

INTERVENTIONS NOW: TARGETS, TACTICS AND TIMING



41TH Annual Meeting of the
American Aging Association

PRE-CONFERENCE
“WINDOW OF OPPORTUNITY FOR ESTROGEN AND
PROGESTIN INTERVENTION DURING AGING AND
ALZHEIMER’S DISEASE”
MAY 31 – JUNE 1, 2012

RENAISSANCE WORTHINGTON HOTEL
FORT WORTH, TEXAS
JUNE 2-4, 2012

SCHEDULE AT A GLANCE

<p style="text-align: center;"><i>FRIDAY</i> <i>JUNE 1, 2012</i></p> <p><i>PRE-PROGRAM</i></p>	<p style="text-align: center;"><u>BRAZOS/CORRIDOR BALLROOM</u></p> <p style="text-align: center;">8:45 AM – 9:00 AM OPENING REMARKS</p> <p style="text-align: center;">9:00 AM – 11:00 AM THE CLINICAL EFFECTS OF HORMONES IN POST-MENOPAUSAL WOMEN</p> <p style="text-align: center;">11:00 AM – 12:30 PM LUNCH ON YOUR OWN</p> <p style="text-align: center;">12:30 PM – 3:00 PM THE EFFECTS OF HORMONES ON COGNITIVE DECLINE IN RODENT MODELS</p> <p style="text-align: center;">3:00 PM – 3:30 PM BREAK</p> <p style="text-align: center;">3:30 PM – 5:30 PM POTENTIAL MECHANISM(S) OF THE LOSS OF EFFECTIVENESS OF HORMONE WITH TIME AFTER THE MENOPAUSE?</p>								
<p style="text-align: center;"><i>SATURDAY</i> <i>JUNE 2, 2012</i></p> <p><i>INTERVENTIONS</i> <i>NOW: TARGETS,</i> <i>TACTICS AND</i> <i>TIMING</i></p>	<p style="text-align: center;"><u>PECOS BALLROOM</u></p> <p style="text-align: center;">8:30 AM – 9:30 AM KEYNOTE ADDRESS: RAJINDAR S. SOHAL</p> <p style="text-align: center;">9:30 AM – 10:10 AM OXIDATIVE STRESS: AN EVOLVING CONCEPT</p> <p style="text-align: center;">10:10 AM – 10:40 AM BREAK</p> <p style="text-align: center;">10:40 AM – 11:40 AM OXIDATIVE STRESS: AN EVOLVING CONCEPT CONT.</p> <p style="text-align: center;">11:40 AM – 1:00 PM</p> <p style="text-align: center;"><u>BRAZOS/CORRIDOR BALLROOM</u> VINCENT CRISTOFALO AWARD LUNCHEON VINCENT CRISTOFALO AWARD LECTURE</p> <p style="text-align: center;">1:00PM - 3:40 PM</p> <table style="width: 100%; border: none;"> <tr> <td style="text-align: center; width: 50%;"><u>PECOS I BALLROOM</u></td> <td style="text-align: center; width: 50%;"><u>PECOS II BALLROOM</u></td> </tr> <tr> <td style="text-align: center;">NEW INVERTEBRATES IN AGING RESEARCH</td> <td style="text-align: center;">ARTHUR BALIN SESSION</td> </tr> </table> <p style="text-align: center;">3:40 PM – 4:00 PM BREAK</p> <table style="width: 100%; border: none;"> <tr> <td style="text-align: center; width: 50%;"><u>PECOS I BALLROOM</u></td> <td style="text-align: center; width: 50%;"><u>PECOS II BALLROOM</u></td> </tr> <tr> <td style="text-align: center;">SUBMITTED PAPERS – 3A</td> <td style="text-align: center;">SUBMITTED PAPERS – 3B</td> </tr> </table> <p style="text-align: center;">5:30 PM – 7:00 PM <u>BALLROOM FOYER</u> COCKTAIL RECEPTION AND POSTER SESSION I</p>	<u>PECOS I BALLROOM</u>	<u>PECOS II BALLROOM</u>	NEW INVERTEBRATES IN AGING RESEARCH	ARTHUR BALIN SESSION	<u>PECOS I BALLROOM</u>	<u>PECOS II BALLROOM</u>	SUBMITTED PAPERS – 3A	SUBMITTED PAPERS – 3B
<u>PECOS I BALLROOM</u>	<u>PECOS II BALLROOM</u>								
NEW INVERTEBRATES IN AGING RESEARCH	ARTHUR BALIN SESSION								
<u>PECOS I BALLROOM</u>	<u>PECOS II BALLROOM</u>								
SUBMITTED PAPERS – 3A	SUBMITTED PAPERS – 3B								

*SUNDAY
JUNE 3, 2012*

*INTERVENTIONS
NOW: TARGETS,
TACTICS AND
TIMING*

PECOS BALLROOM

8:00 AM – 10:00 AM
THERAPEUTIC TARGETS FOR HEALTH SPAN AND LIFESPAN
EXTENSION

10:00 AM – 10:30 AM
BREAK

10:30 AM – 12:30 PM
ADVANCES IN NUTRIENT MODULATION OF AGING

12:30 PM – 2:00 PM
BRAZOS/CORRIDOR BALLROOM
AWARDS LUNCHEON AND
DENHAM HARMAN AWARD LECTURE

2:00 PM – 4:00 PM
PECOS I BALLROOM **PECOS II BALLROOM**
SENSORY AND ORGAN SYSTEMS: TRANSATLANTIC
IMPROVING THE AGING SYMPOSIUM
EXPERIENCE

4:00 PM – 4:30 PM
BREAK

PECOS BALLROOM

4:30 PM – 5:30 PM
PLENARY SESSION: JAMES JOSEPH ADDRESS
ROBERT KRIKORIAN

6:00 PM – 6:30 PM
AGE BUSINESS MEETING

5:30 PM – 7:00 PM
BALLROOM FOYER
COCKTAIL RECEPTION AND POSTER SESSION II

*MONDAY
JUNE 4, 2012*

*INTERVENTIONS
NOW: TARGETS,
TACTICS AND
TIMING*

PECOS BALLROOM

8:00 AM – 10:00 AM
EATING AND AGING

10:00 AM – 10:30 AM
BREAK

10:30 AM – 12:35 PM
PECOS I BALLROOM **PECOS II BALLROOM**
NATHAN SHOCK CENTER STARTING WHEN? TIMING OF
SYMPOSIUM ANTI-AGING INTERVENTIONS

12:35 PM – 1:00 PM
AWARDS CEREMONY FOR TRAINEES

TABLE OF CONTENTS

Acknowledgments	1
Conference Program	9
Poster Session outline	21
Award Presentation Abstracts.....	27
Session Abstracts	29
Poster Session Abstracts	60
Membership Directory	94

ACKNOWLEDGEMENTS

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

National Institute on Aging

Glenn Foundation for Medical Research

The Ellison Medical Foundation

American Federation for Aging Research

The SENS Foundation

The Gerontological Society of America

British Society for Research on Ageing

Wild Blueberry Association of North America

Springer Science & Business Media

Aging Research Network

The Buck Institute for Research on Aging

Institute for Aging and Alzheimer's Disease Research

UNTHSC Preventable Aging Foundation



NATIONAL INSTITUTES OF HEALTH / NATIONAL INSTITUTE ON AGING

Overview

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

Mission

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

Support and conduct high quality research on:

-aging processes

-age-related diseases

-special problems and needs of the aged

Train and develop highly skilled research scientists from all population groups

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

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

Programs

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

www.nia.nih.gov

PLATINUM SPONSOR



GLENN FOUNDATION FOR MEDICAL RESEARCH

The purpose of the foundation, which was founded in 1965 by Paul F. Glenn, is to extend the healthy productive years of life through research on the mechanisms of biological aging.

www.glennfoundation.org

PLATINUM SPONSOR



The Ellison Medical Foundation

The Ellison Medical Foundation supports basic biomedical research on aging relevant to understanding lifespan development processes and age-related diseases and disabilities.

The Foundation particularly wishes to stimulate new, creative, research that might not be funded by traditional sources or that is often under-funded in the U.S

www.ellisonfoundation.org

GOLD SPONSOR

American Federation for Aging Research

For 31 years, the American Federation for Aging Research (AFAR) has been at the forefront of this revolutionary approach to the science of healthier aging. AFAR has played a major role in providing and advancing knowledge of aging and mechanisms of age-related disease by providing grants totaling more than \$132 million in support of researchers in aging and to encourage the training of new scientists and physicians. To learn more about AFAR, visit our website www.afar.org. We also invite you to visit our web site InfoAging.org for the latest information on the biology of aging, common diseases of aging and healthy lifestyles.

www.afar.org

GOLD SPONSOR

SENS Foundation
human regenerative engineering

<http://www.sens.org>

SENS Foundation works to develop, promote and ensure widespread access to rejuvenation biotechnologies which comprehensively address the disabilities and diseases of aging. The Foundation catalyses progress toward a comprehensive panel of rejuvenation biotechnologies through its growing global networks and collaborations, and through key research projects, executed in its own Research Center and numerous affiliated universities, research organizations and other centers of excellence.

GOLD SPONSOR

BSRA ***British Society for Research on Ageing***

The British Society for Research on Ageing

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

www.bsra.org.uk/index.html

SILVER SPONSOR



Wild Blueberry Association of North America
<http://www.wildblueberries.com>

SILVER SPONSOR



SILVER SPONSOR



The goal of the IAADR of the University of North Texas Health Science Center is to serve as a focal organization for leadership in all aspects of research and education into the causes, prevention and treatment of age-related conditions that afflict our population. This goal is achieved through interdisciplinary programs of basic biomedical research, translational research, and clinical studies into the early diagnosis, prevention, and treatment of age-related conditions. The Institute plays an active leadership role in the dissemination of this new knowledge to students, health care professionals and to the lay public.

<http://www.hsc.unt.edu/research/IAADR/>

EXHIBITOR



STUDENT AWARD DONATION



AgeNet (Aging Research Network), is a Canadian charity whose mission is help historically disparate scientific disciplines realize synergies and develop interventions in degenerative disease through funding research and education

STUDENT AWARD DONATION



The Gerontological Society of America is the oldest and largest multidisciplinary scientific organization devoted to the advancement of aging research. Its membership includes more than 5,000 researchers, educators, practitioners, and other professionals. The Society's principal mission is to promote research and education in aging and to encourage their dissemination to others.

STUDENT AWARD DONATION



Preventable Aging Foundation

The mission of the Preventable Aging Foundation is to promote research on mechanisms of biological aging and to facilitate discovery of chemical, nutritional and lifestyle strategies that enhance health and quality of life during aging.

MEMBERSHIP DONATIONS 2012

GENERAL DONORS

Donald Ingram
Holly Brown-Borg
Rochelle Buffenstein
Gregory Fahy
Michael Forster
Donna Holmes
David Slate

JAMES A. JOSEPH/ MARK A. SMITH MEMORIAL FUND

Marlene Joseph
Peggy Harris

RESEARCH FUND

Elliot Bergman
Ian Ross

Student Travel Awards

Mike Anson

Thursday, May 31, 2012 Pre-Meeting

UNT Health Science Center, Medical Education and Training (MET) Bldg., rm. 109/110/111
1000 Montgomery St., Fort Worth

“WINDOW OF OPPORTUNITY FOR ESTROGEN AND PROGESTIN INTERVENTION DURING AGING AND ALZHEIMER’S DISEASE”

Chairs: Meharvan Singh and James W. Simpkins

- 12:30 pm – 2:00 pm** Shuttle service from Worthington Hotel to UNTHSC
- 1:00 pm – 3:00 pm** Pre-Conference Registration (UNTHSC, MET 109/110/111)
- 3:00 pm – 6:00 pm** **PREDOCTORAL FINALIST SYMPOSIUM**
Chair: Heather Bimonte-Nelson, Arizona State University, Phoenix, AZ
- 3:00 pm – 3:15 pm 1. Erin Scott, Georgia Health Sciences University, Augusta, GA
Long-Term Estrogen Deprivation Disrupts Pro-Survival Wnt/ β -Catenin Signaling in the CA1 Hippocampal Region
- 3:20 pm – 3:35 pm 2. Timothy Richardson, University of North Texas Health Science Center, Fort Worth, TX
Estrogen Cytoprotection in Friedreich’s Ataxia via an Antioxidant Mechanism
- 3:40 pm – 3:55 pm 3. Elizabeth Engler-Chiurazzi, Arizona State University, Phoenix, AZ
Don’t Overthink it: Cognitive Benefits of Premarin Depend on Task Complexity in a Rodent Model of Surgical-Menopause
- 4:00 pm – 4:15 pm 4. Jazmin Acosta, Arizona State University, Phoenix, AZ
CEE Exerts Differential Effects on Cognition Depending on Menopause Type: Relation with Androstenedione-Induced Impairments
- 4:15 pm – 4:45 pm** **BREAK**
- 4:45 pm – 5:00 pm 5. Sarah Mennenga, Arizona State University, Phoenix, AZ
Estrone Treatment Impairs Memory and Does Not Impact Number of Basal Forebrain Cholinergic Neurons in the Middle-Aged Rat
- 5:05 pm – 5:20 pm 6. Akram Sidhu, University of North Texas Health Science Center, Fort Worth, TX
Effects of Short-Term Phytoestrogen Supplementation on Motor Performance of Male and Female Mice
- 5:25 pm – 5:40 pm 7. Brittany Branden, Arizona State University, Phoenix, AZ
Cognitive-Impairing Effects of Medroxyprogesterone Acetate in the Rat: Independent and Interactive Effects across Time
- 5:45 pm – 6:00 pm 8. Nioka Chisholm, University of Illinois Urbane-Champaign, Urbana, IL
Long-Term Estrogen and Medroxyprogesterone Acetate Alters Synapses and Tyrosine Hydroxylase in the Aging Prefrontal Cortex
- 6:15 pm** Shuttle service to Worthington Hotel from UNTHSC
- 6:45 pm** Shuttle service to Joe T. Garcia’s restaurant from Worthington Hotel
- 7:00 pm** **DINNER (Joe T. Garcia’s)** <http://www.joets.com>
Shuttle service to Worthington Hotel or Fort Worth Stockyards

Friday June 1, 2012 Pre-Meeting (WORTHINGTON RENAISSANCE HOTEL)

“WINDOW OF OPPORTUNITY FOR ESTROGEN AND PROGESTIN INTERVENTION DURING AGING AND ALZHEIMER’S DISEASE”

Chairs: Meharvan Singh and James W. Simpkins

- 8:00 am – 8:45 am** Pre-Conference Registration and Continental Breakfast at Worthington Hotel
- 8:45 am – 9:00 am** **Welcome and Opening Remarks**
James Simpkins, PhD, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX
- 9:00 am – 11:00 am** **SESSION I**
THE CLINICAL EFFECTS OF HORMONES IN POST-MENOPAUSAL WOMEN
Chair: Meharvan Singh, PhD, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX
- 9:00 am – 9:30 am 9. Pauline M. Maki, PhD, University of Illinois School of Medicine, Chicago, IL
A Scientific Update on the Critical Window Hypothesis: Evidence from Randomized Controlled Trials of Hormone Therapy
- 9:30 am – 10:00 am 10. Mark Espeland, PhD, Wake Forest University Health Sciences, Winston-Salem, NC
The Women’s Health Initiative Memory Study of Younger Women (WHIMS-Y): Rationale, Design, and Preliminary Data
- 10:00 am – 10:30 am 11. Sanjay Asthana, MD, University of Wisconsin School of Medicine, Madison, WI
Cognition and Alzheimer’s Disease: Potential Role of Perimenopausal Hormone Therapy
- 10:30 am – 11:00 am 12. Jane Wigginton, MD, University of Texas Southwestern Medical School, Dallas, TX
Estrogens in Traumatic Brain Injury: A NIFTI Way to RESCUE Patients
- 11:00 pm – 12:30 pm** **LUNCH BREAK (on your own)**
- 12:30 pm – 3:00 pm** **SESSION II**
THE EFFECTS OF HORMONES ON COGNITIVE DECLINE IN RODENT MODELS
Chair: Roberta Brinton, PhD, University of Southern California, Los Angeles, CA
- 12:30 pm – 1:00 pm 13. Heather Bimonte-Nelson, PhD, Arizona State University, Tempe, AZ
Got Hormones? Estrogens, Progestins and Memory During Aging in the Rat Model
- 1:00 pm – 1:30 pm 14. Philip W. Landfield, PhD, University of Kentucky, Lexington, KY
The Complex Role of Glucocorticoids in Aging-Related Decline of Memory

- 1:30 pm – 2:00 pm 15. Martin Kelly, PhD, Oregon Health Sciences University, Portland, OR
A Selective Membrane Estrogen Receptor Agonist Maintains Autonomic Functions in Hypoestrogenic States
- 2:00 pm – 2:30 pm 16. ShaoHua Yang, MD, PhD, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX
Function of Estrogen Receptor Beta as a Mitochondrial Component
- 2:30 pm – 3:00 pm 17. Derek Schreihof, PhD, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX
Estrogen Receptors: Who, Where and When
- 3:00 pm – 3:30 pm **BREAK**
- 3:30 pm – 5:30 pm **SESSION III**
POTENTIAL MECHANISM(S) OF THE LOSS OF EFFECTIVENESS OF HORMONE WITH TIME AFTER THE MENOPAUSE?
Chair: Farida Sohrabji, PhD, Texas A&M University Health Science Center, Bryan, TX
- 3:30 pm – 4:00 pm 18. Farida Sohrabji, PhD, Texas A&M University Health Science Center, Bryan, TX
Bioavailability of IGF-1 Determines if Estrogen is Neuroprotective or Neurotoxic
- 4:00 pm – 4:30 pm 19. Roberta Brinton, PhD, University of Southern California, Los Angeles, CA
Bioenergetic of Reproductive Senescence: Implications for Risk of Alzheimer's Disease
- 4:30 pm – 5:00 pm 20. Darrell W. Brann, PhD, Georgia Health Sciences University, Augusta, GA
Estrogen and the Risk of Dementia and Mortality to Neurological Disease
- 5:00 pm – 5:30 pm 21. Meharvan Singh, PhD, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX
Progesterone, BDNF and Neuroprotection

5:30 pm- 6:00 pm

SESSION IV

GENERAL DISCUSSION

Chairs: Meharvan Singh, PhD and James W. Simpkins, UNT Health Science Center at Fort Worth

The meeting will end with a discussion of the issues on which there was consensus as well as those on which there was controversy or too little scientific data to draw any conclusions. Additionally, a discussion on the direction of clinical and basic science research needed to resolve the remaining issues in the “Window of Opportunity” will occur.

PRE-CONFERENCE ADJOURNS

6:30 pm – 8:30 pm

AAA Conference Registration
AAA WELCOME RECEPTION

Saturday June 2, 2012 Meeting (WORTHINGTON RENAISSANCE HOTEL)

INTERVENTIONS NOW: TARGETS, TACTICS AND TIMING

Chair: Michael J. Forster, PhD

University of North Texas Health Science Center at Fort Worth

- 7:15 am Continental Breakfast
- 8:15 am – 8:30 am **Welcome and Opening Remarks**
Michael Forster, PhD, University of North Texas Health Science Center at Fort Worth
President, American Aging Association
- 8:30 am – 9:30 am **Keynote Address:**

Rajindar S. Sohal, PhD
University of Southern California, Los Angeles, CA

From Oxidative Damage to Redox Stress: Evolution of a Concept
- 9:30 am – 11:40 am **Session I**
OXIDATIVE STRESS: AN EVOLVING CONCEPT
Chair: William C. Orr, PhD, Southern Methodist University, Dallas, TX
- 9:30 am – 9:35 am William C. Orr, PhD, Southern Methodist University, Dallas, TX
Introductory Remarks
- 9:35 am – 10:05 am 22. Henry J. Forman, PhD, University of California, Merced, CA
Signaling by Peroxides and Alkylating Agents - How Specific Thiols are Targeted
- 10:05 am – 10:35 am **BREAK**
- 10:35 am – 11:05 am 23. Pamela Maher, PhD, Salk Institute, La Jolla, CA
Functional Consequences of Age-Dependent Changes in GSH Status in the CNS
- 11:05 am – 11:40 am 24. Malcolm J. Jackson, University of Liverpool, UK
Defective Redox Signaling and Age-Related Loss of Skeletal Muscle
- 11:40 am – 1:00 pm **Vincent Cristofalo Memorial Rising Star Award and Lecture**
Sponsors
The American Federation of Aging Research (AFAR)
Margaret F. Cristofalo

Anne Brunet, PhD
Stanford University, Stanford, CA

The Plasticity of Aging

1:00 pm – 3:40 pm

Session II-A and II-B are Dual Sessions

SESSION II-A

NEW INVERTEBRATES IN AGING RESEARCH

Chairs: Mahadev Murthy, PhD and Nancy Nadon, PhD, National Institute on Aging

SPONSORED BY: NATIONAL INSTITUTE ON AGING

1:00 pm – 1:20 pm

25. Steven N. Austad, PhD, Barshop Institute, San Antonio, TX
Aging in Hydra, Loss of Stemness

1:20 pm – 1:40 pm

26. Diane Bridge, PhD, Elizabethtown College, Elizabethtown, PA
Inducible Aging in Hydra

1:40 pm – 2:00 pm

27. Anthony W. De Tomaso, PhD, University of California, Santa Barbara, CA
Aging and Regeneration in the Basal Chordate, *Botryllus schlosseri*

2:00 pm – 2:20 pm

28. Ayelet Voskonoynik, PhD, Stanford University, Stanford, CA
First Draft of *Botryllus schlosseri* Genome, a Clonal Invertebrate with a Programmed Lifespan

2:20 pm – 2:40 pm

29. William R. Jeffery, PhD, University of Maryland, College Park, MD
The Tunicate *Ciona*: A New Model for Studying Age-related Decay in Regenerative Capacity and Tissue Repair

2:40 pm – 3:00 pm

30. David Mark Welch, Marine Biological Laboratory, Woods Hole, MA
Comparative Biology and Genomics of Aging in Rotifers

3:00 pm – 3:20 pm

31. Jeffry L. Dudycha, PhD, University of South Carolina, Columbia, SC
Daphnia: A Model Crustacean for Genetic Variation of Aging

3:20 pm – 3:40 pm

32. Irv Weissman, MD, Stanford University, Stanford, CA
TBA

SESSION II-B

**TRANSLATION IN ACTION: RECENT ADVANCES
(ARTHUR BALIN SESSION)**

Chair: Mohsen Meydani, Jean Mayer USDA HNRCA at Tufts University and Janko Nikolich-Zugich, University of Arizona College of Medicine

SPONSORED BY: THE GLENN FOUNDATION

1:00 pm – 1:30 pm

33. Jörg Goronzy, MD, PhD, Stanford University, Stanford, CA
T Cell Metabolism and Signaling in Aging – Friends or Foes?

1:30 pm – 2:00 pm

34. Emran Mayer, MD, University of California Los Angeles, Los Angeles, CA
Frontiers in Enteric Neuroscience

2:00 pm – 2:30 pm

35. Sarah Booth, Jean Mayer USDA HNRCA at Tufts University, Boston, MA
Nutrients and Bone Health: Evidence for Additional Role in Heart Health

2:30 pm – 3:00 pm

36. Roger Fielding, PhD, Jean Mayer USDA HNRCA at Tufts University, Boston, MA
Translational Approaches to Clinical Trials of Nutrition and Physical Activity in Older Adults: From Biology to Function

- 3:00 pm – 3:30 pm **BSRA Korenchevsky Award Presentation**
 37. David Bartlett, BSc, MRC-ARUK Centre for Musculoskeletal Ageing Research, University of Birmingham, UK
The Age-Related Increase in Low Grade Systemic Inflammation (Inflammaging) is not Driven by Cytomegalovirus Infection
- 3:40 pm – 4:00 pm **BREAK**
- 4:00 pm - 5:15 pm **Submitted Papers Sessions III-A and III-B are Dual Sessions**
SUBMITTED PAPERS SESSION III-A: MITOCHONDRIA AND METABOLISM IN AGING
Chair: Holly Brown-Borg, PhD, University of North Dakota, Grand Forks, ND
- 4:00 pm – 4:15 pm 38. Alessandro Bitto (N), Drexel University College of Medicine, Philadelphia, PA
Rapamycin Improves Mitochondrial Homeostasis and Delays the Onset of Senescence by Altering p62/SQSTM1 Dynamics
- 4:15 pm – 4:30 pm 39. Nazia Alam, Weill Cornell Medical College, Burke Medical Research Institute, New York, NY
A Novel Peptide that Improves Mitochondrial Function Reverses Diabetes – and Age-Related Visual Decline
- 4:30 pm – 4:45 pm 40. Reyhan Westbrook (N), Southern Illinois University School of Medicine, Springfield, IL
The Impact of Ambient Temperature on Metabolism in Long-Lived Mice
- 4:45 pm – 5:00 pm 41. Simon Johnson (N), University of Washington, Seattle, WA
Affects and Mechanism of a Caloric Restriction Mimetic as an Intervention in a Mouse Model of a Human Mitochondrial Disease
- 5:00 pm – 5:15 pm 42. George Perry, PhD, University of Texas at San Antonio, San Antonio, TX
Impaired Mitochondrial Dynamics and Function Result from LRRK2 Mutations
- 4:00 pm – 5:15 pm **SUBMITTED PAPERS SESSION III-B: HEALTHSPAN: CELLS, TISSUES, ORGANISMS**
Chair: LaDora Thompson, PhD, University of Minnesota, Minneapolis, MN
- 4:00 pm – 4:15 pm 43. Ted Graber (N), University of Minnesota Medical School, Minneapolis, MN
C57BL/6 Neuromuscular Healthspan Scoring System: Assessment of Sarcopenia/Frailty Interventions
- 4:15 pm – 4:30 pm 44. Yun Shi (G), University of Texas Health Science Center at San Antonio, TX
Cell Autonomous and Non-Autonomous Neuromuscular Phenotypes of CuZn-SOD Knockout Mice – an Electrophysiological Study
- 4:30 pm – 4:45 pm 45. Robert Krikorian, University of Cincinnati, Cincinnati, OH
Concord Grape Juice Supplementation and Neurocognitive Function
- 4:45 pm – 5:00 pm 46. Dudley Lamming (G), Whitehead Institute, Massachusetts Institute of Technology, Cambridge, MA
Depletion of mTORC2 Impairs the Health and Longevity of Mice

5:00 pm – 5:15 pm	47. Alexander Kulminski, Duke University, Durham, NC Trade-off in the Effect of the APOE Gene on the Ages at Onset of CVD and Cancer across Ages, Gender and Human Generations
5:30 pm – 7:00 pm	Poster Session I Competition for the Glenn Award and the Nicolai Prize
5:30 pm – 7:00 pm	COCKTAIL RECEPTION
7:00 pm – 8:30 pm	Special Student Session
7:00 pm – 10:00 pm	AGE BOARD OF DIRECTORS DINNER MEETING

Sunday June 3, 2012 Meeting (WORTHINGTON RENAISSANCE HOTEL)

- 7:00 am** **Continental Breakfast**
- 8:00 am – 10:00 am** **Session VI**
THERAPEUTIC TARGETS FOR HEALTH SPAN AND LIFESPAN EXTENSION
Chair: Randy Strong, PhD, Barshop Institute, University of Texas at San Antonio, San Antonio, TX
SPONSORED BY: ELLISON FOUNDATION
- 8:00 am – 8:30 am 48. William Balch, PhD, The Scripps Research Institute, La Jolla, CA
Decoding Proteostasis of Aging
- 8:30 am – 9:00 am 49. Richard A. Miller, MD, PhD, University of Michigan, Ann Arbor, MI
Anti-Aging Drugs in Mice: Two New Ones and an Old Favorite Revisited
- 9:00 am – 9:30 am 50. William C. Orr, PhD, Southern Methodist University, Dallas, TX
Peroxiredoxins and Redox-Sensitive Signaling in Aging
- 9:30 am – 10:00 am 51. Gordon Lithgow, PhD, Buck Institute, Novato, CA
Natural Products that Suppress Protein Aggregation and Slow Aging
- 10:00 am – 10:30 am** **BREAK**
- 10:30 am – 12:40 pm** **Session V**
ADVANCES IN NUTRIENT MODULATION OF AGING
Chair: Barbara Shukitt-Hale, PhD, Jean Mayer USDA HNRCA at Tufts University, Medford, MA
SPONSORED BY: THE GLENN FOUNDATION
- 10:30 am – 11:00 pm 52. Paula Bickford, PhD, University South Florida and VAMC, Tampa FL
Nutritional Interventions and Stem Cell Senescence
- 11:00 am – 11:30 pm 53. Greg Cole, University of California Los Angeles
Supplements and Alzheimer's
- 11:30 am – 12:00 pm 54. Nathalie Sumien, PhD, University North Texas Health Science Center
Timing of Antioxidant Intervention Affects Functional Outcome in Aged Mice
- 12:00 pm – 12:15 pm 55. Shibu Poulouse, Jean Mayer USDA, HNRCA at Tufts University
Protective Effects of Berries on Brain Against Radiation-Induced Tau-Hyperphosphorylation and Ubiquitin Aggregates (abstract #141)
- 12:15 pm – 12:30 pm 55-A. Barbara Shukitt-Hale, PhD, Jean Mayer USDA, HNRCA at Tufts University
Nutritional Interventions in Mobility and Cognition in Aging
- 12:30 pm – 12:40 pm **Special Presentation:**
Felipe Sierra, National Institute on Aging and Dan Perry, Alliance for Aging Research
Brief Update on Trans-NIH Coordination of Aging Research

12:40 pm – 2:00 pm

**AMERICAN AGING ASSOCIATION AWARDS LUNCHEON
(ALL ATTENDEES ARE INVITED TO ATTEND)**

**Awards Presentations:
Denham Harman Award**

1:00 pm – 2:00 pm

PLENARY SESSION: DENHAM HARMAN AWARD LECTURE

**James W. Simpkins, PhD
University of North Texas Health Science Center, Fort Worth, TX**

Wandering in Aging Research: A Life of Running Against the Wind

SESSION VI-A AND VI-B ARE DUAL SESSIONS

2:00 pm – 4:00 pm

**Session VI-A
SENSORY AND ORGAN SYSTEMS: IMPROVING THE AGING
EXPERIENCE**

Chairs: Christian Leeuwenburgh, PhD and Shinichi Someya, PhD, University of Florida

2:00 pm – 2:30 pm

56. Norm Wolf, DVM, PhD, University of Washington, Seattle, WA
Cell Cycling Suppression in a Set of Lens Epithelium Cells

2:30 pm – 3:00 pm

57. Shinichi Someya, PhD, University of Florida, Gainesville, FL
Caloric Restriction, Sirt3, and Age-Related Hearing Loss in Mice

3:00 pm – 3:30 pm

58. Scott Pletcher, PhD, University of Michigan, Ann Arbor, MI
Molecular Dissection of Sensory Modulation of Lifespan in Drosophila

3:30 pm – 4:00 pm

59. Debapriya Dutta, BS, and Christian Leeuwenburgh, PhD, University of Florida, Gainesville, FL
Autophagy Induction with Life-Long and Late-Onset Interventions

2:00 pm – 4:00 pm

**Session VI-B
TRANSANTLANTIC SYMPOSIUM**

Chair: Richard Faragher, PhD, Brighton University, UK

2:00 pm – 2:20 pm

60. Nancy Manley, PhD, University of Georgia, Athens, GA and Claire Blackburn, PhD, University of Edinburgh, Edinburgh, UK
Steroid Receptors and the Control of Thymic Involution

2:20 pm – 2:40 pm

61. Brian Kennedy, PhD, Washington University, Seattle, WA and Dominic Withers, Imperial College, London, UK
S6 Kinase, Aging and Age-Related Disease

2:40 pm – 3:00 pm

62. Dhaval Patel, PhD, King's College London, UK and Mei Zhan, PhD, Georgia Institute of Technology, Atlanta, GA
Sources, Transmission and Effects of Transcriptional Noise in C. elegans Aging

3:00 pm – 3:20 pm

63. Arne Akbar, PhD, University College London, London, UK and Janko Nikolich-Zugich, MD, PhD, University of Arizona, Tucson, AZ
Mechanisms of Reduced T Cell Immunity in Older Adults

3:20 pm – 3:40 pm 64. John Sedivy, PhD, Brown University, Providence, RI and Peter Adams, MD, Beatson Institute for Cancer Research, University of Glasgow, Glasgow, UK
The WNT-Chromatin Axis in Aging

3:40 pm – 4:00 pm 65. Iain Ridgway, MD, University of Bangor, UK and Steven Austad, PhD, University of Texas Health Science Center San Antonio, TX
Mechanisms of Exceptional Longevity in the World's Longest Lived Animal

4:00 pm – 4:30 pm **BREAK**

4:30 pm – 5:30 pm **PLENARY SESSION VII: JAMES JOSEPH ADDRESS**

Robert Krikorian, PhD
University of Cincinnati, Cincinnati, OH

Nutritional Interventions in Cognitive Aging

(Sponsored by: James Joseph and Mark Smith memorial fund, American Aging Association)

6:00 pm – 6:30 pm AAA Business Meeting – **Open to all members**

6:30 pm – 8:00 pm **POSTER SESSION 2**
Competition for Glenn Award and Nicolai Prize

6:30 pm – 8:00 pm **COCKTAIL RECEPTION**

Monday, June 4; Meeting (WORTHINGTON RENAISSANCE HOTEL)

- 7:00 am** **Continental Breakfast**
- 8:00 am – 10:00 am** **SESSION VIII: EATING AND AGING**
Chair: Donald Ingram, PhD, Pennington Biomedical Research Center, Baton Rouge, LA
- 8:00 am – 8:30 am 66. Eric N. Reither, PhD, Utah State University, Logan, UT
Obesity in America: Implications for Aging and Longevity
- 8:30 am – 9:00 am 67. Phil Scarpace, PhD, University of Florida, Gainesville, FL
Anorexic Behaviors with Age: Influence of Leptin and Exercise
- 9:00 am – 9:30 am 68. Mohsen Meydani, DVM, PhD, Jean Mayer USDA HNRCA at Tufts University, Boston, MA
Nutritional Modulation of Aging: Nutrients and Bioactive Compounds
- 9:30 am – 10:00 am 69. Martha C. Morris, ScD, Rush University Medical Center, Chicago, IL
Nutritional Risk Factors and Interventions for Brain Aging and Alzheimer's Disease
- 10:00 am -10:30 am** **BREAK**
- 10:30 am -12:35 pm** **SESSION IX-A AND IX-B ARE DUAL SESSIONS**
- SESSION IX-A: NATHAN SHOCK CENTER SYMPOSIUM**
Chair: Felipe Sierra, National Institute on Aging
- 10:30 am – 10:55 am 70. Veronica Galvan, PhD, UTHSCSA Barshop Institute on Aging, San Antonio, TX
TOR as a Regulator of Brain Aging: Studies of a Vascular Function in a Mouse Model of Alzheimer's Disease.
- 10:55 am – 11:20 am 71. Richard A. Miller, MD, PhD University of Michigan, Ann Arbor, MI
A Shared Molecular Signature for Slow Aging in Mice
- 11:20 am – 11:45 am 72. David J. Marcinek, PhD, University of Washington, Seattle, WA
Acute Pharmacological Intervention Reverses Mitochondrial Deficits and Improves Function in Aged Skeletal Muscle
- 11:45 am – 12:10 pm 73. Jill P. Crandall, MD, Albert Einstein College of Medicine, Bronx, NY
Resveratrol: Therapy for Age-Related Insulin Resistance and Glucose Intolerance?
- 12:10 pm – 12:35 pm 74. Ron Korstanje, PhD, The Jackson Laboratory, Bar Harbor, ME
Studying Aging and Kidney Decline Using Human, Mouse, Zebrafish, and Worms

SESSION IX-B: STARTING WHEN? TIMING OF ANTI-AGING INTERVENTIONS

Chair: Aubrey de Grey, SENS Foundation

- 10:30 am – 10:55 am 75. Christy S. Carter, PhD, University of Florida, Gainesville, FL
Behavior as a Tool for Assessing the Efficacy of Late Life Interventions for Combating Aging
- 10:55 am – 11:20 am 76. Donald K. Ingram, PhD, Pennington Biomedical Research Center, Baton Rouge, LA
Late – Life Calorie Restriction in Rhesus Monkeys
- 11:20 am – 11:45 am 77. Sudhir Paul, University of Texas Houston, Houston, TX
Beneficial Catalytic Autoimmunity to Amyloids Amplified with Age
- 11:45 am – 12:10 pm 78. Gouri Yogalingam, SENS Foundation, Mountain View, CA
Towards a Therapeutic Intervention for Age-Related Macular Degeneration
- 12:10 pm – 12:35 pm 79. Matthew O’Conner, PhD, SENS Foundation, Mountain View, CA
MitoSENS: Allotopic Expression of Mitochondrial Genes Using a Co-Translational Import Strategy
- 12:45 pm – 1:15 pm AWARDS CEREMONY**
- 1:15 pm MEETING ADJOURNS**

Poster Session I
Saturday, June 2, 2012

80	Inhibition of Phosphodiesterase 2 Reverses Behavioral and Synaptic Deficits Caused by Chronic Stress Exposure <i>Ying Xu (P,G), Jiao Sun, Ling Chen, Jianxin Li, Chong Zhang, Hanting Zhang, James O'Donnell</i>
81	Rapamycin Administered Late in Life Extends C57BL/6 Health Span in an Age- and Sex-Specific Manner <i>Kathleen Fischer (P) Vanessa Soto, Lauren Sloane, Alex Bokov, Jon Gelfond, Vivian Diaz, Samantha Rendon, Keith Maslin, Martin Javors, Steven Treaster, Arlan Richardson, Steven Austad</i>
82	Healthspan in Females --- How the Other Half Ages <i>Lauren Sloane (P, G) Vanessa Soto, Keith Maslin, Kathleen Fischer, Arlan Richardson, Steven Austad</i>
83	Mitochondrial DNA Copy Number in Peripheral Blood is Associated with Femoral Neck Bone Mineral Density <i>Jung-Ha Kim (P) and Duk-Chul Lee</i>
84	Systems Biology of Human Aging - Network Model 2012 <i>John Furber (P)</i>
85	Caloric Restriction-Like Regulation of the Neuroendocrine Axis by SirT1 in Pituitary-Specific SirT1 Knock Out Mice (PITSKO) <i>Bonkowski, M.S. (P, G), Bell, E., Davidson, S., Reyes, J., Hunter, T.A., Liberts, S. and Guarente, L.</i>
86	A Web-Based Environment for System-Level Models of Aging <i>Pat Langley (P)</i>
87	Pulsed-Wave Doppler Reveals Attenuated Cardiovascular Aging in the Naked Mole-Rat <i>Kelly Grimes (P)</i>
88	The Naked Mole-Rat Proteasome does not Age <i>Karl Rodriguez (P, G) and Rochelle Buffenstein</i>
89	Aging Compromises Oral Siphon Regeneration in the Protochordate <i>Ciona intestinalis</i> <i>William Jeffery</i>
90	Superior Proteome Stability in the Longest Lived Animal <i>Stephen Treaster (P, N), Keith Maslin, Iain Ridgway, Asish Chaudhuri, Steven Austad</i>
91	Protein Oxidation may be an Important Regulator in the Development of Insulin Resistance <i>Jennalynn Styskal (P), Adam Salmon, Nicholas Musi, Arlan Richardson</i>
92	Growth Hormone Alters Glutathione-S-Transferase Activity and Proteins Expression in Long-Living Ames Dwarf Mice <i>Lalida Rojanathammanee (P), Sharlene Rakoczy, Holly Brown-Borg</i>
93	Age-Related Changes of Dopamine, Noradrenaline, and Adrenaline in Adrenal Glands of Mice <i>Akiko Amano (P), Makoto Tsunoda, Yuki Kishimoto, Toshiro Aigaki, Naoki Maruyama, Akihito Ishigami</i>
94	Reduced Pathogen Uptake by Aged Dendritic Cells: Setting the Stage for Impaired Adaptive Immunity? <i>Megan Smithey (P), Gang Li, Janko Nikolich-Zugich</i>
95	Age-Related Changes in Circulating T cell Subset Phenotype and T Cell Receptor Diversity in Cytomegalovirus Negative Humans <i>Anne Wertheimer, Michael Bennett, Byung Park, Carmine Martinez, Noreen Currier, Jeffrey Kaye, Janko Nikolich-Zugich</i>
96	Dissecting the Mechanisms Underlying the Maintenance and Function of Old Naïve T cells <i>Kristin Renkema (P)</i>
97	Functional Analysis of Age-Related Novel GEF, Zizimin2 in Immunosenescence <i>Mitsuo Maruyam (P)</i>
98	Mechanisms Underlying Skeletal Muscle Weakness with Bedrest <i>Haiming Liu (P, N) and LaDora V. Thompson</i>

*Poster Session I Continued:
Saturday June 2, 2012*

99	Correction of Aberrant Neutrophil Migration in the Healthy Aged Through Inhibition of the p110d Isoform of PI3Kinase <i>Hannah Greenwood (P), Elizabeth Mann, Georgia Walton, Robert Insall, Elizabeth Sapey, Janet Lord</i>
100	Enhancing Autophagy <i>in vitro</i> and in Rodent Hearts Offers Protection from Oxidative Stress Mediated Toxicity in Cardiac Cells <i>Debapriya Dutta (P,N), Jinze Xu, William Dunn, Christiaan Leeuwenburgh</i>
101	Nrf2 - The oxidative Damage Theory of Aging is Born Again, Alive and Thriving <i>Vincent Giuliano (P)</i>
102	Association of Ferritin with anti-Oxidative Status and Insulin Resistance <i>Jee-Yon Lee (P), Duk-Chul Lee, Jee-Aee Im, Ji-Won Lee</i>
103	The Leukocyte Telomere Length is Independently Associated with Physical Function in Elderly Women <i>Jee-Yon Lee (P), Jung-Ha Kim, Hyo-Weon Bang, Jae-Hong Ko, Duk-chul Lee</i>
104	Unmasking Aging in Non-Aging Hydra? <i>Szymon Tomczyk (P) Kathleen Fisher, Steven Austad, Brigitte Galliot</i>
105	Interventions to Determine Which is Most Important in Aging & AD: Redox Shift, Overwhelming ROS or Insufficient Anti-Oxidants <i>Debolina Ghosh (P, N) and Gregory Brewer</i>
106	Effects of Rapamycin on Aging: Similar or Different to Dietary Restriction? <i>Wilson Fok (P) Yiqiang Zhang, Adam Salmon, Arunabh Bhattacharya, William Wood, Yongqing Zhang, Kevin Becker, Carolina Livi, Yidong Chen, Walter Ward, Arlan Richardson, Viviana Perez</i>
107	Enhanced Autophagy Drives Coordinate Expression in Nuclear and Mitochondrial Genomes <i>Chad Lerner (P), Alessandro Bitto, Claudio Torres, Christian Sell</i>
108	The Role of Genes, Aging, and Environment in Healthspan: Insights from Longitudinal Human Data <i>Alexander Kulminski (P), Irina Culminskaya, Konstantin Arbeev, Svetlana Ukraintseva, Liubov Arbeeva, Anatoli Yashin</i>
109	Characterizing Aging in the Colonial acidian, <i>Botryllus schlosseri</i> <i>Roma Munday (P), Max Evoy-Mount, Anthony De Tomaso</i>
110	Age and Sex Specific Differences in C57BL/6 Murine Sleep Patterns <i>Keith Maslin (P), Vanessa Soto, Stephen Treaster, Steven Austad, Kathleen Fischer</i>
111	Conservation of Longevity-Associated Pathways from <i>C. elegans</i> MIT Mutants in the Long-Lived Surf1 Knockout Mice <i>Daniel Pulliam (P, N) Deepa Sathyaseelan, Yuhong Liu, Shauna Hill, Holly Van Remmen</i>
112	The Effect of Temperature on Longevity Interventions in <i>C. elegans</i> <i>Scott Leiser (P, G), George Sutphin, Marissa Fletcher, Anisoara Begun, Melissa Primitivo, Matt Kaeberlein</i>
113	Mutation Burden and Aging <i>Alan Herr (P) and Matt Kaeberlein</i>
114	Life Extension in The Fly: Are the Results Reproducible? <i>Robin Mockett (P) and Amber Nobles</i>
115	Caffeine Extends Life Span, Delays Age-Associated Pathology, and Improves Healthspan in Worms <i>George Sutphin (P, N), Emma Bishop, Melana Yanos, Matt Kaeberlein</i>
116	Expression of Innate Immune Response Markers After Bypass of Senescence in Human Cells <i>S. Kaye Smith (P), Angela Sheerin, Hui-Sun Leong, Badr Ibrahim, Katrin Jennert Burston, Richard Faragher, David Kipling</i>
117	Advanced Paternal Age: Activation of P53 and the Impact on APEN1 Abundance and Distribution in Spermatogenic Cells <i>Jamila Momand (P) and Christi Walter</i>

Poster Session I Continued:
Saturday, June 2, 2012

118	SOD1 Interacts with Macronutrients to Modulate Lifespan in Drosophila <i>Xiaoping Sun (P), Toshimitsu Komatsu, Jinhwan Lim, Mara Laslo, Jason Yolitz, Cecilia Wang, Luc Poirier, Thomas Alberico, Edward Spangler, Sige Zou</i>
119	Takeout Regulates Longevity by Modulation of Juvenile Hormone Signaling <i>Khalil Chamseddin, Siti Nur, Sarah Morris, Santharam Kolli, Johannes Bauer (P)</i>
120	Mutation in a Complex IV Assembly Protein (sco2) Leads to Decreases In Complex IV Activity and Distinct Metabolic Phenotypes <i>Shauna Hill (P), Daniel Pulliam, Deepa Sathyaseelan, Yuhong Liu, Holly Van Remmen</i>

Poster Session II
Sunday, June 3, 2012

121	Age-Associated Declines in WNT3 and Beta-Catenin in the Brain <i>Kiran Chaudhari(P, N) and Nathalie Sumien</i>
122	Genetic Components of the Hypoxic Response for Lifespan <i>Marissa Fletcher (P), Scott Leiser, Anisoara Begun, Matt Kaeberlein</i>
123	Effect of Rapamycin on Lifespan Extension of Inbred C57BL Mice and Potential Mechanisms <i>Yiqiang Zhang (P), Shunhua Lao, Alex Bokov, Yuji Ikeno, Holly VanRemmen, Arlan Richardson, Walter Ward</i>
124	Five Dysfunctional Telomeres Predict Cellular Senescence in Normal Human Cells <i>Zeenia Kaul (P, G)</i>
125	Epigenetic Modifications are an Important Molecular Adaptation During Dietary Restriction <i>Michael Walsh (P), Daniel Pulliam, Lauren Sloane, Holly Van Remmen</i>
126	Growth Hormone Regulates the Expression of DNA Methyltransferase in the Ames Dwarf Mouse <i>Vanessa Armstrong (P, N)</i>
127	Development of a Brain-Specific Raptor Conditional Knock-Out Mouse to Study the Role of Neuronal mTOR in Aging <i>Stacy Hussong (P), Raquel Burbank, Veronica Galvan</i>
128	The Effect and Mechanism of GLP-1 Receptor Agonist in the Treatment of Diabetes-Related Alzheimer's Disease <i>Song Chen (P), Ai-ran Liu, Feng-mao An, Wen-bing Yao, Xiang-dong Gao</i>
129	Analysis of Changes in the Innervation of Mouse Internal Anal Sphincter During Aging <i>Cunfang Wang (P), Michael Houghton, Prasanna Gamage, Mark Yeoman, Bhavik Patel, Richard Ranson, Jill Saffrey</i>
130	Neuron Numbers are Maintained but Nerve Fibre Density Increases in Ageing Mouse Colon <i>Prasanna Gamage (P, G) M Saffrey, Richard Ranson, Bhavik Patel, Mark Yeoman</i>
131	Ultrastructural Analysis of Changes in the Cells of the Mouse Internal Anal Sphincter During Aging <i>M Jill Saffrey (P), Heather Davies, Frances Colyer, Prasanna Gamage, Bhavik Patel, Mark Yeoman, Richard Ranson</i>
132	A Dominant Negative Splice Variant of Er Beta and the Effective Post-Menopausal Estrogen Therapy <i>Jun Ming Wang (P), Raveena Reddy, Jonathan Fratkin, Baoying Zheng</i>
133	Diabetic-Dependent Loss in Nerve Conduction: Role of Myelin Protein, Lipid and Cholesterol Availability And Integrity <i>Ryan Hamilton (P, G), Michael Walsh, Yun Shi, Arunabh Bhattacharya, Alex Bokov, Asish Chaudhuri, Holly VanRemmen</i>
134	Determination of an Oral Dosage of Vitamin C that may Prevent Recurrence of Superficial Bladder Carcinoma <i>Alfred Ordman (P)</i>
135	Cranberry Extract Promotes Longevity and Modulates Stress Response in Caenorhabditis elegans <i>Yuqing Dong (P), Sige Zou, Sujay Guha, Min Cao, Ryan Kane, Anthony Savino</i>
136	The Dietary Effects of High-Fat/ High-Sucrose Feeding in PAPP-A (-/-) Mice <i>Cristal Hill (P), Oge Arum, Michal Masternak, Cheryl A Conover, Andrzej Bartke</i>
137	Cognitive and Psychomotor Impairments of Aged Mice are not Improved by Treatment with Metformin <i>Margaret Rutledge (P), Nopporn Thangthaeng, Nathalie Sumien, Michael Forster</i>
138	2002-2012 Wild Blueberry Health Study Ten Year Report <i>Anthony Wetherell, Roseanne Schnoll, Barrie Sachs, Diana Burns, David Doiron, Howard Raphaelson, Alec Pruchnicki, Dana Mathew, Bruce Kristal, Rozlyn Krajcik, Steve Massardo, Ines Augie, Norman Orentreich, Joseph Vogelmann, Amy Kokesh, James Joseph, KathyHull, Kurt Gerstmann, Richard Coppings, Rolf Martin(P)</i>

Poster Session II Continued:
Sunday, June 3, 2012

139	2002-2012 Wild Blueberry Health Study Report: Maximizing Statistical Power Per Minute of Measurement <i>Rolf Martin (P), Diana Burns, David Doiron, James Joseph, Bruce Kristal, Howard Raphaelson, Joseph Vogelmann, Anthony Wetherell</i>
140	US Multiple-Cause-of-Mortality Data Connect Osteoporosis to ICD10 Illness Categories Associated with Polyphenol Consumption <i>Rolf Martin (P), Howard Raphaelson, J. Wesson Ashford</i>
141	Protective Effects of Berries on Brain Against Radiation-Induced Tau-Hyperphosphorylation and Ubiquitin Aggregates <i>Shibu Poulouse (P, G) Donna Bielinski, Stacey Gomes, Bernard Rabin, Kirsty Carrihill-Knoll, Barbara Shukitt-Hale</i>
142	Controls on Lifespan Extension by Caloric Restriction: Type of Food Restriction, Reproductive Mode, and Evolutionary History <i>Kristin E. Gribble (P, G) and David B. Mark Welch</i>
143	Treatment Compliance to Diabetes Care Among Older Adults: A Cross-Sectional Study <i>Liaquat Khawaja (P)</i>
144	Visceral Adiposity is Associated with Expression of SIRT1 in Peripheral Blood Mononuclear Cells <i>Ji Won Lee (P) Hang kyu Lee, Sang Hui Chu</i>
145	Pro-Longevity Interventions Can Hide Among Commonly Prescribed Medicines <i>Svetlana Ukrainseva (P), Konstantin Arbeev, Liubov Arbeeva, Alex Kulminski, Deqing Wu, Irina Culminskaya, Igor Akushevich, Anatoli Yashin</i>
146	Effects of Turning-Based Treadmill Training on Balance and Turning Performance in Subjects with Chronic Stroke <i>Ray-Yau Wang (P), I-Hsuan Chen, Yea-Ru Yang</i>
147	Simple Syntheses of Diverse Polyphenolic Compounds – Facilitating Dissection of Multiple Modes of Activity <i>L. Searle, G. Dichello, RGA Faragher and E.L. Ostler (P)</i>
148	Synthesis and <i>in vitro</i> Biological Evaluation of Substituted Phenylpiperazine Analogues <i>Maninder Malik (P), Yogesh Mishra, Robert Mach, Robert Luedtke</i>
149	Rapamycin Improves Mitochondrial Function and Insulin Sensitivity in a Mouse Model of Type 2 Diabetes <i>Deepa Sathyaseelan (P), Ryan Hamilton, Michael Walsh, Daniel Pulliam, Yun Shi, Shauna Hill</i>
150	Blood and Tissue Heat Shock Protein 70 Responses are Divergent and Related to Obesity, not Chronological Age <i>Kylie Kavanagh (P), Ashley Wylie, Stephen Kritchevsky</i>
151	The Protective Role of Calcineurin Aβ in ER Stress via Activation of the Unfolded Protein Responses in Astrocytes <i>Yanan Chen (P)</i>
152	Effect of Short-Term CR Initiated at Different Ages on Cognitive and Motor Function and Cellular Oxidative Defensive Systems <i>Nopporn Thangthaeng (P, G), Michael Forster, Nathalie Sumien</i>
153	Cu/ZnSOD Overexpression Extended Lifespan in Sprague-Dawley Rats, but had Little Effect in F344 Rats <i>Lisa Flores, Adam Salmon, Melanie Ortiz, Sara Dube, Nicolas Musi, Wenbo Qi, Shuko Lee, Gene Hubbard, Holly Van Remmen, Arunabh Bhattacharya, Yuhong Liu, James Kirkland, Tamar Pirtskhalava, Tamara Tchkonja, Arlan Richardson, Yuji Ikeno (P)</i>
154	Depot-Specific Patterns in Adipose Tissue Oxidative Stress and Dysfunction in Aging <i>Adam Salmon (P) and Arlan Richardson</i>

*Poster Session II Continued:
Sunday, June 3, 2012*

155	The Naked Truth about Cytoprotection: Lessons from the Naked Mole-Rat <i>Kaitlyn Lewis (P, N) and Rochelle Buffenstein</i>
156	Cytochrome B5 Reductase Overexpression Increases Lifespan and Oxidative Stress Resistance in Fruit Fly <i>Yaning Sun (P, G)</i>
157	Demographic, Behavioral And Physiological Assessment of the Senescence Switch in <i>H. oligactis</i> <i>Samantha Rendon (P, N), Vanessa Soto, Natalia Podlutskaya, Kathleen Fischer, Steven Austad</i>
158	Growth, Development, and Phenotypes of Mice Treated with High Dose Rapamycin, Tacrolimus, or Acetylsalicylic Acid from Neonatal Age D10 <i>Simon Johnson (P)</i>

KEYNOTE ADDRESS

From Oxidative Damage to Redox Stress: Evolution of a Concept

Rajindar S. Sohal, PhD

Department of Pharmacology and Pharmaceutical Sciences
University of Southern California, Los Angeles, California

Causal involvement of reactive oxygen species (ROS) in the aging process has often been accorded a high level of plausibility. The historical view of the mechanism by which ROS may play a role in the aging process is that the age-associated accumulation of irreversibly modified molecular products of ROS attacks is responsible for the senescence-related losses in physiological fitness. Support for this structural damage-based hypothesis was provided by observations, such as the age-associated increases in the rates of ROS production and the steady-state concentrations of oxidatively damaged macromolecules. However, an increasing number of findings appear to be at odds with the validity of the structural damage-based concept of causation of senescence. Specifically, the amounts of structural damage are not always reflected in the severity of physiological losses or in the achieved life span. More recently, the role of ROS in cell physiology has undergone a fundamental conceptual shift. Instead of being considered to be entirely toxic, some ROS have been recognized as essential molecules, involved in cell signaling, via reversible modifications of the redox-sensitive thiols of cysteinyl residues of proteins, as well as in the maintenance of cellular redox state. Accordingly, in the revised concept, referred to as the “redox stress hypothesis” (FRBM 52, 539-555, 2012), senescence is envisioned to involve a pro-oxidizing shift in the cellular redox state, arising from an age-associated increase in ROS production, which results in the over-oxidation of redox-sensitive protein thiols and the disruption of cell signaling thereof. ROS are postulated to play a key role in transitions of cellular states from proliferation to differentiation and senescence. Thus structural damage is considered to play a relatively lesser role in the age-associated functional losses.

VINCENT CRISTOFALO MEMORIAL RISING STAR AWARD AND LECTURE

The Plasticity of Aging

Ann Brunet, PhD

Stanford University, Stanford, CA

Aging, long thought to be solely the byproduct of “wear and tear”, is in fact a highly plastic process regulated by a combination of genetic and environmental factors. My lab is interested in discovering genes that regulate lifespan and in exploring how the products of these genes integrate environmental stimuli that promote longevity, such as dietary restriction. The pathway connecting the insulin signaling pathway to FoxO transcription factors is well known to play a pivotal role in aging from worms to mammals. One part of my lab is focused on understanding how the insulin-FoxO pathway acts to regulate gene expression programs and cellular responses that are important for longevity in mammals. We are particularly interested in the role of longevity genes and pathways, including the insulin-FoxO pathway, in aging neural stem cells. My lab also uses unbiased approaches in the nematode *C. elegans* and in mammalian cells to identify novel pathways that control organismal longevity, particularly in response to dietary restriction. Finally, we are developing the extremely short-lived African killifish *N. furzeri* as a new vertebrate model for aging studies to explore the genetic architecture of longevity in vertebrates.

DENHAM HARMAN AWARD LECTURE

Wandering in Aging Research: A Life of Running Against the Wind

James W. Simpkins, PhD

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

My interest in the aging brain began in the laboratory of Dr. Joseph Meites at Michigan State University where I was able to determine the role of hypothalamic norepinephrine (NE) in reproductive aging. We observed that the NE system failed with age and this caused a cessation of ovulation. We then began to assess the role of endogenous opiate systems in the dysregulation of NE neurons and found that with the loss of estrogens, a decline in opiate inhibition of NE neurons occurred, preventing the pre-ovulatory increase in NE release and therefore in the LH surge. Most intriguing to me was that loss of ovarian estrogens could effectively remove opiate influence on a fundamental reproductive process. Inasmuch as the cardinal feature of the loss of estrogens at the menopause is hot flashes, we assessed effects of opiate withdrawal on menopausal hot flashes in an animal model. We observed hot flashes with opiate withdrawal in rats that mirrored those in menopausal women and this animal model is used today to discover drugs for treatment of hot flashes. In addition, we first reported in 1994, that estrogens are potent neuroprotectants in vitro and in vivo, an observation that has now been replicated hundreds of times. Additionally, we observed that a major component of estrogen neuroprotection is not dependent on known estrogen receptors, allowing us to synthesize a large number of potent neuroprotective non-feminizing estrogens. Finally, the intervention testing program of the NIA has shown that chronic feeding of mice with one non-feminizing estrogen, 17- α -estradiol, appears to extend life span in mice. Collectively, studies emanating from my laboratory have resulted in the production of an animal model for drug discovery for the menopause, and a series of compounds that may prove to be useful in protecting the brain from acute damage and chronic neurodegeneration, resulting in: a recently completed clinical trial on estrogens and traumatic brain injury and one of a few compounds that can enhance longevity.

JAMES JOSEPH AWARD LECTURE

Nutritional Interventions in Cognitive Aging

Robert Krikorian, PhD

University of Cincinnati, Cincinnati, OH

We will review categories of cognitive aging that are characteristic of Western societies. In comparing Western and non-Western cultures, we will identify the dietary factors that contribute to metabolic disturbance other age-related disease conditions and review empirical findings that implicate metabolic disturbance as an important factor promoting age-related neurocognitive decline, including Alzheimer's disease and other types of dementia. We will discuss the findings of recent interventional research suggesting that dietary modification can be effective in improving memory function and, potentially, reducing risk for neurodegeneration.

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. Long-Term Estrogen Deprivation Disrupts Pro-Survival Wnt/ β -Catenin Signaling in the CA1 Hippocampal Region

Erin Scott (P), Quanguang Zhang, Dong Han, Bhavna Desai, Darrell Brann

Georgia Health Sciences University, Augusta, GA

Surgical menopause incurs a doubled lifetime risk of dementia, and prolonged depletion of the neuroprotective ovarian hormone 17 β -estradiol (E2) may contribute. We hypothesized that this phenomenon may be due, in part, to elevation of the neurodegenerative Wnt antagonist Dkk1 and dysregulation of pro-survival Wnt/ β -Catenin signaling in the hippocampus following a period of long-term E2 deprivation (LTED). To study the effects of LTED on Wnt/ β -Catenin signaling and E2 neuroprotection, E2 was administered to ovariectomized rats either immediately or 10 weeks (LTED) following ovariectomy, and global cerebral ischemia (GCI) was performed one week after E2 treatment. Intriguingly, LTED females displayed basal elevation of Dkk1 in the CA1 hippocampal region and a corresponding basal reduction of the Wnt/ β -Catenin product Survivin. GCI exacerbated this pattern, and E2 prevented it only when initiated immediately, but not 10 weeks, following ovariectomy. Furthermore, the acute, GCI-induced Dkk1 elevation and Survivin reduction was found to be mediated by c-jun N-terminal kinase (JNK), and inhibition of JNK afforded neuroprotection in LTED animals. Finally, we extended our surgical menopause findings to physiological menopause by showing that Dkk1 expression co-localized with the apoptotic marker TUNEL in CA1 hippocampal neurons and coincided with loss of E2 neuroprotection in aged, reproductively senescent female rats. As such, this study provides further support for the “critical period hypothesis” of E2 replacement and sheds light on the increased risk of dementia following surgical menopause.

2. Estrogen Cytoprotection in Friedreich's Ataxia via an Antioxidant Mechanism

Timothy Richardson (P) and James Simpkins

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

Estrogen and estrogen-related compounds have been shown to have very potent cytoprotective properties in a wide range of in vitro and in vivo disease models, recently including Friedreich's ataxia (FRDA). The present study demonstrates the cytoprotective effects of 17beta-estradiol (E2) and other estrogen-like compounds in a FRDA fibroblast cell model. In addition, this study illustrates a potential mechanism by which estrogen may be acting to protect this cell model from a L-buthionine (S,R)-sulfoximine (BSO) induced oxidative insult. We demonstrate that phenolic estrogens, independent of any known estrogen receptor (ER), are able to attenuate reactive oxygen species (ROS), prevent lipid peroxidation and protein damage, maintain oxidative phosphorylation and ATP levels and prevent mitochondrial membrane potential collapse, all without restoring intracellular levels of frataxin or GSH. These cytoprotective effects of estrogen appear to be due to a direct overall reduction in oxidative damage to the mitochondria, enabling the FRDA fibroblast mitochondria to match ATP production to energy requirements and better survive oxidative stress. These data support the hypothesis that phenol ring containing estrogens are possible candidate drugs for the delay and/or prevention of FRDA symptoms.

3. Don't Overthink it: Cognitive Benefits of Premarin Depend on Task Complexity in a Rodent Model of Surgical-Menopause

Elizabeth Engler-Chiurazzi (P), Jason Caselli, Keley Schaefer, Carmen Gammage, Heather Bimonte-Nelson

Arizona State University, Phoenix, AZ

Premarin (conjugated equine estrogens, or CEE) is a complex formulation composed of many estrogens, and is a commonly prescribed hormone therapy (HT) in the United States. In women, the literature regarding the cognitive impact of CEE is mixed; uncertainty among clinicians and patients as to the effectiveness of CEE for cognitive outcomes has made characterizing its cognitive impacts an important public health issue. In the rodent, we and others have shown that CEE can sometimes benefit, and sometimes impair, cognition. Yet, in both humans and rodents, the factors that influence whether CEE is beneficial or detrimental for

cognition have yet to be determined. One factor that may impact the realization of cognitive benefits with CEE-containing HTs is the complexity of the cognitive task. Subcutaneously administered 17 β -estradiol, the most potent circulating estrogen in women and rats, is maximally beneficial for cognition when working memory demand is greatest. However, whether similar cognitive demand parameters underlie the necessary requirements for beneficial mnemonic effects of CEE is unknown. The current study evaluated the impact of subcutaneous CEE treatment on spatial working memory in middle-aged, surgically menopausal rats, comparing performance on a simple spatial task (4 arms) and a complex spatial task (8 arms). In each task, animals were required to locate one platform, the location of which was fixed within a day and changed across days, hence requiring working memory. Therefore, in the complex spatial task, the increased complexity included potential interference from more arms, and correlated spatial locations, in which to enter. Each animal was tested on both task versions. Results showed that CEE treatment interacted with task complexity. On the simple spatial task, CEE improved performance; however, on the complex spatial task, CEE was detrimental to performance. These findings could help to clarify the conflicting literature regarding the cognitive impact of CEE.

4. CEE Exerts Differential Effects on Cognition Depending on Menopause Type: Relation with Androstenedione-Induced Impairments

Jazmin Acosta (P)

Arizona State University, Phoenix, AZ

Recently, we used the 4-vinylcyclohexene diepoxide (VCD) rodent model of ovarian follicle-depletion, which mimics transitional menopause, and the traditional rat model of surgical menopause, ovariectomy (OVX), to cognitively test the most commonly-prescribed estrogen therapy in the United States, conjugated equine estrogen (CEE; tradename Premarin). We found that CEE benefited cognition in surgically-menopausal rats, but, in contrast, impaired cognition in transitionally-menopausal rats. We also found a positive correlation between higher androstenedione serum levels, the primary hormone secreted by the residual follicle deplete ovaries, and spatial working memory errors, in VCD rats. In a follow-up study, we examined the hypothesis that androstenedione impairs memory. Middle-aged OVX rats received vehicle or one of two doses of androstenedione, with goal doses resulting in blood levels seen in VCD-treated follicular deplete, ovary-intact animals from our prior study (Acosta et al., 2009, 2010). Rats were tested on a spatial working

and reference memory using the water radial arm maze (WRAM). Androstenedione at the highest dose impaired reference memory performance during learning as well as the ability to handle multiple items of spatial working memory information as memory demand was elevated. Serum androstenedione levels were comparable to the higher serum levels we have shown previously to correlate with impaired working memory, and this correlation was replicated (Acosta et al., 2009, 2010). These findings suggest that androstenedione, a hormone produced by the follicle deplete ovary, is detrimental to spatial learning, reference memory, and working memory.

5. Estrone Treatment Impairs Memory and Does Not Impact Number of Basal Forebrain Cholinergic Neurons in the Middle-aged Rat

Sarah Mennenga (P), Elizabeth Engler-Chuirazzi,

Joshua Talboom, Blair Braden, Candy Tsang,

Madeline Andrews, Heather Bimonte-Nelson

Arizona State University, Phoenix, AZ

Premarin (conjugated equine estrogens) is the most widely prescribed estrogen-based menopausal hormone therapy in the United States, and is comprised of over 50% estrone (E1) sulfate. Following Premarin administration, E1 is the principal circulating estrogen. However, the cognitive and neurobiological effects of E1 in a middle-aged rodent model have not yet been evaluated. We assessed cognitive effects of continuous E1 treatment in middle-aged surgically menopausal rats using a maze battery. We also quantified number of choline acetyltransferase-immunoreactive (ChAT-IR) neurons in distinct basal forebrain regions known to be impacted by the most potent naturally-circulating estrogen in rodents and women, 17 β -estradiol (17 β -E2). On the spatial working memory delayed-match-to-sample water maze, the highest E1 dose impaired memory performance during acquisition and after delay challenge. E1 did not impact ChAT-IR neuron number in the medial septum (MS) or horizontal/vertical diagonal bands. In a comparison study, 17 β -E2 increased MS ChAT-IR neuron number. Findings indicate that E1 negatively impacts spatial working memory and memory retention, but does not increase ChAT-IR neuron number in basal forebrain, as does 17 β -E2. Thus, collectively, data suggest that both 17 β -E2 and Premarin can enhance cognition and increase number of ChAT-IR basal forebrain neurons, while E1 does not induce these effects. Findings from preclinical basic science studies can inform the design of specific combinations of estrogens that could be beneficial to the brain and cognition. Accumulating data suggest

that E1 is not likely to be among these key beneficial estrogens.

6. Effects of Short-Term Phytoestrogen Supplementation on Motor Performance of Male and Female Mice

Akram Sidhu (P), Michael Forster, Nathalie Sumien
University of North Texas Health Science Center, Fort Worth, TX

It is well established that with age, motor function is increasingly impaired and recently menopausal status has been determined to be a predictor of decreased muscle strength and balance. Estrogens have been shown to exert beneficial effects on motor systems in aged individuals and to improve locomotion post spinal injury. Some of the actions of estrogen on motor performance have been hypothesized to be driven by ERB activation in the striatum. Whereas estrogen therapy may be undesirable for males and some females, estrogen-mimetics have the potential to provide beneficial effects without undesirable side-effects. Plant-derived, non-steroidal compounds called phytoestrogens have been widely used as substitutes in anticipation of estrogen-like therapeutic effects, though human and animal data are still controversial regarding the beneficial effects of such compounds and whether the effects generated are differential based on the gender/sex of the subjects. In this study, we hypothesized that short-term supplementation with a phytoestrogen-rich diet would reverse age-related motor impairments in both sexes. Separate groups of young (6 months), middle-aged (12 months) and old (24 months) C57BL/6J mice of each sex were placed on either a phytoestrogen-free diet (N=15-17) or a phytoestrogen-rich diet containing 600 µg/g soy phytoestrogens (N=16-19). After 5 weeks on diet, the mice were subjected to a series of behavioral tests to measure locomotor activity, reflexes, and motor function. Male and female mice exhibited age-associated declines in motor performance, and sex differences were also noticeable. Short-term dietary supplementation of phytoestrogens did not improve spontaneous activity, reflexes or coordinated running. However, old phytoestrogen-supplemented groups took longer to fall from the bridge than their control counterparts, most notably in females. These results suggest that phytoestrogen supplementation may be useful for improving age-related impairments of balance, but not impaired coordination or strength. Further, the results suggest that efficacy may be gender/sex dependent.

7. Cognitive-Impairing Effects of Medroxyprogesterone Acetate in the Rat: Independent and Interactive Effects Across Time

Blair Braden¹ (P), Alexandra Garcia¹, Sarah Mennenga¹, Laszlo Prokai², Stephanie Villa¹, Jazmin Acosta¹, Natalie Lefort¹, Alain Simard¹, Heather Bimonte-Nelson¹

¹Arizona State University, Phoenix, AZ, ²University of North Texas Health Science Center, Fort Worth, TX

RATIONALE: The synthetic progestin medroxyprogesterone acetate (MPA), widely used in hormone therapy (HT) and as the contraceptive Depo Provera, is implicated in detrimental cognitive effects in women. Recent evidence in aged ovariectomized (Ovx) rodents shows that short-term MPA treatment impairs cognition and alters the GABAergic system. **OBJECTIVES:** Using rats, we evaluated the long-lasting cognitive and GABAergic effects of MPA administered in young adulthood (Early-MPA), modeling contraception, and how this early exposure interacts with later MPA treatment (Late-MPA), modeling HT. **METHODS:** Early-MPA treatment involved weekly anti-ovulatory MPA injections (3.5 mg) from 4 to 8 months of age in ovary-intact rats. At 10 months old, rats were Ovx and weekly MPA injections were re-initiated and continued throughout testing for Late-MPA treatment. **RESULTS:** On the water radial-arm maze, all MPA-treated groups showed working memory impairment compared to Controls ($p < 0.05$); Early+Late-MPA rats were impaired on multiple dimensions of working memory ($p < 0.05$). On the Morris maze, Late-MPA rats showed greater overnight forgetting compared to Controls ($p < 0.05$). At study conclusion, MPA was detected in serum in all MPA-treated groups except Early-MPA, confirming treatment and clearance from serum in Early-MPA rats. In animals with detectable serum MPA, higher MPA levels were associated with less dorsal-hippocampal glutamic acid decarboxylase, the synthesizing enzyme for GABA ($p = 0.0059$). **CONCLUSIONS:** Findings suggest that MPA treatment leads to long-lasting cognitive impairments in the rodent, even in the absence of circulating MPA in animals given prior MPA treatment, which may relate to the GABAergic system. Further research defining the parameters of the negative impact of this widely used progestin on brain and cognition is warranted.

8. Long-Term Estrogen and Medroxyprogesterone Acetate Alters Synapses and Tyrosine Hydroxylase in the Aging Prefrontal Cortex.

Nioka Chisholm (P) and Janice Juraska

University of Illinois Urbana-Champaign, Urbana, IL

The onset of menopause in humans is associated with undesirable side effects and many women initiate hormone treatment therapies to alleviate these symptoms. Research indicates that these treatments also affect cognition, and studies in young animals have found that hormone treatment can alter several neuroanatomical measures. However, very little is known about the effects of long-term hormone treatment on the aging female brain. This study examined the effects of hormone treatment on the number of synapses and the volume of tyrosine hydroxylase fibers in the rat medial prefrontal cortex (mPFC). Female Long-Evans rats were ovariectomized at middle age (12-13 months) and placed in one of 4 groups: no replacement, estradiol (E2), E2 and progesterone, or E2 and medroxyprogesterone acetate (MPA). At 20 months animals were sacrificed and the brains were immunostained for synaptophysin and tyrosine hydroxylase. Adjacent sections were Nissl stained to calculate volume which was multiplied by the density of synapses and fibers to calculate total amounts of each measure. Animals receiving estradiol and MPA had significantly more synaptophysin labeled boutons in the mPFC than animals not receiving replacement ($p < .03$) and those receiving estradiol and progesterone ($p < .02$). In addition, treatment with E2 and MPA resulted in a greater density of tyrosine hydroxylase fibers than no replacement animals in layer 1 and layers 2/3 ($p < .05$). There was also a trend for animals receiving estradiol alone to have more synapses than those receiving estradiol with progesterone. In layers 2/3, animals receiving E2 alone also had greater tyrosine hydroxylase densities than no replacement animals ($p < .01$). For layers 2/3, the number of synapses showed a positive correlation with the density of tyrosine hydroxylase fibers ($r = .44$, $p < .05$). These results provide a possible mechanism for the beneficial cognitive effects that have been observed with this hormone treatment (Chisholm & Juraska, 2012).

9. A Scientific Update on the Critical Window Hypothesis: Evidence from Randomized Controlled Trials of Hormone Therapy

Pauline M. Maki, PhD (P)

University of Illinois School of Medicine, Chicago, IL

The “critical window hypothesis” states that use of hormone therapy during the perimenopausal or early postmenopausal period confers favorable cognitive effects whereas later use confers negative effects. Whether hormone therapy alters the risk for Alzheimer’s disease is a critical public health question; the prevalence of Alzheimer’s is increasing because the population is aging, more women than men will die of the disease, and there is no cure. Randomized clinical trials that focus on early markers of Alzheimer’s disease are important because of the high costs associated with primary prevention trials. Verbal memory is the best and possibly earliest neuropsychological predictor of who will convert to Alzheimer’s disease. This presentation will therefore review randomized clinical trial data of hormone therapy and verbal memory. Clinical trials involving younger postmenopausal women provide some evidence for a beneficial effect of estrogen alone therapy on verbal memory, particularly among surgically menopausal women. Clinical trials involving older postmenopausal women show neutral effects of estrogen alone therapy on verbal memory. Verbal memory decreases with combined estrogen and progestin therapy in younger and older postmenopausal women. There is a significant gap in understanding about the effects of other estrogen plus progestin formulations on verbal memory. Recent evidence also suggests a relationship between hot flashes and memory dysfunction in women with moderate to severe hot flashes, but the robustness of this relationship has not yet been evaluated. The KEEPS and ELITE trials will provide important new insights into the cognitive effects of hormone therapy in younger and older postmenopausal women.

10. The Women’s Health Initiative Memory Study of Younger Women (WHIMS-Y):

Rationale, Design, and Preliminary Data

Mark A. Espeland, PhD (P)¹, Sally Shumaker, PhD¹, Susan M. Resnick, PhD²

¹Division of Public Health Sciences, Wake Forest University Health Sciences; ²Laboratory of Behavioral Neuroscience, Intramural Research Program, National Institute on Aging

Conjugated equine estrogens (CEE) therapy has been shown to increase the risk of cognitive impairment when initiated in women ages 65 or older and to result in small but sustained mean decrements in cognitive function and brain volume. However, there is some evidence from other studies suggesting that CEE may protect cognitive functioning in women if initiated during the recent post-menopausal period. The primary objective of the Women’s Health Initiative Memory Study of Younger Women

(WHIMS-Y) is to test whether prior random assignment to CEE-based hormone therapies has a long-term effect on women's cognitive function relative to placebo if initiated among postmenopausal women aged 50-54 years. WHIMS-Y enrolled 1,263 women, who are being followed with annual validated telephone-based assessments conducted by trained, certified, and masked interviewers to measure global cognitive function (primary outcome) and domain-specific functions. These women had entered the Women's Health Initiative (WHI) Hormone Therapy Trials in 1996-1999 during which they had been assigned to active or placebo treatment for an average [range] of 7.0 [3.9, 10.1] years. WHIMS-Y enrollment occurred an average of 7.2 [5.4, 9.2] years after the trials ended, when the women averaged 67.1 [62.9, 71.7] years of age. 39% of the women had a hysterectomy prior to WHI enrollment (median age range of hysterectomy was 35-39 years); the remaining women reported their last regular menstrual period occurred an average of 4.0 years prior to WHI enrollment. Overall, 51% of the women had prior use of hormone therapy. The first two years of planned WHIMS-Y follow-up will be completed in the fall of 2012 and are projected to provide >80% power to detect mean differences of ≥ 0.14 standard deviation units between women who had been assigned to active versus placebo therapy.

11. Cognition and Alzheimer's Disease: Potential role of Perimenopausal Hormone Therapy

Sanjay Asthana, MD (P)

University of Wisconsin School of Medicine, Madison, WI

There is converging evidence from animal, epidemiological and intervention studies that hormone therapy (HT) could enhance cognition and potentially reduce risk for Alzheimer's disease (AD). However, findings from Women's Health Initiative Memory Study (WHIMS) suggested that extended therapy with conjugated equine estrogen (CEE) in older postmenopausal women increased risk for AD and worsened cognition. These findings raised several critical issues concerning HT, including the "critical window" concept positing that initiation of HT during perimenopause could be most effective. This important concept, including other issues concerning HT are being addressed by the KEEPS (Kronos Early Estrogen Prevention Study) and the NIA-funded KEEPS Cognitive and Affective Study (KEEPS-CA). These studies are evaluating differential efficacy of 4 years of therapy with transdermal estrogen and CEE on markers of heart disease, cognition, lipids and thromboembolic

complications in healthy perimenopausal women. This presentation will discuss methodological limitations of prior pivotal HT trials, and discuss the scientific rationale, design and progress of the KEEPS-CA study to date.

12. Estrogens in Traumatic Brain Injury: A NIFTI way to RESCUE Patients?

Jane Wigginton, MD (P)

University of Texas Southwestern Medical School, Dallas, TX

Elevated endogenous and exogenously administered estrogens are neurologically protective in animal models of brain injury in both males and females, noting up to 65% less ultimate injury in those receiving estrogen. Scientific reports reveal that many patients experience declines in blood levels of sex steroids following severe traumatic brain injury (TBI). Normally, uninjured individuals have a cerebrospinal (CSF) sex steroid level that is approximately 1/10 that of their serum level. Reports of CSF levels of sex steroids following TBI were lacking, therefore we conducted the NIFTI study, collecting serial CSF and blood samples from patients with severe TBI (GCS 3-8) who required placement of a therapeutic ventriculostomy. The preliminary analyses of 10 patients, including male and female, both young and older (post-menopausal) revealed rapid changes in all sex steroids, with increasing post-traumatic CSF estradiol levels observed. In all patients, a CSF estradiol/serum estradiol ratio of >1 in at least one paired sample was associated with good recovery. Ratios of up to 13.56 [244 pg/ml (CSF)/18 pg/ml (serum)] were noted in male patients with good outcomes. CSF estradiol levels that were consistently lower than serum levels were associated with mortality. These early findings supported our recently completed, landmark interventional clinical study of a single, early intravenous dose of estrogens in trauma patients who experienced a severe head injury, RESCUE (Resuscitative Endocrinology: Single-dose Clinical Uses for Estrogen-Traumatic Brain Injury).

13. Got Hormones? Estrogens, Progestins and Memory During Aging in the Rat Model

Heather Bimonte-Nelson, PhD (P)

Department of Psychology, Arizona State University, Tempe, AZ 85287

Arizona Alzheimer's Consortium, Phoenix, AZ

This talk will center around a multidimensional approach to studying the effects of clinically-used estrogens

and progestins on memory, using the aging rat model. Recent findings in rodents evaluating the cognitive effects of Premarin, several of its components, and progestins, will be presented. As well, we have shown in two independent studies testing rodents that had undergone chemical-induced ovarian follicular depletion, that higher serum levels of endogenous androstenedione correlated with increased working memory errors on a maze task. These unanticipated findings, leading to our current hypothesis that higher circulating levels of androstenedione impair memory, will be discussed. We very recently took the next step in this line of research, under the tenet that if higher circulating androstenedione is in fact related to poorer memory in the aging female, we would expect to see impairments with its administration to older ovariectomized animals. Findings showed that exogenous androstenedione administration was detrimental to multiple memory domains, including spatial learning, working memory, and reference memory. Further, again, poorer working memory performance was correlated with higher circulating androstenedione levels; this was the third replication of this finding. Thus, incorporated into discussion will be caveats and discoveries shown from rodent model studies, including recent perspectives integrating androgens that yield insight into plausible limitations and parameters governing the impact of clinically-used hormones on cognitive aging. We believe this is especially clinically relevant since the main hormone produced by the follicle depleted ovary is androstenedione. The ultimate goal is to capitalize on basic science findings so that results can translate to novel investigations of clinically-relevant hormone therapies, with an eye toward optimizing for maximal therapeutic cognitive efficacy while minimizing other non-cognitive health-related risk factors. Variables such as temporal parameters, history of transitional versus surgical menopause, type and dose of estrogens and progestins, and memory requirements of the evaluation task, appear to impact cognitive outcome individually, and for some of these variables, in an interactive manner, at least in the aging rat model. How these factors interact, and discovering the mechanism of these effects, is a key future direction for research.

14. The Complex role of Glucocorticoids in Aging-Related Memory Decline

Philip W. Landfield, PhD (P)

University of Kentucky, Lexington, KY

A longstanding hypothesis holds that glucocorticoids (GCs) promote major aspects of unhealthy brain aging, including memory decline (Porter & Landfield, *Nat Neurosci*, 1998). Although much

evidence supports this view, a number of contradictory observations have also been reported (see review, Landfield et al, *Current Alz Res*, 2007). Using extensive gene microarray analyses of rat hippocampus, we tested two predictions of this hypothesis, that 1) GC-dependent genes should preferentially exhibit aging changes; and 2) GCs and aging should shift expression of co-regulated genes in the same direction. Results confirmed the first but not the second prediction, as a majority of co-regulated genes shifted expression in opposite directions with corticosterone (CORT) and aging (Chen et al, in prep). The opposite-direction genes included well-established aging markers, including inflammatory and glial genes (decreased by CORT, increased in aging) and plasticity genes (increased by CORT, decreased in aging). These data reject the simple pro-aging hypothesis and imply that some brain aging changes linked to memory deficits are associated with weakening of GC effects. Conversely, some brain aging markers were associated with same-direction genes. Further, in aging monkeys, microarray analyses showed that increased GC signaling in hippocampus was correlated with metabolic syndrome (Blalock et al, *J Neurosci*, 2010), which has been linked to increased risk of Alzheimer's. Thus, our data support the involvement of GCs in cognitive aging, but suggest a new more complex model in which both selective strengthening and weakening of GC effects in different pathways combine to generate much of the impaired memory phenotype.

15. A Selective Membrane Estrogen Receptor Agonist Maintains Autonomic Functions in Hypo-estrogenic States

Martin Kelly, PhD (P)

Oregon Health Sciences University, Portland, OR

At the start of the new millennium, there were nearly 42 million women over the age of fifty in the United States, and based on a life expectancy of 79.7 years, a woman can anticipate spending one third of her lifetime in the menopausal state. A major problem confronting postmenopausal women is whether or not to take hormone replacement therapy. In spite of the controversy, it is known that estrogens maintain autonomic functions including energy and bone homeostasis, core body temperature, sleep-wake cycles and have been reported to exert a positive influence on mood and affect. Consequently, the availability of selective estrogen receptor modulators (SERMs) that elicit the beneficial effects of 17 β -estradiol (E2) in the central nervous system (CNS) but lack its peripheral risks and unwanted side effects is of considerable clinical interest. None of the

existing SERMs (e.g. tamoxifen and raloxifene) fully meet these criteria. We have developed a novel agonist, STX, which selectively activates a Gq-coupled membrane estrogen receptor (Gq-mER) on estrogen-sensitive CNS neurons, some of which are involved in control of autonomic functions. As proof of principle, we have investigated a number of homeostatic processes that are regulated by the hypothalamus: First, STX, similar to E2, attenuated post-ovariectomy body weight gain and reduced food intake in ovariectomized female guinea pigs. Second, STX, similar to E2, reduced fat pad accumulation in ovariectomized female guinea pigs and rats. Third, STX, like E2, increased tibial bone density in both ovariectomized guinea pigs and rats. Lastly, STX, similar to E2, normalized core body temperature in ovariectomized female guinea pigs. Interestingly, all of these protective estrogenic effects were sustained by every other day (QOD) administration of STX. Therefore, the Gq-mER-coupled signaling pathway appears to be involved in maintaining homeostatic functions and may constitute a novel therapeutic target for treatment of hypo-estrogenic symptoms.

16. Function of Estrogen Receptor Beta as a Mitochondrial Component

ShaoHua Yang, MD, PhD (P) James W. Simpkins, Ph.D

University of North Texas Health Science Center, Fort Worth, TX, Department of Pharmacology and Neuroscience, Institute for Aging and Alzheimer's Disease Research, University of North Texas Health Science Center, Fort Worth, TX. 76107

Estrogen receptors (ERS) are believed to be ligand-activated transcription factors belonging to the nuclear receptor superfamily, which on ligand binding translocate into the nucleus and activate nuclear gene transcription. To date, two ERs have been identified: ER α and ER β . ER α plays major role in the estrogen-mediated genomic actions in both reproductive and nonreproductive tissues, whereas the function of ER β is still unclear. We used immunocytochemistry, immunoblotting, and proteomics to demonstrate that ER β localizes in the mitochondria. In immunocytochemistry studies, ER β was detected in mitochondria in primary neurons, primary cardiomyocyte, and murine hippocampal cell line. Immunoblotting of purified human heart mitochondria show an intense signal of ER β , which was further confirmed by matrix-assisted laser desorption / ionization mass spectrometric analysis. In addition, loss of mitochondrial ER β immunoreactivity was identified in ER β knockdown

cells. A phenotype change characterized as an increase in resistance to oxidative stressors is associated with ER β knockdown. ER β knockdown results in a lower resting mitochondrial membrane potential ($\Delta\psi_m$) and increase in resistance to hydrogen peroxidase-induced $\Delta\psi_m$ depolarization. ER β knockdown cells maintained ATP concentrations despite insults that compromise ATP production and produce less mitochondrial superoxide under oxidative stress. Furthermore, similar mitochondrial phenotype changes were identified in primary neurons derived from ER β knock-out mice. These data demonstrate that ER β localized in mitochondria and function as a mitochondrial function component involved in $\Delta\psi_m$ maintenance.

17. Estrogen Receptors: Who, Where and When Derek Schreihof, PhD (P)

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

Estrogen receptor (ER) expression is implicated in the neuroprotective effects of endogenous and exogenous estradiol (E2). Aging and long-term estrogen depletion (LTED) reduce the benefits of E2 in the brain. Replacement of estrogens early in the menopause is beneficial for brain function whereas delayed replacement is detrimental. Several studies implicate the expression of ER α as a key determinant of the beneficial effects of E2 in the brain. However, both ER β and G-protein coupled ER are also implicated in the neuroprotective actions of E2. Because hormone therapy is contraindicated for many postmenopausal women, complementary and alternative therapies have been gaining popularity. Dietary soy, and soy phytoestrogens, have beneficial effects on the cardiovascular system and are neuroprotective in rodent models of cerebral ischemia. We hypothesized that soy phytoestrogens can mitigate the effects of E2 depletion on ER expression levels in areas of the brain protected by E2 during ischemic injury. Adult female Sprague-Dawley rats were ovariectomized and placed on soy free (SF) or high soy (HS) diets for 4 weeks. A HS significantly increased ER α mRNA levels in the cerebral cortex, hippocampus, and cerebral blood vessels compared to SF rats. HS also increased ER β and GPER in the hippocampus. Similarly, 24 hours after transient focal cerebral ischemia HS increased ER α , ER β , and GPER. However, these changes were not reflected in protein levels. Because estrogen-dependent neuroprotection is lost after 10 weeks of LTED, a second group of rats was ovariectomized and fed SF, HS, or SF+estradiol and protein levels

were examined in the cerebral cortex. There were no differences in whole cell ER α levels, but LTED increased mitochondrial ER α levels, whereas HS did not. Similarly, ER β levels in the mitochondria increased with LTED, but did not reach significance. These results suggest that LTED leads to increased ER expression in the mitochondria and that this effect is partially reversed by soy in the cortex. These results have implications for the ability of ER activation to affect oxidative stress responses in the brain.

18. Bioavailability of IGF-1 Determines if Estrogen is Neuroprotective or Neurotoxic

F. Sohrabji (P), A. Selvamani and R. Balden

Department of Neuroscience and Experimental Therapeutic, Women's Health in Neuroscience Program, Texas A&M Health Science Center, Bryan, TX.

Stroke is a leading cause of mortality and disability, and both the risk and severity of stroke are elevated in older women. The relative stroke protection observed in younger, premenopausal women is usually attributed to ovarian hormones, however, estrogen therapy to older women, paradoxically, increases their risk for stroke. We propose that the impact of estrogen therapy cannot be considered in isolation, instead it should include consideration of other endocrine mediators that collaborate with estrogen to produce neuroprotective effects in younger, relatively disease-free demographics, but are reduced or dysregulated in aging and in conditions that predispose stroke. Due to its modulation of ischemic cell death, the post-stroke inflammatory response, neuronal survival and regeneration, and general somatic health, IGF-1 may be a crucial biochemical marker that determines the neuroprotective “window” of hormone therapy. This is supported by our data that acyclic middle-aged female rats (that have low IGF-1 levels) sustain a more severe infarction as compared to adult female (with higher IGF-1 levels) (Selvamani and Sohrabji 2010a). Estrogen exacerbates stroke severity in the former while it is neuroprotective in younger females. Post-stroke IGF-1 treatment reinstates estrogen's protective effects on middle-aged females, while post stroke treatment with an IGF-1 receptor inhibitor abolishes estrogen's neuroprotective effects in young females (Selvamani and Sohrabji, 2010b). These data support the hypothesis that IGF-1 bioavailability modulates estrogen-mediated neuroprotection. Failure of the insulin/IGF-1 axis is associated with metabolic disease, which increases stroke risk and severity, and may be targeted by other endocrine regulators, acting independently or

cooperatively with estrogen. Low Vitamin D levels, for example, are implicated in numerous chronic inflammatory conditions including cardiovascular disease, cancer, and infectious diseases. In female rats, diet-induced Vitamin D deficiency increases infarct volume and severely impairs sensory motor performance, with a concomitant reduction in circulating IGF-1 levels (Balden et al., 2012). Adoptive transfer of CD4⁺ cells from healthy females facilitated recovery in VDD animals and restored peripheral IGF-1 levels. These studies support the hypothesis that IGF-1 availability is closely associated with stroke outcomes, and underscore the impact of the aging endocrine environment on cerebrovascular disease.

19. Bioenergetic of Reproductive Senescence: Implications for Risk of Alzheimer's Disease

Roberta Diaz Brinton, PhD (P)

Pharmacology and Pharmaceutical Sciences, School of Pharmacy, and Department of Neurology, Keck School of Medicine, University of Southern California, Los Angeles, CA, 90089

Each year ~1.5 million American women ages 45 to 55 enter the perimenopause, a neuroendocrine transition state well known to result in reproductive senescence. Less recognized and explored is the impact of the perimenopause transition on brain regions involved in cognition and vulnerable to Alzheimer's pathology.

Postmenopausal women constitute >60% of the affected Alzheimer population and are those who will bear the greatest burden of this disease. The earliest etiological factors that ultimately lead to late-onset Alzheimer's disease, when prevention is still possible, remains unresolved for the primary victims of the disease, postmenopausal women. While the prevailing perception is that the greater predominance of women with AD is solely due to their longevity, our findings indicate that loss of ovarian hormones has adverse consequences in the brain, including decreases in bioenergetics and mitochondrial function; declines in synaptic transmission and cognitive behavior; activation of inflammatory responses; and the generation of Alzheimer's pathology. Data derived from the aging female brain indicate induction of a compensatory shift from a primarily aerobic glycolysis pathway to a ketogenic/fatty acid β -oxidation pathway which is indicative of a pathway leading to white matter degeneration.

The essential role of mitochondrial bioenergetics and the unique trajectory of compensatory metabolic adaptations in brain enable a bioenergetic-centric strategy for development of

biomarkers to detect the bioenergetic shift in brain to enable early identification of people at risk for developing AD. From a therapeutic perspective, this unique trajectory of alterations in brain metabolic capacity enable disease-stage specific strategies targeting brain metabolism for disease prevention and treatment. Because Alzheimer's is a 20-year prodromal disease, identification of the phenotypes and earliest risk factors that lead to Alzheimer's in those at greatest lifetime risk has the most potential impact to prevent the epidemic of Alzheimer's disease.

20. Estrogen and the Risk of Dementia and Mortality to Neurological Disease

Darrell W. Brann, PhD (P) and Quan-guang Zhang
Georgia Health Sciences University, Augusta, GA

Women who enter menopause prematurely have a significantly increased risk for cognitive decline and dementia, as well as mortality from neurological diseases. The mechanisms underlying this increased risk are poorly understood. Herein, we demonstrate that long-term estrogen deprivation (LTED) induced by bilateral ovariectomy or aging leads to a dramatic increased sensitivity of the normally resistant rat hippocampal CA3 area to ischemic stress, and a corresponding profound increase in Alzheimer's disease (AD)-related proteins, including phospho-amyloid precursor protein, beta-site amyloid- β precursor protein-cleaving enzyme-1 (BACE1), β -amyloid, and phospho-tau. LTED also produced a switch in amyloidogenic processing after ischemic stress from a non-amyloidogenic to an amyloidogenic one. Intriguingly, CA3 hypersensitivity to ischemic stress was gender-specific, as this phenomenon was not observed in males. Of significant note, the CA3 hypersensitivity also extended to β -amyloid-induced cellular stress, as the CA3 region of LTED rats was found to be profoundly hypersensitive to exogenous β -amyloid-induced neurotoxicity. CA3 hypersensitivity to ischemic stress was shown to be mediated by induction of a NADPH oxidase-superoxide-JNK-cJun signaling pathway, which also mediated transcriptional up-regulation of BACE1, as well as hyperphosphorylation of tau and amyloid precursor protein. The increased damage to the CA3 in LTED animals correlated with a worse cognitive outcome after ischemic stress, which was reversed by JNK inhibitor treatment. 17 β -estradiol (E2) treatment at the end of the LTED period could not prevent the CA3 hypersensitivity and AD protein induction, but if E2 was initiated at the beginning of LTED and maintained throughout the entire LTED period it completely prevented the hypersensitivity and AD

protein induction. As a whole, the findings provide insight into the potential mechanisms underlying the increased risk of cognitive decline, dementia and mortality from neurological diseases following premature menopause, and provide important support for the 'critical window' hypothesis of estrogen replacement therapy.

21. Progesterone, BDNF and Neuroprotection

Meharvan Singh, PhD (P)

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

While progesterone can protect brain cells from a wide variety of insults, our understanding of the mechanisms involved is still incomplete. Our laboratory has implicated both the classical progesterone receptor and a membrane progesterone receptor (Pgrmc1) as complementary mediators of progesterone's protective actions. Among the critical downstream sequelae for the cytoprotective protective effects of progesterone is the regulation of both the synthesis and cellular release of the neurotrophin, brain-derived neurotrophic factor (BDNF). In fact, we suggest that the effectiveness of progesterone, but not the synthetic progestin, medroxyprogesterone acetate (MPA), to act as a neuroprotectant is driven by their opposite effects on BDNF signaling. Specifically, while progesterone increases the expression of BDNF, MPA suppresses it. Our laboratory has also implicated members of the ERK/MAPK signaling pathway as key cellular mediators of the effects of progesterone. Specifically, the ERK1/2 and ERK5 signaling pathways have divergent and potentially competing effects on the regulation of BDNF. Collectively, our data suggest that not all progestins are equally effective as neuroprotectants, and that the effectiveness of progesterone to promote brain health is dependent, at least in part, on the availability of key cellular mediators that lead to a positive regulation of BDNF signaling. Finally, as it relates to the proposed "therapeutic window of opportunity" for estrogens to elicit neuroprotective effects, we suggest that an equally finite window of opportunity exists for progesterone. In this regard, we demonstrate that the ability of progesterone to increase BDNF expression diminishes as a function of age. We suggest that understanding the cellular and molecular players that underlie the "therapeutic window" for progesterone's protective effects will yield unique insight into how the therapeutic window may be expanded.

22. Signaling by Hydroperoxides and Alkylating Agents- How Specific Thiols are Targeted

Henry Jay Forman, PhD (P)

University of California, Merced, CA

Understanding of how hydroperoxides (ROOH) and alkylating participate in signal transduction has advanced significantly in the past few years. H₂O₂ and lipid hydroperoxides are clearly the agents through which signaling by what are called reactive oxygen species occurs under physiological conditions. Signaling by alkylating agents, such as 4-hydroxy-2-nonenal (HNE), production of which is increased under pathological conditions, also occurs under normal physiological conditions due to constant low-level lipid peroxidation. Recent identification of specific targets and consideration of the kinetics of the chemical reactions involved is helping to clarify how ROOH and HNE affects many of the signaling pathways that were previously shown to be affected but where the mechanism was obscure. We now know that cysteine residues in the thiolate (ionized, S⁻) form found in many signaling proteins can be specific targets; however, without catalysis by either another component of the target protein or a separate enzyme, the reaction kinetics suggest that oxidation or alkylation is not likely to occur under physiological conditions. Catalysis involved with signaling by ROOH is required because even when an O-O bond is attacked by a good nucleophilic protein thiolate, the RO[•] that would be formed is a very poor leaving group. Thus, a proton donor (to make ROH, a good leaving group) or metal (to make an RO⁻-metal intermediate) can markedly assist in breaking that bond and accelerate the reaction by orders of magnitude. Similar catalysis can also accelerate alkylation. Examples of catalysis are the activation of Nrf2 by alkylation of Keap1, glutathionylation of protein tyrosine phosphatase 1b by H₂O₂ and activation of ASK1 by peroxiredoxin catalyzed oxidation of thioredoxin. The challenge ahead is identification of the enzymes involved.

23. Functional Consequences of Age-Dependent Changes in GSH Status in the CNS

Pamela Maher, PhD (P)

Salk Institute, La Jolla, CA USA

Oxidative stress, inflammation and mitochondrial dysfunction have all been suggested to play key roles in aging and may be linked via alterations in cellular redox homeostasis. The small molecule antioxidant glutathione (GSH) is a tripeptide consisting of the amino acids glutamate, glycine and cysteine. Cells contain up to millimolar concentrations of GSH. Thus, GSH is one of the most important small

molecule antioxidants in somatic cells. Furthermore, the glutathione/glutathione disulfide (GSH/GSSG) pair forms the major redox couple in cells and as such plays a critical role in regulating redox-dependent cellular functions. Indeed, an impairment in GSH status is thought to be the precipitating event in a wide range of age-associated neurological disorders. GSH can also modulate the activity of a variety of different proteins. In addition to its well-known function in protein glutathionylation, it plays critical roles in regulating a number of important enzymes including glyoxalase (Glo-1). Glo-1 is the rate limiting enzyme for the removal of reactive dicarbonyls such as methylglyoxal (MG) and is absolutely dependent on GSH for activity. MG is a potent protein glycation agent that is formed endogenously as a by-product of glycolysis but can also be acquired from the diet. The interaction of MG with amino groups in proteins leads to the formation of irreversible modifications called advanced glycation end products (AGEs). Not only does glycation directly affect protein structure and function but AGE-modified proteins can activate receptors that are associated with both inflammation and oxidative stress. Furthermore, MG itself can reduce GSH levels and damage mitochondria resulting in an increase in oxidative stress. Thus, age dependent decreases in GSH in combination with enhanced oxidative stress and coupled with significant levels of MG and AGEs acquired from endogenous and/or dietary sources could contribute to age-dependent changes in CNS function.

24. Defective Redox Signaling and Age-Related Loss of Skeletal Muscle

Malcolm J. Jackson (P)

Institute of Ageing and Chronic Disease, University of Liverpool, UK

Chronic loss of skeletal muscle mass and function is a major contributor to frailty and weakness in the elderly. The major cause of age-related loss of muscle mass is a decrease in the number of skeletal muscle fibres associated with atrophy and weakness of the remaining fibres. A large number of studies have reported a dysregulation of reactive oxygen species (ROS) homeostasis during ageing that may potentially lead to increased oxidative damage to tissues and/or to defective redox signaling. Skeletal muscle tissue from aged organisms contains increased amounts of oxidative damage, but whether this is the cause of age-related deficits in muscle or a consequence of ageing has been the subject of controversy. Studies from our group have examined the changes in ROS generation that occur with ageing in man and experimental models and have also

utilised a transgenic approach to modify ROS generation in model organisms. Data from these experiments support the hypothesis that aberrant ROS regulation plays a role in the deficits in skeletal muscle and motor neurons that occur during ageing, but also indicate that this does not simply occur through increased oxidative damage to tissues, but is associated with defects in important redox-sensitive adaptive responses to contractile activity.

This research is supported by the Medical Research Council, Biotechnology and Biological Sciences Research Council and United States National Institute on Aging.

25. Aging in Hydra, Loss of Stemness

Tomczyk, S.¹, Podluskaya, N.², Fischer, K.², Galliot, B.¹, Austad, S.² (P)

¹University of Geneva, ²University of Texas Health Science Center San Antonio

Hydra oligactis contains strains that change reproductive mode from asexual to sexual reproduction in response to cool temperatures and other strains that do not. The cold-responsive strain also changes from an apparent nonaging phenotype to an aging phenotype at cooler temperatures whereas the other strain does not. As reported previously and confirmed by us, a characteristic of the aging phenotype is a rapid decline in the number of interstitial cells, a compartment containing multipotent interstitial stem and progenitor cells that produce many of hydra's terminally differentiated cells such as gland cells, neurons, and nematocytes as well as gametes during sexual reproduction. As stem cells in other organisms often have exceptionally effective mechanisms to protect stem cell compartments against accumulating DNA damage. We have been investigating DNA damage and repair dynamics in *H. oligactis* strains of both types, exposed to a variety of genotoxic challenges. We will present preliminary results from the studies.

26. Inducible Aging in Hydra

Daniel E. Martinez, PhD¹ and Diane M. Bridge, PhD (P)²

¹Pomona College, Claremont, CA; ²Elizabethtown College, Elizabethtown, PA

Within the genus *Hydra*, intriguing differences in life history exist. *Hydra vulgaris* does not appear to show increased mortality with age, while *Hydra oligactis* shows increased mortality and physical deterioration following induction of sexual reproduction. A wide

range of manipulations of cell and tissue fate are possible when working with *Hydra*. For example, the germ cells which give rise to gametes can be eliminated, and animals can be maintained indefinitely without them. Cell and tissue fate manipulations, together with the ability to produce transgenic *Hydra*, can be used to address the mechanisms underlying the difference in lifespan in *Hydra vulgaris* and *Hydra oligactis*. Questions of interest include the following. Does an abnormal heat shock response in *Hydra oligactis* play a role in senescence in this species? Is the presence of gametes required for induction of senescence in *Hydra oligactis*?

27. Aging and Regeneration in the Basal Chordate, *Botryllus schlosseri*

Anthony W. De Tomaso, PhD (P)

Department of MCD Biology, University of California, Santa Barbara, CA

Theories which aim to identify the mechanisms of aging can be broadly classified into two groups. The first attributes aging to progressive deterioration in the molecular and cellular machinery which eventually lead to death through the disruption of physiological homeostasis; the wear-and-tear model. The second suggests that life span is genetically programmed, and therefore aging may be derived from intrinsic processes which enforce a non-random, terminal time interval for the survivability of the organism. We are studying an organism that demonstrates both properties: the colonial ascidian, *Botryllus schlosseri*. *Botryllus* belongs to the phylum Tunicata, the sister group to the vertebrates. Besides this close phylogenetic relationship, *Botryllus* has a number of life history traits which make it an excellent model for studies on aging. First, *Botryllus* has a colonial life history, and grows by a process of asexual reproduction during which entire bodies, including all somatic and germline lineages, regenerate every week, resulting in a colony of genetically identical individuals. A colony can be split into multiple pieces and will continue to grow, allowing the characterization of genetic changes over the lifetime of a single genotype. In addition, the stem cells responsible for regeneration can be enriched and characterized for both genetic and functional changes over time. Second, previous studies of lifespan in genetically distinct *Botryllus* lineages suggest that a direct, heritable basis underlying mortality exists that is unlinked to reproductive effort and other life history traits. In this talk I will focus on functional and molecular characterization of the stem cells responsible for

regeneration, as well as studies on parabiosis between old and young individuals. We have found that germline and somatic stem cells are likely separate lineages based on both prospective isolation as well as molecular characterization. In an unpublished study we have identified two distinct molecular signatures which appear to segregate somatic and germline progenitors, and these fingerprints are consistent with FACS-based prospective isolation using labeled lectins, and functional studies are now in progress. We have found that juvenile individuals contain long-lived germline-restricted progenitors and have assessed their engraftment in adult individuals using natural parabiosis. Finally, we have found that stem cells enriched from juveniles show significant differences compared to equivalent populations from adult individuals.

28. First Draft of *Botryllus schlosseri* Genome, a Clonal Invertebrate with a Programmed Lifespan
Voskoboynik A^{1,2} (P), Sahoo D², Pushkarev D³, Koh W³, Neff NF³, Newman AM², Passarelli B³, Corey D², Mantalas G³, Palmeri KJ^{1,2}, Ishizuka KJ^{1,2}, Fan CH³, Penland L³, White RA 3rd, Weissman IL^{1,2*}, Quake SR^{3*}

¹*Institute of Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA, USA.* ²*Department of Developmental Biology, Stanford University, Hopkins Marine Station, Pacific Grove, CA, USA.* ³*Departments of Applied Physics and Bioengineering, Stanford University and Howard Hughes Medical Institute, Stanford, CA, USA.*

*Equal correspondence.

Colonial protochordates like *Botryllus schlosseri* do not maintain their genome in long-lived individuals, but rather in colonies where individuals within the asexual generation have a relatively short lifespan of ~21 days (14 days as a developing bud and 7 days as an adult zooid), while the entire colony has a long lifespan (from a few months to several years). Adult zooids are transient structures which die through massive apoptosis and are remade anew from stem cells every week. Toward the end of its lifespan, both asexual and sexual reproduction will begin to slow and eventually halt, suggesting that the colony dies when it is no longer able to replace tissues and reproduce. When a specific parent colony is experimentally separated into a number of clonal replicates (subclones), they frequently undergo senescence simultaneously, suggesting a heritable factor that determines lifespan in these colonies. To further understand the underlying mechanisms associated with regeneration, tissue homeostasis and longevity, we initiated a *B. schlosseri* genome

project. The estimated size of the *B. schlosseri* genome is 600Mb. Somatic cells contain 32 chromosomes (N=16) and a relatively high percentage of repeats. To accurately assemble the genome, we developed a novel method that allows us to sequence specific 6-8kb genomic fragments by Illumina HiSeq. The assembled Illumina paired-end 100bp reads were further assembled with 400bp Roche 454 Titanium and Pacific Biosciences RS 2-10kb reads, and mapped onto 14 chromosomes, isolated by microfluidics and sequenced individually. Here, we will present the draft genome and transcriptome of *B. schlosseri* and highlight the stem cells and developmental associated genes of this protochordate model that is considered to be the closest invertebrate living relative of vertebrates.

29. The Tunicate *Ciona*: A New Model System for Studying Age-related Decay in Regenerative Capacity and Tissue Repair

William Jeffery, PhD (P)

University of Maryland at College Park

A reduced capacity for tissue repair and regeneration is a hallmark of human aging but the underlying mechanisms are poorly understood. This gap in our knowledge is due in large part to the complexity and long lifespan of vertebrate regenerative models. Here we introduce the tunicate *Ciona intestinalis* as a new model system for studying age related changes in regeneration. Tunicates are chordates that have been inferred as the sister lineage of the vertebrates. *Ciona* has powerful and rapid regeneration: the pigmented ocelli in its siphons are replaced within a week after amputation, and even the brain (CNS) can be entirely reformed within a month after its removal. *Ciona* has a lifespan of a year and grows continuously during this period, allowing its age to be estimated on the basis of size. The *Ciona* genome has been sequenced, there is a large collection of ESTs and transgenic strains, and a protein database is under construction. Most importantly, the ability to accurately replace damaged organs, such as the siphons, their ocelli, and the brain, is dependent on age. In young animals siphon ocelli are replaced rapidly after amputation by processes similar to those employed during vertebrate limb regeneration: the formation of a wound epithelium and blastema, stem cell recruitment from local niches, cell proliferation and differentiation, intercalary replacement, proximal-distal polarity, and fidelity of pattern. Notch is a key signaling system involved in this process. The rate of regeneration fades during the life cycle, and in old animals regeneration is compromised with patterning defects and structural malformations appearing instead of normal ocelli. In summary, the ascidian *Ciona* offers

exceptional potential for studying the relationship between aging and regeneration in a biological context that is likely to be similar to that of the vertebrates. Supported by NIH Grant R01AG037918.

30. Comparative Biology and Genomics of Aging in Rotifers

David Mark Welch¹ (P) and Terry Snell²

¹*Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, Marine Biological Laboratory, Woods Hole MA;* ²*School of Biology, Georgia Institute of Technology, Atlanta GA*

Rotifers are a valuable new invertebrate model to investigate the cellular, molecular, and genetic processes of aging and senescence. As experimentally tractable basal triploblast animals, rotifers provide evolutionary breadth to comparative studies of aging. Additionally, rotifers have 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. We are investigating how closely related species of the well-studied monogonont rotifer genus *Brachionus* differ in their responses to stimuli that alter aging and lifespan, including caloric restriction (CR), antioxidants, pathway inhibitors, nutrient supplements and natural products, and then using differential transcriptomics of the varying phenotypes to dissect the evolutionary genetics of aging. For example, we have found that degree and manner of CR, as well as reproductive mode, all affect lifespan extension to different degrees in different *Brachionus* isolates, suggesting a link between the CR response and evolutionary ecology. To demonstrate the applicability of differential transcriptomics to study aging and the genetic mechanisms of lifespan extension, and to gather baseline gene expression data on the aging process, we collected digital expression data from five life history stages in *B. manjavacas*. There are significant differences in gene expression between different ages and reproductive stages for genes involved in mitochondrial function, ciliary function, apoptosis pathways and cellular stress response, including a 500-fold up-regulation in *Cu/Zn sod* in senescent females relative to eggs or neonates.

31. Daphnia: A Model Crustacean for Genetic Variation of Aging

Jeffrey L. Dudycha, PhD (P)

University of South Carolina, Columbia, SC

We are developing the crustacean *Daphnia* as a new invertebrate model for the biology of aging. *Daphnia* have several features that complement existing invertebrate models. They are cyclic parthenogens, and thus they are a highly genetically diverse group from which naturally-occurring genomes can be clonally replicated in the lab. They have been a major model organism in ecology for more than a century, particularly with respect to demography and phenotypic responses to environmental variation.

They have adult tissue regeneration throughout their lifespan. In addition, they are the focus of a major international genomics consortium, and the complete genome of several species has been sequenced with a dedicated community working to improve genetic and bioinformatic resources. We have taken a multi-faceted approach that exploits features of the *Daphnia* system to advance understanding of aging and develop further tools for working with *Daphnia*. So far, these approaches include using long- and short-lived ecotypes for demographic characterization, for investigation of the age-dependence of stress response, for determination of differential regeneration capacity, and comparative analysis of genes involved in aging. Planned future work includes age-dependent transcriptomic analysis, functional genetics, and epigenetic analyses.

32. TBA

Irv Weissman, MD (P)

Stanford University, Stanford, CA

33. T Cell Metabolism and Signaling in Aging – Friends or Foes?

Jörg Goronzy, MD, PhD (P)

Stanford University, Stanford, CA

T cell-dependent B cell responses in response to vaccines decline with age, suggesting defects in CD4 T cell memory function. Early T cell receptor signaling in CD4 memory T cells from individuals older than 65 years is intact compared to young adults, suggesting that T cell activation is normal. However, elderly T cells are less able to support the differentiation of B cells into plasma blasts in an in vitro system. This defect correlates with the increased transcription of the dual-specific phosphatase (DUSP) 4 on days 2 to 4 after activation. DUSP4 is a nuclear phosphatase that dephosphorylates ERK and JNK and thereby curtails sustained T cell activation.

Increased nuclear DUSP4 shortens expression of CD40L and ICOS and decreases production of several cytokines. Many of these DUSP4 targets are T cell effector molecules involved in B cell differentiation. DUSP4 silencing in memory CD4 T cells improves CD40L, IL-4 and IL-21 expression significantly more in elderly than in young adults. Consequently, the ability of CD4 memory T cells to support B cell differentiation in the elderly is restored. The increased transcription of DUSP4 in elderly memory CD4 T cells is due to increased activity of AMPK. Increasing AMPK activity in young T cells increases the transcription of Egr1 upon T cell activation, which upregulates DUSP4 expression. Phosphorylation of AMPK and expression of Egr1 is increased in elderly T cells. Since AMPK is a sensor of the cellular energy state, the AMPK activation may indicate a lower cellular metabolism in elderly T cell which may be favorable for survival but detrimental for effector function. The identification of a negative signaling feedback loop regulated by the metabolic activity of the elderly T cell opens the opportunity to directly target this pathway to optimize vaccine responses in the elderly.

34. Frontiers in Enteric Neuroscience

Emran A. Mayer (P)

University of California Los Angeles, Los Angeles, CA Oppenheimer Family Center of Neurobiology of Stress, Department of Medicine, David Geffen School of Medicine at UCLA CNS

Even though the gastrointestinal tract is generally viewed primarily as a digestive organ, a new perspective of a much wider role in body homeostasis has been evolving over the past decade. The gut forms a unique interface with the environment: Its surface area is 100 fold greater than our skin, and the gut epithelium separates us from 100 trillion microorganisms which make up the intestinal flora. The gut associated immune system makes up more than 80% of all immune cells within the body, and specialized endocrine cells contain more than 40 signaling molecules which play a central role in ingestive behavior, pain and possibly emotional and cognitive states. In addition to assuring appropriate gut responses to ingested food, the enteric nervous system, made up of ganglionated plexuses of 50 million neurons which are sandwiched in between the gut layers, plays an important role in sensing the luminal environment. Finally the close bidirectional neural and endocrine communications between the intestinal microbiota, the gut and the brain, are likely to play an important role in modulating affective and cognitive processes.

35. Nutrients and Bone Health: Evidence for Additional Role in Heart Health?

Sarah L. Booth (P) and Elizabeth Samelson

Jean Mayer USDA HNRCA at Tufts University, Boston, MA

Certain “bone-associated” nutrients, including calcium, vitamin D and vitamin K, may also play a protective role in the pathogenesis of vascular calcification, although not necessarily through common pathways. In humans, much of the evidence is limited to epidemiologic associations between markers of nutrient status and vascular calcification or bone loss. Calcium supplementation is extensively used for treatment of osteoporosis, usually in conjunction with vitamin D. However, there is a controversy regarding the rate of cardiovascular events among women consuming calcium supplements. Inverse associations have been attributed to a variety of mechanisms, including the positive influence of calcium on blood pressure, serum cholesterol level, and blood glucose. Conversely, there are reports of calcium supplementation with and without vitamin D supplementation being associated with increased CVD risk that is attributed to elevated serum calcium concentrations and increased vascular calcification. In the absence of accurate dietary intake data, it is difficult to determine the total (diet and supplement) calcium intakes at which the the purported adverse cardiovascular effects may occur. Vitamin K has been associated with reduced bone loss and reduced risk for vascular calcification through its role as an enzyme cofactor. Bone matrix proteins, such as Matric Gla protein require vitamin K for function as a negative regulator of mineralization. Findings from randomized clinical trials have not supported vitamin K’s role in reducing age-related bone loss. In contrast, there is growing evidence that vitamin K may have a protective role against progression of vascular calcification. The majority of these studies have co-administration of vitamin K with calcium and/ or vitamin D, so a unique effect of vitamin K is not known. Similar to calcium and vitamin D, the randomized clinical trials have yet to be conducted, particularly those that address the nutrient-nutrient interaction.

36. Translational Approaches to Clinical Trials of Nutrition and Physical Activity in Older Adults: From Biology to Function

Roger Fielding, PhD (P)

Nutrition, Exercise Physiology, and Sarcopenia Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA, USA.

Associate Director, Boston Claude D. Pepper Older Americans Independence Center

The age-related loss of skeletal muscle mass, sarcopenia, is associated with well-characterized functional limitations and physical disability that occur with advancing age. Interventions that target sarcopenia are currently being explored with the hope that they can improve physical functioning and prevent disability in older adults. Although resistance exercise training may attenuate age-related muscle loss in healthy older adults and animals, skeletal muscle growth capacity in response to exercise appears to be limited with more advanced age and frailty. The cellular processes that initiate muscle hypertrophy and the extent to which they are altered with age continue to be investigated. We have examined the ribosomal protein S6 kinase (p70S6K), a member of the protein kinase B/mammalian target of rapamycin (Akt/mTOR) pathway that has been implicated as an important factor in regulating muscle size during overload and disuse atrophy, in both older animals and humans. These proteins are potential regulators of age-associated muscle wasting and may also contribute to the attenuation of muscle hypertrophy that has been reported in older humans and animals in response to exercise interventions. We have examined and will present data on the effects of acute and chronic contractile activity on changes in phosphorylation and expression of members of the Akt/mTOR signaling pathway in skeletal muscle from young and old animals and humans. Our work suggests that dysregulation of intramyocellular lipid storage is a critical mediator of the reduced anabolic capacity observed with advancing age.

37. The Age-Related Increase in Low Grade Systemic Inflammation (Inflammageing) is not Driven by Cytomegalovirus Infection

D.B. Bartlett BSc¹, C.M. Firth MSc², A.C. Phillips PhD³, P. Moss PhD², D. Baylis MBBS⁴, H. Syddall MSc⁴, A.A. Sayer PhD⁴, C. Cooper FMedSci⁴, J.M. Lord PhD¹

¹MRC-ARUK Centre for Musculoskeletal Ageing Research, School of Immunity and Infection, University of Birmingham, UK; ²School of Cancer Sciences, University of Birmingham, UK;

³School of Sport and Exercise Sciences, University of Birmingham, UK; ⁴MRC Lifecourse Epidemiology Unit, University of Southampton, UK

Background

Ageing is accompanied by the development of low grade systemic inflammation, termed 'inflammageing', characterised by raised serum C-reactive protein (CRP) and pro-inflammatory cytokines. Importantly, inflammageing is implicated in the pathogenesis of several age-related diseases including cardiovascular disease, type-2 diabetes and dementia and is associated with increased mortality. The incidence of infection with the persistent herpes virus cytomegalovirus (CMV) also increases with age. Cross-sectional studies have proposed CMV infection as a significant driver of inflammageing, but a definitive case for CMV as a causative agent in inflammageing has not been made.

Methods

We studied longitudinally 249 subjects (153 men, 96 women) who participated in the Hertfordshire Ageing Study at baseline (1993/5, mean age 67.5 years) and at 10 year follow up. At both times anthropometric measurements were made, subjects provided blood samples for analysis of inflammatory status and CMV seropositivity and completed lifestyle questionnaires.

Findings

In the cohort as a whole, serum CRP ($p < 0.02$) and pro-inflammatory cytokines TNF α ($p < 0.001$) and IL-6 ($p < 0.001$) were increased between baseline and follow up sampling whereas levels of the anti-inflammatory cytokine IL-10 were decreased ($p < 0.001$). These changes to cytokine status over time occurred equally in the 60% of subjects who were seropositive for CMV at baseline and follow up, the 8% who were CMV negative at baseline but who became CMV positive by the follow up, and also in the 32% who were CMV seronegative throughout. Factors that positively associated with inflammageing were a sedentary lifestyle.

Interpretation

Inflammageing occurs independently of CMV infection. Vaccination against CMV is unlikely to prevent age-related inflammation and lifestyle factors are the key modifiable variables to be addressed to reduce inflammageing.

38. Rapamycin Improves Mitochondrial Homeostasis and Delays the Onset of Senescence by Altering p62/SQSTM1 Dynamics.

Alessandro Bitto¹(P), Chad Lerner¹, Mina Konigsberg², Daniel Pulliam³, Holly Van Remmen³, Claudio Torres¹, Christian Sell¹

¹Drexel University College of Medicine;

²Universidad Autónoma Metropolitana Iztapalapa, México City; ³Barshop Institute for Longevity and Aging Studies

The Target of Rapamycin (TOR) is an evolutionarily conserved protein kinase that integrates signals from growth factors, cellular nutrients, oxygen and energy availability in order to regulate cell growth and proliferation. Inhibition of the TOR pathway extends lifespan in several model organisms, and represents a potential strategy for anti-aging intervention in humans. We explored the effects of chronic mammalian TOR (mTOR) inhibition in primary human fibroblasts treated with rapamycin, a highly specific TOR inhibitor. Prolonged exposure to rapamycin increased autophagy, reduced the half-life of mitochondrial proteins, and increased the turnover of p62/SQSTM1 and its localization with cytochrome c, suggesting increased mitophagy of damaged mitochondria in human fibroblasts. Consequently, cultures exposed to rapamycin had lower levels of reactive oxygen species and their mitochondria had increased membrane potential and higher resistance to oxidative stress. In the long term, inhibition of mTOR prevented the progressive accumulation of depolarized mitochondria, typical of cells approaching senescence, and decreased the activation of p38MAPK, a protein kinase involved in the establishment of senescence. Rapamycin treatment extended the maximum lifespan of cells (measured as cumulative population doubling, CPDL) by 14% and reduced the presence of senescence-associated markers at high CPDL. Interestingly, lowering p62/SQSTM1 expression increased mitochondrial depolarization to levels comparable to control and promoted the expression of the senescence marker p16INK4a in cells exposed to rapamycin. In conclusion, we find that inhibition of mTOR with rapamycin delays the onset of senescence by improving mitochondrial homeostasis and lowering oxidative stress. Interestingly, we find that rapamycin alters p62/SQSTM1 expression and localization and that p62/SQSTM1 is required to reduce the accumulation of depolarized mitochondria and lower stress levels. Our results identify p62/SQSTM1 as a novel factor involved in the biology of aging and suggest that interventions on its expression and activity could lead to new strategies to slow the aging process.

39. A novel Peptide that Improves Mitochondrial Function Reverses Diabetes- and Age-Related Visual Decline

Nazia Alam¹(P), Aimee Wong¹, Yi Soong¹, Alexander Birk¹, Robert Douglas², Hazel Szeto¹, Glen Prusky¹

¹Weill Cornell Medical College, Burke Medical Research Institute; ²University of British Columbia

Diabetes and aging are leading causes of progressive vision loss, for which there are no effective treatments. Although the etiology of the conditions differ, they share a common pathology of degraded energy metabolism. There is growing evidence that impaired mitochondrial function plays a key role in the advancement of the metabolic conditions that leads to age-related visual decline. However, no study has directly investigated whether improving mitochondrial function will improve visual dysfunction. A potentially efficacious agent is Bendavia (MTP-131), a peptide that selectively targets mitochondria, where it improves mitochondrial function by increasing ATP production and prevents the formation of reactive oxygen species (Szeto & Schiller, 2011). Thus, we treated animal models of diabetes- and age-related progressive visual decline with Bendavia, and quantified visual thresholds longitudinally, using a virtual optokinetic system (Prusky et al, 2004). In a mouse model of diabetic visual dysfunction, in which we combined a diabetic diet with streptozocin (STZ) injections, visual dysfunction emerged 1 week after injections, and was reduced by ~15% within 1 month. Visual decline was halted within 1 week of initiating Bendavia treatment, and gradually improved until normal function was restored within 5 months. Groups fed a diabetic diet alone or treated with STZ only displayed less visual decline, which was also fully restored with Bendavia. Similarly, in our age-related mouse and rat models of visual decline, Bendavia treatment was found to improve and sustain visual function above vehicle control animals, and in some cases, was able to reverse the visual deficits. Thus, visual decline in multiple animal models of metabolic dysfunction can be limited or reversed with a peptide that improves mitochondrial function. Since Bendavia has favorable pharmacokinetics and is well tolerated in several clinical studies, it may be a viable tool to treat human visual diseases related to metabolic dysfunction.

40. The Impact of Ambient Temperature on Metabolism in Long-lived Mice

Reyhan Westbrook¹(P), John Kopchick², Andrzej Bartke³

¹*Southern Illinois University School of Medicine;*

²*Department of Biomedical Sciences, Ohio*

University; ³*Internal Medicine-Geriatrics Research SIU School of Medicine*

Decreased growth hormone (GH) induced signaling is linked to increased lifespan in mice. Mice with mutations causing decreased (GH) action are smaller, and have increased oxygen consumption per unit body weight and decreased respiratory quotient compared to their wild type controls. We hypothesize that the smaller body size of growth hormone signaling deficient mice causes them to radiate heat more rapidly, which in turn, drives lipid oxidizing thermogenesis and results in the increased oxygen consumption and decreased respiratory quotient seen in these long-lived mice. In order to test this hypothesis, we utilized indirect calorimetry to measure twenty-four hour oxygen consumption (VO₂), respiratory quotient and spontaneous locomotor activity at both 23° C (standard) and 30° C (thermoneutral) ambient room temperature in long-lived GH receptor gene disrupted, or knock-out (GHRKO) mice and their respective controls. At 23° C long-lived mice had increased VO₂ and decreased respiratory quotient compared to controls; however at 30° C these differences were abrogated. GHRKO mice had a significant reduction in VO₂ at 30° C compared to 23° C while control animals did not. These results indicate that the alterations in energy metabolism in GHRKO mice are at least in part due to the increased need for thermogenesis to maintain homeothermy. Increased thermogenesis may be a mechanism of increased longevity in GHRKO mice.

41. Affects and Mechanism of a Caloric Restriction Mimetic as an Intervention in a Mouse Model of a Human Mitochondrial Disease

Simon Johnson (P) Anthony Castanza, Vy Nguyen, Pabalu Karunadharma, Valerie Wall, Peter Rabinovitch, Matt Kaeberlein

University of Washington, Department of Pathology, Seattle, WA

Leigh syndrome is a rare genetic disease caused by any of several mutations in proteins required for assembly or function of the electron transport chain in the mitochondrial inner membrane. Manifestations of the disease include developmental delay, optic atrophy, hypotonia, ataxia, breathing abnormalities, necrotic brain lesions, and drastically shortened

lifespan. In mice, Leigh syndrome has been modeled by knockout of NADH dehydrogenase [ubiquinone] iron-sulfur protein 4 (Ndufs4), a component of the multi-subunit respiratory chain complex I. Ndufs4 knockout mice experience many of the phenotypes associated with the human presentation of Leigh syndrome including necrosis and neurological deficit, delayed development, ataxia, and short lifespan. Currently no effective interventions exist for treating mitochondrial disorders of any etiology. Yeast genetic data from our lab suggests that dietary restriction mimetics may provide a novel means of intervention in mitochondrial disease. In this study we examined the therapeutic potential of the drug Rapamycin in treating the Ndufs4 mouse model of Leigh syndrome. Ndufs4 knockout mice experienced a partial rescue of many Leigh syndrome associated phenotypes when treated with Rapamycin. We are exploring three potential mechanisms of Rapamycin's action in this disease model. First, through mTOR inhibition mediated decreases in translation. Second, through upregulation of autophagy and mitophagy. Third, by acting through its immunomodulatory activity, as inflammation is a hallmark of this disease. Rapamycin is currently approved by the FDA for use in human patients as an immunosuppressant used for prevention of organ transplant rejection. This work demonstrates the translational worth of aging research in human diseases.

42. Impaired Mitochondrial Dynamics and Function Result from LRRK2 Mutations

Xiongwei Zhu¹, George Perry²(P), Michael Yan¹, Hisashi Fujioka¹, Jun Liu³, Amy Wilson-Delfosse¹, Shu Chen¹, Gemma Casadesus¹, Xinglong Wang¹

¹*Case Western Reserve University, Cleveland, Ohio;*

²*University of Texas at San Antonio, San Antonio, TX;* ³*Ruijin Hospital*

Mitochondrial abnormalities are a feature of Parkinson's disease (PD), which could be a result of deficient regulation of dynamics/quality control. Common causes of autosomal dominant PD cases are mutations in LRRK2, yet how LRRK2 mutations cause dopaminergic neurodegeneration is unknown. To examine a possible interaction, cell lines overexpressing wild type LRRK2 or the PD-associated LRRK2 mutants R1441C and G2019S were used to examine mitochondrial function, fission and fusion proteins, and mitochondrial morphology to understand how LRRK2 is involved in the regulation of mitochondrial dynamics and function in neurons. Specifically, confocal and electron microscopic analysis demonstrated a significant increase in mitochondria fragmentation and

mitochondria with damaged inner structures in SH-SY5Y cells overexpressing wild type LRRK2 or PD-associated LRRK2 mutants (R1441C and G2019S) compared to cells overexpressing empty vector. LRRK2 WT, R1441C and G2019S overexpression resulted in reactive oxygen species overproduction, decreased mitochondrial membrane potential, reduced ATP level and also increased vulnerability to oxidative stress (H₂O₂) or neurotoxin (MPP+). Overexpression of LRRK2 in differentiated primary cortical neurons also resulted in mitochondrial fragmentation, impaired axonal transport of mitochondria and neurotoxicity. Of note, the mitochondria fission protein, DLP1, was increased in neurons overexpressing LRRK2 (WT, R1441C and G2019S mutants). However, overexpression of dominant negative DLP1 K38A abrogated the mitochondria fragmentation and mitochondria dysfunction. It is thought that enhanced kinase activity underlies the toxic effects of PD-associated LRRK2 mutants. In support of this, mitochondrial morphological and functional deficits were never observed in cells overexpressing either GTP binding-deficient mutant LRRK2 K1347A or kinase dead mutant LRRK2 D1994A. These regions have minimal interaction with DLP1 and these cells do not display increased DLP1 levels. We conclude that LRRK2 is involved in the regulation of mitochondrial morphology through its kinase activity, and LRRK2 mutants cause an imbalance of mitochondrial fission/fusion resulting in mitochondrial fragmentation and neuronal dysfunction.

43. C57BL/6 Neuromuscular Healthspan Scoring System: Assessment of Sarcopenia/Frailty Interventions

Ted Graber (P), Lisa Ferguson-Stegall, Jong-Hee Kim, LaDora Thompson
University of Minnesota Medical School, Minneapolis, MN

Sarcopenia, the age-associated loss of muscle mass and strength, contributes to the etiology of frailty and is thus a major medical concern. The development of a healthspan scoring system that allows researchers to test potential interventions for sarcopenia and frailty prevention in animal models of aging is critical. Therefore, our aim was to develop a neuromuscular healthspan scoring system (NMHSS) that accurately describes healthspan. We selected the male C57BL/6 mouse, a long standing aging model, and examined three age groups: (1) young adults (6-7 months old, 100% survival), (2) old (23-25 months old, 75% survival) and (3) elderly (28+ months old, =50% survival); as well as mice along this age continuum to formulate multiple linear regressions that describe the

aging process. Functional performance and in vitro muscle contractility were our initial determinants for the NMHSS. Specifically, we evaluated Rota-Rod performance, inverted-cling grip strength and in vitro muscle contractility. We found significant age-related differences and correlations with age across the NMHSS outcome measurements ($p = 0.05$). The regression models were moderately predictive of Rota-Rod, grip test and the peak tetanic force of the extensor digitorum longus muscle ($R^2 = 0.52, 0.22$ and 0.46 , respectively). A raw score was derived for each healthspan determinant (score = the mean of the ratios of the actual/average and the actual/predicted values). The NMHSS was then derived as the sum of the individual determinant scores. In conclusion, our neuromuscular healthspan scoring system has the potential to become an important tool with which to rate individual mice in order to be able to derive a relative measure of the neuromuscular functionality of that mouse in comparison to that of the overall population at the given age and/or treatment modality. The ability to assess the efficacy of aging interventions in future studies may be greatly enhanced by this tool.

44. Cell Autonomous and Non-Autonomous Neuromuscular Phenotypes of CuZnSOD Knockout Mice—an Electrophysiological study

Yun Shi (P), Michael Walsh, Daniel Pulliam, Yuhong Liu, Yiqiang Zhang, Carlos Jaramillo, Holly Van Remmen
University of Texas Health Science Center at San Antonio, San Antonio, TX

Neuromuscular junction (NMJ) degeneration and muscle atrophy occur with advancing age and in various neuromuscular disorders. Previously we have demonstrated that mice deficient in Cu/Zn superoxide dismutase (CuZnSOD or SOD1) exhibit age-dependent NMJ degeneration, muscle weakness and functional motor deficits. The purpose of this study was to determine if these changes are associated with alterations in neuromuscular electrophysiological properties. SOD1 knockout mice (KO) showed prolonged distal motor latency, reduced sciatic motor conduction velocity and pathological decrement in compound muscle action potential (CMAP) amplitude with low frequency repetitive nerve stimulation (RNS). These changes are indicative of axonopathy and a reduced safety factor at the NMJ. However, it is unclear whether these abnormalities are due to the absence of CuZnSOD in neurons, skeletal muscle or in both tissues. Next we asked whether this phenotype is a cell-autonomous trait of CuZnSOD deficiency by utilizing tissue specific knockout mice. The neuron-specific SOD1 knockout

mice (NKO) developed a moderate reduction in major hind limb muscles. In contrast, muscle-specific SOD1 knockout mice (MKO) showed no signs of muscle atrophy. However, neither NKO mice nor MKO mice showed aberrations in RNS, suggesting the deficits may be a synergistic effect from multiple cell types. Furthermore, neuronal SOD1 overexpression rescued muscle atrophy, aberrant CMAP parameters in the KO mice. These data indicate that restoration of CuZnSOD expression in neurons alone is sufficient to protect against NMJ deficit due to SOD1 deficiency. In conclusion, the complete neuromuscular phenotype in SOD1 knockout mice is likely caused by deficiency of SOD1 in both muscle and neurons during progression of the phenotype. Our data suggest that the NMJ decay in SOD1 knockout mice is principally due to a pre-junctional, neuronal pathology and that muscle atrophy in these mice may be secondary to this neuronal defect rather than an intrinsic muscle SOD1 deficiency.

45. Concord Grape Juice Supplementation and Neurocognitive Function

Robert Krikorian¹ (P) Erin Boespflug¹, David Fleck¹, Amanda Stein¹, JoLynne Wightman², Marcelle Shidler¹, Sara Sadat-Hossieny¹

¹University of Cincinnati; ²Welch Foods, Inc

Concord grape juice contains anthocyanins and flavanols, flavonoid compounds that have been associated with several health benefits including moderation of oxidative stress and inflammation, improved vascular function and blood flow, increased neuronal signaling, and improved metabolic function. These effects are pertinent to human aging and would be expected to mitigate neurocognitive decline. In a previous trial we found that 12 weeks' supplementation with Concord grape juice improved memory in older adults with Mild Cognitive Impairment (MCI), a risk condition for dementia. In this second trial, we extended the duration of the treatment and performed functional Magnetic Resonance Imaging (fMRI) during a working memory task. We assessed long-term memory function and brain activation before and after the treatment. Twenty-one older adult participants with MCI completed a 16-week intervention during which they consumed either 100% Concord grape juice (n = 10) or placebo beverage (n = 11). A subset of the sample (n = 8) also participated in the fMRI studies. At enrollment, there was no demographic, mood, or anthropometric difference or difference in level of cognitive impairment between the groups. After 16 weeks, we found that participants who consumed

grape juice exhibited reduced semantic interference on a recognition memory task, $p = 0.04$, indicating better ability to discriminate learned items from foils. In addition, we observed relatively greater cerebral activation with fMRI in the grape juice treated subjects in right middle frontal cortex, $p = 0.05$, and in right superior parietal cortex, $p = 0.07$. The findings indicate improved cognitive control in long-term memory and greater neuronal activity in brain regions typically involved in working memory operations. These effects are consistent with the demonstrated benefits associated with consumption of foods rich in flavonoids and provide further evidence that Concord grape juice can enhance neurocognitive function in older adults with memory decline.

46. Depletion of mTORC2 Impairs the Health and Longevity of Mice

Dudley Lamming (P), Pekka Katajisto, Amanda Hutchins, David Sabatini,
Whitehead Institute, Massachusetts Institute of Technology, Cambridge, MA

Rapamycin, an inhibitor of mechanistic target of rapamycin complex 1 (mTORC1), extends the lifespans of many organisms including mice. We have found that rapamycin disrupts a second mTOR complex, mTORC2, in vivo, and that mTORC2 disruption is an important mediator of the effects of rapamycin. In *C. elegans* decreased mTORC2 signaling promotes longevity, but in mammals the effects of decreased mTORC2 signaling may not be as beneficial. We have found that mTORC2 action is required for the insulin-mediated suppression of hepatic gluconeogenesis, and mice lacking hepatic Rictor, an essential protein component of mTORC2, are glucose intolerant. In order to determine the effect of decreased mTORC2 signaling on longevity, we have examined three different mouse models of decreased mTORC2 signaling; mice in which one copy of Rictor has been deleted, mice lacking hepatic Rictor, and mice in which Rictor has been depleted in the adult mouse by means of a tamoxifen-inducible Cre. We find that depletion of Rictor negatively impacts the longevity of male mice in all three mouse models. Surprisingly, the effect of Rictor depletion on longevity is separable from the impact of Rictor on glucose homeostasis, as rictor^{+/-} mice have reduced lifespan but normal glucose homeostasis. Furthermore, we find that mTORC2 may also be critical in the pro-longevity effect of calorie restriction. Our results implicate mTORC2 signaling as critical in the survival of male mice, and suggest

that the inhibition of mTORC2 by rapamycin negatively impacts longevity.

47. Trade-off in the Effect of the APOE Gene on the Ages at Onset of CVD and Cancer Across Ages, Gender, and Human Generations

Alexander Kulminski, (P), Anatoli Yashin, Irina Culminkaya, Liubov Arbeeva, Svetlana Ukraintseva, Konstantin Arbeev
Duke University, Durham, NC

Studies show an important role of pleiotropic genes in complex traits. Recently, we demonstrated that pleiotropy could take a complex form with antagonistic action of the same allele on different diseases that constitutes genetic trade-off (Aging Cell 2011;10:533-541). Here we focus on the same apolipoprotein E (APOE) e2/3/4 polymorphism and ages at onset of cardiovascular diseases (CVD) and cancer to elucidate the role of age and gender across generations in the observed trade-off. We used data on two generations of the Framingham Heart Study (FHS) participants followed up 60 years. Kaplan-Meier screening and Cox regression modeling show that the e4 allele carriers live longer lives without cancer than the non-e4 allele carriers in each FHS generation (relative risk (RR) of cancer onset for pooled generations, RR=0.87, p=0.045). Protective role of the e4 allele against cancer is limited to older ages (RR=0.75, p=0.007; 70+ years) with more pronounced effect in men (RR=0.71, p=0.017). The role of the e4 allele in onset of CVD is age and generation-specific. In the parental generation the e4 allele promotes CVD in younger ages (RR=1.49, p=0.0008; <=75 years) but protects older women (RR=0.69, p=0.025). In the offspring generation the e4 allele promotes CVD in all ages (RR=1.22, p=0.009) with more pronounced role in older women (RR=1.56, p=0.039; 70+ years). These results suggest that the aging-related processes in different generations can readily alter the role of genes in late life in a gender-specific manner suggesting critical role of aging in unraveling genetics of traits in late life.

48. Decoding Proteostasis of Aging

William E. Balch¹⁻⁴ (P), D. Hutt¹, D. Roth¹, M. Chalfant¹, J. Tong¹, A. Pottekat¹, S. Pankow³, C. Bamberger³, J. Coppinger³, S. Beckers⁵, G. Manning⁵, H. Gei⁶, D. Garza⁶, P. Reinhart⁶, J. Yates III³

Departments of Cell Biology¹, Molecular Biology² and Chemical Physiology³, The Skaggs Institute for Chemical Biology⁴, The Scripps Research Institute (TSRI), La Jolla, CA; The Salk Institute, Razavi-

Neuman Center for Bioinformatics⁵, La Jolla, CA; Proteostasis Therapeutics Inc⁶, Boston, MA.

The cell exploits the emergent properties of proteostasis, a program coding for the biological protein fold, to manage the health of the human proteome during development and aging. The proteostasis system includes a multitude of folding chaperones, trafficking pathways and degradation systems that respond to folding stress through cell signaling pathways of high clinical relevance (Science (2008) 319:916; Curr. Opin Cell Biol (2011) 23:126)). Proteome maintenance by the proteostasis network (the PN) is both versatile and dynamic-operating both inside and outside diverse cell, tissue and organ environments (Science (2010) 367:766; Nature (2011) 471:42). Physical and pathological challenges during aging to the kinetics and energetics of proteome function compromise the operation of proteostasis resulting in systemic (e.g., muscle, liver, pancreatic) and neurodegenerative folding diseases, most hallmarks of human aging. By use of proteome-oriented genomic, proteomic and bioinformatic tools we are building a multi-layered view of healthy biological protein folding and the changes that occur in response to disease, pointing towards new therapeutic approaches for healthspan management through maintaining a healthy proteome.

49. Anti-Aging Drugs in Mice: Two New Ones and an Old Favorite Revisited

Richard A. Miller, PhD (P)
University of Michigan, Ann Harbor, MI

The NIA Interventions Testing Program ("ITP") is a multi-institutional collaboration that tests drugs thought likely to extend mouse lifespan by slowing the aging process. We reported in 2009 that rapamycin, an inhibitor of the TOR kinase, could extend mouse lifespan when given late in life, but lifespan data by themselves cannot show whether the improved survival represented deceleration of the aging process, or merely of the forms of neoplastic disease most likely to kill mice. We now have new results showing that rapamycin does indeed retard multiple aspects of aging in several tissues, including liver, heart muscle, endometrium, adrenals, and tendon, and also slows the age-dependent loss of spontaneous activity. These data strongly support the hypothesis that rapamycin slows aging in mice, although they do not exclude the idea that the lifespan extension might represent a more direct effect on neoplastic cells themselves. Rapamycin also led to several undesirable side effects, including cataracts, testicular degeneration and (probably) osteoarthritis. Studies now nearing their conclusion

provide preliminary data for improved survival from two agents not previously studied. One of these is acarbose, which interferes with absorption of carbohydrates from the intestine and is in clinical use as a treatment for some forms of diabetes. The other is 17- α -estradiol, a non-feminizing estradiol that works largely through pathways independent of the classical estrogen receptor. Unlike rapamycin, whose effects are stronger on female mice, both acarbose and 17- α -estradiol seem to have much stronger effects on male mice, although definitive conclusions cannot be drawn until more of the mice have died.

Key collaborators: David Harrison, Nancy Nadon, Randy Strong, J. Erby Wilkinson, David Allison, Susan Brooks, Beatriz Carames, Martin Lotz, Dave Sharp, James Simpkins, Daniel Smith, Lauren Wood, Maria Woodward

Support: National Institute on Aging

50. Peroxiredoxins and Redox-Sensitive Signaling in Aging

William C. Orr, PhD (P)

Southern Methodist University, Dallas, TX

Accumulating evidence suggests that severe disruption in thiol homeostasis and redox state represent major changes that accompany aging. In *Drosophila*, the accelerated decrease in redox in middle-age flies is coincident with acceleration of the death rate. Moreover, enhanced production of the major cellular reducing equivalents, GSH and NADPH, in transgenic flies resulted in the most significant effects on increases in life span in *Drosophila* (>40% increase in longevity).

By virtue of their localized actions on peroxides coupled with oxidation of such key “reducing equivalents” as thioredoxin, the peroxiredoxins are considered to be important players in maintaining redox state. Consequently, we have undertaken a series of studies to explore the potential roles of the peroxiredoxin gene family in modulating life span and health span in the fly model. Orthologs for all six members of the human peroxiredoxin family are present and this presentation will focus on three of these, the mitochondrially localized dPrx3, the broadly localized dPrx5 (mitochondrial, cytosolic and nuclear) and the ER-associated dPrx4.

In a series of physiological and genetic studies we have established that together, the mitochondrially-localized dPrx3 and dPrx5, play a key role in the maintenance of redox homeostasis, with strong effects on both apoptosis and longevity. Their effects

have been implicated in multiple pathways/processes associated with aging and have led us to identify at least one potential target, thioredoxin reductase, mediating these effects. In a similar set of studies, we found that modulating redox state by under and overexpression of dPrx4 affected immune- and stress-response genes as well as longevity. Moreover these effects appeared to be largely mediated through JAK-STAT signaling.

From this redox perspective it appears that the identification of these peroxiredoxin targets could pave the way for the development of interventions that would more effectively modulate regulation of signal transduction during aging.

51. Natural Products that Suppress Protein Aggregation and Slow Aging

Silvestre Alavez¹, Pedro Rodriguez¹, Maithili C. Vantipalli¹, David J. S. Zucker^{1,2}, Ida M. Klang^{1,3}

Milena Price, Karla Mark, and Gordon J. Lithgow¹

¹*Buck Institute for Research on Aging, 8001 Redwood Blvd, Novato, California 94945, USA.* ²*Department of Natural Sciences and Mathematics, Dominican University of California, San Rafael, California, 94901, USA.* ³*Karolinska Institute, Center for Biosciences at NOVUM, Department of Biosciences and Nutrition, Hälsovägen 7, S-141 83 Huddinge, Sweden.*

We have undertaken screen of synthetic and natural compounds to find agents for aging interventions. Since aging can be considered a causal factor in a number of age-related diseases. We hope such screen could yield useful therapeutics. We focused our search on compounds that maintain protein homeostasis. Collapse of protein homeostasis results in protein misfolding cascades and the accumulation of insoluble protein fibrils and aggregates, such as amyloids. A group of small molecules, traditionally used in histopathology to stain amyloid in tissues, bind protein fibrils and slow aggregation *in vitro* and in cell culture. We proposed that treating animals with such compounds would promote protein homeostasis *in vivo* and increase longevity. Here we show that exposure of adult *Caenorhabditis elegans* to the amyloid-binding dye Thioflavin T (ThT) resulted in a profoundly extended lifespan and slowed ageing. ThT also suppressed pathological features of mutant metastable proteins and human β -amyloid-associated toxicity. These beneficial effects of ThT depend on the protein homeostasis network. Other agents that extend lifespan also suppress protein homeostasis such as Lithium. We are testing for common mechanisms of action. This is revealing

tissue non-autonomous signaling between neurons and muscle.

Our results to date demonstrate that pharmacological maintenance of the protein homeostatic network by natural products has a profound impact on aging rates, prompting the development of novel therapeutic interventions against ageing and age-related diseases.

52. Nutritional Interventions and Stem Cell Senescence

Paula Bickford, PhD (P)

*James A Haley Veterans Hospital, Tampa Fl;
Department of Neurosurgery and Brain Repair, USF
Morsani College of Medicine, Tampa, FL*

Nutritional approaches to improve age and age related neurological disorders has been a topic of increasing interest for the past 12 years. Our research group and others has investigated many individual botanical products including blueberry for their potential to improve neurological function in the aged. Recently we have focused on a proprietary blend of natural products that was initially designed to increase stem cell proliferation (NT-020). The formula was developed by screening over 100 natural products for individual activity to stimulate human bone marrow cell proliferation in culture and then the most active individual compounds were combined to examine for synergies. The final formulation demonstrated synergy in vitro and includes blueberry extract (enriched in anthocyanins), green tea (primarily as EGCG), carnosine, and vitamin D3. Several studies were performed to demonstrate the activity of the formulation to increase stem cell function and homing of stem cells to damaged tissues in animal models. We have also demonstrated that NT-020 increases the numbers of stem cells in the neurogenic niche in both normal aging and during ischemic injury. In the case of ischemic injury, NT-020 also increases the recruitment of neural stem cells to the injured tissue and reduces brain damage by 70%. Furthermore, during aging there is a change in the neurogenic niche that may in part play a role in reduced stem cell proliferation with age. We now show that NT-020 can influence this neurogenic niche, in addition to direct effects on stem cell proliferation. Young and aged F344 rats were treated with NT-020 for 30 days (0.5% NT-020 in the diet) and then the serum of these animals was used in cell culture of rat mesenchymal stem cells (MSC's) and rat hippocampal neural stem cells (NSC's). We have demonstrated that the aged serum decreases stem cell proliferation while serum from aged rats treated with NT-020 is similar to young in that there is little

impact on NSC proliferation in vitro. We now show that this effect is also observed in MSC's. Furthermore recent studies have demonstrated that one mechanism involved in the effect of aged serum to reduce MSC proliferation is via WNT/ β -catenin activation. We report here that aged rat serum also increases β -catenin in NSC's and that NT-020 treatment of aged rats reduces β -catenin when compared to that observed in NSC's treated with aged rat serum.

53. Supplements and Alzheimer's

Greg M. Cole (P)

*Dept Neurology, UCLA and GRECC, GLA Veterans
Affairs, Los Angeles*

Alzheimer's disease (AD) has a decades long prodromal buildup of amyloid and tau pathology prior to robust synaptic loss and cognitive decline. Many interventions in animal models of beta-amyloidosis including novel pharmaceuticals and dietary supplements can slow the development of pathology but have failed to improve or slow cognitive decline in diagnosed AD patients suggesting interventions may need to be stage-dependent. Potent natural flavonoids and polyphenols including EGCG, fisetin, resveratrol and curcumin as well as various Asian traditional herbal remedies have great potential to target protein aggregate accumulation, inflammation, oxidative damage and dysregulated signal transduction underlying failed synaptic plasticity and cognitive deficits. Many supplements have the dual advantages of relatively low toxicity and cost that make them suitable for prevention, but in large trials supplements including vitamin E, Gingko and omega-3 fatty acids have had only modest or equivocal benefits in MCI or AD patients. Nevertheless, epidemiology data suggest long-term use of weak anti- β supplement approaches might successfully delay AD onset. For example, the omega-3 DHA continues to show promise, but an apparent lack of efficacy with ApoE4 carriers raises the issue of disease heterogeneity and pharmacogenetic interactions with serious implications for clinical trial plans. Because tau pathology is clearly implicated in disease progression after the clinical onset, our group has developed evidence for the pleiotropic polyphenol curcumin in a post tangle model with cognitive deficits. Working on development of pleiotropic supplement cocktails combining DHA, lipoate and curcumin with enhanced bioavailability, we find both positive and negative interactions with late intervention suggesting the need for careful testing of combinations rather than simply combining lists of

apparently promising individual candidate supplements, a frequent practice of supplement makers. Supplement cocktails have already shown some modest evidence for limited treatment efficacy, but the full potential of supplements remains to be seen.

54. Timing of Antioxidant Intervention Affects Functional Outcome in Aged Mice

Nathalie Sumien, PhD (P) and Michael J. Forster, PhD

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

Antioxidants as an anti-aging intervention strategy have yielded variable outcomes regarding their effectiveness in both human and animal studies. The timing of antioxidant intervention during life is an important factor that may influence the functional outcome and was evaluated in the current study. Mice were supplemented with antioxidants (ascorbate, 1.65 mg/g diet; d- α -tocopheryl acetate, 0.825 mg/g diet, and coenzyme Q₁₀, 0.825 mg/g diet) beginning early or late in life and evaluated for the effectiveness of the regimens on age-related functional declines. We hypothesized that (i) single and/or combinations of antioxidants would improve psychomotor and cognitive 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 cognitive and psychomotor deficits. For either 5 weeks or 12 months, male C57BL/6J mice received a base diet (NIH-31) or one of seven antioxidant-supplemented diets, prior to functional testing beginning at 22 months of age. Long-term antioxidant supplementation failed to prevent age-related impairment of psychomotor and cognitive 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 antioxidant regimens. These effects occurred in the absence of any improvement in performance on a wire suspension test (a measure of strength). Furthermore, short-term antioxidant supplementation improved performance on a Morris water maze test (a measure of spatial learning and memory) and on an active avoidance task (a measure of cognitive flexibility). 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.

55. TBA

TBA (P)

56. Cell Cycling Suppression in a Set of Lens Epithelium Cells

Norm Wolf, DVM, PhD (P)

University of Washington, Seattle, WA

A strange phenomenon exists in the single layer of cells that cover the lens surface. At the very front of the lens in the region known as the Central Zone (CZ) the cells do not divide, apparently throughout adult life in all mammals so far investigated. Immediately next to and lateral to the CZ the single cell layer called the Germinal Zone (GZ) undergoes continual cell division, the descendants of these cells migrating to the equator of the lens where they internalize, lose their nuclei and become the lens fibers. While the CZ has been suggested as a stem cell source we have found no evidence for (or against) this and CZ cells when plated die within 24 hours (unlike GZ cells that grow and flourish there). We have not found gene expressions characteristic of stem cells, nor have we evidence for migration from the CZ to the GZ. By RTPCR, however, we have seen that the cell cycle suppressor, KLF4, is 16X as highly transcribed in the CZ as in the GZ. At present we have been able to induce an antibody to KLF4 to enter the nuclei of CZ cells and cause their survival when plated in vitro, but have not produced their cell division. We will present evidence for the unusual status of these immediately juxtaposed cell groups, indistinguishable by histology, one of which is in continual replication. The other, while negative for senescence (beta galactosidase), dies quickly when plated in vitro and can only be induced to temporarily enter replication in vivo to repair physical wounding.

57. Caloric Restriction, Sirt3, and Age-Related Hearing Loss in Mice

Shinichi Someya, PhD (P)

Departments of Aging and Geriatrics Research, University of Florida, 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. Yet, currently there is no treatment for this age-related disorder. It is well established that caloric restriction (CR) without malnutrition consistently extends both the mean and maximum lifespan of a variety of

species. CR also delays the progression of age-associated diseases such as Alzheimer's disease, Parkinson's disease, cataract, and AHL in rodents. Yet, whether the anti-aging action of CR in mammals is a regulated process and requires specific regulatory proteins such as sirtuins still remains unclear. Sirtuins are NAD⁺-dependent protein deacetylases that regulate lifespan in lower organisms, and have emerged as broad regulators of cellular fate and mammalian physiology. A previous report has shown that lifespan extension by CR in yeast requires Sir2, a member of the sirtuin family, linking sirtuins and CR-mediated retardation of aging. Recently, we have shown that mitochondrial Sirt3 plays an essential role in the CR-mediated prevention of age-related inner ear cell death and hearing loss in mice. In this presentation, I first review the causes, risk factors, and pathogenesis of AHL in animals and humans. I then discuss how CR may slow the progression of AHL in mice, focusing on the role of mitochondrial Sirt3 in the inner ear. Understanding the mechanism of action of CR opens a new field of communication disorder research, and provides the opportunity to define novel therapeutic approaches for human communication impairments.

58. Molecular Dissection of Sensory Modulation of Lifespan in *Drosophila*

Scott Pletcher, PhD (P)

University of Michigan, Ann Arbor, MI

An appreciation of the relationship between sensory input and aging has been spearheaded by research in simple model systems. Apfeld and Kenyon used the nematode, *Caenorhabditis elegans*, to show that suppression of sensory input could extend lifespan. Subsequent work from our lab and others has extended these results to the fruit fly, *Drosophila melanogaster*, and to yeast and revealed an increasingly nuanced relationship between sensory perception and aging. For example, some sensory neurons enhance longevity while others suppress it. Even the well-known relationship between body temperature and lifespan may have a sensory component. It is easy to visualize and measure the output of sensory experiences in terms of rapid behavioral changes, but what has only been made clear recently, but which becomes obvious with just a bit of introspection, is that sensory perception dramatically impacts many aspects of biology. It can initiate physiological changes that influence health and vitality long after the activating stimulus has passed. In this talk, I will survey our results examining the influence of sensory perception on lifespan and measures of health in *Drosophila*. Exposure of *Drosophila* to food-based odorants is

sufficient to reduce lifespan and limit the beneficial effects of dietary restriction; mutations that largely abolish olfactory function result in significantly increased lifespan; a small group of roughly 30 olfactory sensory neurons, which are sensitive to a single ligand, modulate *Drosophila* lifespan and physiology; and taste inputs influence measures of healthy aging, including sleep and daily activity patterns. I will also touch on candidate neuromodulatory mechanisms that may be involved in transducing sensory information to initiate changes in peripheral tissues.

59. Autophagy Induction with Life-Long and Late-Onset Interventions

Dutta D¹ (P), Xu J¹, Notterpek L² and Leeuwenburgh C¹.

¹Department of Aging and Geriatric Research, Division of Biology of Aging and ²Department of Neuroscience, McKnight Brain Institute, University of Florida, Florida

Life-long calorie restriction (CR) has been shown to be highly effective in improving overall organ function and reducing the pathophysiological signs of aging in several organs such as the heart, nerves and muscle. In striking contrast, late-age-onset CR interventions have not been extensively studied. Furthermore, the molecular mechanisms of CR-induced cytoprotective effects remain elusive, with recent evidences suggesting the critical involvement of a cellular digestion process called *autophagy* in mediating its beneficial effects. In addition, the drastic food reduction associated with the traditionally used 40% CR may not be feasible for translation to human studies. We therefore investigated whether a late-age-onset, short term CR intervention of a lower dose (CR 20%) alone, or in combination with the plant polyphenol resveratrol can induce autophagy in the hearts of 26-month old F344xBN rats. We also investigated whether such interventions are protective against oxidative stress induced by doxorubicin, a known oxidant generator. We used 26 month old male F344xBN rats, which were randomly divided into following groups: Control (CON), CR (CR) or CR plus 50mg/kg/day RESV (CR+RESV), fed daily for 6 weeks. Animals were then administered a single IP injection of 10mg/kg doxorubicin or saline control, 24h before sacrifice. Our findings suggest that 20% CR by itself does not induce autophagy, but when combined with resveratrol (CR+RESV), stimulated autophagy in the hearts of late middle-aged rats. Analysis of mitochondrial oxygen consumption in cardiomyocytes revealed increased State III (ADP stimulated) respiration in CR + RESV rats, in

comparison to CON. At the systemic level, serum LDH levels were significantly elevated by doxorubicin administration and only CR + RESV was able to attenuate such an increase. Collectively, a late-life intervention (combinatorial approach of low dose CR and RESV) enhanced basal autophagy in the aged-rodent heart and offers protection against oxidative stress induced toxicity.

60. Steroid Receptors and the Control of Thymic Involution

Chris Cooper¹, Kathy O'Neill², Jerrod L. Bryson¹, Nancy R. Manley¹ (P), and Clare Blackburn² (P).

¹Dept of Genetics, University of Georgia, Athens, GA 30602, USA; ²Center for Stem Cell Research, University of Edinburgh, Edinburgh, UK

Immunosenescence, the decrease in immune system function with aging, is a major contributor to the general decrease in the quality of human health associated with aging. As the body ages, the thymus undergoes involution, causing a drop in naïve T cell availability in the peripheral T cell pool. The central importance of thymic involution in the development of immunosenescence is well established; however, the cellular and molecular mechanisms by which this process occurs are less clear. Androgen signaling has been associated with involution based on increases in thymus size and thymocyte numbers in both castrated mice and those lacking functional androgen receptor (AR). However, to date there is no known cellular target or mechanism by which androgen signaling affects involution. Since androgens act by affecting gene expression within a receiving cell via the androgen receptor (AR), identifying which cell(s) receive the androgen signal is critical to understanding the mechanism by which androgens affect the thymus. Thymic epithelial cells (TECs), express AR and have been shown to increase proliferation and production of specific effectors such as CCL25 in response to castration. In the current collaborative work, we have tested the degree to which age-associated involution is due to the effects of androgens, and the hypothesis that the effects of androgens on the thymus are mediated by direct effects on TECs. Using both gain- and loss-of-function genetic approaches, our data identify TECs as a primary target for androgens in the thymus, and show that the ability to respond to androgens affects maximum thymus size, but does not influence the timing or progression of initial thymic involution. We will present data showing that current models of the role of androgens and sex steroid ablation (SSA) in thymic involution are insufficient to explain the

effects of androgens on thymus size, involution, and rebound.

61. S6 Kinase, Aging and Age-Related Disease
Brian Kennedy, PhD¹ (P) and Dominic Withers² (P)
¹Washington University; ²Imperial College London

62. Sources, Transmission and Effects of Transcriptional Noise in *C. elegans* Aging
Dhaval S. Patel¹(P), Mei Zhan²(P), Eugeni V. Entchev¹, Hyewon Lee², Chang Liu¹, Hang Lu² and QueeLim Ch'ng¹

¹MRC Centre for Developmental Neurobiology, King's College London, UK

²School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, Atlanta GA, USA

Studies in various model systems have revealed that ageing is a process modulated by the interplay between genetic, environmental and stochastic factors. How much each of these factors contributes to the ageing of an individual is still not fully resolved. Our ultimate goal is to dissect and quantitate the part played by each of these factors in the ageing of an individual and build models that can predict the potential longevity of that individual. Our collaborative project studies ageing in *C. elegans*, which has the advantage of being able to grow large isogenic populations of animals in homogeneous environments. Our central aim is to determine how much transcriptional noise in environmentally responsive genes accounts for the heterogeneity of longevity seen in isogenic animals. We have chosen to analyse noise in several genes that are known to respond to changes in two specific environmental cues: food abundance and temperature, both of which are known to affect longevity not only in *C. elegans* but also in other organisms. Crucially, these genes are also members of neuroendocrine signalling pathways that regulate longevity. We have begun to measure the expression levels of these genes under changing environmental and genetic backgrounds in order to determine under which conditions these factors act as sources of transcriptional noise and whether the measured noise correlates with heterogeneity in lifespan. To generate the large-scale data sets needed for analysis we have developed a high-throughput microfluidic device coupled to an automated microscopy and image analysis system.

63. Mechanisms of Reduced T Cell Immunity in Older Adults

Janko Nikolich-Zugich, MD, PhD¹ (P) and Arne Akbar, PhD²

¹Arizona Center on Aging and Department of Immunobiology, University of Arizona College of

Medicine, Tucson, AZ 85718, USA; ²Division of Infection and Immunity, University College London, London, W1T 4JF, England, United Kingdom.

Skin immunity declines with aging in older adults and understanding dysregulation of the immune responses against infection and cancer in this group is becoming paramount to improving their immune defense. In the past project period, we studied the relationship between varicella zoster virus (VZV) specific memory CD4⁺ T cells and CD4⁺Foxp3⁺ regulatory T cells (Tregs) that accumulate after intradermal challenge with a VZV skin test antigen. CD4⁺Foxp3⁻ and CD4⁺Foxp3⁺ T cells increase in parallel in the skin after antigen injection. VZV-specific CD4⁺ T cells were identified with a MHC class II tetramer or by intracellular staining for either IFN- γ or IL-2 after antigen re-challenge *in vitro*. Using both methods, significantly greater fraction of VZV-specific T cells, mainly of a central memory (CD45RA⁺CD27⁺) phenotype, were found at the site of skin challenge compared to the blood of the same individuals. Significant numbers of these cells were Tregs of the CD25^{hi}CD127^{lo}CD39^{hi} phenotype, and these cells did not secrete IFN- γ after antigenic re-stimulation. In addition, increased numbers of these Tregs in the skin were associated with a significantly decreased cutaneous reaction, suggesting that these cells had inhibitory activity *in vivo*. A key observation was that Foxp3⁺ T cells that were CD25^{hi}CD127^{lo}CD39^{hi} were also found within the VZV tetramer population and that these cells were highly proliferative. These results support the concept that CD4⁺Foxp3⁺ regulatory Tregs may be derived from an antigen-specific memory population at the site of an immune response in humans *in vivo*. Investigations of Tregs in the skin of mice infected with model poxviruses revealed also increased representation, but not absolute numbers, of Tregs. Their migration into the site of antigenic challenge was also present. New tetrameric reagents should allow us to interrogate the specificity of these cells in the skin of experimental mice. Differences and similarities between the mouse and the human model will be discussed.

64. The WNT-Chromatin Axis in Aging

Peter D. Adams, MD¹ and John M. Sedivy, PhD²

¹CRUK Beatson Laboratories, University of Glasgow, Glasgow G61 1BD, UK; ²Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence, Rhode Island 02912, USA

The goal of this collaborative study is to understand the contribution of a Wnt-chromatin-cell senescence signaling axis to tissue and organismal ageing.

Previous studies have shown that repression of Wnt signaling in senescence initiates a pathway that culminates in nuclear heterochromatinization, including upregulation and/or relocalization of HP1 and macroH2A proteins. To enhance our understanding of chromatin changes in senescence, we screened a library of over 80 antibodies to distinct histone modifications for those modifications that are altered in cell senescence. By this approach, we identified at several histone modifications that are upregulated in senescent cells *in vitro*. The distribution of these modifications through the genome of senescent cells has been defined by ChIP-seq and their function and contribution to the senescent state is being analyzed. In parallel studies, we have used the method of formaldehyde-assisted identification of regulatory elements (FAIRE) and DNaseI sensitivity assays to analyze the "openness" of chromatin, and ChIP-seq based analysis of mH2A distribution to assess heterochromatinization. These studies have been extended to the single-cell level using immunofluorescence approaches, and to the analysis of aging tissues to investigate the presence of senescent cells as well as processes that reflect chronological aging. Future studies will test the role of repressed Wnt signaling in the initiation of chromatin changes accompanying senescence *in vitro* and aging *in vivo*.

65. Mechanisms of Exceptional Longevity in the World's Longest Lived Animal

Iain Ridgway¹, Steve Austad², Chris Richardson¹, Zoltan Ungvari³, Anna Csiszar³, Danuta Sosnowska³, Eva Philipp⁴

¹Bangor University; ²University of Texas Health Science Center at San Antonio, Barsop Institute for Longevity and Ageing Studies; ³Reynold's Center on Aging; ⁴Kiel University

Bivalve species with exceptional longevity are newly introduced model systems in biogerontology to test evolutionarily conserved mechanisms of aging. Here we tested predictions based on the oxidative stress hypothesis of aging using one of the tropical long lived sessile giant clam species, the smooth giant clam (*Tridacna derasa*; predicted maximum lifespan: > 100 years) and the shorter lived Atlantic bay scallop (*Argopecten irradians irradians*; maximum lifespan: 2 years). In *T. derasa* production of H₂O₂ and O₂⁻ in the gills was lower than in *A. i. irradians*, but we did not find the same pattern in the heart and adductor muscle. Protein carbonyl content in gill and muscle tissues were similar in *T. derasa* and *A. i. irradians*. We observed a positive association between longevity and resistance to mortality induced by exposure to tert-butyl hydroperoxide. In

tissues of *T. derasa* neither basal antioxidant capacities nor superoxide dismutase and catalase activities were consistently greater than in *A. i. irradians*. In conclusion, the aforementioned data are not consistent with the predictions based on the oxidative stress hypothesis of aging. The findings that in tissues of *T. derasa* proteasome activities are significantly increased as compared to those in tissues of *A. i. irradians* warrant further studies to test the role of enhanced protein recycling activities in longevity of bivalves.

66. Obesity in America: Implications for Aging and Longevity

Eric N. Reither, Ph.D (P)

Utah State University, Logan UT

According to David Barker and other scholars, the process of human aging begins early in life – even in utero. To understand the implications of the U.S. obesity epidemic for aging and longevity, it is essential to adopt such an expansive life course perspective. Previously in our nation's history, obesity was relatively rare and typically acquired in midlife. Both the low prevalence of obesity and the relatively short segment of the life course that it affected biological processes limited obesity's impact on population health. Today, the situation is much different. Obesity has become a common chronic condition that is increasingly prevalent among young children and adolescents. Research has shown that obese youth are susceptible to hypertension, lipid disorders, insulin resistance, type-2 diabetes and even observable damage to the heart and blood vessels. A large segment of the U.S. population will carry these health risks over a protracted period of time – several decades in many cases – and this threatens to reduce the quantity and the quality of life for younger generations of Americans. For instance, new demographic models that account for the deteriorating health of younger Americans indicate that rates of obesity-related conditions such as heart disease will increase in the near future. These models are supported by recent evidence that life expectancy has, for the first time in more than a century, stagnated and even begun to decline in certain areas of the U.S. For complex reasons including possible epigenetic mechanisms, reversing the present health trajectory will be difficult, but it is still possible through aggressive public health intervention.

67. Anorexic Behaviors with Age: Influence of Leptin and Exercise

Philip J Scarpace, PhD (P)

Dept. of Pharmacology, University of Florida, Gainesville, FL

Leptin resistance predisposes animals to exacerbated high-fat (HF) induced obesity. With age, the F344 x BN rat displays a steady increase in body weight and adiposity from 3 to 24 months followed by a decline thru 30 months. Despite hyperleptinemia with age, obesity persists, and these aged-obese rats exhibit little anorectic or weight loss responses to leptin. There are reduced hypothalamic leptin receptors and decreased maximal leptin signaling with age. When challenged with a HF diet, aged animals demonstrate an extended period of hyperphagia resulting in exacerbated obesity and fat gain compared with young rats. This observation suggests that leptin resistance reinforces “reward eating” beyond the caloric energy requirements. A minor amount of voluntary wheel running (WR) in aged rats effectively curtails HF consumption and deters dietary weight gain, whereas higher amounts of WR in obese young rats are without beneficial effect. This is associated with increased leptin signaling in the ventral tegmental area (VTA). Conceivably, WR in aged rats substitutes for the positive-reinforcing effects of palatable food, potentially through enhancing leptin action in the VTA. In addition, the simultaneous introduction of a HF diet and WR induces an anorexic behavior in which the animals chose not to eat although food was available at all times. This HF/WR-induced anorexia is preserved across the age span despite the intrinsic decrease in WR activity with age. This phenomenon provides a new model to investigate anorexia behavior in rodents. (Supported by NIH grant AG26159).

68. Nutritional Modulation of Aging: Nutrients and Bioactive Compounds

Mohsen Meydani, D.V.M., Ph.D., Director (P)

Vascular Biology Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA

Aging is associated with the decline of many physiological functions and increased prevalence of chronic diseases. Time dependent, functional deterioration observed in many organisms during aging and age-related diseases may be causally related to several factors including the deleterious action of free radicals, dysregulation of signaling molecules, and changes in epigenetic patterns, which are affected by environmental factors such as

nutrition. There is compelling evidence that nutrition modulates the aging process and age-related diseases. Nutrient sensing complex signaling pathways, composed of mTOR (mammalian target of rapamycin), NAD(+) dependent deacetylases (sirtuins) and AMPK (AMP activated kinase) have been discovered to be metabolic sensors to govern senescence and age-related degenerative diseases. Several bioactive components of food such as resveratrol, curcumin, quercetin and catechins have been shown to modulate activation of these molecular complexes not only through their antioxidant properties but also by altering signaling pathways involved in aging and age-associated disease. In addition, nutrients and bioactive compounds in food can modulate gene expression during aging through epigenetic phenomena by DNA methylation and histone modification. In these processes, nutrients and bioactive compounds of foods modulate methylation of DNA or histone in an age-dependent manner by altering methyltransferases or providing co-factors such as B-vitamins including folate, B12, B6 and methyl donors methionine, choline, betaine. NHANES surveys have also shown that the intake of these and other micro- and macro-nutrients and total caloric intake is inadequate among the elderly. Several factors may be responsible such as: inadequate food intake, changes in normal physiology like dentures, ability to digest and absorb nutrients, increased prevalence of chronic disease as well as socio economic, cultural and polypharmacy factors. Sedentary life styles and reduced basal metabolic rate also contribute to overweight and obesity and visceral accumulation of fat in older adults, which is associated with chronic inflammation, and are well-established causes of several diseases including type II diabetes, atherosclerosis, hypertension thrombosis and other age-associated diseases. To prevent these pathophysiological conditions and add life to the years, adopting a balanced healthy diet (My Plate For Older Adults: <http://now.tufts.edu/articles/eat-well-age-well>) comprised of generous amount of fruits, vegetables and whole grains is the current general recommendation to the elderly along with regular physical activity and several glasses of water every day. Supported by USDA Agriculture Research Service contract #58-1950-7-707.

69. Nutritional Risk Factors and Interventions for Brain Aging and Alzheimer's Disease

Martha Clare Morris, ScD (P)

Rush University Medical Center, Chicago, IL

A growing number of laboratory, animal, and epidemiological studies suggest that antioxidant nutrients, fat composition and B-vitamins are important for the aging brain and the prevention of Alzheimer's disease. With a few exceptions, the randomized clinical trials of dietary components have not resulted in the prevention of cognitive decline. Most of these randomized trials ignore a basic physiological principal of nutrient intake: there is a broad intermediate range of nutrient level that results in optimum physiological function and nutrient levels below or above this intermediate range produce less than optimum or deleterious effects on function. The majority of the randomized trials have allowed multivitamin intake among treated and control groups and otherwise include participants with optimum nutrient intakes. Thus, null results of the trials may be due to the fact that participants are already at optimum levels. This presentation will summarize the biological mechanisms, epidemiological evidence, and clinical trials on the effects of antioxidant nutrients (vitamin E, vitamin C, carotenoids, and flavonoids), dietary fats (saturated, polyunsaturated, monounsaturated, and trans), and B-vitamins (folate, vitamin B12) on the brain and on the development of Alzheimer's disease. The totality and breadth of the evidence to date is strongest for vitamin E, fish and the n-3 fatty acids, fat composition, vitamin B-12, and folate. Clinical trials that have examined the effects of vitamin supplements of vitamin E and folate on cognitive decline demonstrate protective benefits among persons with insufficient levels of these nutrients.

70. TOR as a Regulator of Brain Aging: Studies of Vascular Function in a Mouse Model of Alzheimer's Disease

Veronica Galvan, PhD (P)

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

Cerebral microbleeds, small amounts of blood leaked from blood vessels in the brain, are present in patients with Alzheimer's disease (AD), and are indicative of an underlying vascular pathology. The relevance of microbleeds in AD and other dementias is becoming increasingly apparent with the availability of improved imaging technologies.

We recently showed that chronic feeding of mice with the TOR inhibitor rapamycin, a drug that extends lifespan and delays aging in mice, halts the progression of AD-like memory deficits and reduces Ab accumulation in transgenic human (h)APP mice modeling the disease. To investigate the effects of rapamycin treatment on hemodynamic, vascular and

metabolic functions in brains of hAPP mice we used multi-metric imaging systems (MRI and PET).

Our results demonstrate that hAPP mice have significant vascular dysfunction (reduced cerebral blood flow (CBF) and vascular density) that is not related to metabolic changes, especially in areas that have a prominent role in learning and memory such as hippocampus. Chronic rapamycin treatment restored vascular integrity and function and relieved AD-like cognitive deficits in hAPP mice. *In vivo* 2-photon imaging of brain vessels revealed that rapamycin induced an increase in nitric oxide (NO) in vascular endothelium that was followed by vasodilation. Both rapamycin- and acetylcholine-induced NO release and vasodilation could be blocked by an inhibitor of endothelial NO synthase (L-NAME). Administration of L-NAME reversed the protective effects of rapamycin on brain blood flow and vasculature integrity, indicating that rapamycin preserves vascular integrity and CBF in AD mouse brains through NO signaling. The preservation of vascular integrity and brain blood flow may be key to the maintenance of cognitive function in the hAPP mouse model of AD. Our results suggest that rapamycin, an FDA-approved drug that is already used in the clinic, may have tangible promise as a therapy for AD and possibly for vascular dementias.

71. A Shared Molecular Signature for Slow Aging in Mice

Richard A. Miller (P) and Michael Steinbaugh
University of Michigan, Ann Arbor, MI

Xenobiotic metabolism has been proposed to play a role in modulating the rate of aging. Xenobiotic-metabolizing enzymes (XME) are expressed at higher levels in calorically restricted mice (CR) and in GH/IGF-1-deficient long-lived mutant mice. In this study, we show that many phase I XME genes are similarly upregulated in additional long-lived mouse models, including “crowded litter” (CL) mice, whose lifespan has been increased by food restriction limited to the first 3 weeks of life, and in mice treated with rapamycin. Induction in the CL mice lasts at least through 22 months of age, but induction by rapamycin is transient for many of the mRNAs. Cytochrome p450s, flavin monooxygenases, hydroxyacid oxidase, and metallothioneins were found to be significantly elevated in similar proportions in each of the models of delayed aging tested, whether these are based on mutation, diet, drug treatment, or transient early intervention. The same pattern of mRNA elevation can be induced by 2 weeks of treatment with tert-butylhydroquinone, an

oxidative toxin known to activate Nrf2-dependent target genes. These results suggest that elevation of phase I XMEs is a hallmark of long-lived mice and may facilitate screens for agents worth testing in intervention-based lifespan studies.

Key collaborators: Liou Sun, Andrzej Bartke, Ulas Ozkurede
Support: National Institute on Aging, Ellison Medical Foundation

72. Acute Pharmacological Intervention Reverses Mitochondrial Deficits and Improves Function in Aged Skeletal Muscle

David Marcinek¹ (P), Michael Siegel¹, Shane Kruse¹, Justin Percival¹, Peter Rabinovitch¹, Hazel Szeto²
¹University of Washington, Seattle, WA; ²Weill Cornell Medical College

Impaired mitochondrial energy metabolism plays a key pathogenic role in age-related degenerative disorders. Here we demonstrate a rapid reversal of mitochondrial dysfunction in aged skeletal muscle by acute one hour treatment with the mitochondrially-targeted peptide SS-31. SS-31 is a synthetic tetrapeptide that accumulates in the inner mitochondrial membrane and interacts with cardiolipin and cytochrome c to facilitate electron flux through the electron transport chain. Young (5 month old) and old (27 month old) mice were injected intraperitoneally with either saline or 3 mg/kg of SS-31. Skeletal muscle mitochondrial energetics were measured *in vivo* one hour after injection using a unique combination of optical and ³¹P NMR spectroscopy. Age related declines in resting and maximal mitochondrial ATP production, coupling of oxidative phosphorylation (P/O), and cellular energy state (PCr/ATP) were rapidly reversed one hour after SS-31 treatment. Remarkably, mitochondrial function in the treated old mice was indistinguishable from young mice. SS-31 had no observable impact on mitochondrial energetics in young skeletal muscle. The reversal of mitochondrial deficits in aged mice was accompanied by a substantial improvement in skeletal muscle fatigue resistance measured in the tibialis anterior *in situ*. During a fatigue protocol, SS-31-treated old skeletal muscle showed a significantly lower rate of force decline as well as increased final force output. SS-31 mediated improvements in aged muscle mitochondrial function and fatigue resistance translated to enhanced endurance capacity as measured by treadmill running following 7 days of treatment. In conclusion, we have identified a new paradigm for restoring mitochondrial function and

improving the exercise performance of aged skeletal muscle that is based on acute targeting of mitochondrial energy metabolism. The rapid improvements after acute treatment and high translational potential of SS-31 make this peptide a potentially attractive therapeutic intervention for improving skeletal muscle bioenergetics and exercise tolerance in the aged population.

73. Resveratrol: Therapy for Age-Related Insulin Resistance and Glucose Intolerance?

Jill P. Crandall, MD (P)

Albert Einstein College of Medicine, Bronx, NY

Resveratrol is a plant derived polyphenol mainly known for its antioxidant and phytoestrogenic properties. Interest in this compound has increased in recent years, first from its identification as a chemopreventive agent for cancer, and subsequently from reports that it activates sirtuins and extends the lifespan of lower organisms. Resveratrol has demonstrated promising effects on glucose metabolism in a variety of animal models. Resveratrol has also been shown to increase mitochondrial biogenesis and appears to mimic the beneficial effects of caloric restriction on glucose metabolism. Resveratrol may also have cardioprotective effects, due to its estrogenic and antioxidant properties, and has been reported to enhance the availability of the vasorelaxant nitric oxide.

However, despite the many health claims made on its behalf and its widespread use as a nutritional supplement, formal studies of resveratrol in humans are very limited. As an initial step, we conducted a pilot study of resveratrol treatment to assess its potential to improve glucose tolerance, insulin sensitivity and vascular function. We studied the effects of resveratrol in subjects with impaired glucose tolerance (IGT), who have definite, but not yet severe metabolic dysregulation, which may be most amenable to intervention. These studies have relevance to aging since IGT affects up to 30% of older adults, and constitutes a major risk factor for the development of both diabetes and CVD. Further, although lifestyle modification was exceptionally effective in preventing progression from IGT to diabetes in older participants in the Diabetes Prevention Program, metformin was not, highlighting the need for alternate pharmacologic approaches for older adults with IGT. Results of our preliminary studies provide the first evidence in humans that resveratrol may possess clinically relevant effects on glucose metabolism and vascular function. Future studies should include formal randomized, placebo-

controlled trials and efforts to explore the potential mechanisms for resveratrol's cardiometabolic effects.

74. Studying Aging and Kidney Decline Using Human, Mouse, Zebrafish, and Worm

Ron Korstanje^{1,2} (P)

¹The Jackson Laboratory, Bar Harbor, USA; ²Mount Desert Island Biological Laboratory, Bar Harbor, USA

Renal aging is associated with a decline in renal function. This impairment results from morphological changes that affect glomeruli, tubuli and interstitium. Declining kidney function may lead to chronic kidney disease, a major health problem, especially regarding the growing geriatric population. Multiple pathways seem to play a role in age-related kidney damage. And in experimental animal models, it is becoming increasingly recognized that genetic background influences the scale and progression of renal impairment and structural features of renal aging.

We investigated changes in aged kidneys of 26 mouse inbred strains. After quantifying for differences in albuminuria, mesangial matrix expansion (MME), fibrosis, lipid deposits, and perivascular infiltration, we performed genetic analyses and identified a number of associated genes with each phenotype. Further characterization identified possible causal variants and the mechanisms with which these variants lead to the disease phenotypes

In addition to our studies in the mouse, we use two other animal models. First, we knock down the genes in zebrafish to rapidly test the candidate genes that we find in the mouse and to characterize their roles in kidney development and dysfunction. Second, we knock down the genes in *C.elegans* to determine their effects on longevity and to identify the longevity pathways. Surprisingly, knockdown of several genes leads to kidney dysfunction in the zebrafish while increasing longevity in *C.elegans*.

Integrating mouse, zebrafish, and worm studies accelerates our pursuit of renal aging genes. The genetic tools of the three species complement each other, and information revealed in one species feeds experimental design in the others.

75. Behavior as a Tool for Assessing the Efficacy of Late-Life Interventions for Combating Aging

Christy S. Carter, PhD (P)

University of Florida, Gainesville, FL

The need for inexpensive, non-invasive predictors is essential for understanding responsiveness to late-life interventions and concomitant molecular, cellular, and physiological outcomes. Behavior is such a tool, may be applied in several ways, and has a rich scientific history in psychological disciplines, but has not been realized as an innovative approach in the biology of aging with regards to developing and modeling unique and innovative intervention strategies. We will discuss various approaches for how to apply behavior to the development of interventions for the aging process. We will provide examples of using sophisticated behavioral techniques to determine physiological differences in various domains of aging including brain, muscle and cardiovascular function. For example, producing alterations in exercise tolerance or imposing caloric restriction can change physical activity that is itself a “behavioral mechanism” by which one observes changes in physical, physiological, and cognitive function independent of the underlying physiology of these processes. In addition, one may use a multi-behavioral approach to determine if aging in various physiological systems occurs together or independently. These examples illustrate that one intervention may directly impact only one underlying process, but functionally that one intervention may impact all. Most powerfully, is the ability to use individual behavioral characteristics to predict sensitivity to treatment. Individual and strain differences across a wide variety of behavioral domains including physical activity, cognitive function, and physical function are well-documented in the preclinical literature, even in seemingly genetically identical (i.e. inbred) laboratory animals. Leveraging these behavioral differences in the context of identifying those interventions which may be most effective in improving functional/behavioral outcomes may speak to differences in underlying physiology and allow for the most appropriate approach to intervention.

76. Late-Life Calorie Restriction in Rhesus Monkeys

Donald K. Ingram, PhD¹ (P) George S. Roth, PhD²,
Rafael de Cabo, PhD³, Julie A. Mattison, PhD³

¹*Behavioral Neuroscience and Aging Laboratory, Pennington Biomedical Research Center, LSU System, Baton Rouge, LA 70808;* ²*GeroScience, Inc., Pylesville, MD 21132;* ³*Laboratory of Experimental*

Gerontology, Intramural Research Program, National Institute on Aging, National Institutes of Health, Baltimore, MD 21224 USA

Dietary caloric restriction (CR) is the only intervention repeatedly demonstrated to retard the onset and incidence of age-related diseases, maintain function, and extend both lifespan and healthspan in mammals. In 70 years of study, such beneficial effects have been demonstrated in rodents and lower animals, but prior to 1987, had never been examined in primates. To determine whether CR might eventually be applied to humans, the National Institute on Aging initiated a study of CR and aging in rhesus monkeys (*Macaca mulatta*). Control monkeys receive two meals per day sufficient to attain apparent satiety, while the CR group receives 30% less, adjusted for age and body weight. CR was initiated within three general age groups comprising male and female groups: Young: 1-3 years of age; Adult: 3-14 years; and Old: 15-23 years. Current analysis of survival indicates no significant effect of CR in the old group of monkeys, although some measures of health were improved.

77. Beneficial Catalytic Autoimmunity to Amyloids Amplified with Age

Sudhir Paul (P), Yasuhiro Nishiyama and Stephanie Planque

Chemical Immunology Research Center, Department of Pathology, University of Texas Houston Medical School, Houston, Texas

Over 27 misfolded proteins that form amyloid aggregates with β -sheet character are associated with pathogenic events during aging. Autoantibodies to self-antigens are generally pathogenic. However, age-associated amyloid accumulation has no physiological purpose. We present evidence for this hypothesis: Old humans mount a beneficial catalytic autoantibody response to amyloids driven by misfolded, electrophilic epitopes. We and others showed that hydrolysis of peptide bonds by a nucleophilic catalytic mechanism is an innate function of antibodies. Catalytic autoantibodies that hydrolyzed amyloid β peptide (A β) and transthyretin (TTR) were identified in the blood of healthy humans, panels of monoclonal antibodies and a library of antibody variable (V) domains. A β is the major constituent of amyloid plaques in the brain of patients with Alzheimer’s disease, and tissue deposition of fibrillar TTR underlies the pathogenesis of senile systemic amyloidosis. IgM class catalytic autoantibodies with A β and TTR hydrolyzing activities were increased in old humans compared to

young humans. The catalytic autoantibodies inhibited formation of fibrillar A β , destroyed preformed fibrillar A β , and intracerebral injection of an antibody in a transgenic mouse model cleared A β plaques. The autoantibodies cleaved A β at the Lys16-Leu17 and Lys-Gly bonds. Fibrillar TTR was cleaved into small mass fragments, suggesting multiple antibody-sensitive sites. The physiological tetrameric form of TTR was not cleaved, indicating specificity for an amyloid epitope. Synthetic electrophilic amyloid analogs reacted with secreted antibodies and B lymphocytes, suggesting that catalytic autoantibody synthesis may be driven by the age-associated accumulation of nelectrophilic amyloid—lipid peroxidation end product adducts. The electrophilic epitopes are topographically and chemically foreign to B cells, fulfilling the requirements for inducing autoantibodies capable of nucleophilic catalysis. The studies suggest catalytic autoimmunity as a physiological defense mechanism against amyloids. Supported by the NIH and SENS Foundation.

78. Towards a Therapeutic Intervention for Age-Related Macular Degeneration

Maximus Peto and Gouri Yogalingam (P)

LysoSENS Division, SENS Foundation, 110 Pioneer Way, Mountain View, CA, 94041

Post-mitotic retinal pigment epithelial (RPE) cells perform the critical maintenance function of daily phagocytosis and degradation of outer photoreceptor segments to preserve vision. However, the progressive lysosomal accumulation of lipofuscin or “age pigment” in aged RPE cells compromises the function of lysosomes, which reduces phagocytic activity and contributes to the pathogenesis of age-related macular degeneration (AMD). Lipofuscin appears to be resistant to lysosomal degradation despite the presence of over 40 lysosomal proteases, lipases and glycosidases. Our research is focused on identifying novel enzymes that can degrade N-retinylidene-N-retinylethanolamine (A2E), a major autofluorescent component of lipofuscin. Targeted enzymatic degradation of lysosomal A2E may restore lysosomal function, prevent RPE cell death and preserve vision. So far, our group has identified laccase and manganese peroxidase as two non-human enzymes that can effectively degrade A2E *in vitro*. To further study the therapeutic potential of these enzymes we have developed a cell-based model of AMD, where the lysosomes of RPE19 cells are loaded with A2E. Liposome-mediated transfection of laccase and manganese peroxidase into A2E-loaded RPE19 cells can effectively clear lysosomal A2E storage. Current work is focused on engineering lysosomal targeting signals into these enzymes to

permit their direct uptake into RPE cells and delivery to lysosomes. We are also pursuing alternative therapeutic approaches for treating AMD, including augmenting lysosomal exocytosis to eliminate A2E as well as screening human enzymes for possible A2E degrading activity.

79. MitoSENS: Allotopic Expression of Mitochondrial Genes Using a Co-Translational Import Strategy

Matthew O'Connor, PhD (P)

SENS Foundation, Mountain View CA

The mitochondrion contains its own genome and encodes 13 proteins that are essential for electron transport chain function and ATP synthesis to function properly. Congenital mutations in many of the mitochondrial genes are the cause of serious disease phenotypes including diabetes, blindness, dementia, ataxia, epilepsy, and many other neurological disorders. Somatic mutations also accumulate in the mitochondria with normal aging. Allotopic expression of mitochondrial genes in the cell's nucleus is one approach to rescuing mitochondrial mutations. In our strategy, we utilize 5' and 3' elements to target the mRNA to the mitochondria; this approach is a variation of allotopic expression and is hypothesized to result in the co-translational import of these proteins into the mitochondria. This approach is hypothesized to overcome the obstacles experienced by many groups in the import of allotopically expressed proteins into the mitochondria, such as clogging of the mitochondrial import machinery by hydrophobic proteins. Thus far, we have stably transfected 5 of the 13 mitochondrial genes into the nuclear genome of human cell lines and are characterizing the expression and function of these exogenously expressed genes. We will discuss current progress and future plans for replacing and/or making redundant the entire mitochondrial genome. We will also discuss potential applications of the MitoSENS approach in treating mitochondrial diseases, as well as the diseases and pathologies of aging.

80. Inhibition of Phosphodiesterase 2 Reverses Behavioral and Synaptic Deficits Caused by Chronic Stress Exposure

Ying Xu (P.G), Jiao Sun, Ling Chen, Jianxin Li, Chong Zhang, Hanting Zhang, James O'Donnell
West Virginia University, Morgantown, WV

Chronic stress and neuronal vulnerability are interrelated events contributing to cognitive disorders. Phosphodiesterase 2 (PDE2) exerts the action on cognitive processes via the control of intracellular cAMP and cGMP signaling. But how PDE2 affects stress-induced cognitive behaviors and its relevance to neuronal remodeling remain unknown. This study explored the effects of PDE2 inhibitor Bay 60-7550 on stress-induced learning and memory dysfunction by measures related behavioral, morphological and molecular changes. Chronic stress led to learning and memory deficits evidenced by increases in PDE2 message and decreases in related synaptic responses. Inhibition of PDE2 improved the performance of mice in water maze, object recognition and location memory tasks, which were prevented by treatment with CaMKII inhibitor myc-AIP, NMDA receptor (NMDAR) antagonist MK801, and the PKG or PKA inhibitors KT5823 or H89. Bay 60-7550 was also found to regulate stress-induced structural remodeling in CA1 of hippocampus such as increases in dendritic branching and dendritic spine density. However, the neuroplasticity initiated by Bay 60-7550 was not seen in the presence of Myc-AIP, MK801, KT5823 and H89. The participation of CaMKII and NMDAR was confirmed by the subsequent study, which suggested that pre-treatment with myr-AIP and MK801 blocked the effects of Bay 60-7550 on cAMP and cGMP levels. Furthermore, Bay 60-7550 reduced stress-induced ERK activation, reversed the decreased plasticity-related proteins and transcription factors induced by stress, such as c-Fos, Egr-1, BDNF, Elk-1 and CREB. These findings indicate that the effect of PDE2 inhibitor on stress-induced memory impairment possibly by modulating neuroplasticity related CaMKII-NMDAR-PKA/PKG signaling.

81. Rapamycin Administered Late in Life Extends C57BL/6 Health Span in an Age- and Sex-Specific Manner

Kathleen Fischer¹ (P) Vanessa Soto², Lauren Sloane², Alex Bokov³, Jon Gelfond⁴, Vivian Diaz², Samantha Rendon⁵, Keith Maslin⁶, Martin Javors⁷, Steven Treaster⁶, Arlan Richardson¹, Steven Austad⁸

¹*Barshop Institute and Dept. of Physiology UTHSCSA;* ²*Barshop Institute, UTHSCSA;* ³*Barshop Institute and Dept. Biostatistics & Epidemiology, UTHSCSA;* ⁴*Dept. Biostatistics & Epidemiology*

UTHSCSA; ⁵*Cellular and Structural Biology, UTHSCSA;* ⁶*Dept. of Molecular Medicine, UTHSCSA;* ⁷*Dept. of Psychiatry, UTHSCSA;* ⁸*Barshop Institute and Dept. of Cellular & Structural Biology, UTHSCSA*

Inhibition of the mTOR signaling pathway by rapamycin has been shown to extend life in genetically heterogeneous mice and C57BL/6 mice. To determine whether rapamycin treatment delays age-associated declines in health span, we performed a single-blinded study using C57BL/6 mice fed encapsulated rapamycin or control diet starting at 19 months of age. We tested these mice with a series of functional assays chosen for their similarity to measures of aging and frailty in humans. In some assays (e.g. rotarod, stride length) rapamycin treated animals performed significantly better than age-matched controls, but improvements in rapamycin-treated animals occurred at specific ages (and/or treatment durations). Similarly, some changes in performance were observed in both males and females (e.g. rotarod), whereas other in assays we observed sex-specific responses to rapamycin treatment (e.g. metabolism, activity). In all cases, rapamycin either improved or did not change performance in any of the assays we used: we found no adverse health consequences associated with rapamycin treatment.

82. Healthspan in Females --- How the Other Half Ages

Lauren Sloane¹ (P, G) Vanessa Soto², Keith Maslin², Kathleen Fischer², Arlan Richardson², Steven Austad²

¹*University of Texas Health Science Center San Antonio;* ²*Barshop Institute University of Texas Health Science Center San Antonio*

Evaluation of healthspan has become increasingly important with the growing population of elderly in the US. Healthspan, a comprehensive index of health during aging, has been examined in mouse populations in an attempt to identify robust and reproducible measures of age-related change. Our current and previous work has assessed male C57BL/6 mice. This present study examines female C57BL/6 mice at 4 different ages (4, 20, 28 and 32 months of age) on a wide array of proposed healthspan assays (metabolic rate, gait analysis, grip strength, body composition) to evaluate overall healthspan. This same array of assays was previously completed in a male cohort of mice of the same strain and at the same ages. Much of the aging literature has been focused on male mice. Therefore, our objective was to characterize female aging and healthspan,

particularly in the older ages, and ultimately compare these results to the male cohort. Preliminary analyses suggest females and males show similar, parallel trends in grip strength, body composition, balance, motor function, and gait analysis across the ages, though differences exist between the sexes. Through identification of age-related changes, tools can be developed to assist research scientists and clinicians to identify relevant patterns of aging, whether related to frailty or progression of disease states. Furthermore, measuring these variables in aging rodents can lead to development of models that explore the physiology and etiology of complex age-related phenomena and provide critical insights into the mechanisms associated with age-related decline.

83. Mitochondrial DNA Copy Number in Peripheral Blood is Associated with Femoral Neck Bone Mineral Density

Jung-Ha Kim¹ (P) and Duk-Chul Lee²

¹*Department of Family Medicine, Chung-Ang University Healthcare System;* ²*Department of Family Medicine, Severance Hospital, Yonsei University*

Objective. It has been suggested that mitochondrial dysfunction is related to aging and metabolic disorders. However, there are few studies of the relationship between bone mineral density (BMD) and mitochondrial content in humans. We investigated the relationship between BMD and mitochondrial DNA (mtDNA) copy number in peripheral blood of postmenopausal women. **Methods.** The present study included 146 postmenopausal women. Enrolled subjects were not taking any medications and did not have any disorders that altered bone metabolism. We measured bone mineral density using dual-energy X-ray absorptiometry and leukocyte mtDNA copy number using real-time polymerase chain reaction. In addition, anthropometric evaluations and biochemical tests were performed. **Results.** Patients with osteopenia or osteoporosis had lower mtDNA copy numbers than normal subjects ($P < 0.0001$). Femoral neck BMD was negatively correlated with age ($r = -0.01$, $p = 0.04$) and with serum levels of adiponectin ($r = -0.22$, $p = 0.01$) and osteocalcin ($r = -0.31$, $p = 0.0001$). Serum levels of 25-OH Vitamin D ($r = 0.32$, $p < 0.0001$) and mtDNA copy number ($r = 0.36$, $p < 0.0001$) were positively correlated with femoral neck BMD. Multiple regression analysis showed that mtDNA copy number ($\beta = 0.156$, $p < 0.001$) was an independent factor associated with femoral neck BMD after adjustment for age, BMI, waist circumference, WHR, blood pressure, HOMA-IR, hs-CRP, adiponectin, osteocalcin, homocysteine, lipid profiles, 25-OH

vitamin D, and regular exercise. However, mtDNA copy number was not related to lumbar BMD. **Conclusion.** Low mtDNA content in peripheral blood is related to decreased femoral neck BMD in postmenopausal women. Our findings suggest that mitochondrial dysfunction may be a potential pathophysiologic mechanism of osteoporosis in postmenopausal women.

84. Systems Biology of Human Aging - Network Model 2012

John Furber (P)

Legendary Pharmaceuticals

This network diagram is presented to aid in conceptualizing the many processes of aging, the causal chains of events, and the interactions among them. Consideration of this network indicates 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

The many observable signs of human senescence have been hypothesized by various researchers to result from several primary causes. Inspection of the biochemical and physiological pathways associated with age-related changes and with the hypothesized causes reveals several parallel cascades of events that involve several important interactions and feedback loops. This network model includes both intracellular and extracellular processes. It ranges in scale from the molecular to the whole-body level. Environmental effects and proposed interventions are highlighted around the margins of the network. Important pathways include:

- Nuclear mutations, telomere shortening, chromosome breaks, chromatin alterations, and epigenetic DNA adducts change gene expression.
- Extracellular proteins become damaged by glycation, oxidation, crosslinking, and lytic enzymes, resulting in mechanical stiffness, weakness, inflammation and altered environmental niches for cells.
- Lysosomes accumulate lipofuscin, which impairs autophagic turnover of macromolecules and organelles.
- Mitochondrial DNA mutates.
- Lamin-A splice-variant, progerin, accumulates in the nuclear scaffold, impairing cell division.
- Nuclear envelope pore proteins become oxidized, allowing inappropriate traffic of other proteins into and out of the nucleus.
- Oxidized aggregates in cytoplasm become crosslinked, resist turnover, inhibit proteasome activity, increase redox poise, and physically interfere with intracellular transport, especially in axons. Inhibited proteasomes reduce turnover of damaged molecules and of expired molecular signals.

- Inflammatory cascades, promoted by damaged molecules and sick cells, further damage tissues.
- Neuroendocrine and immune systems degrade.
- ER stress: Misfolded proteins accumulate in ER.

85. Caloric Restriction-Like Regulation of the Neuroendocrine Axis by SirT1 in Pituitary-Specific SirT1 Knock Out Mice (PITSKO)

Bonkowski, M.S. (P. G), Bell, E., Davidson, S., Reyes, J., Hunter, T.A., Liberts, S. and Guarente, L.
Department of Biology, Massachusetts Institute of Technology, Cambridge, MA., U.S.A., 02139.

There is a great deal of evidence which suggests a strong role for SirT1 in mediating the effects of calorie restriction (CR). Modulation of the neuroendocrine axis is a common trait in most long-lived mouse mutants and mice on calorie restriction. Here, we provide evidence that SirT1 regulates the neuroendocrine axis in mice, specifically in the pituitary.

Normal mice on CR have suppressed growth and reproductive hormonal axes, compared to *ad libitum* (AL) controls. CR mice also have reduced body size, pituitary and testis weights, sperm counts, and serum IGF-1, LH, and FSH. Surprisingly, CR mice have reduced pituitary SirT1 protein levels. We hypothesize that CR reduces pituitary SirT1 and suppresses the neuroendocrine axis.

Therefore, we developed pituitary-specific SirT1 knockout (PitSKO) mice. PitSKO mice are dwarfed with reductions in pituitary size, pituitary stores of GH, LH, FSH and serum levels of IGF-1, LH, and FSH. These mice also have reduced testis size, sperm counts, and fertility. Analysis of pituitary transcripts using RT-PCR found that PitSKO mice have reduced as GH, and LH gene expression. Interestingly, these transcripts are also reduced in the long-lived Ames and Snell dwarf mice, which harbor mutations in their Pit1 and Prop1 genes. Next we found that pituitary SirT1 co-precipitates with Pit1 *in vitro* and *in vivo*. We also observe that Pit1 is differentially acetylated in PitSKO mice compared to WT controls. Using luciferase reporter assays, we've determined that SirT1 is a Pit1 coactivator. These data suggest that increased SirT1 drives growth and reproduction under AL conditions, and mice on CR have decreased SirT1 and resulting Pit1 activity, leading to a smaller less-fertile mouse.

86. A Web-Based Environment for System-Level Models of Aging

Pat Langley (P)
Arizona State University, Phoenix, AZ

Aging poses substantial challenges in systems biology, since there is mounting evidence that it results from interactions among a diverse array of factors. Efforts to codify, visualize, and utilize our growing knowledge about these processes would all benefit from computational assistance. In this presentation, we report progress toward an interactive modeling environment that addresses these issues. Our software supports three main capabilities: (1) Accessing and viewing content about the quantities and hypothesized causal influences that contribute to aging. Because much of our knowledge about these phenomena is qualitative, our models take this form. Users can inspect this information in both textual and graphical modalities. (2) Entering and editing content about the quantities and causal links involved in aging. Because models are stated in a modular format, users can update this information incrementally as new results become available. Moreover, they can alter models using either textual or graphical operations. (3) Generating comparisons between model predictions and empirical observations. Because models comprise a set of qualitative causal influences between entities, reasoning involves simple chaining along causal paths. Users can view graphically not only whether a prediction agrees with an observation, but also visualize the causal chain that produced the explanation. The modeling environment is accessible through the World Wide Web using standard browsers. For this reason, it provides a natural platform for the community development of formal models about aging processes. The framework's Web-based character also holds potential for education about senescence. We have tested our prototype by constructing initial models of aging mechanisms in lysosomes and mitochondria. Based on our experiences, we are extending the environment to incorporate features such as zooming in and out of the graphical display and viewing links to articles that provide evidence for specific causal claims.

87. Pulsed-Wave Doppler Reveals Attenuated Cardiovascular Aging in the Naked Mole-Rat

Kelly Grimes (P)
University of Texas Health Science Center at San Antonio, San Antonio, TX

Naked mole-rats (NMRs) are extraordinarily long-lived rodents with a maximum lifespan of >31 years. They maintain good health and reproductive potential for at least 75% of their lifespan. We hypothesized that age-related changes in cardiac function will be attenuated in NMRs in comparison with mice. Pulsed-wave Doppler was used to quantify systolic

and diastolic function as well as pulse wave velocity (PWV) in three age cohorts of male and female NMRs (2-3, 8-10 and 17-21 years old; n=6/cohort/sex). Systolic function was preserved from the youngest to oldest cohorts of both males and females, comparable to data collected in mice. Unlike mice that show a pronounced increase in vascular stiffness with age, PWV, a measure of arterial stiffness remained unchanged with age in both sexes of NMRs, suggesting that NMRs maintain vascular youthfulness. Diastolic function decreases slightly with age in both male and female NMRs. We found a 12% decline in diastolic function over 19 years from young to old NMRs (a 19 year difference), whereas early to late ventricular filling (E/A ratio) of 18 month old mice when compared to 4 month old mice, has declined by more than 25%. This suggests that NMRs have a slower rate of age-related change compared to mice, and may have certain defenses to prolong cardiac health. Furthermore, we have found some individual female NMRs with E/A ratios of <1.0, indicative of a preclinical state of diastolic dysfunction. Despite having impaired LV relaxation, the females show no outward signs of increased morbidity. In humans, the incidence of diastolic dysfunction is known to increase with age and is a risk factor for serious heart disease. By using the NMR as a model for healthy cardiac aging, we may gain new insights into heart disease and the potential mechanisms that may delay its progression.

88. The Naked Mole-Rat Proteasome does not Age

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

The proteasome is responsible for the controlled cleavage of nascent and both short- and long-lived proteins. Proteins targeted for degradation include misfolded or oxidatively damaged proteins that accrue with age. A decline in proteasome activity with age may contribute to the enzyme malfunction, protein aggregation that is associated with age-related diseases. The longest-lived rodent, the naked mole-rat (Bathyrergidae; *Heterocephalus glaber*), maintains robust, cancer-free health for at least 75% of its 32 year lifespan suggesting that the normal decline in protein homeostasis, routinely observed in other animals, is either attenuated or delayed. To test the contribution of the proteasome to proteostasis in these long-lived animals, we compared proteasome activity in whole cell and sub-fractionated lysates from liver, brain and spleen tissues between naked mole-rats and physiologically age-matched mice. We observed a significant, global increase in proteasome

chymotrypsin-like activity and trypsin-like activity in naked mole-rat lysates when compared to mouse samples. While the increase was limited to the cytosol of fractionated lysates in naked mole-rat liver tissue, spleen fractions instead showed a significant doubling of activities in the nuclear fractions. Further, in brain fractionated lysates, the increase in proteasome activity compared to mice was seen in the microsomal fraction. When we examined proteasome activity across different age groups, age-related changes in proteasome activity were not evident in even the >24y, naked mole-rat age cohort; in contrast the two oldest age cohorts of mice (24m and 33m) show a significant decline. These findings suggest that naked mole-rat proteasomes may have regulatory mechanisms that maintain high efficiency even at older ages. Studying and understanding proteostasis in the naked mole-rat can lead to important insights on how the cells in these long-lived animals handle increased stress and protein damage to maintain long-term cell survival in their tissues and ultimately a longer life.

89. Aging Compromises Oral Siphon Regeneration in the Protochordate *Ciona intestinalis*

William Jeffery

Marine Biological Laboratory, Woodshole, MA

The protochordate *Ciona intestinalis*, a member of an animal group (tunicates) that is the closest living relatives of vertebrates, shows powerful capacities for regeneration and has a highly developed molecular toolkit. We study the oral siphon, which contains a suite of eight ocelli; each consisting of an epidermal pit, a neural ganglion, and an underlying cup of mesenchyme derived pigment cells. The oral siphon and its pigmented ocelli can be completely replaced in either a month or about a week, respectively, after their removal or damage. Normal oral siphon regeneration is based on cell proliferation and differentiation in a blastema, recruitment of stem/precursor cells from local niches, precise fidelity of patterning through several cycles of amputation and replacement, proximal-distal polarization, and resembles vertebrate limb regeneration in other features. However, the rate of regeneration gradually fades during the life span, which is about one year, and regeneration is markedly compromised in the oldest animals. Old animals consistently fail to replace oral siphons to more than 20% of their original length, even after two months following amputation. Defects in oral siphon ocelli also appear after siphon removal in old animals. First, there is an over-differentiation of pigment cell precursors, although the producing

stem/precursor cells still originate from local niches. Second, the normal pattern of spacing between ocelli is lost. Third, an increase in the number of ocelli from the normal number of eight up to twelve to sixteen ocelli is observed. Third, malformed multiple ocelli appear with duplicated or triplicated pigment cups and misplaced components. Finally, old animals are unable to replace their ocelli at all after two consecutive amputation and regeneration cycles. These defects in siphon replacement serve as a foundation for studying the relationships between aging, tissue repair, and regeneration in this new protochordate model. Supported by NIH Grant R01AG037918.

90. Superior Proteome Stability in the Longest Lived Animal

Stephen Treaster¹ (P, N), Keith Maslin¹, Iain Ridgway², Asish Chaudhuri³, Steven Austad³

¹*Molecular Medicine, University of Texas Health Science Center at San Antonio;* ²*Ocean Sciences, Bangor University;* ³*Barshop Institute for Longevity and Aging Studies, University of Texas Health Science Center at San Antonio*

Maintenance of protein structure and function has been implicated in the aging process in a variety of model organisms. We are utilizing a range of bivalve mollusk species, with lifespans ranging from under a decade to over five hundred years, in a comparative study to investigate the hypothesis that long life requires superior proteome stability. These ages can be individually determined by counting growth rings in the shell. This experimental system provides a unique opportunity to study closely related organisms with vastly disparate longevities, and their relative proteome stabilities. Specifically, we are testing their ability to maintain structure and function under various stressors. Structure is measured by the fluorescent BisANS probe, which binds to hydrophobic regions of proteins exposed as they unfold, incorporation being directly related to compromised tertiary structure. Protein aggregation and preservation of GAPDH function under stress was also measured. Stressors included TBHP as an oxidative stress, and urea as an unfolding agent. To date, we find that stress induced protein unfolding decreases with increasing lifespan. This stability corroborates with superior GAPDH function and decreased protein aggregation under the same stressors. Taken together, these data support the hypothesis that proteome stability is a determinant of longevity. The macromolecules underlying this stability may be relevant for human disease and aging.

91. Protein Oxidation may be an Important Regulator in the Development of Insulin Resistance

Jennalynn Styskal^{1,2}, Adam Salmon^{1,2}, Nicholas Musi², Arlan Richardson^{1,2}

Barshop Institute for Longevity and Aging Studies¹, UT Health Science Center, San Antonio TX²

Oxidative damage accumulation is a proposed mechanism regulating aging processes and disease development. Proteins are sensitive to such oxidative stress, which can cause accumulation, altered conformation and function. Methionine sulfoxide reductase A (MsrA), important in antioxidant defense, is unique in that it specifically repairs protein oxidative damage. Mice lacking MsrA (*MsrA^{-/-}*) and mice over expressing MsrA (*MsrA^{Tg}*) are phenotypically similar to wildtype (WT) mice under normal conditions, but have altered susceptibility to oxidative stress, suggesting that excess methionine oxidation may not be occurring under these conditions. Increasing adiposity has been associated with increased oxidative damage in several disease models. On a high fat (HF) diet, *MsrA^{-/-}* mice become more insulin resistant than WT mice whereas *MsrA^{Tg}* mice are protected. This also correlates to insulin-stimulated signaling sensitivity. 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 to test the effect of MsrA on a specific oxidative stress-induced insulin resistance. By utilizing these models, this study will test the hypothesis that MsrA can regulate the development of insulin resistance by repairing oxidative damage in proteins involved in the insulin signaling pathway *in vitro*. In this study, skeletal muscle isolated from *MsrA^{-/-}*, *MsrA^{TG}*, and WT mice was tested for resistance to oxidative stress. Insulin signaling protein phosphorylation correlates with *in vivo* signaling observations, determined by western blot after insulin stimulation. Our hypothesis is that the level of protein oxidation can be correlated with the degree of insulin resistance in this system. Because oxidation of proteins can lead to a decline in their function, future studies will focus on both functions of the insulin signaling proteins isolated from these models as well as oxidation status of these proteins.

92. Growth Hormone Alters Glutathione-S-Transferase Activity and Proteins Expression in Long-Living Ames Dwarf Mice

Lalida Rojanathammanee (P), Sharlene Rakoczy, Holly Brown-Borg

University of North Dakota, Grand Forks, ND

Ames dwarf mice are deficient in growth hormone (GH), prolactin and thyroid-stimulating hormone and live significantly longer than their wild type siblings. The lack of GH is associated with stress resistance and increased longevity. However, the mechanism underlying GH's actions on cellular stress defense have yet to be elucidated. In this study, wild type or Ames dwarf mice were treated with saline or GH (WT saline, Dwarf saline, Dwarf GH) two times daily for 7 days. Liver tissues were collected 1 hour after of the last injection and mitochondria were isolated. The body and liver weights of Ames dwarf mice were significantly increased after 7 days of GH administration. Mitochondrial protein levels of the glutathione-S-transferase (GST) isozymes, K1 and M4 (GSTK1 and GSTM4), were significantly higher in dwarf mice (Dwarf saline) when compared to wild type mice (WT saline). Growth hormone administration downregulated the expression of GSTK1 (Dwarf GH 96.19 ± 3.85 vs Dwarf saline 112.70 ± 4.05 ; $p < 0.05$). and GSTM4 (Dwarf GH 105.60 ± 1.81 vs Dwarf saline 134.5 ± 3.95 ; $p < 0.001$) proteins in dwarf mice. We further investigated GST activity from liver whole cell lysates using different substrates. GST substrate specific activity (BSP, DCNB, 4HNE) was significantly reduced in GH-treated dwarf mice. In addition, GH treatment attenuated the activity of thioredoxin and glutaredoxin in liver mitochondria of Ames mice. These data indicate that GH plays a role in stress resistance by altering the functional capacity of the GST system through the regulation of specific GST family members in long-living Ames dwarf mice. It also affects the regulation of thioredoxin and glutaredoxin, factors that regulate post-translational modification of proteins and redox balance, thereby further influencing stress resistance and lifespan.

93. Age-Related Changes of Dopamine, Noradrenaline, and Adrenaline in Adrenal Glands of Mice

Akiko Amano¹ (P), Makoto Tsunoda², Yuki Kishimoto³, Toshiro Aigaki¹, Naoki Maruyama³, Akihito Ishigami³

¹*Cellular Genetics, Tokyo Metropolitan University;*

²*Graduate school of Pharmaceutical Sciences, The University of Tokyo;* ³*Tokyo Metropolitan Institute of Gerontology*

[Background] Catecholamines are generic name for biogenic amine that consist of dopamine, noradrenaline (norepinephrine), and adrenaline (epinephrine), and play important roles as neurotransmitters and hormones. The catecholamines were reported to decrease with age in brain and plasma in some species. However, there are some arguable results. To investigate the effect of aging on adrenal catecholamine synthesis, we examined whether age-related changes occur in mice adrenal catecholamine levels and the expressions of synthetic enzymes for catecholamines. [Methods] Adrenal glands were collected from male C57BL/6NCR mice at 6, 12, and 24 months old. Adrenal catecholamines were measured using high sensitivity an automatic semi-microcolumn liquid chromatographic determination and peroxyoxalate chemiluminescence reaction. Total mRNA expressions of tyrosine hydroxylase (TH) and dopamine beta hydroxylase (DBH) in mice adrenal were measured by quantitative real time-PCR. [Results and Discussions] The dopamine levels in mice adrenal glands at 6 months old were higher than those at 24 months old. The TH mRNA ratio at 24 months old was lower than those at 12 months old. Increased dopamine levels might decrease TH activity and mRNA ratio at old mice by negative feedback. In contrast, the ratios of dopamine to noradrenaline showed age-related downward trend. The DBH mRNA ratio at 24 months old was lower than those at 12 months old. Decreasing DBH activity supposed to be factors for decreasing the ratios of dopamine to noradrenaline in old mice. The adrenaline levels and the ratio of noradrenaline to adrenaline at 24 months old showed significant lower than those at 6 months old. These results might be caused increasing degradation of adrenaline or decreasing enzyme activity for adrenaline synthesis. These results suggest that age-related decline of catecholamine synthesis system occur in mice adrenal glands. Detailed mechanisms for these results need to be investigated.

94. Reduced Pathogen Uptake by Aged Dendritic Cells: Setting the Stage for Impaired Adaptive Immunity?

Megan Smithey (P), Gang Li, Janko Nikolich-Zugich
University of Arizona, Arizona Center on Aging, Tucson, AZ

The age-associated decline in T cell immunity to infection has been documented across multiple pathogens, yet the relative contributions of the aged priming environment vs. lymphocyte-intrinsic defects remain unclear. Numerous reports have demonstrated the importance of the CD8a+ dendritic cell (DC)

subset in both (1) the early establishment of *Listeria monocytogenes* infection in the spleen following intravenous inoculation of mice, as well as (2) serving as the critical DC population required for effective priming of the CD8 T cells responsible for clearance of the bacteria. To distinguish the influence of the aging environment from cell intrinsic defects influencing the CD8 T cell response to *Listeria* infection, adult naïve OT-1 TCR transgenic CD8 T cells were transferred into adult or old recipient mice infected with recombinant *Listeria monocytogenes* expressing OVA antigen (Lm-OVA). We found that adult OT-1 CD8 expansion was reduced in aged recipient mice, and this correlated with numeric, phenotypic and functional defects selectively affecting CD8a+ DC. The mechanism targeting *Listeria* into CD8a+ DC at early time points post-infection has been identified as a combination of blood serum proteins and platelets. Ex vivo cell sorting experiments found that uptake of Lm by the CD8a+ DC subset was markedly reduced in old mice at early time points following infection. Further, pre-coating Lm-OVA with young mouse serum prior to inoculation was able to restore bacterial uptake by aged CD8a+ DC to the levels seen in adults, suggesting that early problems establishing Lm-OVA infection within the CD8a+ DC subset of aged mice may set up a less effective T cell priming environment. These preliminary data may elucidate a key early defect in old animals underlying overall impaired immunity to Lm, and potentially many other pathogens which also rely on the CD8a+ DC subset for effective CD8 T cell priming.

95. Age-Related Changes in Circulating T Cell Subset Phenotype and T Cell Receptor Diversity in Cytomegalovirus Negative Humans

Anne Wertheimer¹, Michael Bennett², Byung Park³, Carmine Martinez², Noreen Currier², Jeffrey Kaye⁴, Janko Nikolich-Zugich⁵

¹University of Arizona, Arizona Center on Aging;

²University of Arizona, Department of Immunobiology; ³Oregon Health Sciences University, Knight Cancer Institute; ⁴Oregon Health Sciences Center, Layton Aging and Alzheimer's Center;

⁵University of Arizona, Arizona Center on Aging & Dept of Immunobiology

The impact of intrinsic aging upon the peripheral blood T-cell pool remains incompletely understood. This impact must be distinguished from the influence of latent persistent microorganisms, particularly the cytomegalovirus (CMV), which is believed to cause and/or exacerbate some of the age-related changes. In a cohort of 150 CMV-negative individuals, aged 21-100 years, we have found that aging correlated

strictly with the absolute loss of naïve CD8 T cells, but did not affect memory CD8 T cell numbers. In CMV+ individuals (244, range 21-96 years), the loss of naïve T cells was not greater than the loss associated with aging alone. However, CMV+ individuals exhibited an absolute increase in memory CD8 cells, due to an accumulation of the effector/effector memory phenotype cells. The circulating CD4 subset was much less affected by either CMV infection or aging. Analysis of the T-cell receptor diversity in CMV- subjects showed that the number and magnitude of CD8+ T-cell clonal expansions (TCE) increased with aging, and this increase was inversely proportional to the loss of naïve T cells. These findings provide important insight into the age-related changes in the peripheral blood pool of older adults and suggest potential therapeutic targets for immune rejuvenation.

96. Dissecting the Mechanisms Underlying the Maintenance and Function of Old Naïve T cells

Kristin Renkema (P)

University of Arizona, Tucson, AZ

Today, people aged 65 years and older number about 33 million in the United States; this age group is projected to more than double by 2030. Alarming, infectious diseases remain amongst the leading causes for death in people over 65 years old. Widespread defects in the immune system occur with age, leading to increased susceptibility to disease and decreased vaccine efficacy. The CD8+ T cell functions as the cytotoxic killer of the immune system; these cells are essential for the clearance of viruses and intracellular bacteria. We have found that CD8+ T cells display decreased numbers and function with aging. However, the mechanisms underlying these immune defects are not understood. We have developed a unique mouse model to dissect the extracellular and cellular processes that contribute to the aged immune cell phenotype during immune homeostasis, in order to study these cells at a resting state. Interestingly, we have found that significant changes occur in resting old CD8+ T cells, and that these changes may contribute extensively to the decreased ability to function once the cells are activated. Specifically, we found that old CD8+ T cells acquire a memory phenotype with age without being exposed to activation; we hypothesize that the loss of naïve phenotype may be due to a combination of homeostatic signals. These findings are vitally important to understanding the mechanisms underlying the decreased immune responses in the elderly.

97. Functional Analysis of Age-Related Novel GEF, Zizimin2 in Immunosenescence

Mitsuo Maruyam (P)

National Center for Geriatrics and Gerontology

With advancing age, various physiological functions are getting fragile and dysfunction. Immunological responses, for instance lymphocyte activation and antibody or cytokine secretion, are also gradually impaired and settled in immunosenescence. We have identified Zizimin2 as a functional molecule that is highly expressed in murine splenic germinal center B cells after immunization with T-cell-dependent antigen. The following structural and functional analysis demonstrated that Zizimin2 was a new family member of Dock (dedicator of cytokinesis), Dock11, which is the guanine nucleotide exchange factor (GEF) for Cdc42, a low-molecular-weight GTPase. And CZH2 domain in C-terminal of Zizimin2 protein was capable to bind and activate Cdc42. Interestingly, its gene and protein expression is restricted in immune system, such as dendritic cells, B and T lymphocytes or spleen, thymus and lymph nodes as tissues. This information strongly suggested that Zizimin2 has a potentially functional role in immune responses and especially in immunosenescence, since we also found the specific Zizimin2 expression was linked with aging in mice. It has been known that Cdc42 facilitates formation of filopodia, which is protrusion on cell surface. Over-expression of full length Zizimin2, localized at plasma membrane, induced filopodia formation. CZH2 domain, which localized in cytoplasm, has dominant negative effect for filopodia formation. These results suggest that interaction of Zizimin2 and Cdc42 through CZH2 domain is required for induction of filopodia formation. We next analyzed the functions of Zizimin2 on GM-CSF stimulated murine bone marrow derived dendritic cells (BMDC). Especially, we introduce the correlation between the activation signals for BMDC and the expression and localization of Zizimin2 protein. Consequently, stimulation of Fc γ receptor and Toll-like receptor 4 triggered Zizimin2 up-regulation and Cdc42 activation in BMDC. These data suggest that Zizimin2 is an immune-related and age-regulated GEF, which facilitates filopodial formation through activation of Cdc42, which results in activation of lymphocyte migration.

98. Mechanisms Underlying Skeletal Muscle Weakness with Bedrest

Haiming Liu (P, N) and LaDora V. Thompson

Department of Physical Medicine and Rehabilitation, University of Minnesota, Minneapolis, Minnesota

Skeletal muscle strength diminishes following periods of bedrest, especially in older adults. The mechanisms underlying the loss in muscle strength from bedrest are multifactorial. Maximum specific force (force/cross-sectional area) in single skeletal muscle fibers is dependent on the number of cross bridges per half sarcomere and the fraction of cross bridges in the force generating state (a change in the ratio of myosin cross bridges in the weak- and strong-binding state during contraction). In the current study, we tested the hypothesis that lower specific force generated by type II fibers from unweighted rats resulted from both structural changes in myosin and a decrease of number in cross bridges. Permeabilized semimembranosus muscle fibers from adult rats, some of which were hindlimb-unloaded for 2 weeks, were studied for Ca²⁺-activated force generation. Fibers were also spin labeled specifically at myosin Cys707 to assess the structural distribution of myosin during maximal isometric contraction during electron paramagnetic resonance spectroscopy. Myosin heavy chain (MHC) content per half sarcomere, an estimate of the number of cross bridges available for force generation, was determined by densitometric analysis and comparison to a standard curve of known MHC concentrations. Fibers from unweighted rats generated 31% less specific force than fibers from weight bearing rats. Electron paramagnetic resonance analyses showed that the fraction of myosin heads in the strong-binding structural state during contraction was 35% lower in fibers from the unweighted rats. MHC content per half sarcomere decreased 26% compared to unweighted rats. These data indicate that altered myosin structural distribution during contraction and a decrease in MHC content per half sarcomere play a role in muscle weakness. Understanding these cellular mechanisms is critical in designing therapies to prevent inactivity induced muscle weakness.

99. Correction of Aberrant Neutrophil Migration in the Healthy Aged Through Inhibition of the p110 δ Isoform of PI3Kinase

Hannah Greenwood¹ (P), Elizabeth Mann¹, Georgia Walton¹, Robert Insall², Elizabeth Sapay¹, Janet Lord¹

¹University of Birmingham; ²Beatson Institute for Cancer Research

Aberrant neutrophil migration and the subsequent potential for reduced pathogen clearance have been proposed as a major contributing factor to the increased rates of bacterial infection observed in the elderly population. Specifically, chemokinesis (random movement) appears to be maintained with increasing age, however, chemotaxis (directional

movement) is not. In addition, our understanding of the pathways involved in the control of migration remains incomplete. PI3Kinase is thought to act as a cellular ‘compass’ governing migration and providing directionality via signalling through multiple isoforms of the p110 catalytic subunit. Neutrophils express all 4 of these isoforms p110 α , β , γ and δ however, it appears to be γ and δ that are most heavily implicated in directional migration. Here we confirm a significant reduction in migratory accuracy in response to the chemo-attractants IL8 ($p \leq 0.001$) and fMLP ($p = 0.015$) in the healthy aged with a significant correlation between age and reduced migratory accuracy ($R^2 = 0.459$). Following this, we assessed PI3Kinase activation by western blot in both the basal state and in response to chemokine stimulation. We observed constitutive activation of PI3 kinase in the cells of older donors compared with a transient phosphorylation in the young. Crucially treatment with inhibitors specific for each PI3Kinase isoform, revealed that inhibition of the p110 δ isoform was able to restore migration in the neutrophils from old donors and induce a ‘young migratory phenotype’. In conclusion, we have been able to establish that altered signalling through the PI3Kinase isoform p110 δ is a major controlling factor contributing to reduced neutrophil chemotaxis in the aged and found this defect to be amenable to pharmacological correction.

100. Enhancing Autophagy *in vitro* and in Rodent Hearts Offers Protection from Oxidative Stress Mediated Toxicity in Cardiac Cells

Debapriya Dutta¹(P,N), Jinze Xu¹, William Dunn², Christiaan Leeuwenburgh¹

¹Department of Aging and Geriatrics, University of Florida; ²Department of Anatomy and Cell Biology, University of Florida, Gainesville, FL

Aging is associated with a decline in heart functioning and the progressive accrual of macromolecular oxidative damage mediated by mitochondrially derived oxidants has been proposed to be a major player. This highlights the importance of removing dysfunctional mitochondria, particularly in the post-mitotic heart. Dysfunctional mitochondria are removed by a process termed autophagy. We therefore investigated whether pharmacological or nutritional enhancement of autophagy offers protection against mitochondria-mediated oxidative stress in a cardiomyocyte cell line (HL-1) and in late middle-aged rat hearts, respectively. In HL-1 cells, we mimicked mitochondrial oxidative stress by using Antimycin A (AMA), which increased mitochondrial superoxide generation and nuclear DNA oxidation; and decreased mitochondrial membrane potential and

cellular respiration. Treatment of cells with mTOR inhibitor rapamycin lead to a strong autophagy induction and protected against the cytotoxic effects of AMA, assessed both at the cellular and mitochondrial level. Autophagy inhibition attenuated the cytoprotective effects of rapamycin. For *in vivo* experiments, we investigated whether a late-life intervention of a low dose calorie restriction (CR 20%) alone, or in combination with Resveratrol can induce autophagy in the hearts of late middle-aged rats and whether it is protective against Doxorubicin, a known mitochondrial stressor. 26 month old male F344xBN rats were randomly divided into three groups: Control (CON), CR (CR) and CR plus 50mg/kg/day Resveratrol (CR+RESV), fed daily for 6 weeks. Animals were then administered a single IP injection of 10mg/kg Doxorubicin or saline control, 24h before sacrifice. Our findings suggest that CR and Resveratrol, only when combined, stimulated autophagy in late middle-aged rat hearts. Analysis of mitochondrial oxygen consumption revealed increased State III respiration in CR+RESV rats. Systemically, CR+RESV attenuated Doxorubicin mediated increase in serum lactate dehydrogenase. In conclusion, interventions aimed at enhancing basal autophagy offers protection and could be a potential therapeutic strategy against mitochondria-mediated oxidative stress.

101. Nrf2 - The oxidative Damage Theory of Aging is Born Again, Alive and Thriving

Vincent Giuliano (P)

agingsciences.com

The oxidative damage or free radical theory of aging is perhaps the oldest and most venerable of tens of such theories, going back some 60 years. It suggests that taking anti-oxidants could be a practical anti-aging intervention, and millions of people do that now. But the theory has hit serious speed bumps, such as the realization that free radicals play essential roles in many important physiological processes including cellular energy production, signal transduction, cell-cycle regulation, and immune function. Further, the body has its own complex antioxidant defense system for protection against unwanted free radicals. The newer research suggests taking antioxidant supplements could both interfere with free radicals when and where they are needed and sabotage the body’s own natural antioxidant defense system. Taking antioxidant supplements could therefore be dangerous or life-shortening. Discovery of the Keap1-Nrf2 pathway has provided an entirely new perspective on both the theory of aging and taking antioxidant supplements. The pathway acts on dozens or hundreds of genes

activating the body's own antioxidant defense system, stress-protective genes and inhibits the expression of NF-kappaB and consequent inflammation. The "antioxidants" that do provide demonstrable health benefits, like curcumin, green tea, fish oil and resveratrol, do so not by direct chemical antioxidant action as once thought. Instead, they act primarily by activating the Keap1-Nrf2 pathway and exercise their actions by activating multiple genes and associated pathways. The author will report on key findings in three long research review articles he has recently published in his blog: The pivotal role of Nrf2: Part 1 – a new view on the control of oxidative damage and generation of hormetic effects, Part 2 – foods, phyto-substances and other substances that turn on Nrf2, and Part 3– Is promotion of Nrf2 expression a viable strategy for extending human healthspan and lifespan?

102. Association of Ferritin with Anti-Oxidative Status and Insulin Resistance

Jee-Yon Lee¹ (P), Duk-Chul Lee¹, Jee-Aee Im², Ji-Won Lee¹

¹*Department of Family Medicine, Severance Hospital, Yonsei University;* ²*Sport and Medicine Research Center, INTOTO Inc.*

Background Ferritin is known to be associated with insulin resistance (IR) and oxidative stress; however, recent studies have shown that there is an association between ferritin and anti-oxidative status. To date, the biphasic response of ferritin to oxidative stress has not been fully evaluated. Thus, we investigated the association between ferritin and IR and anti-oxidative status in obese and non-obese women. methods We evaluated the homeostasis model assessment of insulin resistance [HOMA-IR] and total anti-oxidant status (TAS) in a total of 111 healthy women between the ages of 32 and 68 years. Results In all of the study subjects, ferritin levels were positively correlated with age, BMI, TAS and HOMA-IR. In the subgroup analysis, ferritin levels were correlated with age and TAS in the non-obese group and with insulin and HOMA-IR levels in the obese group. On stepwise multiple linear regression analysis, ferritin was found to be independently associated with TAS in the non-obese group and independently associated with HOMA-IR in the obese group. Conclusions Our findings suggest ferritin is associated with IR in obese women and with anti-oxidative status in non-obese women. Further studies are warranted to elucidate the precise role of ferritin in obesity.

103. The Leukocyte Telomere Length is Independently Associated with Physical Function in Elderly Women

Jee-Yon Lee¹ (P), Jung-Ha Kim², Hyo-Weon Bang², Jae-Hong Ko², Duk-chul Lee¹

¹*Department of Family Medicine, Severance Hospital, Yonsei University;* ²*Department of Family Medicine, Chung-Ang University Medical Center*

It has been reported that declined physical function has close relationship with age-related disease and high mortality in older people. Since telomere length reflect of aging, physical function and telomere length may have relationships. However results of previous studies were inconsistent. Therefore the purpose of this study was to investigate the relationship between physical function and telomere length in elderly women. A total of 117 elderly women were included. We measured physical function by using physical performance score as the sum of walking speed score, chair stand score and tandem standing score. The leukocyte telomere length was measured using quantitative real-time PCR method. Physical performance score was negatively correlated with age($r=-0.42$, $p<0.01$), telomere length($r=0.28$, $p=0.002$), waist circumference($r=-0.23$, $p=0.01$), body fat($r=-0.19$, $p=0.04$), triglyceride($r=-0.20$, $p=0.03$), geriatric depression scale-15 scores($r=-0.27$, $p<0.01$) and positively correlated with Korean mini-mental state examination($r=0.20$, $p=0.03$) and lean/fat mass($r=0.25$, $p=0.01$). With a stepwise multiple regression analysis, telomere length was found to be an independent factor associated with physical performance score after adjustment for confounding variables. This study suggests physical function is associated with telomere length in elderly women. Further studies are warranted to elucidate the precise mechanism of this association.

104. Unmasking Aging in Non-Aging Hydra?

Szymon Tomczyk¹ (P) Kathleen Fisher², Steven Austad², Brigitte Galliot¹

¹*University of Geneva;* ²*Barshop Institute for Longevity and Aging Studies, University of Texas Health Science Center at San Antonio, San Antonio, TX*

Over the past years studies performed in short-lived invertebrate model organisms as the fruit fly and the nematode provided major advance in understanding the cellular and molecular base of senescence (Martin, FASEB J. 2010). Although inarguably valuable, these model systems have substantial drawbacks and establishing new invertebrate model system for aging studies might lead to the discovery

of novel genes and pathways implicated in human aging (Austad, J. Gerontol. A Biol. Sci. Med. Sci. 2009). Hydra, a freshwater cnidarian polyp, provides a potentially fruitful model system for aging studies as Hydra maintained asexual do not show any signs of aging, whereas some Hydra species that undergo sexual differentiation rapidly age, as sexual Hydra oligactis (H.o.) that ages in about 3 months (Brien, Biol. Rev. Cambridge Phil. Soc. 1953; Martinez, Exp. Gerontol. 1998; Yoshida et al. Gene 2006). Hydra has three distinct populations of stem cells, which are constantly active throughout the animal's life. In aging Hydra oligactis Yoshida et al. showed that most non-epithelial cell types are lost, suggesting some cross-talk between the induction of gametogenesis, the down regulation of somatic stem cells and the occurrence of senescence. To study aging and non-aging phenotypes in this species, we induced aging in cohorts of H.o. polyps by transferring them to 10°C, a cold temperature sufficient to induce sexual differentiation in few weeks. Indeed we observed anatomical alterations, dramatic decrease in fitness and finally degeneration in about 90% of sexual animals. To further characterize the aging process we established conditions to monitor a battery of reliable biochemical, molecular, cellular and physiological markers. Preliminary cellular analyses indicate that the activity of somatic stem cells, either proliferation or nerve differentiation, steadily decline when the animals age. Further analyses will identify when aging becomes irreversible and what are the molecular actors involved in this process.

105. Interventions to Determine Which is Most Important in Aging & AD: Redox Shift, Overwhelming ROS or Insufficient Anti-Oxidants
Debolina Ghosh (P, N) and Gregory Brewer
Southern Illinois University, Springfield, IL

The NAD(P)H/NAD(P) and GSH/GSSG redox systems control the redox environment for cell survival and protection against excessive reactive oxygen/nitrogen species (ROS/RNS). Previously we found in aging and younger 2 month AD-transgenic mouse neurons an oxidative redox deficit, lower NADH regenerating capacity and GSH levels, even before ROS-mediated macromolecular damage and elevated ROS levels. These redox deficits were reversible with an NAD⁺ precursor. Based on these observations, our aim here was to determine which of these age and AD-related changes are upstream: the NAD(P)H/NAD(P) oxidative redox shift, depletion in GSH or large increases in ROS. We simultaneously determined GSH depletion and ROS elevation in live neurons across the age-span by titrating with an

inhibitor of γ -glutamylcysteine synthetase (GCL). GCL is transactivated by the redox sensitive Nrf2-ARE transcription factor that activates a number of anti-oxidant enzymes. Interestingly, we observed that both Nrf2-ARE and GCL mRNA and protein levels show an age-related deficit in non-Tg and 3xTg-AD brain. However, unlike the NADH/NAD redox state in whole brain and NADH regenerating capacity in neurons, which showed deficits in AD mice from an earlier age, GCL and Nrf2-ARE levels were the same in both genotypes at 2 months. We also treated neurons with a Nrf2-ARE inducer and observed elevated Nrf2-ARE and GCL mRNA expression in both non-Tg and 3xTg-AD neurons at middle age. We also observed that co-treatment of middle aged neurons of both genotype with the Nrf2-ARE inducer and the NAD precursor increased NAD(P)H and provided neuroprotection against A-beta toxicity. The present data suggest that an oxidative redox shift is upstream of lower GSH (re)generation and higher ROS levels and that aging sensitizes neurons to AD-like neurodegeneration. The combination of an NAD precursor and a Nrf2-ARE inducer provides greater neuroprotection than either alone by restoring redox balance and GSH levels in susceptible aging neurons.

106. Effects of Rapamycin on Aging: Similar or Different to Dietary Restriction?

Wilson Fok¹ (P) Yiqiang Zhang¹, Adam Salmon¹, Arunabh Bhattacharya¹, William Wood², Yongqing Zhang², Kevin Becker², Carolina Livi¹, Yidong Chen¹, Walter Ward¹, Arlan Richardson¹, Viviana Perez³

¹University of Texas Health Science Center at San Antonio, San Antonio, TX; ²National Institute of Aging; ³Oregon State University, Portland, OR

Rapamycin (Rapa) has been found to extend lifespan in *S. Cerevisiae*, *Drosophila*, and mice. Base on epistasis studies in invertebrates, it has been hypothesized that Rapa acts similar to dietary restriction (DR). To test this hypothesis, we used male C57BL6 mice fed ad libitum (AL), DR mice fed 60% of the ad libitum diet, and Rapa mice fed ad libitum supplemented with encapsulated Rapa (14 ppm; the same dose used by the studies that showed Rapa increased the lifespan of mice) in the food. Microarray and metabolomics analysis were conducted to compare the similarities or differences in the transcriptome, metabolome, and signaling pathways associated with DR or Rapa interventions for six months, starting at two months of age. Our previous data indicated that there were no differences in body composition, except fat mass, in which only DR treatment had a significant decrease in fat mass. Both treatments had decreased mTOR signaling

(measured by phosphorylation of S6) and increased levels of autophagy (measured by the ratio of LC3II/LC3I). From our microarray analysis of the liver, DR has a more dramatic effect on genes than Rapa. Principal component analysis showed that there was no overlap between Rapa and DR but there was some shared overlap between AL and Rapa. The gene analysis showed that the overlap of genes between DR and Rapa relative to AL is small (563 genes, 24% of all significantly changed genes). We found similar results in our pathway analysis as well. The metabolome highlights even larger differences between DR and Rapa relative to AL. 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 and that the differences observed indicates Rapa is more different than similar to DR.

107. Enhanced Autophagy Drives Coordinate Expression in Nuclear and Mitochondrial Genomes

Chad Lerner (P), Alessandro Bitto, Claudio Torres, Christian Sell

Drexel University, Philadelphia, PA

Coordination between mitochondrial turnover and the expression of mitochondrial genes in both the nucleus and mitochondrial genome are required to maintain proper mitochondrial function. However, the precise mechanisms that ensure this coordination are not well defined. We find that mTOR activity influences signaling from mitochondria to the nucleus via changes in autophagy and p62/SQSTM1 turnover. Reducing mTOR activity by culturing human fibroblast cells in the presence of nanomolar concentrations of rapamycin increases the interaction between p62/SQSTM1 and Keap1 and promotes Nrf2 accumulation. The accumulation of Nrf2 leads to increased NRF1 levels, increased expression of nuclear encoded mitochondrial genes such as TFAM and of mitochondrial genes involved in oxidative phosphorylation. This effect appears to be due to a rapid turnover of p62/SQSTM1 complexes serving to sequester Keap1. These results reveal a portion of the intracellular signaling network that couples mitochondrial turnover with mitochondrial renewal in order to maintain homeostasis within the cell. Modulators of mTOR activity such as IGF-1 and rapamycin would be expected to impact this signaling network to increase or decrease mitochondrial turnover and gene expression. We predict that these shifts in mitochondrial dynamics contribute to changes in lifespan that occur in response to altered mTOR signaling.

108. The Role of Genes, Aging, and Environment in Healthspan: Insights from Longitudinal Human Data

Alexander Kulminski (P), Irina Culminskaya, Konstantin Arbeeve, Svetlana Ukraintseva, Liubov Arbeeve, Anatoli Yashin

Duke University, Durham, NC

The optimism on revealing genetic underpinnings of late-life traits by genome-wide association studies (GWAS) is tempered. We use the candidate-gene and genome-wide genotyping resources on two human generations followed up to 60 years in the Framingham Heart Study original (FHS) and Offspring (FHSO) cohorts to characterize the role of lipid genes, the apolipoprotein E (APOE) e2/3/4 polymorphism and the APOB rs1042034 (C/T) SNP, in total cholesterol (TC) and cardiovascular disease (CVD) across chronological ages and generations. The analyses of the risks of onsets of CVD were conducted at different FHS/FHSO examinations to determine the role of the cohort attrition due to developing CVD and mortality. The APOE e4 allele is associated with shorter lifespan without CVD compared to the non-e4 genotypes in younger women in the original FHS cohort, whereas older women carrying the e4 allele in the FHS live longer CVD-free life. However, older women carrying the e4 allele in the FHSO have shorter CVD-free lifespan. Minor allele homozygous (rs1042034 CC) men are at highly significant risk of early onset of CVD compared to men carrying major allele in the FHS (RR=2.18, p=4.5×10⁻⁵ when followed from baseline) but not in the FHSO. The results hold regardless of longitudinal attrition of these samples. TC does not mediate these associations. The strength of the association of the APOE e4 allele and the rs1042034 CC genotype with TC in the FHSO declines with chronological age in the same individuals. An opposite trend on strengthening of the effect of the e4 allele on TC in the same range of chronological ages is observed in the FHS. The results suggest that aging in changing environment can be a key player in genetic predisposition to healthspan. More detail analyses beyond those offered in standard GWAS can substantially advance the progress in the field.

109. Characterizing Aging in the Colonial acidian, *Botryllus schlosseri*

Roma Munday (P), Max Evoy-Mount, Anthony De Tomaso

Department of Molecular, Cellular and Developmental Biology, University of California Santa Barbara

Botryllus schlosseri is a colonial chordate that grows using a process of asexual reproduction called budding, which eventually gives rise to a colony of genetically identical individuals called zooids. *B. schlosseri* offers a unique model for aging, as a single genotype can be repeatedly isolated into sub-clones, each of which can be raised separately. It has been suggested that many clonal animals are “non-aging” and even potentially immortal, but *B. schlosseri* individuals have a lifespan that can range from 3 months to > 7yrs. It has been shown that under both field and laboratory settings *B. schlosseri* can be subject to nonrandom senescence, where independent sub-clones that were separated months prior die within 48 hrs of each other. Most theories that explain senescence have been divided between the programmed and stochastic theories of aging. Programmed theories presuppose there are biological clocks functioning throughout the lifespan of an individual, which would induce changes in gene expression that affect the systems responsible for maintenance and repair. Stochastic theories see environmental impacts inducing cumulative damage at various levels as the primary cause of aging. In *B. schlosseri*, nonrandom senescence suggests a cellular timekeeper functioning independent of environmental influence. Since *B. schlosseri* can be cultured under both laboratory and field conditions, it offers a unique perspective to delineate between these two modes of aging. In our study we will take weekly measurements and monthly tissue samples of both field and laboratory animals, while recording environmental parameters. This will allow us to track putative biological and cellular signs of aging under different environmental conditions over the lifespan of multiple individuals. Through the use of biomarkers previously described in other aging models, we hope to establish potential indicators that will allow us to track aging through core processes like growth, reproduction and cellular maintenance.

110. Age and Sex Specific Differences in C57BL/6 Murine Sleep Patterns

Keith Maslin (P), Vanessa Soto, Stephen Treaster, Steven Austad, Kathleen Fischer

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

Sleep fragmentation increases with age and is a common health problem in aging populations. Most commonly studied in relation to sleep apnea, sleep fragmentation in humans is associated with the disruption of many body functions ranging from glucose metabolism to cognition. In an effort to assess the effects of rapamycin on healthspan in C57BL/6 mice we used a non-invasive assay to

monitor animal activity. Pack et al (2006) developed a high-throughput model for phenotyping sleep in mice that validates the use of continuous activity monitoring as a method to measure sleep, with sleep defined as any bout of inactivity of \approx 40 seconds. Based on their model, we measured activity across a 24 hour light/dark cycle to assess sleep patterns and sleep fragmentation. We report here that C57BL/6 mice that were assessed at 25 and 30 months of age show significant sex differences in sleep fragmentation. In addition, we show sex- and age-related differences in both sleep and activity, confirming previous reports. While in aging humans there are no significant differences in EEG activity, during REM and slow-wave sleep, there are significant sex differences in sleep fragmentation. The mechanisms underlying age- and sex-related differences in sleep fragmentation warrant further investigation.

111. Conservation of Longevity-Associated Pathways from *C. elegans* MIT Mutants in the Long-Lived Surf1 Knockout Mice

Daniel Pulliam¹ (P, N) Deepa Sathyaseelan², Yuhong Liu², Shauna Hill¹, Holly Van Remmen¹

¹University of Texas Health Science Center at San Antonio/Barshop Institute; ²Barshop Institute, San Antonio, TX

Mitochondrial dysfunction has been long been implicated in aging and age-related diseases. However, inhibition of the electron transport chain (ETC) through genetic manipulation or RNA interference has been shown to increase the lifespan of yeast, *C. elegans*, *Drosophila*, and mice. In *C. elegans* this lifespan extension is influenced by reactive oxygen species, the mitochondrial unfolded protein response (mtUPR), hypoxia inducible factor 1 (HIF1), AMP-activated protein kinase (aak-2/AMPK), and mitochondrial biogenesis. Here we explore whether mechanisms resulting in increased lifespan in *C. elegans* mit mutants are conserved in the long-lived Surf1 knockout mouse (Surf1^{-/-}). In which deletion of SURF1, an ETC complex IV assembly protein, results in a 50-80% decline in complex IV activity. Complex IV inhibition from lack of SURF1 assembly protein is associated with an activation of the mtUPR, a retrograde signaling response resulting in an increase in mitochondrial specific chaperones and proteases aimed at refolding misfolded proteins and degrading damaged proteins. Specifically the mtUPR-associated proteins Hsp60, ClpP, Lon protease are increased from 34-55% in brain, skeletal muscle and liver in Surf1^{-/-}. Thioredoxin 2 is also increased by 75%, 118% and 800% in skeletal muscle, brain and liver (respectively). In heart,

despite an increase in the mtUPR transcription factor chop, none of the mtUPR proteins were upregulated with the exception of ClpP (66%). Furthermore, the mtUPR is also increased in fibroblasts isolated from Surf1^{-/-} and wild-type mice following treatment with paraquat, a superoxide radical generator. Interestingly, the increase in mtUPR activation corresponded to cell survival with paraquat treatment. In addition to the mtUPR activation in Surf1^{-/-} mice, there are tissue-specific increases in other pathways implicated with longevity in *C. elegans* mit mutants including mitochondrial biogenesis and AMPK activation. Our data suggest that mitochondrial alterations induce activation of cellular response pathways that could contribute to the increased longevity in this model.

112. The Effect of Temperature on Longevity Interventions in *C. elegans*

Scott Leiser (P. G), George Sutphin, Marissa Fletcher, Anisoara Begun, Melissa Primitivo, Matt Kaeberlein
University of Washington, Seattle, WA

The use of model organisms in longevity studies has provided countless insights into the biology of aging. For many model organisms, particularly poikilotherms such as fruit flies and roundworms, the interaction between temperature and lifespan is of greater importance than in homeotherms such as mammals. In the roundworm *Caenorhabditis elegans*, lifespan varies inversely with temperature, but the temperature for most experiments is selected for the ease of completion (e.g. 20° allows for bench-top culture and 25° allows for shorter experiments) rather than optimized for the intervention under investigation. Our recent work investigates the effect of temperature on the lifespan of various worms with disruptions in known aging-related pathways. Using 15°, 20°, and 25°C, the results show that while decreasing temperature increases lifespan in every intervention tested, the extent of the increase varies widely between strains. We identify interventions that have opposing effects on lifespan at different temperatures. Interestingly, 20°C, the most commonly used temperature in *C. elegans* research, showed the most variability in longevity. We believe that lifespan is highly sensitive to small changes in temperature near 20°C and may be impacted by incubators with variability or fluctuations in temperature. Our current work aims to determine common mechanisms for the variability in temperature-dependent lifespan changes. We are initially focusing on the role of heat stress response on lifespan at high temperature and the role of vulval integrity defects on lifespan at low temperature.

These experiments will provide insight into the effect of temperature on the lifespan of *C. elegans*, and will help determine which temperatures are best used for specific conditions and strains. We hope to also identify which of these mechanisms are likely to be conserved in other model organisms.

113. Mutation Burden and Aging

Alan Herr (P) and Matt Kaeberlein
University of Washington, Seattle, WA

The essence of life is the organization and regulation of genetic information encoded in DNA, honed by mutation, selection, sex, and recombination. To maintain the evolutionary innovations of the past, biological organisms invest heavily in a wide variety of DNA repair mechanisms that promote accurate DNA replication during cell division and removal of DNA damage. Mutations nevertheless accumulate in somatic cells and have been proposed to contribute to aging along with other forms of damage. What level of random mutation burden compromises homeostasis of dividing cell populations? Does mutation burden influence the aging of post-mitotic cells? We have previously shown that haploid yeast cell populations collapse when mutation rates exceed an error threshold of one inactivating mutation per essential gene per replication cycle. Here, we show that viable haploid mutator strains with sub-lethal mutation rates exhibit a reduced replicative lifespan, as measured by the number of times a mother cell can bud. We also show that diploid yeast cells are subject to an error-threshold. Using strains that have combinations of defects in polymerase nucleotide selectivity, proofreading, and post-replicative mismatch repair (MMR), we are defining the upper limit of mutagenesis in diploid yeast. These findings set the stage for determining whether inherited mutation burden synergizes with aging-associated damage to accelerate cellular aging.

114. Life Extension In The Fly: Are The Results Reproducible?

Robin Mockett (P) and Amber Nobles
University of South Alabama, Mobile, AL

This study was conducted in order to establish the reproducibility of life extension previously reported in *Drosophila melanogaster* overexpressing several different genes individually, as a prelude to testing for additivity of effects in flies overexpressing multiple genes simultaneously. The GAL4-UAS expression system was used to drive the expression of several genes involved in antioxidative defense, protein repair or removal of oxidized molecules in the nervous system or globally. Results were

obtained for flies expressing (i) *Drosophila* glutamate-cysteine ligase, catalytic subunit (GCLc), (ii) autophagy-related gene 8a (Atg8a), (iii) target of rapamycin, FRB inhibitory fragment (TORFRB), (iv) human superoxide dismutase 1 (hSOD1) or (v) bovine methionine sulfoxide reductase A (bMSRA). No appreciable life extension was observed in flies of either sex in either of two different genetic backgrounds, regardless of the spatial distribution of gene expression. These results underscore the importance of corroborating *Drosophila* longevity results in independent laboratories, as is done for dietary supplements administered to mice.

115. Caffeine Extends Life Span, Delays Age-Associated Pathology, and Improves Healthspan in Worms

George Sutphin (P, N), Emma Bishop, Melana Yanos, Matt Kaerberlein
University of Washington, Seattle, WA

The longevity of an organism is influenced both by internal (genetic) and external (environmental) factors. With respect to internal factors, a significant effort is being made to identify novel pharmacological agents that extend life span by targeting genetic pathways with a defined role in the aging process. On the external side, the molecular mechanisms responsible for the positive influence of environmental interventions, such as dietary restriction and mild stress, are being widely explored. The environment experienced by humans in modern societies already contains countless compounds that may influence longevity. Understanding the role that the most prevalent of these compounds play in the aging process will be important to predicting and interpreting the outcome of introducing new interventions. Caffeine is the most widely used psychoactive drug worldwide. Prior studies in flies, worms, rodents, and humans indicate that caffeine may positively impact age-associated neurodegenerative diseases, such as Alzheimer's. We observe that caffeine is also capable of extending life span and improving healthspan in *Caenorhabditis elegans*, a finding confirmed in a recently published screen looking for FDA-approved compounds capable of extending worm life span. We also find that caffeine delays pathology in a nematode model of polyglutamine disease. Life span extension using caffeine shows clear epistatic interaction with two known longevity interventions: dietary restriction and reduced insulin signaling. This presentation details our findings related to the use of caffeine to delay aging and age-related pathology in worms.

116. Expression of Innate Immune Response Markers After Bypass of Senescence in Human Cells

S. Kaye Smith¹ (P), Angela Sheerin², Hui-Sun Leong¹, Badr Ibrahim², Katrin Jennert Burston², Richard Faragher², David Kipling¹

¹*School of Medicine, Cardiff University;* ²*School of Pharmacy and Biomolecular Sciences, University of Brighton, UK*

Human cells are protected from immortalisation by intrinsic proliferative lifespan barriers (PLBs), the best characterised of which is cellular senescence. Senescent cells may contribute to ageing by their inability to proliferate, and though an altered pattern of protein secretion known as the senescence-associated secretory phenotype (SASP). It has been speculated that the SASP may also serve to attract immune cells and stimulate clearance of senescent cells. Here we analysed the transcriptional profiles of primary human corneal endothelial cells (HCEC), and HCEC cultures forced to bypass senescence by overexpression of cyclin-dependent-kinase 4 (CDK4), to model dysregulation of the CDK4-p16-pRb axis. HCEC-CDK4 cultures display an extended proliferative lifespan before reaching a telomere-driven PLB designated Mint-CDK4. We compared the transcriptional profiles of proliferating, quiescent and senescent HCEC, and HCEC-CDK4 both during their proliferative phase and after onset of Mint-CDK4. Clustering analysis revealed 125 transcripts that were differentially upregulated at Mint-CDK4. This cluster was enriched with interferon-stimulated genes (ISGs). Cells at Mint-CDK4 secreted elevated amounts of SASP proteins including IL-6, IL-8 and IL-1 α , and low levels of interferon- β . Treatment of early-passage HCEC-CDK4 with recombinant IL-1 α induced the majority of the Mint-CDK4-associated transcripts, including the ISGs. Separate experiments revealed that ISG expression is an inherent response of HCEC to IL-1 α . The ISG response upregulates proteins involved in antigen processing, transport to the ER, and display (via MHC Class I). Such a response could function to enhance the presentation of antigens (such as proteins carrying neoplastic sequence changes) in cells that have escaped senescence via expression of oncogenes or disruption of tumor suppressor genes. We speculate that the SASP response evolved to provide a 'dual-pronged' response to OIS; the canonical SASP promoting a generalized inflammatory response to attract effector cells of the immune system, and the ISG highlighting specific cells containing potentially neoplastic lesions for clearance.

117. Advanced Paternal Age: Activation of P53 and the Impact on APEN1 Abundance and Distribution in Spermatogenic Cells

Jamila Momand (P) and 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. Previously, 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. Spermatogenic cells isolated from Apex1 heterozygous mice 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. 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 wild type 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 previously been shown to play a role in regulating Apex1 expression. In pachytene spermatocytes obtained from p53 null mice, APEN1 expression was reduced by 40% relative to wild-type mice. Also, the activation of TRP53 was significantly increased in pachytene spermatocytes obtained from older aged animals relative to young. However, northern blot analysis revealed that the abundance 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. 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.

118. SOD1 Interacts with Macronutrients to Modulate Lifespan in Drosophila

Xiaoping Sun (P), Toshimitsu Komatsu, Jinhwan Lim, Mara Laslo, Jason Yolitz, Cecilia Wang, Luc

Poirier, Thomas Alberico, Edward Spangler, Sigé Zou

Laboratory of Experimental Gerontology, National Institute on Aging, Baltimore, MD

Lifespan is modulated by various genetic and environmental factors. Dietary macronutrients are among the most potent environmental factors that modulate lifespan. Superoxide dismutase 1 (SOD1) is a major cytosolic enzyme responsible for scavenging superoxides. Here we tested the hypothesis that SOD1 interacts with dietary macronutrients to modulate lifespan using *Drosophila* as the model organism. We found *sod1* reduction blunted lifespan extension by the high sugar-low protein (HS-LP) diet but not the low-calorie diet relative to the standard diet. The HS-LP and low-calorie diets both reduced target-of-*rapamycin* (TOR) signaling, but only the HS-LP diet increased oxidative damage. *sod1* knockdown did not affect phosphorylation of S6 kinase, suggesting that SOD1 acts in parallel with or downstream of S6 kinase. Surprisingly *rapamycin* decreased lifespan in *sod1* mutant but not wild-type males fed the standard, HS-LP and low calorie diets, whereas antioxidant N-acetylcysteine only increased lifespan in *sod1* knockdown males fed the HS-LP diet, relative to diet-matched controls. Our findings suggest that SOD1 is required for lifespan extension by protein restriction only when dietary sugar is high, and support the context-dependent role of reactive oxygen species (ROS) in aging and caution the use of *rapamycin* and antioxidants in aging interventions. This work supported by the IRP of the NIA, NIH.

119. Takeout Regulates Longevity by Modulation of Juvenile Hormone Signaling

Khalil Chamseddin, Siti Nur, Sarah Morris,

Santharam Kolli, Johannes Bauer (P)

Southern Methodist University, Dallas, TX

In order to understand the molecular mechanisms of longevity regulation, we recently performed a screen designed to enrich for genes common to several longevity interventions. Using this approach, we identified the *Drosophila melanogaster* gene *takeout*. *takeout* is upregulated in a variety of long-lived flies, and extends life span when overexpressed. Here, we investigate the mechanisms of *takeout*-dependent longevity. *takeout* overexpression specifically in the fat body is sufficient to increase fly longevity and is additive to the longevity effects of dietary restriction. *takeout* long-lived flies do not show phenotypes often associated with increased longevity, such as enhanced stress resistance or metabolic abnormalities. However, males exhibit greatly diminished courtship behavior, leading to a reduction

in fertility. Interestingly, takeout contains a binding domain for Juvenile Hormone, a fly hormone that plays a role in the regulation of developmental transitions. Importantly, the longevity and courtship phenotypes of takeout overexpressing flies are reversed by treatment with the Juvenile Hormone analog methoprene. These data suggest that takeout is a key player in the tradeoff-switch between fertility and longevity. takeout may control fertility via modulation of courtship behavior. This regulation may occur through Juvenile Hormone binding to takeout and a subsequent reduction in Juvenile Hormone signaling activity.

120. Mutation In a Complex IV Assembly Protein (sco2) Leads to Decreases In Complex IV Activity and Distinct Metabolic Phenotypes

Shauna Hill (P), Daniel Pulliam, Deepa Sathyaseelan, Yuhong Liu, Holly Van Remmen

Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio

Cytochrome c oxidase (COX) is an essential transmembrane protein complex in the mitochondrial respiratory electron chain. Mutations in genes responsible for the assembly of COX are associated with Leigh syndrome, cardiomyopathy, spinal muscular atrophy and other fatal metabolic disorders in humans. Paradoxically, mice lacking the COX assembly protein SURF1 show increased longevity associated with upregulation of mitochondrial biogenesis and stress response pathways despite significant reductions in COX activity. Here we asked whether a mouse model of cytochrome c oxidase deficiency due to a mutation in the sco2 gene, a copper chaperone that is required for the activity of COX would have similar molecular and physiologic changes. A complete knockout of the sco2 gene in mice is embryonic lethal, however mice harboring a mutated sco2 knock-in (KI) allele that is commonly found in human patients with sco2 mutations is viable, and despite the 30-60% reduction in COX activity, no significant phenotypic abnormalities are readily apparent. Interestingly, these mice have a decrease in lean mass and increase in fat mass. Preliminary evidence suggests that these mice are insulin resistant and glucose intolerant as compared to wild-type mice. The sco2 KI/KI mice also have decreased running endurance on the treadmill suggesting that these mice have muscle weakness. Interestingly, the COX-deficient mice do not have changes in the blood lactate levels suggesting that these mice do not upregulate glycolysis to compensate for decreased rates of respiration. This is counter to studies done in another

COX deficient Surf1^{-/-} mice, illuminating the complex nature of mitochondrial dysfunction on physiology. Results from this study will further our understanding of the role of complex IV in physiological outcomes due to mitochondrial dysfunction.

121. Age-Associated Declines in WNT3 and Beta-Catenin in the Brain

Kiran Chaudhari (P, N) and Nathalie Sumien

Pharmacology and Neuroscience, Institute for Aging and Alzheimer's Disease Research, UNT Health Science Center, Fort Worth, Texas.

The canonical wingless (wnt) pathway has been well characterized in the context of developmental processes and synaptic differentiation. Brain aging is associated with synaptic loss and impaired neurogenesis, and most likely involves an impaired wnt pathway. Recent studies support the potential role of wnt signaling pathway in the brain of adult mice. Increased β -catenin activity, an important component of the pathway, decreases amyloid deposition and the wnt pathway has been tied to various pathologies of Alzheimer's Disease. Furthermore, increased wnt activity by lithium improved brain function. The role of wnt in age-related brain deficits have not been evaluated, therefore this study aimed at characterizing the effect of age on canonical wnt pathway activity in different regions of the brain. It was hypothesized that wnt pathway components will be decreased with age. The brains from young (4 month) and old (24 month) C57BL6/J male mice (n=4) were dissected into six regions (cerebral cortex, cerebellum, midbrain, brainstem, striatum, and hippocampus) and homogenized in order to determine the levels of Wnt3 and beta-catenin, 2 major players of the canonical pathway, by western blot analysis. All immunoblot images were quantified densitometrically using UVP Vision Works software. The band(s) intensity was normalized against the housekeeping gene beta-actin. Preliminary results revealed declines in wnt3 and beta-catenin levels in cerebral cortex, hippocampus and cerebellum. These trends will become significant as more mice are run in this study. These preliminary data suggest a down-regulation of the wnt signaling pathway with age, and could account for the decline in motor and cognitive function associated with aging. Further studies will determine the extent of this association, and whether manipulating the pathway can reverse age-related brain dysfunction.

122. Genetic Components of the Hypoxic Response for Lifespan

Marissa Fletcher (P), Scott Leiser, Anisoara Begun, Matt Kaeberlein

University of Washington, Seattle, WA

Adapting to fluctuating oxygen environments is important for the survival of cells and organisms. The response to low oxygen (hypoxia) plays a major role in acute injury (e.g. ischemia/reperfusion), tumor formation and growth, and has recently been implicated in aging. Previous work has indicated that subjecting *Caenorhabditis elegans* to hypoxic conditions significantly increases worm lifespan. The lifespan increase is observed when worms are exposed to hypoxia either for life starting from a late larval stage or for just one day at the same larval stage. The low oxygen environment activates the hypoxic response pathway, mediated by the hypoxia-inducible factor (HIF-1). Previous data show that stabilization of HIF-1 protein, via genetic mutation or alterations in atmospheric oxygen, is sufficient to extend lifespan. However, many aspects of this pathway remain unknown, including the mechanism for increased longevity, the difference between genetic and environmental manipulations of HIF-1, and the relevant targets of the HIF-1 transcription factor. Our project examines the genetic components that are responsible for the extended lifespan in hypoxia. We measured the lifespans of various interventions in aging-related pathways under both normoxic and hypoxic conditions. Our results suggest that most aging-related pathways tested have no effect on hypoxia-induced lifespan extension. However, the data show that the forkhead transcription factor, DAF-16, is required for hypoxia to increase lifespan, and that hypoxia causes DAF-16 to enter the nucleus. Interestingly, the results indicate that hypoxia decreases lifespan when oxidative stress response factor, SKN-1, is overexpressed, suggesting that SKN-1 may compete with HIF-1 for transcriptional or other cofactors. Collectively, the results show that hypoxic treatment increases lifespan in worms, but that the hypoxic pathway is intertwined with other stress responsive pathways in the context of aging.

123. Effect of Rapamycin on Lifespan Extension of Inbred C57BL Mice and Potential Mechanisms

Yiqiang Zhang (P), Shunhua Lao, Alex Bokov, Yuji Ikeno, Holly VanRemmen, Arlan Richardson, Walter Ward

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

Down regulation of mTOR signaling by rapamycin has been shown to extend lifespan in genetically heterogeneous mice. In the present study, we investigated whether down regulation of mTOR by rapamycin extends lifespan in an inbred strain of mice, C57BL6, which has been widely used for aging research. We also investigated the potential mechanisms by which rapamycin extends lifespan. We found that feeding mice a rapamycin diet (14 mmp) extended the lifespan of C57BL6 mice, both male and female, whether initiated at 4 or 19 months of age. Body weight does not change after 6 months on rapamycin diet. However, we found the size of a few organs decreased significantly in rapamycin-fed mice, including the heart and testes. In rapamycin-fed mice, mTOR signaling decreased significantly in several tissues including adipose tissue, heart, liver and kidney, but not brain (cortex, midbrain, and hippocampus), indicated by the levels of phosphorylation of ribosome subunit S6. Correspondingly, we found in rapamycin-fed mice autophagy increased in the same tissue which mTOR signaling was down regulated while did no change in the tissue which mTOR signaling was not altered, indicated by the LC3II/LC3I ratio. We further found that proteasome activity decreased in several tissues which have increased autophagy, including heart and liver. Our data is the first to show that rapamycin has a differential effect on protein degradation; autophagy is up regulated in most tissues while proteasomal activity is reduced. It is of interest in the future to determine if the differential effect of rapamycin on protein degradation is important in the lifespan extension observed with rapamycin.

124. Five Dysfunctional Telomeres Predict Cellular Senescence in Normal Human Cells

Zeenia Kaul (P, G)

The Research Institute at Nationwide Children's Hospital, Columbus, OH

Telomeres are the nucleoprotein structures that protect chromosome ends. When this protective function is lost, a DNA damage response (DDR) occurs at chromosome termini that can be visualized by the co-localization of DDR proteins and telomeres in telomere-dysfunction induced foci (TIFs). Normal replicative senescence is associated with progressive telomere shortening in primary fibroblasts, and is thought to result from telomere DDR-induced p53 tumour suppressor protein signalling. We previously established a sensitive and quantitative technique for detecting spontaneous telomere dysfunction in human metaphase cells (meta-TIF assay). In this study, we utilized the meta-TIF assay to investigate spontaneous telomere dysfunction in primary human

cells. Normal diploid skin and stromal fibroblasts, and epithelial cells were isolated from breast tissues of five healthy donors who had undergone reduction mammoplasty, and the cells were passaged until replicative senescence occurred. Meta-TIF assays showed that a small but detectable number of dysfunctional telomeres were present in young dividing cells and that the number of TIFs increased as a function of increasing population doublings. Spontaneous telomere dysfunction resulted primarily from post-replicative processes affecting only one of a sister-chromatid pair. Although telomere length was inversely correlated with the probability that a chromosome end would become dysfunctional, we found instances where long telomeres were associated with a DDR, indicating that telomere length is not the only factor responsible for end-protection. Using the meta-TIF data, we calculated that around five-dysfunctional telomeres per cell were present in G1-phase arrested senescent cultures. Applying the same calculations to meta-TIF assay data from a panel of seventeen immortalized and cancer derived cell lines we found that immortalized cells with a functional p53 allele always had fewer than five G1-phase dysfunctional telomeres per cell, suggesting five dysfunctional telomeres is the threshold required to induce p53-dependent senescence in human cells.

125. Epigenetic Modifications are an Important Molecular Adaptation During Dietary Restriction

Michael Walsh¹ (P), Daniel Pulliam¹, Lauren Sloane², Holly Van Remmen¹

¹*Department of Cellular and Structural Biology, UTHSCSA;* ²*Barshop Institute, UTHSCSA, San Antonio, TX*

Numerous age-related changes in gene transcription have been reported in liver and other tissues, and dietary restriction can prevent many of these age-related alterations in gene expression. It is likely that epigenetic mechanisms like histone modifications contribute to these transcriptional changes. The N-terminal tails of histones are subject to a variety of covalent modifications, including acetylation, methylation and ubiquitination, and these modifications can have differential effects on gene transcription. We set out to determine age-related changes in acetylation, a common histone modification associated with gene activation. We found a decrease in total acetylation at histone H3 lysine 9 (H3K9) in the livers of 31 month old mice fed ad libitum compared to young mice, with no changes in acetylation at H4K12 or H4K16. Interestingly, dietary restriction prevented the age-related decline in acetylation at H3K9 and

independently reduced acetylation at H4K16. Since dietary restriction prevented the decrease in total H3K9 acetylation, we hypothesized that epigenetic modifications are an important molecular adaptation during dietary restriction. To test this hypothesis, we asked if we could phenocopy the effects of dietary restriction in old mice by increasing acetylation at H3K9 using the histone deacetylase inhibitor sodium butyrate (NaBu). Sixteen month old mice were fed a diet containing 5% NaBu for eight months. This treatment increased histone acetylation in the liver and other peripheral tissues. Old mice fed NaBu had reduced fat mass over time and were more glucose tolerant, consistent with known effects of dietary restriction. However, the old mice fed NaBu did not show increased insulin sensitivity as found in dietary restricted mice; rather, NaBu reduces gluconeogenesis as measured by a pyruvate tolerance test. Further studies will determine the cause of these age-related changes in histone acetylation and the molecular and functional outcomes of altered histone acetylation.

126. Growth Hormone Regulates the Expression of DNA Methyltransferase in the Ames Dwarf Mouse

Vanessa Armstrong (P, N)

University of North Dakota School of Medicine and Health Sciences, Grand Forks, ND

Methylation reactions are important for the establishment and maintenance of epigenetic methylation tags on DNA and histone molecules that are critical to the development and life-long function of an organism. Our previous work has shown the maintenance DNA methyltransferase 1 (Dnmt1), and de novo DNA methyltransferase 3a (Dnmt3a) in liver tissue, are differentially expressed transcriptionally and translationally in the Ames dwarf liver compared to its wild-type counterpart. Ames dwarf mice lack growth hormone contributing to their small size and increased longevity. We postulated that the differences in Dnmt1 and Dnmt3a between dwarf and wild type mice might be due to growth hormone. To test this hypothesis, six-month-old dwarf mice were injected with growth hormone and compared to age-matched groups of saline injected dwarf and wild-type mice using liver tissue and primary hepatocytes. Transcriptional differences were seen across all three in vivo injection groups in Dnmt1 (p=0.0054) with a modest increase in transcription from growth hormone injection. As expected from our previous work, Dnmt1 protein levels were decreased in dwarf saline-injected mice compared to wild type saline injected mice (p<0.01). However, Dnmt1 protein levels were markedly increased with growth hormone

treatment in dwarf mice exceeding protein levels in wild type saline-injected mice ($p < 0.05$). Primary dwarf hepatocytes also showed significant increases in Dnmt1 protein levels with growth hormone treatment ($p = 0.0001$). No significant differences in Dnmt3a mRNA levels were observed across in vivo injection groups in liver tissue ($p = 0.1202$) but liver protein levels were different between all three in vivo groups ($p = 0.0345$). These results suggest that Dnmt expression can be significantly regulated by growth hormone transcriptionally and post-transcriptionally and may play an important role in the longevity of the Ames dwarf mouse.

127. Development of a Brain-specific Raptor Conditional Knock-out Mouse to Study the Role of Neuronal mTOR in Aging

Stacy Hussong (P), Raquel Burbank, Veronica Galvan

University of Texas Health Science Center at San Antonio

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 expression of the mTOR complex 1 protein, Raptor, in adult mouse neurons. Conditional knock-out mice that carry homozygous floxed alleles for Raptor and the transgene for the doxycycline-inducible, neuronal-specific Cre recombinase were generated. At 2.5 months of age, the mice were treated with doxycycline. The penetrance of Raptor knock-out in different brain regions was determined. In addition, preliminary neuronal and motor function tests were carried out. PCR results showed DNA recombination was present in all brain regions regardless of doxycycline induction but not in the liver. However, Western blot data showed reduced Raptor protein in the brain regions of doxycycline-treated animals. These data along with the preliminary behavior studies confirm that this Raptor neuronal-specific, conditional knockout mouse will be a useful tool to study the role of neuronal mTOR complex 1 in aging. Future experiments will determine if decreased mTOR signaling in the mouse nervous system is sufficient to extend lifespan and improve healthspan. Included in the evaluation of healthspan will be measures of neurological function determined by behavioral and physiological analyses.

128. The Effect and Mechanism of GLP-1 Receptor Agonist in the Treatment of Diabetes-Related Alzheimer's Disease

Song Chen¹ (P), Ai-ran Liu², Feng-mao An¹, Wen-bing Yao¹, Xiang-dong Gao¹

¹China Pharmaceutical University; ²Medical School, Southeast University

Diabetes mellitus (DM) is considered to be a risk factor for age-dependent Alzheimer's disease (AD), the latter of which has even been considered as type 3 diabetes. Several physiopathological features including hyperglycemia, advanced glycation end products (AGEs), oxidative stress and dysfunctional insulin signaling relate DM to AD. In our study, high glucose-, AGEs-, oxidative stress-induced neuronal injury and intracerebroventricular-streptozotocin (ICV-STZ) animals as the possible models for diabetes-related AD were employed to investigate the effects of GLP-1 receptor agonist. Our study demonstrated that GLP-1/exendin-4 (Ex-4, a long-acting GLP-1 receptor agonist) could exert a protective effect against reduced viability of PC12 cells caused by high glucose/AGEs/oxidative stress. Bilateral ICV-STZ administration was used to produce impaired insulin signaling in the brain, and rats treated with Ex-4 had better learning and memory performance in the Morris water maze test compared with rats treated with saline. Additionally, we demonstrated that Ex-4 reversed ICV-STZ-induced abnormal tau hyperphosphorylation through down-regulation of glycogen synthase kinase 3 β (GSK-3 β) activity, a key kinase in both DM and AD. To elucidate the mechanisms of the protection of GLP-1/Ex-4 against high glucose/AGEs, we evaluated phosphorylated GSK-3 β (Ser9) and phosphorylated tau (AT8) in embryonic rat hippocampal neurons/PC12 cells. We found that high glucose/AGEs induced hyperphosphorylation of tau and activation of GSK-3 β , and treatment with GLP-1/Ex-4 could significantly reverse abnormal dephosphorylated/phosphorylated of GSK-3 β and tau. To further investigate the upstream signaling pathway involved in GLP-1/Ex-4 effects on high glucose-induced neurotoxicity, we pretreated PC12 cells with a specific inhibitor of PI3 kinase (LY294002) and a loss of GLP-1/Ex-4-mediated protective effects in PC12 cells could be observed. Our findings suggest that GLP-1 receptor agonist may prove of therapeutic value in the treatment of AD especially DM-related AD, and provide new experimental evidence for GSK-3 β as the potential target of GLP-1 receptor agonist treatment of diabetes-related AD.

129. Analysis of Changes in the Innervation of Mouse Internal Anal Sphincter During Aging
Cunfang Wang¹ (P), Michael Houghton¹, Prasanna Gamage¹, Mark Yeoman², Bhavik Patel², Richard Ranson³, Jill Saffrey¹

¹The Open university, UK; ²Brighton University, UK; ³Northumbria University, UK

The intrinsic neurons of the intestine (enteric neurons) play a crucial role in the regulation of gastrointestinal functions. Age-associated decreases in enteric neurons have been reported in mouse, rats, guinea-pig and humans. However, very few studies have investigated the impact of age on the density of nerve fibres in the intestine. Here, the density and distribution of nerves expressing different molecules that are involved in neural signalling in the ano-rectum are being examined. Key functional groups of neurons that influence contractility of the internal anal sphincter include intrinsic inhibitory motor neurons, which co-express neuronal nitric oxide synthase (nNOS) and the inhibitory neuropeptide vasoactive intestinal polypeptide (VIP), intrinsic sensory neurons, which are cholinergic and co-express the peptide calcitonin gene-related peptide (CGRP); and excitatory motor neurons, which are also cholinergic, but which co-express the excitatory neuropeptide, substance P (SP). In addition neuronal proteins, including the calcium binding protein calretinin (CR) and PGP 9.5, are used as markers of some cholinergic neurons and all enteric neurons, respectively. In this work we describe the distribution and densities of nerve fibres expressing these markers within the circular muscle and mucosal layers of the ano-rectum of male C57BL/6 mice at 3, 12, 18 and 24 months of age. Neuronal subpopulations were identified in tissue sections using conventional immunofluorescence techniques and confocal microscopy.

130. Neuron Numbers are Maintained but Nerve Fibre Density Increases in Ageing Mouse Colon
Prasanna Gamage¹ (P, G) M Saffrey¹, Richard Ranson², Bhavik Patel³, Mark Yeoman³

¹The Open university, UK; ²Northumbria University, UK; ³Brighton University, UK

Ageing is associated with a functional decline in the gastrointestinal (GI) system. This is evidenced by an increase in the incidence and severity of debilitating conditions such as faecal incontinence and chronic constipation with age. Normal functioning of the gut depends on the coordinated activity of many cell types including intrinsic neurons, known as enteric neurons, which consist of several functionally distinct subtypes. Although some previous studies have

shown a neuronal loss in the gut with age, the functional subtypes of neurons affected have not been determined. We therefore investigated neuronal subpopulations in the myenteric plexus of distal colon from C57BL/6 mice at 3-4, 12-13, 18-19 and 24-25 months of age. Neurons were identified by immunolabelling wholemounts using antisera to calbindin, nNOS and Hu C/D to identify sensory, inhibitory and total neurons respectively. We found no statistically significant changes in total neuronal numbers or in the numbers of either of the subpopulations investigated with increasing age. However the density of myenteric neurons decreased between 3-4 and 12-13 months, because of gut growth. There was evidence of neuronal atrophy, such as swollen nerve fibres, in both sensory and inhibitory neurons in 18-19 and 24-25 month animals. Therefore, the results of this study suggest that ageing does not result in a loss of these neurons in mouse colon at the ages studied. However, physiological data show that some intrinsic colonic reflexes change during ageing in these mice¹. Therefore we speculate that the decline in gut functionality with age may be in part due to changes in the properties of neurons, rather than neuronal loss.

1. Honey M.; Yeoman M. S.; et al.(2011) *Neurogastroenterology & Motility*: 23(S1) 48.

This work was supported by an Open University Charter Studentship to Prasanna.P.K.M. Gamage, and by BBSRC 'Ageing bladder and bowel' grant numbers BB/G015988/1 to MJS and RR and BB/G015147/1 to MY.

131. Ultrastructural Analysis of Changes in the Cells of the Mouse Internal Anal Sphincter During Aging

M Jill Saffrey¹ (P), Heather Davies¹, Frances Colyer¹, Prasanna Gamage¹, Bhavik Patel², Mark Yeoman², Richard Ranson³

¹The Open University, UK; ²University of Brighton, UK; ³Northumbria University, UK

Ageing is associated with increased incidence of gastrointestinal disorders, including constipation and fecal incontinence. The causes of these disorders are complex and multifactorial, but are likely to include cellular changes in the gut during aging. The gut is a complex system comprising many different cell types, including smooth muscle cells, neurons, glial cells, Interstitial cells of Cajal and epithelial cells. Analysis of aging of the cells of the gut is therefore challenging. We are investigating the changes that occur in the cells of the mouse internal anal sphincter (IAS) during aging, because this part of the gut plays

a crucial role in regulation of defecation. The muscle of the IAS is innervated by neurons of the intrinsic enteric ganglia, which, along with interstitial cells and fibroblast-like cells, play an essential role in the regulation of smooth muscle function. As part of a study to characterise age-related changes in the IAS, we are analysing the ultrastructural properties of cells of the C57BL/6 mouse anal sphincter at 3-4 and 24-25 months of age. Some samples from animals of intermediate ages will also be analysed. Possible changes in the organisation of the different cells within the IAS and their inter-relationships, as well as evidence for age-associated organelle changes and degenerative changes in different cells types are being examined. This work will provide important information about how the different component cells of the IAS may change with increasing age. Supported by BBSRC 'Ageing bladder and bowel' funding: BBSRC grant BB/G015988/1 to MJS & RR and BB/G015147/1 to MY.

132. A Dominant Negative Splice Variant of ERbeta and the Effective Post-Menopausal Estrogen Therapy

Jun Ming Wang (P), Raveena Reddy, Jonathan Fratkin, Baoying Zheng

University Mississippi Medical Center, Jackson, MS

Background: The molecular mechanisms for the discrepancy in outcome of initiating estrogen therapy (ET) around peri-menopause or several years after menopause in women are unknown. Our recent work in OVX female rats in which ET was initiated either 6 days (Early ET, analogous to 4 months post-menopause in humans), or 180 days (Late ET, analogous to 11 years post-menopause in humans) after ovariectomy suggest that the ovarian hormone-deficient state during menopause increases the expression of this dominant negative isoform, ERbeta2, which leads to reduced efficacy of ET. The current work is to identify the age-dependent ERbeta2 expression in dentate gyrus in autopsy of subjects in three different ages: younger than 35 (3 subjects), 45-60 (4 subjects), and older than 75 of age (4 subjects). Re-cuts were made from the formalin-fixed, paraffin embedded tissue blocks and immunoperoxidase stains for human ER beta and its splicing variant, human ERbeta2 were performed. The testicular sections used as control reacted appropriately. The numbers of ERbeta and ERbeta2 expression cells in dentate gyrus were analyzed using unbiased stereology module of SlideBook. Results: Our results showed that, in each mm², there were 733 ± 126 ERbeta-IR and 530 ± 761 ERbeta2-IR in dentate gyrus of women age younger than 35, 499 ± 61 ERbeta-IR and 735 ± 193 ERbeta2-IR in women

age between 45 and 60 of age, and 1076 ± 272 ERbeta-IR and 931 ± 338 ERbeta2-IR positive cells in women older than 75. Conclusions: These preliminary results showed a significant higher number of ERbeta2, the dominant negative splice variant of ERbeta in dentate gyrus of women more than 20 years in post-menopause, suggesting that the dominant negative splice variant of ERbeta may play a key role in post-menopausal estrogen therapy.

133. Diabetic-Dependent Loss in Nerve Conduction: Role of Myelin Protein, Lipid and Cholesterol Availability And Integrity

Ryan Hamilton (P, G), Michael Walsh, Yun Shi, Arunabh Bhattacharya, Alex Bokov, Asish Chaudhuri, Holly VanRemmen

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

Diabetic peripheral polyneuropathy is a major consequence of elevated blood glucose which leads to dysfunction of the lower limbs. In humans and mice this pathology includes neuronal dysfunction, Schwann cell dysfunction, axonal degeneration and chronic motor and sensory neuron demyelination. Therefore, we hypothesize that altered myelination and impaired nerve conduction velocity in diabetes occurs as a result of changes in myelin protein and cholesterol/lipid integrity (availability and oxidation/aggregation). To test this, we isolated sciatic nerves from 5 month C57Bl/KS littermate controls and diabetic mice for Western and EM analysis after assaying sciatic nerve conduction velocity (NCV). Diabetic mice demonstrated a reduction in NCV (0.73 ± 0.05-* fold), an increase in g-ratio (1.13 ± 0.01 fold, axon/fiber diameter) and a reduction in myelin thickness (0.73 ± 0.01-* fold). Sciatic nerve Western blot analysis showed increased levels of myelin basic protein (1.75 ± 0.30-* fold), reduced levels of peripheral myelinating protein 22 (0.57 ± 0.09 fold-*), decreased levels of the rate limiting cholesterol synthesis enzyme hydroxy methyl glutaryl coenzyme A reductase inhibition (1.62 ± 0.05-* fold), and myelinating transcription factor krox-20 (1.57 ± 0.12-*) consistent with increased myelin basic protein. Myelin basic protein carbonyls (2.85 ± 0.88-* fold) in sciatic nerve, and isoprostanes in spinal cord (1.28 ± 0.05-**) were increased indicating increased oxidative damage associated with demyelination. Rate limiting lipid synthesis (ACC 0.67 ± 0.04-* fold), lipid/cholesterol trafficking (ApoE, 0.50 ± 0.12-* fold), and lipid/cholesterol uptake (LDL-R, 0.59 ± 0.07-* fold) were reduced. These data suggest that reduced myelin thickness and nerve conduction velocity associated with diabetes may be associated with the

following findings: 1) an imbalance between myelin proteins and lipids/cholesterol synthesis, 2) a severe reduction in lipid/cholesterol trafficking and uptake, and 3) modifications of myelin protein and lipids that disrupt both hydrophobic and electrostatic interactions.

134. Determination of an Oral Dosage of Vitamin C That May Prevent Recurrence of Superficial Bladder Carcinoma

Alfred Ordman (P)

Beloit College, Beloit, WI

In 1994, my group published in AGE the determination of an optimal dosage of vitamin C (AA), which produces the highest serum level before the kidneys remove it. That dosage is 500 mg BID, resulting in 0.1mM AA in the serum. This dosage may reduce free radicals and chronic disease, as proposed by AGE founder Denham Harman. AA also kills cancer cells. The mechanism hypothesized is generation of extracellular hydrogen peroxide catalyzed at the cancer cell membrane. In vitro, this requires above 1mM AA. For treating most cancers in vivo, this can be achieved transiently through intravenous AA. This enhances the effectiveness of many chemotherapies. For superficial bladder carcinoma (SBC), after surgical removal of the cancerous cells, chemotherapy is instillation of Bacillus Calmette-Guerin (BCG). The recurrence rate with this therapy is 85%. Recurrence routinely requires bladder replacement. But prior to our discovery that AA must be consumed twice daily to maintain high levels, high dosage vitamins including AA reduced recurrence to 40%. Despite the concern of many physicians that a high intake of AA might increase the risk of kidney stones, individual trials and metastudies indicate that AA supplements actually decrease the risk of kidney stones. The major inconvenience of taking AA supplements found in 2010 is that 10% of those consuming them experience LUTS. I investigated the oral dosage of AA that maintains the highest level of AA in the bladder. Varying the level and interval between dosages, I found AA above 2g BID were not absorbed. That dosage provided urinary concentrations of 6 mM, higher than needed to kill cancer cells. By consuming 2g BID two days each week, one may minimize the risk of LUTS while maintaining bladder [AA] likely to kill recurring cancer cells.

135. Cranberry Extract Promotes Longevity and Modulates Stress Response in *Caenorhabditis elegans*

Yuqing Dong¹ (P), Sige Zou², Sujay Guha¹, Min Cao¹, Ryan Kane¹, Anthony Savino¹

¹*Clemson University*; ²*NIA*

Nutraceuticals are known to have numerous health and disease preventing properties. Recent studies suggest that extracts containing cranberry may have anti-aging benefits. However, little is known about whether and how cranberry by itself promotes longevity and healthspan in any organism. Here we examined the effect of a cranberry only extract on lifespan and healthspan in *Caenorhabditis elegans*. (*C. elegans*) Supplementation of the diet with cranberry extract increased lifespan in *C. elegans*. Cranberry also increased tolerance of *C. elegans* to heat shock, but not to oxidative stress or ultraviolet irradiation. In addition, we tested the effects of cranberry extract on brood size and motility and found that cranberry did not influence these behaviors. Our mechanistic studies indicated that lifespan extension induced by cranberry requires the insulin/IGF signaling pathway and DAF-16. We also found that cranberry extract (CBE) promotes longevity through osmotic stress resistant-1 (OSR-1) and one of its downstream effectors UNC-43 but not through SEK-1, a component of the p38 MAP kinase pathway. We found, however, that SIR-2.1 and JNK signaling pathways are not required for cranberry to promote longevity. Our findings indicate that cranberry supplementation confers increased longevity and stress resistance in *C. elegans* through pathways modulated by daf-16 and osr-1. This study reveals the anti-aging property of widely consumed cranberry and elucidates the underpinning mechanisms. Our findings provide a foundation for developing effective botanical interventions with cranberry to promote healthy aging in humans.

136. The Dietary Effects of High-Fat/ High-Sucrose Feeding in PAPP-A (-/-) Mice

Cristal Hill¹ (P), Oge Arum¹, Michal Masternak², Cheryl A Conover³, Andrzej Bartke¹

¹*Southern Illinois School of Medicine*; ²*Burnett School of Biomedical Sciences, University of Central Florida*; ³*Endocrine Research Unit-College of Medicine Mayo Clinic*

Longevity and aging are influenced by insulin-like growth factor-1(IGF-1) and insulin signaling, which share common intracellular signaling pathways. Bioactive IGF-1 increases growth in cancers and may contribute to metabolic diseases such as insulin resistance. Enhanced availability of IGF-1 is

promoted by cleavage of IGF-binding proteins (IGFBPs) by proteases, including the pregnancy-associated plasma protein-A (PAPP-A). However, the effects of diets that promote obesity and increase pro-inflammatory cytokines, leading to increased expression of PAPP-A, remain to be elucidated. We hypothesized that PAPP-A(-/-) mice should be more resistant to the damaging effects of a high-fat/high-sucrose diet. Four experimental groups of 12-14 months-of-age PAPP-A(-/-) (n=10) and normal-littermate (n=10) females were fed either a low-fat (11% kcal)/low-sucrose (73.1% kcal-cornstarch) diet or a high-fat (58% kcal)/high-sucrose (25.5% kcal) diet for 12 weeks. GTT revealed that PAPP-A(-/-) mice fed a high energy diet are more glucose tolerant than normal-littermates fed a low energy diet (P<0.05). Neither genotype showed altered insulin sensitivity when fed either diet, as reflected in the ITT. The high energy diet increased IGF-1 levels in PAPP-A(-/-) mice compared to littermates(-/-) fed a low energy diet (P<0.002). There was no diet effect on IGF-1 levels in normal-littermates when fed either diet. The data above suggest that the PAPP-A(-/-) female mice are resistant to the effects of high-fat/high-sucrose feeding. These mice live 30% longer than their normal littermates and have decreased bioactive IGF-1 on normal diets. Furthermore, a high energy diet does not affect the IGF-1 levels in PAPP-A(-/-) mice when compared to normal littermates possibly altering insulin/IGF-1 signaling. In addition, high energy fed PAPP-A(-/-) females have improved ability to assimilate glucose during GTT compared to their normal littermates. Thus, PAPP-A has a role in IGF-1 and insulin signaling, as well as in diet-induced metabolic dysfunction; and therefore PAPP-A expression may alter longevity. Cytokine data are currently being analyzed to further investigate the effects of diet in both genotypes.

137. Cognitive and Psychomotor Impairments of Aged Mice are not Improved by Treatment with Metformin

Margaret Rutledge (P), Nopporn Thangthaeng, Nathalie Sumien, Michael Forster
University of North Texas Health Science Center, Fort Worth, TX

Metformin, a biguanide, has been suggested as a plausible caloric restriction mimetic (CRM) based on its ability to confer a similar gene-expression profile, extend life-span, and delay age-associated losses of physiological function. The purpose of the current study was to assess the functional consequences of metformin in mice, when relatively short-term treatment was initiated at various ages. Male C57BL/6J mice (aged 4 mo, n=32; 11 mo, n=32; 22

mo, n=36) were assigned to either a metformin group (MET, administered 2 mg/ml in drinking water) or a control group (CON), and a battery of behavioral tests was initiated after the first month of treatment. The MET groups generally weighed slightly less, ingested less food, and drank less fluid than CON mice, but no difference in blood glucose was observed. Significant age-related declines for most variables were evident in the behavioral test performance of both groups, with no indication of a significant MET-related improvement. In a Morris water maze test, the CON and MET groups failed to differ during acquisition at any age, whereas deficient performance in recall was evident in the old MET group when they were tested after a 7-day delay. Additionally, a mild but significant impairment in visual acuity was observed in MET-treated mice. A survey of biochemical assays targeting activity of enzymes involved in redox homeostasis was performed on liver, cerebral cortex, and hippocampus. In the cortex of middle-aged and old mice, metformin was associated with reduced superoxide dismutase activity; no other assays yielded significant effects. In this study, metformin did not improve psychomotor or cognitive performance, and thus failed to mimic the beneficial effects of short-term caloric restriction. Instead, our results suggest that metformin may be associated with negative effects on long-term memory and visual acuity.

138. 2002-2012 Wild Blueberry Health Study Ten Year Report

Anthony Wetherell¹, Roseanne Schnoll², Barrie Sachs³, Diana Burns⁴, David Doiron⁴, Howard Raphaelson⁴, Alec Pruchnicki⁵, Dana Mathew⁶, Bruce Kristal⁷, Rozlyn Krajcik⁸, Steve Massardo⁸, Ines Augie⁸, Norman Orentreich⁸, Joseph Vogelman⁸, Amy Kokesh⁹, James Joseph¹⁰, Kathy Hull¹¹, Kurt Gerstmann¹², Richard Coppings¹³, Rolf Martin¹⁴ (P)
¹*Defence Science & Technology Laboratory (Retired), UK;* ²*Dept. of Health & Nutr, Sci, Brooklyn College/CUNY, Bklyn, NY 11210;* ³*Happy Rainbows Herbs, Teas and Crystals, Sherman, CT 06784;* ⁴*Mansfield Senior Center, Mansfield, CT 06268;* ⁵*St. Luke's Roosevelt Hospital, New York, NY 10019;* ⁶*New Fairfield, CT 06812;* ⁷*Weymouth, MA 02189;* ⁸*Orentreich Foundation for the Adv of Sci, Cold Spring, NY 10516;* ⁹*Raleigh, NC 27604;* ¹⁰*Human Nutr Res Ctr on Aging at Tufts University, Boston, MA 02111;* ¹¹*New Fairfield Senior Center, New Fairfield, CT 06812;* ¹²*New York, NY 10003 and Sherman, CT 06784;* ¹³*Lane College, Jackson, TN 38301;* ¹⁴*MMT Corporation*

Background: Inspired by the 1999 results of Joseph et al. (J. Neurosci;19(18):8114-21) and by Jim Joseph himself, we began evaluating wild blueberry health effects via the Internet in 2002. Methods: Recruitment, design, measurements, information-OMICs and statistics are described at Blueberrystudy.com, in patents 6,712,615 and 8,095,480, and in these annual proceedings from 2003-2012. Results: Decision-speed improved 4.2% within one week and maintained this gain over four weeks [N=97; p=0.024]. Self-reported sharpness, energy, aches, peacefulness, mood, sleep quality and overall health all improved within four weeks [N=99; p for each<0.02]. Odds of word recall score improvement vs. decline were 7 to 1. Year-after-year word recall scores generally improved by 0.5-1% per year until scores of 100% correct were obtained. The rate of year-after-year improvement was unrelated to the number of measurements each year. Individuals measured memory score changes as small as 0.5% per year within their personal data at 95% confidence by conducting 150 or more measurements/year. In ten years no adverse effect was attributed to blueberries. Glutathione-S-transferase, a detoxification enzyme related to resistance to Alzheimer's and Parkinson's diseases, was detectable in 0/12 participants before and 5/12 after two months of daily blueberry consumption (1 cup/day). Serum glutamic oxaloacetic transaminase increased slightly by 18% (p=0.03 without correction for multiple comparisons). Significant decreases (p<0.05) were observed for carcinoembryonic antigen, gamma-glutamyl transferase, interleukin-1 β , total cholesterol and uric acid. Conclusions: Wild blueberries can be safely consumed each day for many years. Word recall scores for long-term participants improved steadily until reaching 100% correct. Internet measurements can be more precise than laboratory measurements because averaging scores over time removes good-day/bad-day effects. Participants located primarily in CT, land of steady habits, were willing to conduct 10 to 180 measurements each year in exchange for blueberries, in major part because of their dedication to gold-standard science.

139. 2002-2012 Wild Blueberry Health Study Report: Maximizing Statistical Power Per Minute of Measurement

Rolf Martin¹ (P), Diana Burns², David Doiron², James Joseph³, Bruce Kristal⁴, Howard Raphaelson², Joseph Vogelman⁵, Anthony Wetherell⁶
¹MMT Corporation; ²Mansfield Senior Center, Mansfield, CT 06268; ³Human Nutr Res Ctr on Aging at Tufts University, Boston, MA 02111; ⁴Weymouth, MA 02189; ⁵Orentreich Foundation for the Adv of

Science, Cold Spring, NY 10516; ⁶Defence Science & Technology Laboratory (Retired), UK

Background: To our knowledge, Blueberry Health Study participants have collected more performance data over more years with greater statistical power than have been reported elsewhere. Individual participant's data sets document year-to-year score changes as small as 0.5% to 1% at 95% confidence if 150 or more repeated measurements are conducted each year. It thus seems appropriate to devote part of our ten-year report to methods that made both this power and compliance longevity possible. Methods: During planning in 2001, JJ insisted we have solid controls for changes in participant motivation. His concern and ours was that motivation changes over time would inevitably cause scores to reflect both expectations and intervention effects. Participants might concentrate more, even if blind to their scores, according to current expectations and desire to improve. Even if expectations and motivation did not change, we would never know unless such changes were carefully measured. AW recommended measuring expectations by asking online participants to report how much they expected their scores to improve during and between measurement sessions. JJ agreed and we began collecting one month of pre-intervention, repeated baseline measurements. AW gave us another critical recommendation: measure only vertical finger movements, not horizontal, because horizontal movements add too much variability to response times. We have since learned that this advice cuts within-person standard deviations to less than half, boosting power proportionately. RM minimized between-stimulus delays to increase almost ten-fold the number of responses collected per minute and shorten measurement sessions to less than five minutes. Motivation changes were also measured independently by monitoring decision-speed errors. Client-side JavaScript was added to warn participants whenever error rates rose and also to check computer performance after each keystroke, to ensure relatively accurate measurement of response times. For email guidance sent to participants, information-OMICs and additional power-enhancement methods, please visit Blueberrystudy.com/Methods.

140. US Multiple-Cause-of-Mortality Data Connect Osteoporosis to ICD10 Illness Categories Associated with Polyphenol Consumption Rolf Martin¹ (P), Howard Raphaelson², J. Wesson Ashford³

¹MMT Corporation; ²Mansfield Senior Center, Mansfield, CT 06268; ³Stanford / VA Aging Clinical Research Center, Palo Alto, CA 94304

Background: Laboratory and human epidemiological and intervention studies indicate that polyphenol-rich foods may significantly reduce age-associated disease. For example blueberry supplements doubled survival after lab-induced heart injury to rats (Ahmet et al. 2009/PMID:19936253) and reduced stroke damage by half (Sweeney et al. 2002/12509072). Brown rice and blueberries are also associated with 16% and 23% diabetes risk reductions (Sun et al. 2010/20548009; Wedick et al. 2012/22357723). Following the report by Zhang et al.(2011/21912699) that blueberry supplements prevent experimental osteoporosis, we examined NCHS-CDC Multiple-Cause-of-Mortality (MCO) records to prepare a preliminary map of possible polyphenol-associated illness categories. Methods: 2005-2009 MCO records were scanned with market research software developed at Eric Marder Associates to select osteoporosis (ICD10 M81) records and tally all comorbid conditions for ICD10 categories A-Z within M81 records and separately within all records. Results were obtained in 5-year ranges from 75 to 100 to avoid chance associations based on age. Below age 75, osteoporosis and Alzheimer's records are too few for adequate power. Nursing home records were tallied separately to control place of mortality. Results: The following illness categories are overrepresented in osteoporosis-containing records (compared to all records within each age bin) and are therefore associated with osteoporosis and possible polyphenol deficiency: ICD10-B (infections/parasites) odds ratio 1.81/p=0.0005; D (neoplasms/blood/immunity) 2.29/p<0.00001; E (endocrine/nutritional/metabolic) 1.70/<0.00001; E11 (non-insulin dependent diabetes) 1.17/0.00003; E6 (obesity) 1.77/0.0005; F (mental/behavioural disorders including hyperactivity and autism) 1.52/<0.00001; G (nervous system/Alzheimer's/Parkinson's diseases) 1.42/<0.00001; G1/G2/G3/F03 (Alzheimer's/related dementias) 1.42/<0.00001; H (vision/hearing/adnexa) 7.17/<0.00001; I (circulatory/heart disease and stroke) 1.12/<0.00001; J (respiratory) 1.25/<0.00001; J44 (COPD) 1.47/<0.00001; J45 (asthma) 3.56/0.02; K (digestive) 2.31/<0.00001; L (skin/infections) 1.78 p<0.00001; M06 (rheumatoid arthritis) 5.46 p=0.039; N0 (nephritis) 1.50/0.045; R/S/T/W/X/Y (misc/injury/poisoning/accidents/external) 1.31-2.74/<0.012. Conclusions: Demonstrated relationships between polyphenols and osteoporosis and between osteoporosis and other ICD10 categories suggest that low polyphenol consumption may contribute to a majority of ICD10 illness categories.

141. Protective Effects of Berries on Brain Against Radiation-Induced Tau-Hyperphosphorylation and Ubiquitin Aggregates
Shibu Poulouse¹ (P, G) Donna Bielinski¹, Stacey Gomes¹, Bernard Rabin², Kirsty Carrihill-Knoll², Barbara Shukitt-Hale¹

¹USDA - ARS, HNRCA at Tufts University;

²Department of Psychology, University of Maryland Baltimore County, Baltimore, MD

Aging is the universal risk factor for most of the chronic and acute pathologies in the central nervous system (CNS). Aging is also associated with increased oxidative stress and inflammation in the brain, leading to a cascade of altered signaling and ultimately to the progressive degeneration and death of neurons. Overactive tau kinases in brain and resultant intracellular inclusions that consists of paired helical filaments (PHF) with abnormally hyperphosphorylated tau (PHF-tau) has been a clinical hallmark of Alzheimer's disease as well as other diseases of CNS origin. Blueberries and strawberries contain an array of phytochemicals, which have the potential to ameliorate oxidative stress and/or inflammatory assaults on brain cells. In this context we have investigated whether feeding rats with blueberry- or strawberry-supplemented diets, followed by irradiation with high energy and charge (HZE) 56Fe particles, a model for accelerated aging, would elicit protective effects in the brain. Irradiation disrupted autophagy related proteins in the hippocampus and striatum and caused substantial hyperphosphorylation of tau proteins in both striatum and hippocampus. Supplementing rat diets with blueberries or strawberries, prior to irradiation, protected these brain regions against hyperphosphorylation of tau proteins; it also reduced the accumulation of polyubiquitinated aggregates by inhibiting the phosphorylation of mTOR, activating other proteins such as Beclin 1 and ATG7, and enhancing the turnover of MAP1B-LC3 as well as P62/SQSTM1. The results offer new insights on the potential role of blueberries and strawberries in disease prevention and brain health.

142. Controls on Lifespan Extension by Caloric Restriction: Type of Food Restriction, Reproductive Mode, and Evolutionary History
Kristin E. Gribble (P) and David B. Mark Welch
Marine Biological Laboratory, Woodshole, MA

While caloric restriction (CR) is the only intervention known to increase lifespan in a wide range of taxa, the origin of, and mechanisms controlling, this phenomenon remain unknown. We are investigating how closely related species of the well-studied

monogonot rotifer genus *Brachionus* from different environments vary in their responses to stimuli that alter lifespan, including caloric restriction (CR), and are using differential transcriptomics of the diverse phenotypes to dissect the genetics and evolutionary origins of aging. We find that different mechanisms control the response to chronic caloric restriction (CCR) and intermittent fasting (IF) and that diverse evolutionary pressures are acting on aging pathways in sexual and asexual females. Asexual females increased lifespan by 50% under both CCR and IF, but sexual females increased lifespan under IF only. Lifetime fecundity increased with increasing CCR and IF for both reproductive types, though the proportion of lifespan devoted to reproduction remained constant under CCR and significantly increased under IF for asexual females. Tests of CCR and IF on five isolates of *Brachionus*, originating from different lakes in Spain with varying historical resource dynamics, provided evidence that evolutionary history can shape the lifespan response to CR. While some isolates had no change in lifespan or reproduction with CCR at 10% of *ad lib.* feeding or with IF, others showed an increase in lifespan with both 10% CCR and IF, and had an increase in lifetime fecundity under CCR. In yet other isolates, longevity increased under CCR but significantly decreased due to IF, and reproduction declined in both CR treatments. Future studies will examine the differential transcriptomics of isolates shown to have significant positive, lack of, and negative response to CCR or IF, to determine which genes are involved in CR-mediated lifespan extension.

143. Treatment Compliance to Diabetes Care Among Older Adults: A Cross-Sectional Study Liaquat Khowaja (P)

Aga Khan University

Introduction Diabetes has been increasing worldwide and this disease usually occurs among the older age (over 35 years) population. Appropriate self-care practices including lifestyle modifications and medication compliance are critical to satisfactory control and manage diabetes. The objective of this study was to assess current treatment patterns, treatment compliance and reasons for noncompliance in patients with type 2 diabetes in primary care diabetes centers in Pakistan. **Methodology** A cross-sectional study was conducted to collect data through structured interviews based on pre-tested questionnaire. Total 183 patients including 46% males and 54% females from the ages 35 and over were randomly selected for the study from a population of patients attending 5 primary care

diabetes centers. Information was collected on socio-demographic characteristics, duration of diabetes and compliance to physician's advices. **Results** Overall, median age for male subjects was 53.8 years, significantly older ($p=0.008$) than females (47.7 years). The mean duration of diabetes among respondents was 9.9 ± 6.3 years. Only 28% reported full compliance as per physician's advice for timely medication, routine exercise, timely lab investigation and follow up for next visit. Nearly half (47%) of the subjects were taking medication for diabetes related complications. Lack of financial resources, lack of knowledge, lack of supportive services and fear of needle were the main reasons for not following physician's advice. We also found that people with low literacy levels were less likely to manage their condition effectively compared to people with higher educational level ($p<0.005$). **Conclusion** To manage the diabetes both physicians and patients should attempt to improve compliance, which could lead to better disease management. Based on the findings, a public health intervention and information campaign is needed to change behaviours of persons with diabetes. Further, physicians can also educate patients by identifying potential risks of non-compliance and educate them accordingly.

144. Visceral Adiposity is Associated with Expression of SIRT1 in Peripheral Blood Mononuclear Cells

Ji Won Lee¹ (P) Hang kyu Lee², Sang Hui Chu²

¹*Yonsei University College of Medicine Severance Hospital;* ²*Clinical Nursing Science Yonsei University College of Nursing*

Objective: The sirtuin1 (SIRT1) is activated during calorie restriction and has been related to energy balance through glucose or lipid metabolism. These findings suggest that SIRT1 may play a role in the pathophysiology of visceral obesity. Therefore, we investigated the relationship between SIRT gene expression in circulating peripheral blood mononuclear cells (PBMCs) and abdominal visceral adiposity as measured by computed tomography (CT). **Patients:** We recruited 43 men and women without histories of diabetes or cardiovascular disease. **Measurements:** Biomarkers of metabolic risk factors, body composition by computed tomography were assessed. SIRT1 levels were measured by RT-PCR using peripheral blood mononuclear cells. **Results:** The SIRT1 levels negatively correlated with the BMI, waist circumference, visceral fat areas and HOMA-IR levels and positively correlated with adiponectin. By step-wise multiple regression analysis, visceral fat area and HOMA-IR were selected as explanatory variables accounting for

SIRT1 levels, explaining 37% of the variance in SIRT1 levels. Conclusions: Our findings suggest that SIRT1 may be a mediator that links insulin resistance, visceral obesity to cardiovascular risk factors. This work was supported by a National Research Foundation of Korea grant funded by the Korea government (MEST) (2011-0013909).

145. Pro-Longevity Interventions Can Hide Among Commonly Prescribed Medicines

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

Accumulating evidence suggests that some of the commonly prescribed medicines may have significant beneficial effects on longevity and/or markers of physiological aging that span beyond of their immediate therapeutic action. Such effects can be modulated by genotype and age at treatment and can readily be estimated from available longitudinal human data. The goal of this study was to evaluate the influence of candidate pro-longevity medicines (cholesterol-lowering drugs, ACE inhibitors, thyroid drugs) on long-term survival in humans, taking into account individual genotype and age at treatment, and paying attention to potential trade-offs (such as improved survival at some ages but worsened at other, for the same treatment). For these analyses we used data from Framingham Study containing rich genetic and phenotypic information on about 15,000 of individuals. The candidate medicines were selected based on current knowledge about their biological effects and on results of our own preliminary studies. We found that, for carriers of particular genotypes, beneficial effects of these medicines on survival are substantial and sometimes exceed those observed in studies of other candidate pharmacological interventions into aging/longevity, such as with rapamycin, resveratrol, metformin and spermidine. For other genotypes, the pro-longevity effect of a medicine disappears and even becomes negative. Similarly, the age at treatment may non-monotonically influence the effect of a medicine. While anti-aging/pro-longevity interventions that work in all people and at all ages may exist, our study suggests that some of already approved medicines could be used as pro-longevity interventions on individual basis.

146. Effects of Turning-Based Treadmill Training on Balance and Turning Performance in Subjects with Chronic Stroke

Ray-Yau Wang (P), I-Hsuan Chen, Yea-Ru Yang
National Yang-Ming University

Background. Ability to turn safely is an essential aspect of functional ambulation. Given the inter-limb asymmetry and additional balance requirement, turning performance is compromised in subjects after stroke. However, the specific training program for enhancement of turning performance has not yet been established. **Objectives.** This study aimed to investigate effects of turning-based treadmill training on muscle strength, balance, and turning performance in post-stroke subjects. **Methods.** Eighteen subjects with chronic stroke (onset > 6 months) were randomized into an experimental (n=9) and a control (n=9) group. Subjects in the experimental group underwent 12-session of turning-based treadmill training, whereas subjects in the control group received regular treadmill training for 12 sessions. The muscle strength, balance, and turning performance were measured at pre-training and post-training. The muscle strength of lower extremities was measured by the hand-held dynamometer. Functional balance was indicated by Berg Balance Scale. Dynamic balance ability was assessed using the Balance Master system. Turning performance including velocity and cadence were assessed while turning toward the affected and unaffected side. **Results.** The turning-based treadmill training resulted in significant increase in maximal excursion of dynamic balance and turning velocity. Both the regular and turning-based treadmill training resulted in an improvement in functional balance and a decrease in cadence during turning. However, the muscle strength, except the hip flexors, did not change significantly in both groups. **Conclusion.** Our results indicate the turning-based treadmill training is beneficial for turning and balance performance. Therefore, the turning-based training is suggested to be one strategy to improve turning ability in patients with chronic stroke.

147. Simple Syntheses of Diverse Polyphenolic Compounds – Facilitating Dissection of Multiple Modes of Activity

L. Searle, G. Dichello, RGA Faragher and E.L. Ostler (P)

School of Pharmacy and Biomolecular Sciences, University of Brighton, Brighton, UK. BN2 4GJ. email: e.ostler@brighton.ac.uk.

There is growing evidence that many of the deleterious processes of aging may be modulatable by simple chemical compounds. The potential to develop orally available anti-degenerative medicines has consequently excited intense interest from researchers in recent years. A wide variety of polyphenolic natural products, often isolated from “super-foods”, have been identified as potential lead

compounds for anti-aging therapeutics. Such compounds have included examples of flavonoids, lignans and stilbenes. A common motif in such compounds is a central (trans-substituted) double bond positioned between two phenolic groups. It has been suggested that such compounds may exert positive effects via antioxidant activity. However, it should not be forgotten that many of these have multiple other activities, including oestrogenic effects, and some can even interrupt DNA synthesis and repair via intercalation. Consequently, there is a need to dissect the diverse bioactivities of potential therapeutic compounds via detailed structure-activity relationship (SAR) studies. Accordingly we have established simple, robust and high-yielding syntheses to facilitate access to a broad range of structural variants of compounds containing this motif. Our syntheses rely on simple and rapid (<24h) reactions without the need for air sensitive reagents or complex purification strategies. Characterisation of the *in vitro* activity of the compounds synthesised to date is ongoing. Available compounds, synthetic methods and quantified activities will be presented.

148. Synthesis and *in vitro* Biological Evaluation of Substituted Phenylpiperazine Analogues

Maninder Malik¹ (P), Yogesh Mishra¹, Robert Mach², Robert Luedtke

¹University of Texas Health Science Center at San Antonio, San Antonio, TX; ²Washington University, St. Louis, MO

Purpose: Substituted phenylpiperazines of varying intrinsic efficacy (antagonists, partial agonists and full agonists) were found to attenuate L-dopa-associated abnormal involuntary movements (AIMs) in 6-hydroxydopamine (6-OHDA) unilaterally lesioned male Sprague Dawley rats, which is an animal model of L-dopa-induced dyskinesia (LID). In the course of our studies, we noticed that antagonists, partial agonists and full agonists at dopamine D3 receptors were all effective in attenuating AIMs. However, adverse side effects were observed when testing compounds a) of <100-fold D3 vs. D2 receptor subtype selectivity or b) designated as antagonists by the cyclase assay. Therefore, in an attempt to obtain compounds, which exhibit high selectivity for D3 vs. D2 dopamine receptors, a panel of new compounds was synthesized and tested for affinity and intrinsic efficacy at human D2Long or D3 dopamine receptor subtype. Method: A filtration-binding assay was used to characterize the binding properties of novel dopaminergic compounds at human D2 and D3 dopamine receptors. Competition curves were performed using 125I-IABN and nonspecific binding was defined using (+)

butaclamol. The intrinsic efficacy of these novel dopaminergic compounds was determined using a forskolin-dependent adenylyl cyclase inhibition assay with transfected HEK 293 cells expressing either the human D2Long or D3 dopamine receptor subtype. Result: Novel substituted phenylpiperazine analogues exhibited lower affinity and selectivity but higher intrinsic efficacy at D2Long and D3 dopamine receptor subtype. Conclusion: In our previous studies fewer side effects were observed when compound was designated as antagonist by the cyclase assay. Since, substituted phenylpiperazine analogues exhibit higher internal efficacy at D3 dopamine receptor it might represent a new class of potential pharmacotherapeutic agents for the treatment of L-dopa-associated dyskinesia in patients with Parkinson's Disease.

149. Rapamycin Improves Mitochondrial Function and Insulin Sensitivity in a Mouse Model of Type 2 Diabetes

Deepa Sathyaseelan (P), Ryan Hamilton, Michael Walsh, Daniel Pulliam, Yun Shi, Shauna Hill
Barshop Institute for Longevity and Aging Studies, San Antonio, TX

The effect of Rapamycin (RAPA) on lifespan extension in mammals is well established. Even though RAPA ameliorates many age-associated diseases in animal models, studies have shown that administration of RAPA increases insulin resistance in normal mice suggesting that treatments based on mTOR inhibition will cause exacerbation of diabetes. The current study was designed to test the effect of RAPA on insulin sensitivity in db/db mice, a mice model for diabetic dyslipidemia. Administration of RAPA for 9 months, starting at 2 months of age, significantly reduced body weight and fat mass in female db/db mice compared to db/db mice fed the control diet (Eudragit). Levels of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1a) and mitochondrial proteases Lon and ClpP were significantly down-regulated in the white adipose tissue of db/db mice and administration of RAPA restored the levels of PGC-1a and Lon, but not ClpP, in the adipose tissue of db/db mice. Levels of antioxidant enzymes glutathione peroxidase 1 (Gpx1), peroxiredoxin 3 (Prdx3) and superoxide dismutase 1 (SOD1), but not mitochondrial superoxide dismutase 2 (SOD2), were lower in the white adipose tissue of db/db mice and RAPA feeding up-regulated SOD2 levels while levels of Gpx1, Prdx3 and SOD1 remain unaffected. Insulin receptor substrate-1 (IRS-1), Akt and acetyl CoA carboxylase protein levels were lower in the white adipose tissue of db/db mice and RAPA up-regulated

levels of these proteins, suggesting improved insulin sensitivity. RAPA also increased fatty acid β -oxidation and lipolysis in the adipose tissue of db/db mice consistent with the decreased fat mass. Finally, insulin sensitivity were significantly improved by RAPA treatment in db/db mice. Collectively, our study demonstrates for the first time that RAPA improves glucose tolerance and insulin sensitivity in female db/db mice that may be modulated through changes in mitochondrial function in the white adipose tissue.

150. Blood and Tissue Heat Shock Protein 70 Responses are Divergent and Related to Obesity, Not Chronological Age

Kylie Kavanagh (P), Ashley Wylie, Stephen Kritchevsky

Wake Forest University School of Medicine, Winston-Salem, NC

Heat Shock Protein (HSP)70 protects cells from the accumulation of damaged proteins, maintains cellular homeostasis, and is thought to prevent age-related functional decline. It is unclear whether high or low levels indicate optimal health, and how circulatory and tissue levels relate. We assayed HSP70 levels in skeletal muscle (SkM) and peripheral blood mononuclear cells (PBMNCs) from 40 healthy female vervet monkeys (9-20yrs, lifespan \approx 26yrs) under both basal and stressed conditions. Isolated PBMNCs were suspended in media with supplemental O₂/CO₂ for 4 hours at 37C (basal), or 1 hour at 42C and 3 hours at 37C (stress). HSP70 release into the media was quantified by ELISA. SkM biopsies were minced and incubated similarly, with HSP70 quantification by immunoblotting. Adiposity and metabolic measures were also assessed. There was a strong association between waist circumference and fasting insulin ($r=0.45, p=0.004$) concordant with the known relationship between central obesity and insulin resistance (IR). Age was related to worsened glycemic control (HbA1c%; $r=0.36, p=0.02$) but not to any HSP70 endpoint. In both tissues, HSP70 induction was significantly related to central obesity and IR, but in opposing directions. In SkM, both basal and stressed HSP70 was negatively associated with central obesity ($r=-0.39, p=0.01$ and $r=-0.33, p=0.04$) consistent with HSP70 deficits reported in human and animal studies of IR and diabetes. However, the induction of HSP70 release from PBMNCs was significantly positively associated with insulin levels ($r=0.39, p=0.01$). Basal SkM and PBMNC HSP70 were related ($r=0.41, p=0.008$), however there was no correlation between tissues stress responses. This study highlights how different

cell types respond to stress, and that circulating levels of HSP70 do not estimate tissue stress responses. We also demonstrate that obesity and IR, not age, predict HSP70 response. These data help explain controversy in the literature regarding HSP70 measures and underscore obesity and IR as early contributing factors to its dysregulation.

151. The Protective Role of Calcineurin A β in ER Stress via Activation of the Unfolded Protein Responses in Astrocytes

Yanan Chen (P)

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

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 PERK-like ER kinase (PERK). This is followed by phosphorylation of the eukaryotic initiation factor-2 α (eIF2 α), which attenuates protein synthesis. Recently, our lab reported that calcineurin (CN) strengthened the early UPR by binding to PERK and enhancing its autophosphorylation (Bollo et al PLoSOne 5 (8): e11925). Here, we report that CN-A β , the astrocyte specific isoform of CN, protects astrocytes from ER stress, likely by enhancing the phosphorylation of PERK. First, immunofluorescent staining of mouse brain sections showed that CN-A β is localized in astrocytes. Second, cultured astrocytes deficient in CN-A β exhibited a significantly higher level of cell death and eIF2 α phosphorylation at rest compared to wildtype siblings. Oxygen and Glucose Deprivation (OGD) treatment did not further increase cell death or eIF2 α phosphorylation in CN-A β $-/-$ cells. In vivo, Rose Bengal (RB)-induced ischemic stroke caused larger infarct lesions in CN-A β $-/-$ mice compared with wildtypes. On the other hand, overexpression of CN-A β tagged with GFP significantly increased the viability of astrocytes that are exposed to OGD for 1 hour. Furthermore, immunoprecipitation experiments with anti-PERK showed that CN-A β was co-immunoprecipitated with PERK. In vitro kinase assays with recombinant proteins GST-cytoPERK and CN-A β demonstrated that PERK autophosphorylation is increased in presence of CN-A β . Taken together, we suggest that CN-A β is bound to PERK and enhances the early UPR. This is a new function for an old phosphatase that remarkably,

increases autophosphorylation of PERK, thereby enhancing early cell survival during stress.

152. Effect of Short-Term CR Initiated at Different Ages on Cognitive and Motor Function and Cellular Oxidative Defensive Systems

Nopporn Thangthaeng (P, G), Michael Forster,

Nathalie Sumien

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

Caloric restriction (CR) has been reported to extend lifespan and confer health benefits, regardless of whether it is initiated early in life or implemented for short periods during middle-age. Both effects may involve an ability to promote redox homeostasis and attenuate oxidative damage. The purpose of the current study was to determine if the effect of short-term CR to improve cognitive or psychomotor function was correlated with the activity of enzymes regulating cellular redox homeostasis in different brain regions. Three age groups of C57BL/6J males, young (3 mos; n = 38), middle-aged (12 mos; n = 37), and old (19 mos; n = 44) were assigned to either ad libitum (AL) or 30% CR. After eight weeks, the mice were tested for psychomotor and cognitive functions. Upon completion of the test battery, the activity of glutamate-cysteine ligase (GCL), glutathione reductase (GR), glutathione peroxidase (GPx), superoxide dismutase (SOD), glutathione S-transferase (GST) and glutaredoxin, thioredoxin reductase (TrxR) was measured. Age-related declines in all measures of behavioral performance of the AL-fed mice were evident. Caloric restriction improved performance on bridge walking regardless of the initiation age, whereas for the other tasks, CR-related improvement occurred mainly after it was initiated at 12 or 19 months. Overall, CR failed to yield consistent effects on enzymatic activities for all regions of the brain within any of the age groups. The current results suggest that whereas CR yields a reducing shift in the redox state of glutathione and can indeed modify activity of enzymes involved in redox homeostasis, the latter effect would seem to be largely independent of the ability of CR to improve psychomotor and cognitive performance.

153. Cu/ZnSOD Overexpression Extended Lifespan in Sprague-Dawley Rats, but had Little Effect in F344 Rats

Lisa Flores¹, Adam Salmon¹, Melanie Ortiz¹, Sara Dube¹, Nicolas Musi², Wenbo Qi¹, Shuko Lee³, Gene Hubbard¹, Holly Van Remmen¹, Arunabh Bhattacharya¹, Yuhong Liu¹, James Kirkland⁴, Tamar Pirtskhalava⁴, Tamara Tchkonina⁴, Arlan Richardson¹, Yuji Ikeno¹ (P)

¹Barshop Institute/UTHSCSA; ²UTHSCSA; ³Audie Murphy VA Hospital; ⁴Mayo Clinic

Recently, our laboratory made the surprising observation that overexpressing Cu/ZnSOD [Tg(SOD1-SD)+/0] in Sprague Dawley (SD) rats resulted in a significant increase in lifespan and a reduction in age-related pathologies. The purpose of this study is to determine why overexpressing Cu/ZnSOD increases lifespan in SD rats. The Tg(SOD1-SD)+/0 rats showed lower levels of oxidative damage to DNA and lipids in vivo, and higher resistance to oxidative stress in vitro. Both Tg(SOD1-SD)+/0 and wild-type rats showed an age-related increase in body fat, and the Cu/ZnSOD overexpression did not attenuate adiposity. Interestingly, Tg(SOD1-SD)+/0 rats showed a significant increase in insulin sensitivity at a young age and lower plasma glucose levels at an old age. To further investigate the role of Cu/ZnSOD overexpression on aging, we generated transgenic rats with F344 overexpressing Cu/ZnSOD. Tg(SOD1-F344)+/0 rats showed similar levels of Cu/ZnSOD overexpression to Tg(SOD1-SD)+/0 rats. The Tg(SOD1-F344)+/0 rats showed lower levels of oxidative damage to lipids in vivo, however, neither Tg(SOD1-F344)+/0 nor wild-type rats showed any age-related changes in body fat, insulin sensitivity, and plasma glucose levels. Furthermore, Tg(SOD1-F344)+/0 rats showed little increase in lifespan compared to wild-type rats. Our research is very exciting because these results indicate that the overexpression of Cu/ZnSOD could be more protective against oxidative stress, attenuate aging, and age-related diseases under obese conditions in mammals. (Supported by grant from the VA Merit Review, American Federation for Aging Research, and Glenn Foundation)

154. Depot-Specific Patterns in Adipose Tissue Oxidative Stress and Dysfunction in Aging

Adam Salmon (P) and Arlan Richardson

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

The dynamic endocrine and secretory functions of adipose tissue play significant roles in processes that control metabolism and healthspan. With age, changes in adipose tissue promote dysfunctional lipid metabolism and redistribution of fat depots. Aging is associated with a loss of subcutaneous adipose tissue and accumulation of fat in intra-abdominal visceral adipose tissue and in ectopic sites such as muscle and liver. Fat accumulation at these sites promotes development of several age-related pathologies including insulin resistance, cardiovascular disease,

cancer and cognitive decline. However, the basic molecular mechanisms that cause age-related adipose tissue dysfunction are unknown. Among rodent tissues, oxidative damage is relatively high in adipose tissue; thus, aging-associated increases in oxidative stress may be a plausible primary cause of adipose dysfunction. In this study, we tested whether aging promotes significant alterations in the antioxidant defense of adipose tissue. We studied white adipose tissue from intra-abdominal visceral depots and subcutaneous depots collected from C57BL/6 mice between 6 and 32 months of age. After mice reached adulthood, aging was associated with a significant decline in mass of all fat depots with the effects most dramatic among subcutaneous depots. We also found significant age-dependent increases in expression of genes associated with the breakdown of lipids in these tissues. Surprisingly, we found changes in antioxidant expression and activation of stress-signaling in adipose tissues that suggest age-associated increases in oxidative stress. Furthermore, the degree of age-related changes differed among visceral and subcutaneous fat depots. Together, these findings suggest that a primary cause of adipose tissue dysfunction with age is a growing inability to effectively deal with accumulating oxidative stress. Future studies will address whether alleviation of oxidative stress can preserve healthy functional adipose tissue with age.

155. The Naked Truth about Cytoprotection: Lessons from the Naked Mole-Rat

Kaitlyn Lewis¹ (P. N) and Rochelle Buffenstein²

¹University of Texas Health Science Center at San Antonio; ²University of Texas Health Science Center at San Antonio/Barshop Institute

Most models of extended longevity (e.g., long-lived flies, rodents) exhibit pronounced resistance to cytotoxins. This is most evident in the longest-lived rodent, the naked mole-rat that lives an order of magnitude longer (32y) than similar sized mice and demonstrates a profound resistance to a broad spectrum of toxins in vitro. Throughout this very long lifespan, naked mole-rats experience very little decline in physiological and biochemical markers with age and cancer has never been reported. We hypothesize that the nuclear factor-erythroid 2-related factor-2 [Nrf2] signaling cytoprotective pathway plays a larger role in this profound toxin and cancer resistance. Nrf2 is a transcription factor ubiquitously expressed and is conserved from worms to humans. Nrf2 degradation is regulated by kelch-like ECH-associated protein 1 [Keap1], which targets Nrf2 for ubiquitination and subsequent degradation via the proteasome. After a stressful insult (i.e. toxin, ROS),

interactions between Nrf2 and Keap1 are inhibited and free Nrf2 is able translocate into the nucleus, bind to the antioxidant response element [ARE] and thereby activate the transcription of >600 cytoprotective molecules, including those involved in detoxification, glutathione metabolism, and proteasome subunits. Largely studied with regards to cancer, Nrf2 has also been shown to interact with p53 and p21, playing a role in modulation of the cell cycle and cancer progression. We find that naked mole-rats have constitutively elevated levels of Nrf2-signaling in vitro and in vivo, and ask if the upregulation of this pathway is responsible for the broad resistance against xenobiotics and observed changes in cell proliferation. Both in vitro and in vivo results from naked mole-rats reveal increased resistance to toxins compared to shorter-lived rodents. Moreover, there are several indications that naked mole-rats show rapid Nrf2 upregulation and signaling, suggesting that this highly conserved mechanism may contribute to prolonged healthspan, cancer resistance, and longevity.

156. Cytochrome B5 Reductase Overexpression Increases Lifespan and Oxidative Stress

Resistance in Fruit Fly

Yaning Sun (P. G)

National Institute on Aging

Free radicals, mainly reactive oxygen species (ROS), have been proposed as a main proximate cause of aging. Cytochrome b5 reductase (Cytb5R) has been shown to play an important role in the free radical defense system in both plasma membrane and mitochondria. Studies in yeast demonstrate that overexpression of NQR1, a yeast homologue of Cytb5R, significantly extends both chronological and replicative lifespan in yeast. However, it is unclear whether and how Cytb5R promotes longevity in higher eukaryotes. Here we investigate the function of Cytb5R in modulating lifespan in the fruit fly, *Drosophila melanogaster*. Using the Gal4-UAS system, we test the effect of Cytb5R overexpression on lifespan, stress resistance, and changes in expression of aging-related genes. We found that overexpression of Cytb5R significantly increased mean lifespan (>10%) of female but not male flies fed a standard sugar-yeast-based diet (10% sugar, 10% yeast). We also found that overexpression of Cytb5R increased mean lifespan (>10%) of both male and female flies fed a calorie restriction diet (2.5% sugar, 2.5% yeast). Moreover, Cytb5R overexpression increased flies' resistance to paraquat-induced oxidative stress. Our mechanistic studies indicated that Cytb5R overexpression increased the expression of stress response genes, including genes in Jun kinase (JNK)

signaling, such as JNK, pucker and lethal (2) essential for life, in the Nrf2 pathway, such as mitochondrial peroxiredoxins (Prx5037 and Prx5) and glutathione S-transferase D1, and in the sirtuin pathway, including dSir2. In summary, our findings suggest that Cytb5R interacts with JNK, Nrf2 and/or sirtuin pathways to promote longevity. Thus, Cytb5R could be a novel target for pro-longevity interventions. (This work was supported by the IRP of the NIA, NIH).

157. Demographic, Behavioral And Physiological Assessment of the Senescence Switch in *H. oligactis*

Samantha Rendon (P, N), Vanessa Soto, Natalia Podlutskaya, Kathleen Fischer, Steven Austad
University of Texas Health Science Center at San Antonio, San Antonio, TX

Hydra, a genus of freshwater polyps found on every continent except Antarctica, is a simple cnidarian that has been studied for its remarkable regenerative abilities for more than two centuries. Several long-term studies have suggested that hydra do not undergo senescence (Brien 1953; Martinez 1998). Their putative immortality might be attributable to their three distinct stem cell lineages: ectodermal epithelial stem cells, endodermal epithelial stem cells, and interstitial stem cells that allow for continual tissue renewal (Bosch 2008). Hydra species reproduce either by asexual budding or by the production of gametes. Typically, sexual reproduction occurs in times of environmental stress (Thorp & Covich 2001). One species of hydra, *H. oligactis*, appears exceptionally promising for elucidating mechanisms of aging. There are several known strains of this species. One strain (Japanese) has been reported not to age when reproducing through asexual budding at 18°C, but ages quickly when switches to sexual reproduction at 10°C (Yoshida et al 2006); other strains (European) do not reliably switch reproductive mode under this condition. Thus as an experimental system, this species can be used to determine the relative contributions of reproductive mode versus temperature in the switch from a non-aging to an aging phenotype. In order to determine the relative contributions of reproductive mode versus environmental temperature in the senescence switch of *H. oligactis*, we performed longitudinal studies on populations of both European and Japanese strains at normal rearing temperature (18°C) and at 10°C. Senescence, operationally defined as an increase in mortality rate with age, a decrease in budding (for animals still reproducing asexually) or regeneration rates, was assessed using demographic, behavioral

and physiological markers. Additional markers consist of any age-related decrease in contraction response or prey capture rate. These measures in sum reveal whether there is a general age-related decline in function in the population.

158. Growth, Development, and Phenotypes of Mice Treated with High Dose Rapamycin, Tacrolimus, or Acetylsalicylic Acid from Neonatal Age D10

Simon Johnson (P)
University of Washington, Seattle, WA

Rapamycin and acetylsalicylic acid are two drugs that have been demonstrated to increase murine lifespan and delay or modulate the appearance and progression of age related diseases. In addition to their promise as interventions in age-related pathologies, both of these drugs have been examined as interventions in human diseases not directly associated with normal aging. Rapamycin has shown promise as an intervention in models of human genetic diseases, including laminopathies and hereditary neurodegenerative diseases, while acetylsalicylic acid has been well described as a preventative treatment in cardiovascular disease and chronic inflammatory disorders of both age related and hereditary origins. While these drugs have been studied as interventions in multiple mouse disease models the affects of these compounds on murine growth and development has not been thoroughly examined. The affects of these drugs on development will indicate their potential as therapeutic interventions in human genetic disorders which become symptomatic early in life, as well as providing insight into any affects longevity interventions may have during development. In this study we are examining the affects of these drugs and the drug FK-507, an immunosuppressant which targets the same molecule as Rapamycin but affects Calcineurin signaling and not mTOR, on weight gain, activity, strength, coordination, and blood cytokine profile of mice treated from a young age.