a most abundant peptide produced in adipose tissue. Adipopectin has been known to be associated with lipid metabolism, glucose metabolism and insulin resistance. Furthermore adiponectin plays a role as an anti-inflammatory, anti-atherogenic, and cardioprotective property. We investigate the effect of adiponectin on physical performance in apparently healthy community dwelling old women. This study conducted as a part of the Yonsei Aging Study (YAS) (Lee et al., 2010). YAS was designed as a survey to investigate the factors related to depression, cognitive function and physical performance in community dwelling old people in Korea. A total of 136 women, aged more than 60 years (male mean age 73.9±7.0), were included in this study. We measured anthropometric and cardio-metabolic parameters including body composition, lipid profile and insulin-like growth factor1 (IGF-1), cortisol, dehydroepiandrosterone-sulfate (DHEA-S) and adiponectin. Depression was assessed with the short-form GDS consisting of 15 questions. Cognitive function was assessed by the Korean version of Mini-Mental State Examination (MMSE). Physical performance was assessed by gait speed, chair stand test, and tandem standing test. The sum of the three components was used as the final physical performance score.
Physical performance scores were significantly correlated with age (r=-0.44, p<0.001) MMSE score (0.24, p<0.01), adiponectin (r=0.28, p<0.005), GDS (r=-0.275, p<0.005), high density lipoprotein cholesterol (r=0.20, p<0.05), cortisol (r=-0.30, p<0.005), C-reactive protein (r=-0.19, p<0.05), fat mass (r=-0.23, p<0.01), lean body mass (r=0.22, p<0.01), waist circumference (-0.25, p<0.005), BMI (r=-0.18, p<0.05), Mean values of adiponectin was differed with statistical significance (p<0.005) between 4 groups, divided by the quartile scores of physical performance. Serum adiponectin level was independently associated with physical performance score after adjustment of confounding factors with stepwise regression procedure. In conclusion, this result suggested that serum adiponectin concentration may be a contributing factor for physical performance in old women.

150. Long-Lived SURF1 Knockout Mice with Decreased Mitochondrial Complex IV Activity Exhibit Increased Insulin Sensitivity

Deepa Sathyaseelan (P), Daniel Pulliam, Adam Salmon, Lauren Sloane, Holly Van Remmen
Barshop Institute for Longevity and Aging Studies, University of Texas Health Science Center at San Antonio, San Antonio, TX

Preservation of mitochondrial function and reduced generation of reactive oxygen species are correlated with increased lifespan and healthspan. Recent studies in invertebrates and rodents have challenged this paradigm by demonstrating that several mitochondrial electron chain alterations are associated with increased longevity. For example, mice lacking SURF1 (Surfeit locus protein 1), a 30 kDa inner mitochondrial membrane protein essential for assembly of electron transport chain complex IV (Cytochrome c oxidase, COX), have a 50–80% decrease in COX activity compared to control littermates, yet increased longevity. Maintenance of mitochondrial function is essential for insulin sensitivity, which is one important correlate of longevity. In this study, we asked whether SURF1-/- mice have alterations in insulin sensitivity that might contribute to the increased longevity in these mice. Compared to wildtype littermates, SURF1-/- mice displayed reduced body weight that could be attributed to decreased fat mass. While loss of SURF1 had little effect on glucose clearance, it significantly improved insulin sensitivity. We also found an increase in the activation of AMP-activated protein kinase (AMPK) and subsequent acetyl CoA carboxylase (ACC) inhibition in multiple tissues suggesting increased fatty acid oxidation. This was paralleled with an increase of hormone-sensitive lipase (HSL) phosphorylation level in the adipose tissue of the

SURF1 null mice. Together these data suggest a novel crosstalk between electron transport chain dysfunction and AMPK activation that causes increased fatty acid oxidation and insulin sensitivity and suggest increased insulin sensitivity as a potential mechanism for increased longevity in these mice.

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151. Differential Effects of Mild Treadmill Exercise on Inactivity-Induced Decline in Power in Soleus Single Fibers with Age

Jong-Hee Kim (P, G), Xiaolin Zheng, LaDora Thompson
University of Minnesota, Minneapolis, MN

The purpose of this study was to investigate the effects of mild treadmill exercise on inactivity-induced alterations in contractile properties (e.g., diameter, force, velocity, and power) of single fibers from the soleus muscle. Adult (5-12 months), old (24-31 months), and very old (32-40 months) F344BNF1 rats were randomly assigned to three experimental groups: weight bearing control (WB), non-weight bearing (NWB), and non-weight bearing exercise (NWBX). NWB rats were hindlimb unweighted for two weeks representing inactivity. The NWBX rats were hindlimb unweighted for two weeks and received treadmill exercise for 15 minutes, 4 times a day. The alterations in single fiber contractile properties to the NWB condition were age-specific. Two weeks of NWB result in a reduction in fiber diameter and isometric maximal force (Po) in the very old rats (-12% and -40%, respectively). Specific force (Po/CSA) significantly declined in both the old (-36%) and very old (-23%) rats. Absolute peak power and peak power normalized to CSA were significantly reduced in all three age groups. In contrast, NWB did not induce a change in velocity. The response to the mild exercise program was age-specific, too. Exercise attenuated the inactivity-induced reduction in peak power in adult (+9.6%, 63% level of WB group) and old (+29%, 100% level of WB group) animals. Interestingly, in the very old animals that received exercise there was a further reduction in Po/CSA (-29%, 55% level of WB group) and peak power (-49%, 30% level of WB group). These results suggest that mild treadmill exercise is beneficial in preventing inactivity-induced muscle dysfunction in old animals. However, this exercise program is detrimental in the very old age group.

152. Mitophagy and Mitochondrial Protein Turnover in Aging

Simon Johnson (P, N), Matthew Kaeberlein, Peter Rabinovitch
University of Washington, Seattle, WA

Mitochondria are dynamic and heterogeneous organelles. The regulation of mitochondrial structure and func tion is determined by a complex balance of mitochondrial fission, fusion, mitophagy, and protein turnover within the mitochondria. The importance of mitochondrial fission and fusion in human disease is evident. Point mutations in mitofusin 1 (MFN1) and optic atrophy 1 (OPA1), proteins which regulate fusion of the outer and inner mitochondrial membranes, lead to the neurodegenerative diseases Charcot-Marie-Tooth syndrome and dominant optic atrophy, respectively. In addition, PINK1 and Parkin have recently been shown to regulate mitofusin and disruption of these regulators has been implicated in the development of heritable forms of Parkinson's disease. In tissue aberrant mitochondrial turnover can lead to an accumulation of damaged and dysfunctional mitochondria, a loss of tissue respiratory capacity, and an increase in oxidative stress, all of which contribute to the aging process. While proper mitochondrial turnover is clearly an important factor in tissue homeostasis, the specific role of mitochondrial turnover in aging tissues remains to be determined. Using a novel staining and imaging technique I observed that aged mouse heart and liver are characterized by an accumulation of large, high reactive oxygen species (ROS) producing mitochondria. To determine the nature of these high-ROS producing mitochondria I developed a method for imaging mitophagy in living tissue using mitochondrial dyes, the autophagic marker LC3-GFP, and a lysosomal probe. Combined with this technique we examined age related changes in mitochondrial fission and fusion regulating proteins, and carefully examined age related changes in mitochondrial size and shape by electron microscopy. This data is providing us with insight into how the populations of mitochondria within a tissue change with age at a level of resolution not yet described.

153. Nectarine Extract Reduces Oxidative Damage and Promotes Longevity in Drosophila melanogaster

Xiaoping Sun (P), Olga Boyd, Peter Weng, Thomas Alberico, Mara Laslo, Bradley Kern, Sige Zou
NIA, Laboratory of Experimental Gerontology, Baltimore, MD

Consumption of diet rich in botanicals, such as vegetables and fruits, has been shown to provide numerous health benefits to humans partly due to the high antioxidant capacities and other bioactivities possessed by botanicals. Emerging evidence indicates that botanicals can improve the survival and healthspan in animals. However, few fruits are known to have prolongevity properties, let alone the underlying mechanisms. Here we investigate whether supplementation of nectarine, a globally consumed fruit, can promote lifespan and healthspan using the fruit fly Drosophila melanogaster as the model organism. We fed wild type Canton-S or sod1 mutant flies the standard, dietary restriction (DR) or high fat diet supplemented with 0-4% nectarine extract. We measured lifespan, food intake, locomotor activity, fecundity, gene expression changes, and oxidative damage in these flies. SOD1 is a major enzyme responsible for scavenging reactive oxygen species in the cell, and sod1 mutations result in high levels of oxidative damage in the cell. We have found that supplementation of 4% nectarine extended lifespan, increased fecundity and decreased expression of some metabolic genes, including a key gluconeogenesis gene PEPCK, and oxidative stress response genes, including peroxiredoxins, in female wild type flies fed the standard, DR or high fat diet. By measuring the level of a lipid oxidation marker, 4-hydroxynonenal-protein adduct, we have demonstrated that nectarine reduced oxidative damage in wild type females fed the high fat diet. In addition, nectarine improved the survival and reduced oxidative damage in female sod1 mutant flies. Together, these findings suggest that nectarine promotes longevity and healthspan partly through modulating glucose metabolism and reducing oxidative damage. Future studies will be directed to more understanding the molecular mechanisms underlying the prolongevity effect of nectarine.

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154. Role of Nrf2 in Murine Embryonic Fibroblast Immortalization and Lifespan

Bethany Carboneau (P, N)[1], Evi M. Mercken[1], Laura Jódar[2], Julia Ariza[2], José M. Villalba[2] and Rafael de Cabo[1]

[1]Laboratory of Experimental Gerontology, National Institute on Aging, NIH, Baltimore, MD; [2]Departamento de Biología Celular, Fisiología e Inmunología, Facultad de Ciencias, Universidad de Córdoba, Spain

Nuclear factor E2-related factor-2 (Nrf2) is a transcription factor which serves as cellular sensor of oxidative and electrophilic stress generated from endogenous reactions and exogenous agents, and is one of the main regulators of intracellular redox balance. Low Nrf2 activity is usually associated with carcinogenesis, but Nrf2 has been also considered as an oncogene due to its ability to increase survival of transformed cells. Since alterations in intracellular redox balance are involved both in the cessation of cell growth that accompanies senescence and in the uncontrolled growth associated with tumorigenesis, we aimed to investigate the impact of Nrf2 genetic deletion on cellular immortalization and lifespan of murine embryonic fibroblasts (MEFs). We report here that genetic deletion of Nrf2 promotes MEFs immortalization due to an early loss of p53-dependent gene expression. However, compared with their Wt counterparts, immortalized Nrf2-/- MEFs exhibited decreased growth rates, lower cyclin E levels and impaired expression of NQO1 and cytochrome b_5 reductase. Moreover, SirT1 levels were also significantly reduced in Nrf2-/- MEFs and these cells exhibited shorter lifespan. Our results underscore the dual role of Nrf2 in protection against carcinogenesis and in the delay of cellular aging.

155. Timing of Antioxidant Intervention During Life Determines Functional Outcome

Nathalie Sumien (P), Uzoma Ikonne, Theresa, Carbonaro, Jacques D. Nguyen, Kiran Chaudhari, and Michael J. Forster

University of North Texas Health Science Center, Fort Worth, TX.

Both human and animal studies have yielded variable outcomes with regard to the effectiveness of dietary supplementation with small molecule antioxidants as an anti-aging intervention strategy. The current study evaluated the possibility that the timing of antioxidant intervention during life is a variable that may influence functional outcomes. Mice were supplemented with antioxidants (ascorbate, 1.65 mg/g diet; d-α-tocopheryl acetate, 0.825 mg/g diet, and coenzyme Q_{10}, 0.825 mg/g diet) beginning early or later in life, and these regimens were evaluated for their effect on age-related decline of psychomotor functions. We hypothesized that (i) single and/or combinations of antioxidants would improve psychomotor performance if supplemented during late life when age-related deficits were already present and, (ii) long-term supplementation beginning earlier in life would prevent the onset of age-related psychomotor deficits. C57BL/6J mice received a base diet (NIH-31) or one of seven antioxidant-supplemented diets for either 5 weeks (short-term)

or 12 months (long-term), prior to functional testing beginning at 22 months of age. Long-term antioxidant supplementation failed to prevent age-related impairment of psychomotor performance as measured in a comprehensive battery of tests. However, a significant improvement in bridge-walking performance (a measure of balance) and running performance on a rotating rod (a measure of coordination) was evident when older mice with pre-existing impairments received the short-term regimens of supplementation. These effects occurred in the absence of any improvement in performance on a wire suspension test (a measure of strength). There was no clear indication of beneficial interactions among the antioxidants when supplemented in different combinations. These results provide a clear indication that timing of antioxidant intervention during aging is a critical determinant of the functional outcome.

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