28th ANNUAL MEETING OF THE AMERICAN AGING ASSOCIATION
12th ANNUAL MEETING OF THE AMERICAN COLLEGE OF CLINICAL GERONTOLOGY
Washington Athletic Club, Seattle, Washington June 4-8, 1999

"Models I: New Prospects for Identifying Longevity Mechanisms in Invertebrates"
Chair: Robert Arking

INVITED PAPERS

1. Pamela Larsen: **GENETIC PATHWAYS AFFECTING LONGEVITY IN CAENORHABDITIS ELEGANS.**
2. Robert Shmookler-Reis: **UNBIASED CHROMOSOMAL MAPPING OF LOCI GOVERNING LONGEVITY IN C. ELEGANS.**
3. Trudy MacKay: **QTL MAPPING OF AGING GENES IN DROSOPHILA MELANOGASTER.**
4. John Phillips: **CRITICAL CELL TYPES FOR LONGEV**

"Models II: Emerging Vertebrate Models of Aging"
Chair: George Roth

5. James F. Nelson: **CAN EXPRESSION ARRAYS HELP IDENTIFY LONGEVITY GENES IN OLD AND NEW MODELS OF RETARDED AGING?**
6. Mathias Jucker: **MOUSE MODELS TO STUDY MECHANSIMS OF BRAIN AGING AND ALZHEIMER'S DISEASE.**
7. Steven Austad: **A CONSIDERATION OF AGING IN SELECTED AVIAN SPECIES.**
8. Caleb Finch: **FISH AS MODELS FOR NEGLIGIBLE SENESCENCE.**
9. Michael Hayek: **PHYSIOLOGICAL CHANGES WITH AGE IN THE DOG; A POTENTIAL MODEL FOR AGING RESEARCH.**
10. Mark A. Lane: **NONHUMAN PRIMATE MODELS IN BIOGERONTOLOGY.**

SUBMITTED PAPERS

11. Glenn S. Gerhard: **ZEBRAFISH (DANIO RERIO) AGING AND OXIDATIVE STRESS.**
12. J.A. Mattison: **STUDIES OF AGING IN AMES DWARF**

"Mechanisms I: New Approaches to Investigating Oxidative Stress in Aging"
Chairs: Richard Weindruch and Kenichi Kitani

INVITED PAPERS

13. Nikki Holbrook: **AGE-RELATED ALTERATIONS IN PROLIFERATION-ASSOCIATED AND STRESS-ACTIVATED SIGNALING PATHWAYS.**
14. Richard Weindruch: **HISTOLOGICAL ASSESMENT OF OXDIATIVE DAMAGE IN AGING SKELETAL MUSCLE.**
15. Mark Mattson: **OXIDATIVE DISRUPTION OF NERVE CELL ION HOMEOSTASIS: A PIVOTAL EVENT IN NEURODEGENERATIVE DISORDERS.**
16. James Joseph: **OXIDATIVE STRESS PROTECTION AND VULNERABILITY: THE YIN AND YANG OF BRAIN AGING.**

SUBMITTED PAPERS

17. D. Busbee: **PROTECTION FROM GLUTATHIONE DEPLETION BY A GLYCONUTRITIONAL.**
18. A. Z. Reznick: **MUSCLE ATROPHY AND OXIDATIVE STRESS IN IMMOBILIZATION AND AGING.**
19. Jung Suh: **(R)-α-LIPOIC ACID SUPPLEMENTATION OF OLD RATS DECREASES AGE-DEPENDENT ACCUMULATION OF IRON AND ASCORBATE DEPLETION IN BRAIN.**
20. K.Kitani: **ENHANCING EFFECT OF RASAGILINE ON SUPEROXIDE DISMUTASE (SOD) AND CATALASE (CAT) ACTIVITIES IN DOPAMINERGIC SYSTEM IN THE RAT.**
21. **The Hayflick Lecture**
E. Wang: FUNCTIONAL GENOMICS STUDY OF HUMAN LONGEVITY: GENES, BIOCHIPS AND HIGH THROUGHPUT TECHNOLOGY.

"Mechanisms II: Emerging Paradigms and New Perspectives"
Chairs: Judd Aiken and Debbie McKenzie

INVITED PAPERS

22. Greg Morin: **AVERTING CELLULAR SENESCENCE THROUGH TELOMERE MANIPULATION.**
23. David Sell: **STATUS OF GLYCATION MARKERS IN HUMAN AND RODENT STUDIES.**
24. Debbie McKenzie: **MECHANISM OF AMYLOID FORMATION IN NEURO-DEGENERATIVE DISEASES.**
25. Raymond J. Monnat, Jr.: **WERNER SYNDROME RESEARCH: THE LAST CENTURY AND IN THE NEXT.**

SUBMITTED PAPERS

26. A.D.N.J. de Grey: **BAD CODON USAGE: A NOVEL TECHNIQUE TO AID THE MITOCHONDRIAL IMPORT OF TRANSGENE-ENCODED HYDROPHOBIC PROTEINS.**
27. N.S. Gavrilova: **THE FUTURE OF HUMAN LONGEVITY: A MORE REALISTIC APPROACH FOR LIFE EXPECTANCY FORECASTING.**
28. L.A. Gavrilov: **CHILDREN BORN TO OLDER MOTHERS DO NOT INHERIT MATERNAL LIFESPAN.**
29. R. James: **THE CLONING AND CHARACTERIZATION OF A MOUSE SMC GENE.**
30. Abstract Not Presented.
31. M. Aker: **REACTIVATION OF SILENCED ALLELES ON THE INACTIVE X CHROMOSOME IN AGING.**
32. T.E. Jones: **ALTERATIONS IN RESPONSE TO AGING OF EXCITATION-CONTRACTION COUPLING PROTEINS IN SKELETAL MUSCLE.**
33. K. Wheaton: **MODULATION OF KINASE ACTIVITY CAN ALTER THE SENESCENT PHENOTYPE.**
34. A.C. Cosmas: **CARDIAC OVERLOAD AS A PATHOPHYSIOLOGICAL EVENT: CORRELATES TO THE "THRESHOLD AGE."**
35. V.H. Brophy: **ANALYSIS OF THE RELATIONSHIP BETWEEN PARAOXONASE ACTIVITIES AND GENOTYPE.**
36. K. Agostinuccil: **VITAMIN E AND AGE ALTER LIVER MITOCHONDRIAL MORPHOMETRY.**
37. N. Van Zeeland: **AGE-ASSOCIATED ELECTRON TRANSPORT SYSTEM ABNORMALITIES CONCURRENT WITH INTRA-FIBER ATROPHY IN RAT SKELETAL MUSCLE FIBERS.**
38. J. Wanagat: **AGE-ASSOCIATED CHANGES IN CARDIAC FUNCTION, STRUCTURE AND MITOCHONDRIAL GENETIC AND ENZYMATIC ABNORMALITIES IN THE FISCHER 344 X BROWN NORWAY F_1 HYBRID RAT.**
39. J.M. Long: **COMPARISON OF HIPPOCAMPAL GLIA NUMBER IN C57BL/6J AND 129/SvJ MICE OF DIFFERENT AGES.**
40. M.A. Pahlavani: **AGE-RELATED DECLINE IN ACTIVATION OF CALCIUM/CALMODULIN-DEPENDENT PHOSPHATASE CALCINEURIN AND KINASE CAMK-IV IN RAT T CELLS.**
41. ABSTRACT NOT PRESENTED
42. A.B. Ordman: **BACKGROUND FOR A CONSENSUS ON THE VALUE OF CONSUMING VITAMIN C AND E SUPPLEMENTS.**
43. ABSTRACT NOT PRESENTED
44. E.A. Porta: **SEQUENTIAL HISTOCHEMICAL STUDIES OF LIPOFUSCIN IN HUMAN CEREBRAL CORTEX FROM THE FIRST TO THE NINTH DECADE OF LIFE.**
45. J. Lee: **DIETARY RESTRICTION AND 2-DEOXY-D-GLUCOSE PROTECT NEURONS IN THE BRAIN AGAINST EXCITOTOXIC, OXIDATIVE AND ISCHEMIC INJURY: EVIDENCE FOR THE INVOLVEMENT OF STRESS PROTEINS.**
46. Z.M. Guo: **DIETARY RESTRICTION ATTENUATES AGE-RELATED DECREASE IN REPAIR OF SPECIFIC GENES.**

47. J. Li: **TELOMERIC SHORTENING IN AGING RAT LENS AND DELAY BY CALORIC RESTRICTION.**
48. N.S. Wolf: **CATARACT AS A BIOMARKER OF AGING: CALORIC RESTRICTION DELAYS BOTH.**
49. M.E. Lopez: **AGE AND FIBER TYPE AFFECT THE PHENOTYPE OF AGE-ASSOCIATED ELECTRON TRANSPORT SYSTEM ABNORMALITIES IN RHESUS MONKEY SKELETAL MUSCLE.**
50. N.L. Bodkin: **THE INSULIN RESISTANCE SYNDROME AND ITS DELAY AND MITIGATION BY LONG-TERM CALORIE RESTRICTION: IMPLICATIONS FOR CALORIE RESTRICTION-MIMETIC THERAPY.**
51. A. Black: **BODY COMPOSITION AND BONE MINERAL DENSITY DURING INITIATION OF CALORIC RESTRICTION IN YOUNG AND AGED RHESUS MACAQUES.**
52. A.M. Handy: **A NONHUMAN PRIMATE MODEL OF MENOPAUSE: EVALUATION OF REPRODUCTIVE CYCLING AND HORMONES.**
53. K. Poydence: **DEVELOPMENT AND UTILITY OF A MULTICENTER, LONGITUDINAL DATABASE OF AGING IN NONHUMAN PRIMATES.**
54. T. Moscrip: **AGE-RELATED DECLINE IN FOOD INTAKE AND MOTIVATION FOR FOOD IN RHESUS MONKEYS.**
55. P. Mascarucci: **ENDOTOXIN-INDUCED CYTOKINE RESPONSES IN RHESUS MONKEYS: EFFECTS OF AGE AND CALORIE RESTRICTION.**
56. D.J. Waters: **PET DOGS AS A MAMMALIAN MODEL TO STUDY THE ASSOCIATION BETWEEN AGING AND SPONTANEOUS CANCER DEVELOPMENT.**
57. E.C. Petrie: **EFFECTS OF ADVANCED AGING ON PLASMA CATACHOLAMINE RESPONSES TO THERMAL STRESS.**
58. H. Li: **FAMILY CARING FOR ELDERS IN HOSPITAL: A MODEL DERIVED FROM MULTIPLE PERSPECTIVES.**
59. A. Weiss: **SYSTEMIC GROWTH HORMONE DOES NOT PREVENT MUSCLE ATROPHY IN DENERVATED HINDLIMBS OF AGED MICE.**

"Manipulations I: Innovative Approaches for Altering Aging Processes"
Chairs: Roger McCarter and George Martin

INVITED PAPERS
60. M. J. Forster: **ANTI-AGING ACTIONS OF NOVEL NITRONES.**
61. Anthony Cerami: **PHARMACEUTICAL TREATMENTS TO PREVENT AND REVERSE GLYCATION PRODUCTS.**
62. Barbara Drinkwater: ABSTRACT NOT RECEIVED
63. George Martin: **EMERGING PARADIGMS AND NEW PERSPECTIVES FOR HUMAN GENETICISTS INTERESTED IN THE BIOLOGY OF AGING.**

SUBMITTED PAPERS
64. A. Donati: **AGE-RELATED CHANGES IN DOLICHOL (D) LEVELS AND AUTOPHAGIC-PROTEOLYTIC (A/P) ACTIVITY IN RAT LIVER.**
65. K. Eric Paulson: **THE NITRONE-SPIN TRAP PBN ALTERS THE CELLULAR RESPONSE TO H2O2: CALCIUM-DEPENDENT ACTIVATION OF THE EGF RECEPTOR/ ERK PATHWAY.**
66. Wenzhen Duan: **DIETARY RESTRICTION AND 2-DEOXYGLUCOSE ADMINISTRATION IMPROVE BEHAVIORAL OUTCOME AND REDUCE DEGENERATION OF DOPAMINERGIC NEURONS INMODELS OF PARKINSON'S DISEASE.**

ANNUAL LUNCHEON AND AWARDS:

1999 Nicolai Awardee – **Julie Ann Mattison**
"Studies Of Aging In Ames Dwarf Mice: Effects Of Caloric Restriction." from the Department of Physiology, Southern Illinois University, Carbondale, IL 62901.

1999 Nicolai Awardee – **Keith Wheaton**
"Modulation Of Kinase Activity Can Alter The Senescent Phenotype." from the Department of Medical Biochemistry and Southern Alberta Cancer Research Centre, University of Calgary, Calgary Alberta T2N 4N1

1999 Nicolai Awardee – **Marisol E. Lopez**
"Age And Fiber Type Affect The Phenotype Of Age-Associated Electron Transport System Abnormalities In Rhesus Monkey Skeletal Muscle." from the Department of Animal Health and Biomedical Sciences, Education and Clinical Center, University of Wisconsin-Madison, Madison WI 53706

1999 Glenn Awardee – **Whenzuan Duan**
"Dietary Restriction And 2-Deoxyglucose Administration Improve Behavioral Outcome And Reduce Degeneration Of Dopaminergic Neurons In Models Of Parkinson's Disease." from the Sanders-Brown Research Center on Aging and Department of Anatomy & Neurobiology, University of Kentucky, Lexington, KY 40536

1999 runner-up Glenn Awardee – **Jeffrey M. Long**
"Comparison of Hippocampal Glia Number In C57BL/6J And 129/SvJ Mice Of Different Ages." from The Gerontology Research Center, National Institute On Aging, NIH, Baltimore, MD 21224

1999 Hayflick Lecturer – **Eugenia Wang**
"Functional Genomics Study Of Human Longevity: Genes, Biochips And High Throughput Technology." from the Bloomfield Center For Research in Aging, Lady Davis Institute, Jewish General Hospital, Department of Medicine, McGill University, Montreal, Quebec, Canada.

1999 Harman Lecturer – **Caleb E. Finch**
"Evolution Of Alzheimer's Disease." from the Gerontology Center and Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0191

"Manipulations II: Novel Approaches to Nutritional Modulation of Aging"
Chairs: Paula Bickford and James Joseph

INVITED PAPERS
67. George Roth: **CALORIC RESTRICTION MIMETICS: GREAT TASTE, LESS FILLING, LONGER LIFE.**
68. Simin Meydani: **ANTIOXIDANTS AND INFLUENZA INFECTION IN THE AGED.**
69. Paula Bickford: **DIETS HIGH IN ANTIOXIDANTS CAN REVERSE AGE-RELATED DECLINES IN CEREBELLAR b-ADRENERGIC RECEPTOR FUNCTION AND MOTOR LEARNING.**

SUBMITTED PAPERS
70. B. Shukitt-Hale: **PREVENTION AND REVERSAL OF AGE-RELATED MOTOR AND COGNITIVE BEHAVIORAL DEFICITS WITH ANTIOXIDANT DIETS.**
71. Eric T. Shigeno: **MITOCHONDRIAL DECAY INCREASES OXIDATIVE STRESS IN THE AGING RAT HEART: REVERSAL BY DIETARY SUPPLEMENTATION WITH (R)-α-LIPOIC ACID.**

CONSENSUS CONFERENCE
"DO VITAMINS E AND C PRODUCE BENEFICIAL EFFECTS ON AGING AND AGE-RELATED DISEASE?"
Organized by Mohsen Meydani

Invited Participants: Bruce Ames, Balz Frei, Paul Jacques, Mark Levine, Simin Meydani, Meir Stampher, M.D., Marit Traber, Rick Weindruch

Bruce Ames: **Oxidants, antioxidants, and degenerative diseases of aging**
Meir Stampfer: **Vitamins E and C in the prevention of age-related diseases**
Paul Jacques: **Vitamin E and C in the aging eye**
Richard Wiendruch: **Free radicals, aging and the antioxidant role of vitamins E and C in mammalian species**
Mark Levin: **How much vitamin C do we need?**
Balz Frei: **Vitamins E and C and oxidative stress**
Maret Traber: **Vitamins E and C metabolism, natural vs. synthetic**
Simin Nikbin Meydani: **Vitamins E and C and aged immune system**

1

GENETIC PATHWAYS AFFECTING LONGEVITY IN *CAENORHABDITIS ELEGANS*. P. K. K. Leong and P. L. Larsen *, Molecular Biology Program and Division of Biogerontology, University of Southern California, CA 90089.

The primary goal of the present study is to test whether an overall decline in metabolic rate is a major mechanism for the increase adult longevity in the *daf*-2 mutants of *Caenorhabditis elegans*. The *daf*-2 gene encodes an insulin receptor-like protein and is speculated to regulate metabolism in this nematode. In this study metabolic rate is defined as the rate of oxygen consumption and was measured by indirect calorimetry for the 4-day-old adults of N2 (wild type) and several alleles of *daf*-2 with different adult life span. A significant ($p<0.001$) increase in metabolic rate for N2 from 15°C to 22.5°C was observed and the thermal response is as expected for *C. elegans*, a poikilotherm. The *daf*-2 (m41) mutant adults, on the other hand, exhibited a weak but significant ($P=0.046$) decrease in metabolic rate from 15°C to 22.5°C. We designate this observation of either an absence of or a negative thermal response of metabolic rate as the temperature-insensitive metabolic rate (Tim) phenotype. Tim is likely associated with a decrease of DAF-2 signaling at 22.5°C in the *daf*-2 (m41) mutant because this allele has been shown to display temperature-sensitivity for the life span extension (AGE) phenotype. Comparisons between N2 and four *daf*-2 alleles revealed that the metabolic rates of all four *daf*-2 alleles were significantly lower than those of N2 ($P<0.001$) at 15°C. There was, however, no significant difference among the four *daf*-2 alleles (ANOVA of slope; Variance Ratio=0.58, F 0.05(1,60)=4.0, P.0.25) despite a wide range of median adult life span from 25.8 days to 44.3 days exhibited by these alleles. The lack of difference in metabolic rates revealed by the *daf*-2 alleles at 15°C demonstrate that metabolic rate is independent of the adult life span for the *daf*-2 alleles examined.

To test whether the Tim phenotype is regulated by the same genes as the Age phenotype, the metabolic rates for the *daf*-2 (m41) and the *daf*-16 (m26); *daf*-2 (m41) mutants were measured. Mutations in the *daf*-16 gene can completely suppress the life span extension phenotype of many *daf*-2 alleles. *Daf*-16 is placed downstream of *daf*-2 in the genetic pathways involved in extending adult life span for *C. elegans*. The metabolic rate for the *daf*-16 (m26); *daf*-2 (m41) double mutant was very similar to that for *daf*-2 (m41) and significantly ($p<0.0010$ lower than that for N2 at both 15°C and 22.5°C. In addition, the Tim phenotype that was observed in the *daf*-2 (m41) mutant was also exhibited by the *daf*-16 (m26); *daf*-2 (m41) double mutant. The similarities in the metabolic rates and their thermal response between the *daf*-2 (m41) and the *daf*-16 (m26); *daf*-2 (m41) mutants demonstrate that the *daf*-16 gene, although affects adult life span, is not involved in the pathway that regulates adult metabolic rate in the *daf*-2 (m41) mutant. We therefore conclude that for the *daf*-2 (m41) mutant, increase in adult life span and regulation of adult metabolic rate are controlled by different genetic pathways.

2

UNBIASED CHROMOSOMAL MAPPING OF LOCI GOVERNING LONGEVITY IN C. ELEGANS. Srinivas Ayyadevara, John J. Thaden and Robert J. Shmookler Reis*, Depts. of Medicine, Geriatrics, and Biochemistry & Molec. Biology, Univ. of Arkansas for Med. Sci., and Central Arkansas Veterans Health Care System & GRECC, 4300 West 7th Street - Research 151, Little Rock, AR 72205. email: ReisRobertJS@exchange.uams.edu.

We conducted two new inter-strain crosses and created a heterogeneous recombinant inbred (RI) population from each, for a total of 4 crosses analyzed to date. The longest-lived 1% of worms in each RI population and comparable numbers of unselected young worms were genotyped. Data were then analyzed by maximum-likelihood scanning of a binary trait using composite interval mapping. We have now observed a total of 12 loci with highly significant LOD scores, well above the "0.05 threshold for genome-wide scans. Since genetic mapping in inter-strain crosses can only detect loci which are dimorphic in the parental strains, each cross can identify a subset of the total life- span-affecting loci. From the number of loci mapped in multiple crosses, we estimate that C. elegans harbors at most a few dozen polymorphic genes with a comparable effect on life span. We have now initiated back-crosses to construct multiple congenic (near-isogenic) lines for each QTL region in a parent of contrasting phenotype. Several of these back-crossed lines have been tested for longevity and differ significantly from the recurrent parent.

3

QTL MAPPING OF AGING GENES IN DROSOPHILA MELANOGASTER. Trudy F. C. Mackay, Department of Genetics, Box 7614, North Carolina State University.

We have mapped Quantitative Trait Loci (QTL) affecting genetic variation in life span between two inbred laboratory strains of D. melanogaster, using a population of 98 recombinant inbred (RI) lines derived from the parental strains. The mean adult life span of the homozygous RI lines was determined in each of five laboratory environments: standard culture conditions, high and low temperature, and heat shock and starvation stress. In addition, each of the RI lines was crossed to both parental strains, and the mean adult life span determined in two genetic backgrounds and high and low larval density environments. The RI lines were genotyped for insertion sites of highly polymorphic, high copy number roo transposable element markers, providing a dense (4 cM) molecular marker map. QTL were mapped within each sex and environment using a composite interval mapping procedure. A minimum of 26 QTL was detected; all were sex-, environment- and/or cross-specific. Ten of the QTL had sexually antagonistic or antagonistic pleiotropic effects in different environments. Degrees of dominance of life span QTL varied widely, from additive, partially dominant, fully dominant, to overdominant. The locations of life span QTL often coincided with those of candidate genes affecting longevity. These data provide support for the pleiotropy theory of senescence, and the hypothesis that variation for longevity might be maintained by opposing selection pressures in males and females and variable environments. However, the magnitude of genotype-environment interaction exhibited by life span QTL will complicate efforts to understand the genetic basis of variation in longevity in natural populations.

4

CRITICAL CELL TYPES FOR LONGEVITY IN DROSOPHILA. J.P. Phillips*[1], T.L. Parkes[1], M. Martin[1], K. Kirby[1], G.L. Boulianne[2], and A.J. Hilliker[1], [1]Dept of Molecular Biology & Genetics, University of Guelph, Guelph, Ont., Canada, [2]Div. of Neurology & Pgm in Dev. Biol., Hospital for Sick Children, Toronto, Ont., Canada.

The process of lifespan determination in multicellular organisms is the manifestation of events that occur in specific cell types. Loss of function in certain cell types can have relatively greater impact on lifespan of the whole organism than loss of function in other cell types. Our interest is in (10 identifying genes that are critical in sustaining normal lifespan and in (2) identifying those cell types in which modified or enhanced expression of specific genes can extend lifespan.

Reactive oxygen (Ro) has been widely implicated in mechanisms of senescence and life span determination. That motorneurons (MNs) are a critical nexus in the relationship between RO metabolism and aging is suggested by recent investigations of FALS, a paralytic, life-shortening disease involving loss of MNs in the brain and spinal cord and associated with multinational defects in CuZn superoxide dismutase (SOD). To determine if RO metabolism in MNs is an important determining factor in normal aging and life span determination, we utilized enhancer-trap technology coupled with the yeast GaL4/UAS regulatory system to generate transgenic Drosophila with auxiliary expression of SOD targeted to adult MNs.

The results demonstrated that expression of SOD in MNs not only restores the lifespan of Drosophila SOD-null mutants, but reduces presenescent mortality and extends the maximum lifespan of wildtype flies by more than 40%. In contrast, MN-directed expression of catalase (CAT), the catalytic partner of SOD in the metabolic reduction of superoxide to water, ameliorates the extensive post-eclosion mortality exhibited by CAT-null mutants but has no effect on adult lifespan in wildtype flies. Interestingly, the lifespan extending effect of supplementary SOD expression in MN is abolished by joint MN-directed expression of CAT. These results, and those of other experiments, including MN-targeted expression of human FALS Sod, and the targeted expression of those genes in other specific cell types, suggest that motorneurons are a critical cell type in lifespan determination in Drosophila.

5

CAN EXPRESSION ARRAYS HELP IDENTIFY LONGEVITY GENES IN OLD AND NEW MODELS OF RETARDED AGING? James F. Nelson*, University of Texas Health Science Center at San Antonio.

The calorie-restricted phenotype has offered tantalizing clues but no proof of factors that may retard aging in rodents. Several new vertebrate candidates for models of extended life span have recently emerged and will be briefly evaluated. These include the dwarf (dw/dw mouse) and the melatonin-treated rodent. If these and other genetically and pharmacologically manipulated rodents are substantiated as independent models for extended life span, they substantially increase discriminatory power to identify longevity assurance genes and underlying mechanisms. Multiple models of longevity offer the prospect of identifying those factors shared by all models and, presumably, most likely to be associated with extended life. A major question becomes how to efficiently and comprehensively probe these models to identify shared mechanisms. The arrival of expression array technology is fortuitous if it can be demonstrated that it has adequate sensitivity to identify the magnitude of differential gene expression that distinguishes models with extended life span from control values. Our results using commercial arrays to compare levels of gene expression during aging and calorie restriction indicate the affirmative and will be presented.

6

MOUSE MODELS TO STUDY MECHANSIMS OF BRAIN AGING AND ALZHEIMER'S DISEASE. Mathias Jucker*, Neuropathology, Institute of Pathology, University of Basel, Schönbeinstr. 40, CH-4003 Basel, Switzerland.

Brain aging and Alzheimer's disease (AD) have in the past been studied using a variety of animal models, with nonhuman primates and rats being the most popular. With the rapid evolution of mouse genetics and transgenic technology, murine models have gained increased attention because they permit to study the genetic contribution of a given age-related trait and of specific molecular mechanisms.

To identify genetic factors involved in brain aging we are studying behavioral and structural brain changes with aging among inbred mouse strains. In C57BL/6J mice performance in the Morris water maze is largely maintained with aging and stereological analysis revealed no significant age-related changes in neuron number, synaptic boutons number, or glial number in hippocampal subregions. A correlation was however observed between the number of synaptic boutons and maze performance (Calhoun *et al.*, Neurobiol. Aging 19: 599, 1998: Long *et al.*, ibid 19: 497, 1999). These results provide a basis for investigations into other inbred mouse strains that show more pronounced age-related impairments in learning and memory, such as 129Sv/J mice. The continuation of such structure-function analysis in aging inbred mice offers the potential of conducting GTL linkage analysis to identify the genetic loci underlying age-related changes in structure and function.

To study specific molecular mechanisms of AD pathophysiology we have analyzed transgenic mice, which overexpress mutated human amyloid precursor protein under the control of a neuron-specific promoter (APP23 mice). These mice develop amyloid plaques in neocortex and hippocampus progressively with age (Sturchler-Pierrat et al., PNAS 94: 13287, 1997). To study the impact of amyloid plaque formation on neurodegeneration, we have used modern stereological techniques to demonstrate that amyloid plaque formation is accompanied by neuron loss, synaptic changes and alterations in the cholinergic system (Calhoun *et al.*, Nature 395:755, 1998: and submitted). We have also shown that amyloid plaque formation and neuron loss is associated with robust activation of microglia consistent with an important role of microglia activation in AD pathogenesis. Las but not least, we have found vascular amyloid in APP23 mice and thus, have demonstrated that neuronal overexpression of APP can result in cerebrovascular amyloid and related angiopathy.

7

A CONSIDERATION OF AGING IN SELECTED AVIAN SPECIES. Steven N. Austad*, University of Idaho.

Research on fundamental mechanisms of aging has traditionally focused on short-lived vertebrates, because individuals may be feasibly followed from birth to death. However, such species have demonstrably poor defenses against underlying aging processes. An alternative experimental approach is to seek to identify mechanisms associated with high-quality defenses against aging processes. Birds have such defenses. Despite mass-specific metabolic rates up to twice those of similar-sized mammals, birds live on average 3-times as long as mammals. As a consequence some species consume 5-fold more oxygen per gram per lifetime as humans. Therefore birds make ideal models of effective defenses against reactive oxygen species. In addition, birds' body temperature is about 3° C higher than that of mammals and their blood contains 2-5 times higher glucose concentration. Birds must therefore also possess extraordinarily effect defenses against the formation of advanced glycation end-products. I will discuss which bird species seem most accessible for laboratory research on aging and summarize recent findings.

8

FISH AS MODELS FOR NEGLIGIBLE SENESCENCE. Caleb E. Finch*, Gerontology Center and Dept. Biological Sciences, University of Southern California, Los Angeles, CA 90089-0191.

Fish are relatively little used as models for aging and offer opportunities to study mechanisms that allow very long-life spans with gradual-to-negligible senescence. Most research on vertebrate models of aging has focussed on laboratory rodents which have life spans of 2-3 years and share many attributes of aging in humans and primates. Some fish have relatively short life spans of 3-5 years (guppy and platyfish). However, for models that approach human centenarians, certain deep-dwelling fish may be useful. Several species of rockfish (genus Sebastes) from the North Pacific live more than one hundred years, as evaluated by annual growth rings of the otolith. Reproductive age changes appear to be minimal. Data on the life history traits for these and other long-lived species was gathered to determine the sustainable commercial catch. The high degree of similarity of the amyloid beta peptide sequence in at least one fish to that in humans and primates is reason enough to examine brain aging in these fish. (Finch CE, 1998, Variations in senescence and longevity include the possibility of negligible senescence. J Gerontol 53A: B235-239).

9

PHYSIOLOGICAL CHANGES WITH AGE IN THE DOG; A POTENTIAL MODEL FOR AGING RESEARCH. M.G. Hayek*, S.P. Massimino and G.D. Sunvold. Research and Development, The Iams Company, Lewisburg, OH 45338.

The population of senior companion animals is increasing in the United States. With this increase in numbers, there is a heightened interest in understanding the changing physiology and nutritional needs of these aging companion animals. Defining these needs will aid in providing nutritional diets suited for this particular life stage. One consequence of these studies is the recognition that the dog may make a good model for aging research. Also, the design of nutritional strategies to affect these physiological changes may be applicable to other species, particularly humans. Three areas where this can be noted are the age-related changes seen with the immune response, glucose metabolism and alterations of microbial populations of the gastrointestinal tract. First, similar to other species, the dog experiences a decrease in immune response with age. This suppression is observed in the decreased ability of the dog's lymphocytes to proliferate with age as well as decreased ability to produce antibodies to sheep red blood cells. Nutritionally, it has been shown that antioxidants have the potential to reverse this decline in the dog. Secondly, similarly to other species, the dog has a decreased ability to metabolize glucose with age. Particularly, blood glucose levels remain elevated long after a meal in aged dogs compared to their younger counterparts. A nutritional strategy that can alleviate this age-related increase is to alter the type of carbohydrate present in a meal. Lastly, microbial populations in the gastrointestinal tract differ in older dogs with a higher prevalence of pathogenic populations. This may be altered providing specific fiber to promote healthy bacterial growth. Continued research with the geriatric dog will not only benefit the pet population, but these nutritional strategies may have applications for humans as well.

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NONHUMAN PRIMATE MODELS IN BIOGERONT-OLOGY. M. A. Lane*. Intramural Research Program, Gerontology Research Center, National Institute on Aging, NIH. Baltimore, MD. 21224.

Nonhuman primates offer a unique opportunity to study physiological aging in species more closely related to humans. A major advantage is that nonhuman primates can be used to validate findings obtained in other, shorter-lived species to determine to what extent aging phenomena discovered in these species may be relevant to primate, particularly human, aging. Nonhuman primates share about 90% of the human genome and exhibit similarities in many aspects of physiology including morphology, behavior and reproductive biology. In recent years the use of nonhuman primate models in biogerontology has been steadily increasing. This presentation will survey relevant primate aging findings in the areas of neurobiology, cardiovascular disease and diabetes, skeletal and reproductive aging and caloric restriction. When possible, emphasis will be placed on comparing nonhuman primate findings to those obtained in rodent models and humans. In addition, the development and potential utility of a database comparing primate and nonprimate models for the development of markers of aging will be discussed along with advantages and limitations of primate models.

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ZEBRAFISH (DANIO RERIO) AGING AND OXIDATIVE STRESS. Glenn S. Gerhard[1]*, Richard Stewart[1], Elizabeth J. Kauffman[1], Keith C. Cheng[1,2,3], and Robert G. Allen[4], Department of Pathology[1], The Jake Gittlen Cancer Research Institute[2], and the Department of Biochemistry and Molecular Biology[3], Penn State College of Medicine, Hershey, PA 17033 and Lankanau Medical Research Center[4], Wynnewood, PA 19096.

The zebrafish has now been established as an important vertebrate model in developmental biology research with the creation of several thousand genetic mutants that are leading to the identification of genes that control development. In order to use such a genetic approach to identify genes relevant to aging and age-related disease, the basic gerontological characteristics of the zebrafish, such as life span and age-related pathology, must first be defined. Several characteristics also make the zebrafish a compelling model system for aging studies, including a robust life span and the manifestations of mammalian-like "gradual" senescence, the ability to modulate the rate of aging by either temperature reduction (TR) or caloric restriction (CR), as well as expanding genetic and biological resources. We have initiated several preliminary studies to establish the zebrafish as a model for aging, including a nearly completed life span study using an outbred zebrafish cohort and an inbred (golden sparse) cohort. The groups have been housed at similar densities and fed on a twice per day "ad libitum" schedule, with water temperatures maintained at 25 +/- 2°C. As expected, the outbred group is longer lived with the 50th percentile survivorship at approximately 3 years and several individuals likely to survive for more than 5 years. A common morphological change in old fish is the development of a bent back phenotype. Parallel to our studies on longevity, fish have been subject to TR and CR to test our hypothesis that oxidative stress is a common mechanism by which TR and CR exert their putative longevity modulating effects. Metabolic rate (oxygen utilization per unit weight) was unchanged by 70% CR but was 40% lower with 10°C TR. Lipid peroxidation potential, measured by thiobarbituric acid reactive substances, was unchanged by short term TR, but increased by CR, possibly due to differential dietary antioxidant consumption. Ultrastructural analysis of skeletal muscle fibers revealed a decrease in intracellular lipid storage droplets by TR. We have also cloned the entire zebrafish mitochondrial genome as three overlapping fragments, as well as cDNAs encoding the zebrafish genes for glutathione peroxidase, gamma glutamyl cysteine synthetase, and catalase. These genes show exquisite amino acid sequence conservation between highly divergent organisms and will enable more detailed studies on aspects of oxidative stress in zebrafish aging. We believe that the zebrafish will fill an important biological gap that currently exists between invertebrate and vertebrate models of aging.

12 ***NICOLAI AWARD***

STUDIES OF AGING IN AMES DWARF MICE: EFFECTS OF CALORIC RESTRICTION. J.A. Mattison*, C. Wright, R.T. Bronson†, G.S. Roth#, D. Ingram#, A. Bartke Department of Physiology, Southern Illinois University, Carbondale, IL 62901 †Human Nutrition Research Center on Aging, Tufts University, Boston, MA 02111 #National Institutes of Health, National Institute on Aging, Bethesda, MD 20892

Ames dwarf mice, who are small and deficient in growth hormone (GH), prolactin, and thyroid stimulating hormone (TSH), live significantly longer ($P<0.0001$) than their normal siblings. In our original report, the difference in average lifespan was more than 350 days for males and more than 470 days for females ($P<0.0001$).

While Ames dwarf mice live much longer than their normal siblings do when maintained under standard laboratory conditions, it was of interest to examine their response to caloric restriction (CR). CR has repeatedly been shown to increase lifespan in rodents and other non-rodent species (fish, spiders). The possibility exists that dwarf mice are voluntarily caloric restricted, eating less naturally, and therefore have characteristics in common with CR animals. We are testing the hypothesis that this possible natural caloric restriction will negate any benefits of an imposed CR on lifespan.

Male and female Ames dwarf mice and their normal counterparts have been fed ad libitum (AL) or a 30% CR diet for 18 months. Animals are monitored daily and weighed weekly. For all four groups, the CR mice weigh significantly less than their AL fed counterparts (normal females: -42%, normal males: -23%, dwarf females: -18.8%, and dwarf males: -22.2%).

We also compared daily food consumption in dwarf and normal mice. Female dwarf mice consume significantly more food per gram body weight than normal females and a similar tendency is evident for the males. Although they receive 30% less food, the CR mice are eating the same amount as AL mice per gram body weight.

Food consumption (grams of food/gram body weight/day ± SE)

Group	AL	CR
Normal male	0.121 ± 0.008	0.105
Normal female	0.100 ± 0.006*#	0.11
Dwarf male	0.138 ± 0.008*	0.117
Dwarf female	0.133 + 0.009#	0.12

*#significant difference between the two groups ($p< 0.01$)

In addition to studying the effects of CR, we are examining histopathological changes in aging dwarf versus normal mice. Results available to date suggest that the incidence of tumors does not differ between the two groups but tumors appear to develop later in dwarf than in normal mice. Apparently age-related pathological changes in Ames dwarf mice resemble those in normal mice except for the age of onset.

There are several plausible contributors to the longer lifespan evident in dwarf mice. They are diminutive in size (approximately one-third of normal) and it has been recognized that smaller animals within a given species live longer than their larger counterparts. Ames dwarfs have significantly lower levels of insulin-like growth factor I (IGF-I) and GH/IGF-I deficiency may be directly related to the longer life because GH transgenic mice have a reduced lifespan and exhibit several traits of premature aging. The lower blood glucose and thyroid hormones in dwarf mice may be contributing to a slower metabolism. In addition, dwarf mice have a lower basal body temperature. These are all traits that resemble a CR state. However, AL dwarfs are not lean and can become obese.

We conclude that Ames dwarfs are not CR mimetics although they share many characteristics. The longer life evident in dwarfs is due to factors other than caloric intake. It remains to be determined whether CR will delay aging and cause a further life extension in Ames dwarf mice.

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AGE-RELATED ALTERATIONS IN PROLIFERATION-ASSOCIATED AND STRESS-ACTIVATED SIGNALING PATHWAYS. Yusen Liu and Nikki J. Holbrook*. Laboratory of Biological Chemistry, National Institute on Aging, Baltimore, MD 21224

Exposure of cells to environmental insults can activate multiple signaling cascades that serve to alter the pattern of gene expression and contribute to determining cell fate. Although many of the pathways activated are restricted to stress, there is significant overlap with

signaling pathways that serve to regulate cell proliferation. This is particularly true in the case of oxidants and thiol modifying reagents, where growth factor receptors play an important role in transmitting the stress-initiated signals. The ability of a cell to mount the appropriate response to external signals is critical for maintaining homeostasis during stress and there is increasing evidence in lower organisms linking stress tolerance to longevity. Research in our laboratory has been driven by the hypothesis that the ability to activate these responses declines with age, and using a variety of different model systems we and others have provided evidence to support this view. Our recent efforts have focused on the activation of two distinct signaling pathways, p70 S6 kinase, and the extracellular-regulated kinase (ERK) mitogen-activated protein kinase in response to proliferative and/or stress signals in primary rat hepatocytes from young versus aged hosts. We have demonstrated that there is an age-related decline in the activities of both of these pathways and have been exploring the mechanisms contributing to these alterations.

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HISTOLOGICAL ASSESMENT OF OXDIATIVE DAMAGE IN AGING SKELETAL MUSCLE. TA Zainal[1], TD Pugh[2], TD Oberley[3] and R. Weindruch[4*]—University of Wisconsin (Nutritional Sciences[1], Institute on Aging[2], Pathology and Laboratory Medicine[3] and Medicine[4]) and VA Hospital Pathology and Laboratory Service[3] and GRECC[4], Madison, WI 53705

The loss of skeletal muscle mass during aging, often referred to as sarcopenia, is of great public health significance. The accrual of oxidative damage in skeletal muscle with age as a result of increased oxidative stress has been hypothesized to contribute to the development of sarcopenia. Age-dependent increases in markers of oxidative damage to DNA, lipids, and proteins determined biochemically in mammalian skeletal muscle have been reported, thereby supporting a role of oxidative damage in the etiology of sarcopenia. To date, however, the accumulation of oxidative damage in mammalian skeletal muscle during aging has not been investigated from a histological standpoint, such that the localization of the accrual of oxidative damage is not well defined.

Caloric restriction (CR), which retards aging and diseases in laboratory rodents, attenuates many age-dependent biochemical and physiological changes in skeletal muscle. We have employed histological techniques to quantify oxidative damage in skeletal muscles from rhesus monkeys (vastus lateralis) and C57Bl/6 mice (upper hindlimb) varying in age and diet (control vs. CR).

An immunogold electron microscopic technique utilizing an antibody raised against 4-hydroxy-2-nonenal (HNE)-modified proteins was used to quantitate and localize the age-dependent accrual of oxidative damage in rhesus monkey skeletal muscle. Using rhesus monkeys ranging in age from 2-34 years, HNE-modified proteins increased 360% maximally. Comparing control vs. CR monkeys from an ongoing CR study at the UW-Madison, levels of HNE-modified proteins in skeletal muscle from the CR group were 50% less than control group values. Interestingly, oxidative damage localized largely to myofibrils. Accumulation of lipid peroxidation-derived aldehydes, such as malondialdehyde (MDA) and 4-hydroxy-2-alkenals (4-HDA), was measured biochemically and also increased with age.

For histological quantitation of nuclear oxidative damage, we are developing a technique using an antibody which recognizes 8-hydroxyguanine, 8-hydroxyguanosine and 8-hydroxy-2'-deoxyguanosine. Using skeletal muscle from 30 month-old mice, preliminary data suggest that nuclei in muscle from mice on CR displayed lower amounts of immunoreactivity than those from controls. The frequency distributions are very different with more nuclei from the control mice being densely stained.

Together, these data suggest that oxidative damage increases with age in skeletal muscle. Further, this increase is blunted by long-term CR. These are the first data to show that the age-associated accrual of oxidative damage is attenuated in primates by CR. These are also, to our knowledge, the first histological data evaluating oxidative damage as a function of age.

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OXIDATIVE DISRUPTION OF NERVE CELL ION HOMEOSTASIS: A PIVOTAL EVENT IN NEURODEGENERATIVE DISORDERS. Mark P. Mattson*, Wenzhen Duan, Ward A. Pedersen and Carsten Culmsee. University of Kentucky, Lexington, KY 40536.

Studies of animal and cell culture models of Alzheimer's disease (AD), Parkinson's disease (PD), stroke and amyotrophic lateral sclerosis (ALS) suggest that increased levels of oxidative stress (membrane lipid peroxidation, in particular) may disrupt neuronal ion homeostasis and energy metabolism, by impairing the function of membrane ion-motive ATPases and glucose and glutamate transporters (*J. Neurosci.* 15: 6239-6249; *J. Neurosci.* 17: 1046-1054). Such oxidative and metabolic compromise may thereby render neurons vulnerable to excitotoxicity and apoptosis (*J. Neurosci.* 17: 5089-5100). Studies of the pathogenic mechanisms of AD-linked mutations (amyloid precursor protein and presenilin mutations) strongly support central roles for increased oxidative stress and perturbed cellular calcium homeostasis in disease pathogenesis (*Physiol. Rev.* 77: 1081-1132; *Nature Med.* 5: 101-107). Interestingly, oxidative stress induces the expression of both death-promoting proteins (e.g., Par-4 and caspases) and life-promoting proteins (e.g., neurotrophic factors and stress proteins) (*Nature Med.* 4: 957-962; *Restorative Neurol. Neurosci.* 9: 191-205). Our data suggest that events occurring locally in synaptic terminals of dendrites and axons play a major role in initiating the neurodegenerative process in AD and related disorders (*J. Neurochem.* 69: 273-284; *Exp. Neurol.* 153:35-48; *Brain Res.* 807: 167-176). Experimental findings and data from studies of postmortem human brain tissue suggest that synapses are particularly sensitive to adverse effects of oxidative stress.

In recent studies we have shown that dietary restriction can increase resistance of neurons to degeneration in animal models of AD, PD and stroke (*Ann. Neurol.* 45: 8-15; and in press). The mechanism whereby dietary restriction benefits neurons in the brain appears to involve a "preconditioning" cellular response resulting in upregulation of stress proteins such as HSP-70 and GRp-78. We have recently developed cell culture and in vivo "models" of dietary restriction, and find that neurons subjected to chemically-induced energy restriction exhibit reduced levels of oxidative stress and improved calcium homeostasis following exposure to excitotoxic and oxidative insults relevant to AD, PD and stroke. Collectively, the data suggest that oxidative stress and perturbed calcium homeostasis are convergence points in the pathophysiology of different age-related neurodegenerative conditions. Moreover, our findings suggest that dietary modifications, particularly reduced calorie intake, will greatly reduce one's risk for age-related neurodegenerative disorders.

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OXIDATIVE STRESS PROTECTION AND VULNERABILITY: THE YIN AND YANG OF BRAIN AGING. J. A. Joseph*, N. Denisova, B. Shukitt-Hale, D. Fisher and I. Cantuti-Castelvetri. USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111.

An abundance of data indicate that there is increased vulnerability (IV) to oxidative stress (OS) in aging and that this increase contributes to the deleterious effects on neuronal function seen in aging and age associated neurodegenerative diseases.. Results from our laboratory indicate that in striatal slices obtained from young (6 mo) and old 22-24 (mo) rats there was a greater age-related vulnerability to oxidative stress induced by hydrogen peroxide exposure in the slices as assessed via GTPase activity and reductions in oxotremorine-enhancement of K+-evoked increases in dopamine release. Recent examinations indicated that these age-related increases were also observed when DA was infused into the zona compacta and motor and cognitive behavior assessed. In an effort to determine the factors involved in this IV in aging, we examined whether alterations in membrane lipid constituencies and qualitative distribution in receptor subtype would contribute to increased OS sensitivity in aging. Mechanistic examinations using PC-12 cell models suggested that alterations in membrane lipid composition (e.g., sphingomyelin and cholesterol concentrations) showed that exposure of the cells to H2O2 or dopamine (DA) reduced the ability of these cells to clear (buffer) Ca2+ and this effect could be enhanced by increasing the plasma membrane concentrations of sphingomyelin

(SPM) and cholesterol to the same levels as those seen in aged neuronal membranes. These increases were associated with decreased glutathione levels under both control and OS conditions, as well as alterations in both ERK and JNK expression. Subsequent experiments indicated that the SPM metabolite, sphingosine-1-phosphate (SPP) produced the greatest decreases in glutathione, as well as OS IV, but SPP effects were antagonized via glutathione repletion. Differential OS IV was also found when COS-7 cells transfected with one of 5 muscarinic receptor subtypes and exposed to DA. M1 - M2 - or M4-transfected cells showed OS IV which was expressed as greater decrements in Ca2+ clearance while those transfected with M3 or M5 receptors did not and that these differences, and that the patterns of ERK and JNK activation varied among vulnerable and non-vulnerable receptors. These findings suggest that areas of the brain containing high concentrations of the vulnerable M subtypes (e.g., striatum and hippocampus) may be subject to greater neurodegeneration in aging and neurodegenerative disease. Taken together, these results suggest that there are both membrane and receptor considerations in OS IV in aging that could contribute to the behavioral decrements.

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PROTECTION FROM GLUTATHIONE DEPLETION BY A GLYCONUTRITIONAL. D. Busbee[1*], R. Mouneimne[1], R. Burghardt[1], C. Gauntt[2], B.H. McAnalley[3], and H.R. McDaniel[3]. [1]Department of Veterinary Anatomy and Public Health, Texas A&M university, College Station, TX 77843. [2]Department of Microbiology, UTHSC, San Antonio, TX 78284. [3]Mannatech™, Inc. Coppell, TX 75019.

We evaluated the capacity for an exogenously added mixture of complex plant saccharides, a glyconutritional (GN), to protect rat hepatic cells *in vitro* or mouse splenocytes *in vivo* from depletion of glutathione by a sulfhydryl-binding agent (rat cells) or coxsackie virus B3 (mouse splenocytes). Rat cells were treated with the GN or received carrier medium only. GN-treated cells showed protection against depletion of intracellular glutathione by patulin, a sulfhydryl-binding agent. Cells exposed to the GN for 15 hours prior to patulin treatment showed no increase in glutathione protection above that demonstrated by cells treated 3 hours. These data indicate that a complex mixture of plant saccharides exerts a protective effect on liver cells against chemically initiated depletion of intracellular glutathione. CD-1 male mice were treated with coxsackie virus, CVB3, with subsequent viral-initiated pancreatitis, weight loss, and depletion of intracellular glutathione. Mice treated by IP-injection with the GN at the time of CVB3 infection and weekly thereafter exhibited an 8% weight gain but showed normal titers of anti-CBV3 globulin. Splenocytes from infected mice were compared over 8 months post-infection. CVB3-infected mice exhibited a significant loss of splenocyte glutathione, while CVB3-infected mice receiving IP GN exhibited no loss of splenocyte glutathione over 248 days post-infection. The glutathione protection mechanism is not at this time understood. It could result from protection of glutathione, enhanced de novo synthesis of glutathione, or increased regeneration of oxidized glutathione. The data suggest that the GN could act to protect cells from damage by oxidative radicals.

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MUSCLE ATROPHY AND OXIDATIVE STRESS IN IMMOBILIZATION AND AGING. N. Zarzhevsky and A. Z. Reznick.* Musculoskeletal Laboratory, Department of Anatomy and Cell Biology, Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, 31096 Haifa, Israel.

With advancing age, skeletal muscles show signs of muscle degeneration and loss of muscle mass. This phenomenon has been termed sarcopenia of old age. The etiology of sarcopenia in aged humans and animals is not clear and has been attributed partially to disuse and the immobilization typical of old peoples.

In the past several years, models of immobilization of hind-limb skeletal muscles of old animals have been developed. These models include immobilization of the right hind limb by a plaster-of-Paris cast or by external fixation using a rigid external frame with two wires going through the femur and tibia of the immobilized animals. In these studies it has been shown that muscle atrophy due to immobilization is associated with increased muscle damage that is accompanied by elevation of protein oxidation and lipid peroxidation. The increased muscle damage was assessed both morphologically and biochemically. At the biochemical level, the activity of acid phosphatase was determined as a criterion for an increase in lysosomal catabolic activity, and in addition, the activity of creatine phosphokinase was assessed as a measure of muscle general energetic status. Indeed, immobilization caused significant changes in the activity levels of these two enzymes. The extent of muscle damage in young and old rats was compared, and it was found that in most parameters, muscles of young animals were affected more than muscles of old animals. However, the capacity for recovery from immobilization-associated damage was by far greater in the young animals compared to the old ones.

In an attempt to elucidate the molecular mechanisms responsible for the above-observed oxidative stress, the role of cytokines such as tumor necrosis factor-a (TNF-α) has been investigated. Using skeletal muscle derived L-6 cells; it has been possible to show that TNF-α causes the induction of the transcription factor nuclear factor (NF)κB, which is regulated by the redox status of these cells. NF-κB, in turn, is responsible for the activation of several genes, including inducible nitric oxide (NO) synthase, and the increase of NO in muscle cells. The elevation of NO has been suggested to trigger the protein degradation machinery, leading eventually to muscle atrophy and weight loss. Comparison of several muscle pathologies supports the notion that cytokines initiate the signal transduction pathways via increase of oxidative stress, which culminates in muscle protein catabolism. Elucidating this cascade of events may help to alleviate some of the muscle maladies as well as the sarcopenia of old age.

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(R)-α-LIPOIC ACID SUPPLEMENTATION OF OLD RATS DECREASES AGE-DEPENDENT ACCUMULATION OF IRON AND ASCORBATE DEPLETION IN BRAIN. Jung Suh, Alma Rocha, Eric Shigeno, Balz Frei, and Tory M. Hagen. Linus Pauling Institute Oregon State University, 571 Weniger Hall, Corvallis, OR 97331-6512

Increased levels of free iron in the brain are associated with heightened oxidant production and are believed to causally contribute to age-related neurodegenerative disorders. Lipoic acid is a potent thiol antioxidant capable of crossing the blood-brain-barrier that has also been shown to chelate iron in vitro. In this study, we investigated the putative therapeutic effect of (R)-a-lipoic acid in lowering the age-related accumulation of iron in the brain as well as its effects on tissue antioxidant status. To test this hypothesis, male Fischer 344 rats (F344) of increasing age (2, 12, 24, and 28 months) were fed an AIN-93M diet with or without 0.2% (w/w) (R)-a-lipoic acid for two weeks. Based on food consumption, rats of all age groups received about 4 mg/day of lipoic acid. Following sacrifice, rat brains were removed and subdivided into 3 regions; fore-, mid-, and hindbrain, which were analyzed for iron concentration by atomic emission spectroscopy and ascorbate by HPLC with electrochemical detection. Results showed an age-dependent, yet heterogeneous, increase in tissue iron levels with the largest accumulation in the hind- followed by fore- and then midbrain (4.6-, 2.4-, and 1.5- fold in 28 versus that for 2 mo old animals, respectively). As a parameter for cellular antioxidant status, tissue ascorbate levels in the three brain sub-regions were measured. Results showed an age-related decline in ascorbate levels in all regions of the brain, with the largest decrease observed in hindbrain, followed by forebrain and midbrain (63%, 47%, and 16% in 28 versus 2 mo old rats, respectively). There was a significant inverse correlation between tissue ascorbate and iron levels with age (r^2 = 0.35 and 0.87 *in fore- and hindbrain*, respectively). Lipoic acid supplementation caused a 60% reduction in the total tissue iron in the forebrain, but no change in iron status was observed in the mid- or hindbrain versus unsupplemented age-matched controls. Consistent with the iron data, a substantial restoration of ascorbate levels was observed only in the fore-brain. Tissue ascorbate levels in the mid- and hindbrain of old supplemented rats were not significantly different from levels found in their age-matched controls. These differences in the response to lipoic acid supplementation suggest that there may be regio-specific variations in lipoic acid uptake, metabolism and/or function. In summary, our results show an age-dependent increase in brain iron associated with an age-related decline in ascorbate levels. This suggests a novel mode of protection of lipoic acid by direct chelation of free metal ions, in addition to its antioxidant properties, in particular sub-regions of the brain.

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ENHANCING EFFECT OF RASAGILINE ON SUPEROXIDE DISMUTASE (SOD) AND CATALASE (CAT) ACTIVITIES IN DOPAMINERGIC SYSTEM IN THE RAT. M.C. Carrillo[1,2], C. Minami[2], K.Kitani*[2], W. Maruyama[2], K. Ohashi[2], T. Yamamoto[2], M.Naoi[2,3], S Kanai[4], M.B.H. Youdim[5]. [1]National Univ. Rosario,Suipacha, Rosario, Argentine. [2]National Institute for Longevity Sciences, 36-3, Gengo, Moriokacho, Obushi, Aichi 474-8522, [3]Institute of Applied Biochemistry, Mitake, Gifu, [4]Tokyo Metropolitan Inst. Gerontol, Tokyo, Japan. [5]Pharmacology Unit, Rappaport Faculty of Medicine, Technion, Haifa, Israel.

Rasagiline N-propargyl-1(R)- aminoindan is a selective irreversible MAO-B inhibitor and also possess an anti-apoptotic activity. Both of the effects have been previously shown for deprenyl. Deprenyl also has an effect to increase SOD and CAT activities selectively in brain dopaminergic regions1). Because of similarities of pharmacology of both drugs, we attempted to examine whether rasagiline also increases antioxidant enzyme activities.

Male F-344 rats (8.5-month-old) were divided into three groups (each 4 rats). Two experimental groups were infused subcutaneously with rasagiline saline solutions by means of osmotic minipumpus implanted subcutaneously on the back of rats. Control animals were also similarly implanted with saline filled minipumps. Three and a half weeks later, animals were sacrificed and interested tissue samples removed. CAT and SOD activities were determined as reported previously1). Two doses of rasagiline (0.5 mg / kg / day, 1.0 mg / kg / day) significantly increased CAT activities about 2 fold in substantia nigra (S.N), striatum (ST), but not in hippocampus (HP). Interestingly in both renal cortex (RC) and medulla (RM), CAT activities were significantly increased. Both Mn- and Cu, Zn-SOD activities were increased 2 to 4 fold in SN, ST and RC, Heart (H). Observations on the heart and kidneys are also new, since we and others have never examined the effects of deprenyl on these organs. Although both doses of rasagiline were similarly effective, the 0.5 mg / kg dose was more effective than the two fold greater dose (1.0 mg /kg / day).

Several groups including our group2) have reported an extension of survival of deprenyl-treated animals of different species including rats, mice, hamsters and dogs3, 4). Although the mechanism(s) of the life extension effect of deprenyl remains unelucidated, we have raised the possibility that effects of deprenyl on life span and antioxidant enzymes may causally be related3, 4). It would be an interesting trial to see the effect of rasagiline on survivals of animals.

1) Carrillo M.C. et al. Exp Neurol. 116:286-294, 1992.
2) Kitani K et al. Life Sci 52:281-288,1993.
3) Kitani K et al. Ann New York Acad Sci 786: 391-409, 1996.
4) Kitani K et al. Ann New York Acad Sci 854:291-306, 1998

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THE HAYFLICK LECTURE

FUNCTIONAL GENOMICS STUDY OF HUMAN LONGEVITY: GENES, BIOCHIPS AND HIGH THROUGHPUT TECHNOLOGY. E. Wang*, H. Riol*, J. F. Qian*, G.-H. Zhang*, X. Y. Ye*, A. Agarwal-Mawal*, Y. S. Wang*, L. Bathum# & B. Jeune#, *The Bloomfield Center for Research in Aging, Lady Davis Institute, Jewish General Hospital, Department of Medicine, McGill University, Montréal, Québec, Canada; *Division of Family Medicine, Taichung Veterans Hospital, Taiwan, ROC; #Division of Geriatrics, Department of Medicine, University of Odense, Denmark

Investigating the determination of life span of a human being is probably one of the ultimate tasks in the gerontologic research quest. Whether one favors the theory of programmatic and/or stochastic regulation, one can not deny the fact that a complex system is at work in determining how we become old, and how long we live. With the advent of the Human Genome era, we are fortified with tools, technology, and systems to allow us to address the complexity of the aging process from a multigenic and multifactorial approach. We can venture from single-gene analysis to the comprehensive profiling of the regulation of families of gene. The now popular high-throughput technology provides us a platform and means to screen the activation or repression of hundreds of genes' expressions at a glance. This report will describe my experience so far using the functional genomic approach to investigate the complexity of genetic and environmental factors determining human life span, largely studying the extremely old population. I will describe our experience in setting up a large centenarian-based pedigree population, the technology development for simultaneous isolation of DNA, RNA, and protein samples, and preliminary results of investigating the regulation of candidate gene expression, focussing primarily on genes functionally involved in the process of apoptosis, to test our hypothesis that extreme longevity in man may be associated with a beneficial lymphocyte suicidal program to get rid of damaged or infected cells. In addition, I will discuss our technology development, how to set up a robotic system to manufacture biochips for gene screening using the samples from the above population. Finally, I will describe two computer programs involved in the data mining task of analyzing the information derived from the high-density microarray biochips. I will demonstrate how computerized system analysis allows us to formulate new hypotheses based on information about the gain or loss of hundreds of gene expressions, as well as new strategies to study the complex traits of the individualistic and heritable characteristics of human life span determination. This presentation will be a progress report of the first phase of our long road in studying human longevity, i.e., the set-up of our biological and technology systems aimed at the gene discovery task of identifying centenarian-associated candidate gene expressions. Identification of these genes will pave the way to our ultimate goal, which is to aspire to live as long as Madame Calmont, till 122 years old, with minimal functional senescence.

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AVERTING CELLULAR SENESCENCE THROUGH TELOMERE MANIPULATION. Gregg Morin*, Geron Corporation, 230 Constitution Dr., Menlo Park, CA 94025.

Cultured normal human cells have a finite life span that is determined by their ability to fully replicate the termini, or telomeres, of their chromosomal DNA. The RNP reverse transcriptase telomerase is required for replication of the repetitive DNA that constitutes telomeres. Most normal human cells express no telomerase and their telomeric DNA shortens upon cell division ultimately leading to cellular senescence, but cancer cells of all types overwhelmingly express telomerase as a means to stabilize their telomeric DNA and become immortal. Normal human skin fibroblasts and retinal pigment epithelial cells transfected with the catalytic component of human telomerase, hTERT, have now more than doubled their expected life span in culture. These cells retain growth control mechanisms typical for normal human cells and have no apparent changes associated with malignant transformation. These cells exhibit a trend towards increased mean TRF length and decreasing telomerase activity levels but the telomerase activity in the these cells remains at sufficiently high levels to maintain stable long telomeres. These experiments confirm the link between telomere structure and replicative life span and indicate that telomere manipulation could provide a novel approach to develop therapeutics for age-related diseases.

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STATUS OF GLYCATION MARKERS IN HUMAN AND RODENT STUDIES. David Sell[1]*, David Burke, Richard Miller, and Vincent Monnier[1]. [1]Institute of Pathology, Case Western Reserve University, Cleveland, OH 44106 and The Geriatrics Center, University of Michigan, Ann Arbor, MI 48109.

In recent years considerable interest has been in the development of markers of aging in order to study mechanisms of aging and its interventions. Quite commonly these markers have been isolated and studied in collagen and lens proteins due to their ubiquitousness, slow turnover and longevity. A number of adducts and cross-links have been found to increase with age including those of glycation (furosine), oxidation (methionine sulfoxide, o-tyrosine), glycoxidation (pentosidine, CML, GOLD, vesperlysines), lipoxidation (CML), and nonoxidative post-Amadori modifications (i.e., derivatives of 3-deoxyglucosone and methylglyoxal such as CEL and MOLD). In our work, we have observed a significant ($P<.0001$) increase in ornithine in human skin. Although its exact origin is not known, ornithine probably originates from fragmented modifications of arginine such as argpyrimidine and the imidazolones. In application of markers, longitudinal determination of glycation (furosine) and glycoxidation (CML, pentosidine) rates in skin collagen of ad libitum (AL) and caloric restricted (CR) mice of the C57BL/6NNia strain showed that glycation rate could predict early death in AL and CR mice. Similarly, pentosidine and CML formation rates could predict early death in AL and

CR mice, respectively. Further studies with genetically heterogeneous mice [i.e., progeny of (BALB/cJ X C57BL/6J) F1 mothers crossed to (C3H/HeJ X DBA/2J) F1 fathers] showed that genes on mouse chromosomes 1, 2, and 8 have suggestive associations with CML, furosine, and pentosidine, respectively. Preliminary data, though not fully conclusive, suggest that a locus on chromosome 12 may influence both longevity and furosine accumulation in parallel, with some genotypes associated with both extended longevity and low levels of furosine in tail tendon collagen. These data suggest that an age-related deterioration in glucose tolerance may occur at late age which is associated with early demise of mice.

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MECHANISM OF AMYLOID FORMATION IN NEURODEGENERATIVE DISEASES. Debbie McKenzie* and Judd Aiken. Department of Animal Health and Biomedical Sciences. University of Wisconsin-Madison. 1656 Linden Dr. Madison, WI 53706.

Amyloid formation is a critical step in the pathogenesis of many neurodegenerative diseases, especially Alzheimer's Disease (AD) and Creutzfeldt-Jakob disease (CJD). The etiology of both diseases is still not well-understood but, in both cases, involves the toxic accumulation of aberrant forms of normal host-encoded proteins, βA4 in AD and PrP in CJD. The major difference between the normal and aberrant forms of these proteins is conformational with the abnormal forms having a higher B-sheet content than the normal isoforms. These conformational changes also result in the normal forms being monomeric and relatively soluble while the abnormal, disease-specific proteins are aggregated and exceedingly insoluble. Jarrett and Lansbury proposed that amyloidogenesis occurs via nucleation-dependent polymerization. Cell-free conversion assays have demonstrated that aggregates or "seeds" of βA4 and PrP are capable of directing the conversion of the normal isoform to the cellular isoform.

We have extended these studies, taking advantage of the infectious nature of PrP, to determine whether co-factors facilitate or enhance the conversion process. In this study, disruption of PrP^{Sc} (as monitored by the loss of proteinase K resistance) by guanidine hydrochloride (GdnHCl) resulted in decreased infectivity. Upon dilution of the GdnHCl, both PrP protease resistance and infectivity were restored. The addition of transition metals, copper and zinc, facilitated restoration of both infectivity and protease resistance of PrP in a subset of samples that did not renature by the simple dilution of the GdnHCl. These data suggest that transition metals may play an important role in the early steps of PrP amyloidogenesis.

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WERNER SYNDROME RESEARCH: THE LAST CENTURY AND IN THE NEXT. Raymond J. Monnat, Jr.*; Department Of Pathology and Genetics, University of Washington, Seattle, WA 98195.

Werner syndrome (WRN; McKusick/MIM #277700) is an uncommon autosomal recessive disease that results from mutational inactivation of the chromosome 8p WRN gene. The WRN gene encodes one member of the human RecQ helicase protein family that may play diverse roles in cellular nucleic acid metabolism. Clinical and biological interest in WRN has focused on resemblance of the WRN clinical phenotype to premature aging in association with genetic instability and an elevated risk of neoplasia. WRN patients are at increased risk of several specific neoplasms, most notably soft tissue and osteosarcomas, thyroid carcinoma, an unusual form of melanoma and meningiomas. Somatic genetic instability may represent a common mechanistic link between the WRN phenotypes of premature aging and this elevated cancer risk. I will review our understanding of the structure and function of the WRN protein, the mutational basis for WRN, evidence for genetic instability in WRN patients, and the unanswered questions that serve as a focus for WRN research at the University of Washington and in other labs in the U.S., Japan and Europe.

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BAD CODON USAGE: A NOVEL TECHNIQUE TO AID THE MITOCHONDRIAL IMPORT OF TRANSGENE-ENCODED HYDROPHOBIC PROTEINS. A.D.N.J. de Grey, University of Cambridge, Cambridge, UK

The mitochondrial theory of aging remains compelling, despite increasing acceptance that mutant mtDNA never becomes at all abundant in any tissue — a conclusion challenged only by novel PCR variants whose quantitative accuracy is widely doubted. The theory's survival is largely because impressive evidence continues to accumulate that mitochondria are indeed the most important site of free radical damage in vivo; a prominent recent example is that only the mitochondrial isoform of superoxide dismutase is vital in mice. A possible mechanism whereby low levels of mutant mtDNA may cause disproportionate systemic damage was proposed recently [de Grey ADNJ, 1998, J. Anti-Aging Med.1 (1): 53-66].

Mutant mtDNA is preferentially amplified during mitochondrial turnover in non-dividing cells such as muscle fibres. This may confound attempts to reverse mtDNA decline by introduction or amplification of wild-type mtDNA. An alternative which escapes this problem is "nuclear mtDNA" — suitably modified transgenic copies of the mtDNA's 13 protein-coding genes, whose products would enter mitochondria by the same pathway as the hundreds of naturally nuclear-coded mitochondrial proteins. Most objections to the efficacy and/or feasibility of this approach have been rebutted, but a serious difficulty remains: the mitochondrial protein import machinery cannot handle proteins as hydrophobic as the 13 of interest.

Some mitochondrial protein import is cotranslational. However, there appears to be little regulation of whether a particular molecule is imported co- or post-translationally: it appears to be largely random. Hydrophobic proteins are thought to be unimportable because the most hydrophobic domains cannot be unfolded; they may thus be more amenable to co- than post-translational import, since a domain that has not yet been constructed cannot refuse to unfold. In support of this idea, overexpression of a factor involved in nuclear-cytoplasmic transport improves import of hydrophobic proteins [Corral-Debrinski M et al., 1999, Mol. Microbiol. 31(5): 1499-1511]; the mechanism may be that faster completion of export allows the mRNA to be transported to distant mitochondria before much translation has occurred.

A protein might be more cotranslationally imported if it is constructed more slowly, allowing import to keep up. This might happen if the mRNA includes many rare codons. There is thus a strong case for a synonymous substitutions to introduce rare codons into "nuclear mtDNA" transgenes.

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THE FUTURE OF HUMAN LONGEVITY: A MORE REALISTIC APPROACH FOR LIFE EXPECTANCY FORECASTING. N.S. Gavrilova*, L.A. Gavrilov, G.N. Evdokushkina, V.G. Semyonova, Center on Aging, NORC/University of Chicago, 1155 East 60th Street, Chicago, IL 60637.

Recent scientific debates on the future of human longevity and its possible biological limits has revealed a great need for direct biological evidence for such longevity limit if it really exists (Gavrilov, Gavrilova, 1998, *Science*, 281: 1611-1615). For this purpose the familial transmission of human longevity from parents to daughters (more than 4,000 cases for adult daughters born in 1800-1880) was studied, since

The familial transmission of human longevity from mother to daughter is essentially non-linear with very weak resemblance before maternal life span of 85 years (regression slope of daughters life span on maternal life span, $b = 0.04 \pm 0.02$, $n = 3{,}756$ cases, $p = 0.05$) and very high additive heritability for longer lived mothers ($b = 0.53 \pm 0.26$, $n = 484$, $p < 0.05$). This indicates that maternal age of 85 years could be considered as a demarcation line for women longevity. Women who live above this age are fundamentally (biologically?) different from other women in the sense that their daughters live significantly longer. Thus life expectancy at 85 years could be a biological limit for validity of extrapolative approach in forecasting of human life expectancy for women.

Similar study of familial transmission of human longevity from father to daughter revealed a demarcation point at 80 years, suggesting that this age might represent a limit for validity of extrapolative approach to male life expectancy. The familial transmission of human longevity from father to daughter is also non-linear with very weak resemblance before paternal life span of 80 years ($b = 0.03 \pm 0.02$, $n = 3{,}842$, $p = 0.18$) and very high additive heritability for longer lived fathers ($b = 0.36 \pm 0.16$, $n = 763$, $p < 0.05$).

These results are also consistent with the predictions of evolutionary theory of aging and mutation accumulation theory in particular that the additive genetic variance for human life span should increase with parental longevity (Gavrilova et al., 1998, *Human Biology*, 70: 799-804).

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CHILDREN BORN TO OLDER MOTHERS DO NOT INHERIT MATERNAL LIFESPAN. L.A. Gavrilov*, N.S. Gavrilov, G.N. Evdokushkina, V.G. Semyonova. Center on Aging, NORC/University of Chicago, 1155 East 60th Street, Chicago, IL 60637.

Familial resemblance in life span between children and parents was studied by many researchers for a century, but no attention was paid so far to the possible effects of parental age at childbirth on familial transmission of longevity. In this study we have tested the hypothesis that familial resemblance between offspring and parental longevity is higher for children born to younger parents as expected both for genetic reason (higher genetic diversity of young parents) and for cultural reasons (higher overlapping between parental and offspring life cycles). For this purpose the high quality data (more than 15,000 records) on European royal and noble families (more than 900 families) were collected computerized and analyzed.

We have found that familial transmission of human longevity is a function of parental age at childbirth. In particular, both daughters and sons born to older mothers (above age 35) do not demonstrate any inheritance of maternal life span. The regression slope of son's life span on maternal life span is 0.11 $\pm$ 0.05 (n= 3,274 cases, $p<0.05$) when mother is young (15-34 years) and statistically insignificant (-0.01 $\pm$ 0.12, n = 707, p = 0.95) for older mothers (35 years and above). The regression slope of daughter's life span on maternal life span is 0.22 $\pm$ 0.08 (n = 1,479 cases, $p < 0.01$) when mother is young (15-34 years) and statistically insignificant (-0.05 $\pm$ 0.17, n = 306, p = 0.77) for older mothers (35+ years). These estimates were calculated for the range for maternal life span of 75-95 years. The scientific importance and practical implications of this observation are discussed.

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THE CLONING AND CHARACTERIZATION OF A MOUSE SMC GENE. Rosalina James* and Christine Disteche, University of Washington, Dept of Pathology; Seattle, WA, 98195

Structural Maintenance of Chromosomes (SMC) is an evolutionarily conserved family of proteins. SMCs are functional in the condensation and proper segregation of DNA as cells proceed through the cell cycle. They have been shown to be involved in DNA recombination, chromosome condensation, sister chromatid cohesion, and dosage compensation. The high sequence conservation of SMC genes between species suggests that SMCs are equally important proteins in mammalian chromosome dynamics. Study of molecular mechanisms underlying the assembly of genomic material in cells is fundamental to understanding age-related processes such as aneuploidy.

My project involves the cloning and characterization of a mouse SMC gene, Sb1.8. I have cloned and sequenced the mouse Sb1.8 cDNA. High conservation (95% identity) was found between human and mouse cDNA sequences. Ubiquitous expression of Sb1.8 message was seen in multiple adult mouse tissues and throughout embryogenesis. Polyclonal antibodies generated against the mouse SMC were applied to western blots. The expected band size of 140kDa was found in nuclear, but not in cytoplasmic protein extracts. However, immunofluorescence results with these antibodies suggest the SB1.8 protein may also localize to the cytoplasm in mouse cells. This conflicting localization data indicates the antibodies used may not be specific for SB1.8 protein. Finally, full-length and truncated SB1.8 have been expressed with a myc epitope tag in mouse and human cells. The fusion proteins were detected by immunofluorescence methods. Using antibodies against the myc tag, signal was seen in both the nuclei and cytoplasm of mouse fibroblasts and human HeLa cells. Fewer cells were found to express the fusion proteins (~10%) than a control construct containing β-galactosidase (~30-40%), suggesting overexpression of SB1.8 may be toxic to cells. In addition, a number of cells stained with the myc antibodies appeared to be undergoing apoptosis.

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ABSTRACT NOT PRESENTED

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REACTIVATION OF SILENCED ALLELES ON THE INACTIVE X CHROMOSOME IN AGING. M. Aker* and C.M. Disteche, Dept. of Pathology, University of Washington, Seattle, WA 98195

To test the hypothesis that epigenetic control of gene expression is altered with aging, we have set up a system to detect low levels of misexpression of silenced genes in a mouse model of aging. Promoter hypo- and hyper- methylation that lead to aberrant gene expression have been observed in neoplasias. Similarly, loss of chromatin structure and demethylation of promoter regions may lead to reactivation of expression of silenced genes with aging.

In female somatic cells, one of the two X chromosomes is inactivated in early embryogenesis and the inactive state stably inherited in further cell divisions. Epigenetic changes associated with X inactivation include DNA methylation and changes in chromatin structure. There have been conflicting reports of reactivation of silenced X-linked genes during aging. To collect data on frequencies of reactivation, we are constructing transgenic mice with reporter sequences on the X chromosome.

We have inserted a promoterless gene, E. coli b-galactosidase/ neomycin phosphostransferase, behind endogenous X chromosome promoters in male embryonic stem cells. Transgenic female mice heterozygous for four reporter constructs on the X chromosome are being bred. Clonal populations of cells from heterozygous females will display either expression or silencing of the reporter molecule. Monoallelic silencing will be followed with passaging of cells in culture or in vivo in female transgenic mice with aging. Reactivation will be detected as expression of the reporter molecule at the resolution of the single cell.

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ALTERATIONS IN RESPONSE TO AGING OF EXCITATION-CONTRACTION COUPLING PROTEINS IN SKELETAL MUSCLE. Terry E. Jones*, Diana J. Bigelow; University of Kansas, Lawrence, KS 66045-2106.

The decreased force of contraction and increased time-to-peak contraction in aged skeletal muscle may be related to changes in sarcoplasmic reticulum calcium release. The putative mechanical coupling between the excitation-contraction coupling proteins, the ryanodine receptor (RyR) calcium release channel and the dihydropyridine receptor (DHPR) suggests an optimal ratio of these proteins exist. The purpose of this study was to determine if there is an age-dependent change in this ratio, which might explain the decrease seen with, aging in contraction parameters directly attributed to calcium release. We tested a purified heavy sarcoplasmic reticulum (HSR) fraction from the hind limb skeletal muscle of the mammalian-aging model, the Fisher 344 x Brown Norway rat. Antibodies directed against the skeletal muscle isoform of the RyR (Ryr1) and DHPR (DHPRo<1) were used to determine the relative immunoreactive contents of the RyR and DHPR in HSR. The functional probes [^{3}H]ryanodine (for RyR) and [^{3}H]PN200-110 (for DHPR) were utilized to detect the content of functional RyR and DHPR, respectively. The immunoreactive content of the RyR is stable in 14, 22, and 30 mo. old animals compared to that from 5 mo. old adults. Although, at senescence (34 mo. old animals) the relative immunoreactive content of the RyR is 48$\pm$14% ($p<0.05$) compared to that from 5 mo. old adults. The immunoreactive content of DHPR progressively decreases approximately 1% per month ($p<0.036$) in the ages tested and at 34 mo. old was 75$\pm$10% of the immunoreactive content of that of 5 mo. old adults. With respect to functional probe binding, the maximal binding of [^{3}H]ryanodine to HSR is stable through the five ages tested averaging 19.4$\pm$.8 pmol/mg protein. The maximal binding of [^{3}H]PN200-110 to HSR is stable in 5, 14, 22, and 30 mo. old animals averaging 20.7$\pm$1.4 pmol/mg protein. In the 34 mo. old HSR the binding of [^{3}H]PN200-110 decreased 43% to 9.0 pmol/mg protein. The 52% loss of immunoreactive RyR without loss of functional probe binding at senescence suggests conformational changes or post-translational modifications occur that could effect the antibody recognition site of RyR. In contrast, the DHPR shows a 25% loss of immunoreactive protein and 57% loss of functional probe binding in senescence suggesting protein expression level alterations and/or conformational or post-translational changes effecting the antibody recognition and functional probe binding sites. The differential response to aging of these two receptors responsible for calcium release necessary for muscle contraction could disrupt the DHPR:RyR ratio which in turn could

contribute to diminished skeletal muscle function that accompanies aging.

33 *NICOLAI AWARD*

MODULATION OF KINASE ACTIVITY CAN ALTER THE SENESCENT PHENOTYPE. Keith Wheaton*, Edward Parr, Muthupalaniappan Meyyappan, and Karl Riabowol, Department of Medical Biochemistry and Southern Alberta Cancer Research Centre, University of Calgary, Calgary, Alberta T2N 4N1

The limited proliferative lifespan of Human diploid fibroblasts (HDFs) serves as a model of replicative senescence, and has been shown to parallel aging in the whole organism. Senescent HDFs have distinct morphology, are unable to proliferate even in the presence of mitogens and arrest in a G1 like state. The exact nature of the blunted growth response to mitogens remains unclear, but may involve aspects of DNA damage induced arrest and changes in regulation of mitogenic signal transduction pathways. One of the changes seen in senescent HDFs that may influence cell growth is lack of immediate early gene expression, particularly the genes c-fos and egr-1. Our lab has previously shown that down regulation of these genes during aging is due to lack of the serum response factor (SRF) activity, and that an age specific phosphorylation event modifies SRF DNA binding activity. Thus, we are examining kinases, which may have altered activity or specificity with aging. A combined approach of kinase assays, EMSA and phosphopeptide mapping has identified kinases which may differentially phosphorylate SRF in high passage cells. Specifically, PKC isoforms in the nucleus of senescent cells may play a pivotal role in regulating SRF binding to the serum response element (SRE). Thus, the down regulation of many serum responsive transcripts with age is likely due to differential regulation of specific kinases in old cells. Consistent with this idea, we have found that specific combinations of kinase inhibitors can directly alter the cell cycle kinetics of senescent cells and concomitantly alter expression levels of genes known to be altered in replicative senescence, such as D-type cyclins. These studies provide direct support for the idea that subtle alterations in mitogenic kinase cascades are central to the initiation and maintenance of the senescent phenotype.

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CARDIAC OVERLOAD AS A PATHOPHYSIOLOGICAL EVENT: CORRELATES TO THE "THRESHOLD AGE." Arthur C. Cosmas, School of Allied Health, The University of Connecticut, Storrs, CT 06269. Tel. (860) 486-0050; Fax: (860) 486-1588 and Thomas G. Manfredi, University of Rhode Island, Exercise Science Program, Kingston, RI 02881. Tel. (401) 874-5439;Fax: (401) 874-5630.

The pathophysiologic role assumed by oxidative stress appears to be compromised by the presence of a subset of cytoprotective stress-resistance genes that code for a group of protective proteins such as the heat shock group of stress proteins (HSPs) that repair or degrade damaged proteins. In fact, oxidative stress in the form of exercise induced hypoxia may be an important element in preconditioning the heart against a prolonged ischemic overload. As the heart ages, however, common hemodynamic events such as hypertension and ischemia cause a decrease in the induction of proto-oncogenes and stress-resistance genes or an acceleration in the expression of stress genes. As a result, the aging cardiomyocyte, normally compromised in its ability to respond to oxidative stress, becomes confronted with a signal in the form of a hemodynamic overload that intensifies the degenerative alterations that normally characterize aging even though the heart "appears" to continue to compensate. Myocardial mitochondria are profoundly affected by an increase in oxidative stress. If they become dysfunctional they may promote the expression of proto-oncogenes that code for the proliferation of a population of mitochondria that have "modulated" in size in an attempt to accommodate to the oxidative stress. This myocardial response to prolonged ischemic overload appears to be age dependent once the "Critical Age" has been exceeded. The mitochondrial profiles within the cardiomyocyte appear to be dramatically different in young as compared to older hearts and may have relevance as to whether exercise induced cardiac overload will be interpreted as a "pressure overload" or a "volume overload." Left ventricular adaptation to volume overload decreases with age and appears to be replaced by the development of a persistent elevation in end-diastolic pressure and wall stress characteristic of pressure overload. These disruptive changes are initially associated with increased myocardial contractility, but if the stress persists, the hyperfunctional heart will eventually make the transition toward failure.

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ANALYSIS OF THE RELATIONSHIP BETWEEN PARAOXONASE ACTIVITIES AND GENOTYPE. Victoria H. Brophy*, Rebecca J. Richter, Amber C. Harms, Gail P. Jarvik, and Clement E. Furlong. Department of Medicine, Division of Medical Genetics, University of Washington, Seattle, WA 98195

Atherosclerosis is the primary cause of death in the United States with the risk of disease rising with age. Paraoxonase (PON1) is tightly associated with HDL particles and is believed to play a protective role against atherosclerosis by metabolizing oxidized lipids. PON1 also hydrolyzes the bioactive oxon forms of organophosphate pesticides such as parathion, diazinon, and chlorpyrifos. Two polymorphisms have been identified in the coding sequence of human PON1. Position 192 may be either Arg or Gln, while position 55 may be Met or Leu. Several previous studies have found that the presence of the Arg_{192} allele raises the risk of cardiovascular disease in some populations. The studies, however, examined the genotype of the PON1 gene only, not the level of expression of the protein. In this study, we have determined the expression level of PON1 and examined the relationships between the genotype and PON1 level of the individuals. We used a two-dimensional enzyme analysis with the substrate pair paraoxon and diazoxon to determine PON1 status of patient plasma samples. This analysis reveals both the genotype and the expression level of PON1. The genotypes inferred by this assay agreed with that determined by PCR analysis for 99% (149 out of 150) of the samples, confirming the reliability of this new method for inference of $PON1_{192}$ genotype. We found that the expression level, as determined by paraoxonase and diazoxonase activities, varies widely among individuals and within a genotype. Diazoxonase activity varied five-fold (3,936-21,000 U/L) among $PON1_{Q192}$ homozygotes, six-fold (2,710-17,167 U/L) among $PON1_{Q/R192}$ heterozygotes, and four-fold (2065-9161 U/L) among $PON1_{R192}$ homozygotes. Thus, genotype alone is not sufficient for determining PON1 status.

Previous studies have found that individuals having Met alleles at position 55 have lower levels of PON1 mRNA and paraoxonase activity. We found considerable overlap in activity level among the $PON1_{55}$ genotypes. Of the 150 individuals whose $PON1_{192}$ status was detected by two-dimensional analysis, 70 (47%) were $PON1_{Q192}$ homozygotes. Of these, 19 (27%) were $PON1_{LL55}$, 32 (46%) $PON1_{L/M55}$ and 19 (27%) were $PON1_{MM55}$. Analysis of the population of $PON1_{QQ192}$ showed that while the average PON1 activity (diazoxonase) was 13767 U/L (± 4040) for $PON1_{LL55}$ and 9557 U/L (± 2890) for $PON1_{MM55}$, a given $PON1_{M55}$ homozygote could have more than twice the activity of a $PON1_{L55}$ homozygote. Thus, while statistically the $PON1_{L55}$ homozygotes had greater PON1 activity than the $PON1_{M55}$ homozygotes, genotyping alone was far from informative for predicting PON1 levels.

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VITAMIN E AND AGE ALTER LIVER MITO-CHONDRIAL MORPHOMETRY. K.Agostinucci1*, T.G.Manfredi[1], A. Cosmas[2], K. Martin[3], S.N.Han[3], D. Wu[3], J. Sastre[4], S.N.Meydani[3], M.Meydani[3]. [1]Exercise Science Lab., Univ.of Rhode Island, Kingston, RI, [2]School of Allied Hlth., Univ.of Connecticut, Storrs, CT, [3]JM USDA-Human Nutr.Res.Ctr.on Aging at Tufts Univ., Boston, MA, [4]Univ.of Valencia, Spain.

Cell oxidative damage caused by free radicals during cell respiration plays a major role in tissue dysfunction. A lack of vitamin E and selenium results in liver enlargement of mitochondria, suggesting mitochondrial (Mt) fusion and an inability to divide. The purpose of this study was to assess the effects of vitamin E supplementation (500 IU/kg diet) on old C57BL/6 mice liver Mt morphometry and Mt levels of oxidized glutathione (GSSG). Measurements of Mt area, short and long axis, and size distributions were taken from control and vitamin E supplemented mice. Transmission electron microscopy and image analysis was used to measure fixed Mt from liver homogenized samples (Table follows).

	C	E	OE	CvsE	EvsOE
age (days)	760/3.8	758/2.9	827/17.9		
mito.area (u2)	.797/.45	.592/.43	.582/.25	p<.0001	NS
major axis (u)	1.06/.29	.889/.3	.944/.21	p<.0001	p<.002
minor axis (u)	.904/.22	.766/.26	.755/.16	p.0001	NS

C=old control, E=old vitamin E, OE=older Vitamin E; mean/S.D.

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AGE-ASSOCIATED ELECTRON TRANSPORT SYSTEM ABNORMALITIES CONCURRENT WITH INTRA-FIBER ATROPHY IN RAT SKELETAL MUSCLE FIBERS. Jonathan Wanagat, Nathan Van Zeeland*, Pranali Pathare, Judd M. Aiken. University of Wisconsin-Madison, Madison, WI 53706

Sarcopenia, the loss of skeletal muscle mass associated with aging, contributes to the physical frailty observed in aged individuals. The etiology of sarcopenia, like many age-related pathologies, may be attributed to numerous factors including contraction-induced injury, deficient satellite cell recruitment, motor unit decline, increased oxidative stress and age-related mitochondrial abnormalities. Our studies suggest that mitochondrial DNA (mtDNA) deletions are linked to fiber atrophy and fiber loss. In this model, a random deletion event occurs, presumably due to oxidative stress, removing a portion of the mitochondrial genome. This truncated, mutant mtDNA possesses a replicative advantage in comparison to the larger, wild type mtDNA and, thus, accumulates regionally. An energy deficit within the affected region spurs a nuclear compensatory response increasing mitochondrial replication. This increased accumulation of abnormal mitochondria results in an electron transport system (ETS) abnormal phenotype. By this mechanism, the ETS abnormal region gradually expands while the fiber concurrently becomes less functional, atrophies and is eventually lost from the skeletal muscle fiber population.

Our present study is aimed at elucidating the relationship of ETS abnormalities to fiber atrophy by applying cellular analyses. The rectus femoris muscles were removed from Fisher 344 x Brown Norway F_1 hybrid rats aged 5, 18 and 38 months. Whole muscle weights were obtained and a 50% reduction was observed from 18 to 38 months. Serial, transverse cryomicrotome sections, spanning a 2,000 mm region of the rectus femoris muscle, were obtained for histologic analyses. *In situ* histochemical staining for succinate dehydrogenase (SDH) and cytochrome c oxidase (COX) activity was performed on serial muscle sections from 5, 18 and 38-month-old rats. The successive sections were analyzed for the presence of fibers displaying ETS abnormal phenotypes, including negative staining for COX or hyperreactive staining for SDH. Fibers displaying these ETS abnormal phenotypes were not present in any of the 5-month-old rats while as many as 122 fibers were ETS abnormal in a 38-month-old animal. Intra-fiber atrophy was examined by measuring cross sectional area (CSA) of ETS abnormal fibers throughout the length of the fiber, both within the ETS abnormal region (EAR) and in the phenotypically normal region. In ~25% of the ETS abnormal fibers analyzed, the mean CSA within the EAR was statistically smaller than the mean CSA in the phenotypically normal region of the same fiber. The length of each EAR was also measured within each ETS abnormal fiber and was found to display an age-related increase. These findings suggest a role for age-related mitochondrial abnormalities in the development of sarcopenia.

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AGE-ASSOCIATED CHANGES IN CARDIAC FUNCTION, STRUCTURE AND MITOCHONDRIAL GENETIC AND ENZYMATIC ABNORMALITIES IN THE FISCHER 344 X BROWN NORWAY F_1 HYBRID RAT. Jonathan Wanagat*, Judd M. Aiken University of Wisconsin-Madison Madison, WI 53706

Myocardium from Fischer 344 x Brown Norway F_1 hybrid rats of ages 5, 18 and 36-38 months was examined for mitochondrial genetic and enzymatic abnormalities. Age-associated deletions of the mitochondrial genome as well as electron transport system abnormalities were identified and related to global cardiac aging by assessment of cardiac function and cardiac histological structure. Mitochondrial genomes lacking large sections of primary sequence were detected in tissue homogenates from right and left ventricular myocardium and this mtDNA deletion abundance increased with age. *In situ* histochemical staining of serial, cryomicrotome sections of myocardial tissue revealed individual cardiomyocytes displaying abnormal activities of both cytchrome c oxidase and succinate dehydrogenase. These histochemically abnormal cardiomyocytes increased in number with age and were localized primarily in the left ventricular myocardium. In addition to chronological relationships, the mitochondrial abnormalities were related to left ventricular functional and histological changes assessed by in vivo hemodynamic measurements and determinations of percent area of fibrosis, respectively. In vivo hemodynamic measurements showed age-related changes of left pressures in hearts with significant hypertrophy. Histological evaluation of fibrotic change demonstrated the greatest percent area of fibrosis in the left ventricular subendocardium and a focal distribution of replacement and interstitial fibrosis in each of the hearts examined at 38 months of age. The presence of these age-related functional, structural and mitochondrial genetic and enzymatic abnormalities in the Fischer 344 x Brown Norway F_1 hybrid rat heart suggests the possible role of mitochondrial dysfunction, secondary to mtDNA mutations, in age-related cardiomyocyte loss and cardiac aging.

39 ***GLENN AWARD RUNNER-UP***

COMPARISON OF HIPPOCAMPAL GLIA NUMBER IN C57BL/6J AND 129/SvJ MICE OF DIFFERENT AGES. Long, J.M.*, Hengemihle, J.M., Moon, E.K., Mouton, P.R., Ingram, D.K., Gerontology Research Center, National Institute on Aging, NIH. Baltimore, MD 21224.

The purpose of the present study was to examine age-related changes in hippocampal glia number in male 129/SvJ and C57BL/6J mice. The recent advent of transgenic mice provides powerful in vivo models to assess many aspects of age-related neurodegenerative diseases, including neuromorphometrics. Strain differences exist in murine brain aging, therefore, it is important to have baseline age-related neuromorphometric data on each strain that comprises the genetic background of transgenic mouse models. 129/SvJ and C57BL/6J represent two such strains. The hippocampus is an important brain structure to examine for possible age-related changes because it is involved in memory formation and because it has proven to be highly vulnerable to neurodegeneration. These data represent a subset of a larger ongoing project that examines the total number of microglia, astrocytes, neurons and synaptic boutons in the hippocampus of these two strains. Design-based stereological techniques were used to estimate total glia number in hippocampal subregions of male mice of both strains at different ages: young (3-5 mo.), adult (13-17 mo.), and aged (26-28 mo.). Mice were anesthetized, perfused, and each brain was serially sectioned. Utilizing systematic-random sampling, approximately 10 sections were samples for immunocytochemical staining for astrocytes and 10 sections for microglia. The antibodies employed were GFAP and Mac-1 respectively. Estimates of total astrocyte and microglia number were assessed using the optical fractionator technique assisted by a computer-based system.

In C57BL/6J mice, no statistically significant age differences were found in the number of glia in the hippocampal subregions sampled. 129/SvJ mice had a significant age-related increase in the number of microglia in the dentate gyrus. Strain differences in glia number were observed among different subregions and glia cell types. This on-going project will provide important baseline information that can be used to evaluate various genetic manipulations using mouse models. The strain differences observed thus far underscore the need to conduct such evaluations.

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AGE-RELATED DECLINE IN ACTIVATION OF CALCIUM/CALMODULIN-DEPENDENT PHOSPHATASE CALCINEURIN AND KINASE CAMK-IV IN RAT T CELLS. M.A Pahlavani* and D.M Vargas, Geriatric Research, Education, and Clinical Center, South Texas Veterans Health Care System, Audie L. Murphy Veterans Hospital, and Department of Physiology University of Texas Health Science Center San Antonio, TX 78284.

We have previously shown that the DNA binding activity of the transcription factor NFAT which plays a predominant role in IL-2 transcription decreases with age (Pahlavani *et al.*, *Cell. Immunol.* 165: 84, 1996). Because the transactivation (dephosphorylation and nuclear translocation) of the NFAT-c (cytoplasmic component of the NFAT complex) is mediated by the calcium/calmodulin (Ca^2+/CaM)-depen-

dant phosphatase, calcineurin (CaN), and because Ca^2+/CaM-dependent kinases (CaMK-II and IV/Gr) have been shown top lay a critical role in calcium signaling in T cells, it was of interest to determine what effect aging has on the activation and the levels of these calcium regulating enzymes. The induction of calcineurin phosphatase activity and CaMK-II and IV/Gr activities were studied in splenic T cells isolated from Fischer 344 rats at 6, 15, and 24 months of age. In addition, the changes in the protein levels of these enzymes were measured by Western blot. The calcineurin phosphatase activity and CaMK-II and IV kinase activities were at maximum after the cells were incubated with anti-CD3 antibody for 5 to 10 minutes. The induction of calcineurin activity by anti-CD3 and by calcium ionophere (A23187) declined 65% and 55%, respectively, between 6 and 24 months of age. The induction of CaMK-IV activity but not CaMK-II activity by anti-CD3 was significantly less (by 54%) in T cells from old rats compared to T cells from young rats. The decline in the activation of these enzymes with age was not associated with changes in their corresponding protein levels. These results indicate that alteration in the activation of CaN and CaMK-IV may lead to the age-associated decline in T cell function.

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ABSTRACT NOT PRESENTED

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BACKGROUND FOR A CONSENSUS ON THE VALUE OF CONSUMING VITAMIN C AND E SUPPLEMENTS. Alfred B. Ordman*, Biochemistry Program, Beloit College, Beloit, WI 53511.

Since Dr. Denham Harman first proposed the free radical theory of aging, substantial evidence has accumulated to demonstrate the safety and potential health benefits of taking vitamin C and E in supplement form. The purpose of this study has been to review the available medical and scientific studies published to date. The underlying antioxidant mechanisms of vitamin C and E are becoming clear. Clinical trials have demonstrated potential efficacy of supplements in reducing the risks of particular age-associated diseases, especially cancer, atherosclerosis, cataracts, loss of immune function, and neural damage. Various risk factors which may accompany taking high dosages of these micronutrients have been addressed, including prooxidant activity and kidney stones. Five key studies support the selection of optimal dosages of these two vitamins - approximately 500-mg of vitamin C twice a day and 200 to 400 iu of mixed tocopherols daily. One key question not yet addressed is the synergistic effects of these two micronutrients.

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ABSTRACT NOT PRESENTED

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SEQUENTIAL HISTOCHEMICAL STUDIES OF LIPOFUSCIN IN HUMAN CEREBRAL CORTEX FROM THE FIRST TO THE NINTH DECADE OF LIFE. Eduardo A. Porta*, Sebastian Benavides and Alberto J. Monserrat. Dept. Path. Univ. Hawaii, Sch. Med., Honolulu, Hi 96822, USA, and Dept. Path. Univ. Buenos Aires, Sch. Med. Argentina.

The typical golden-yellow autofluorescence of in situ lipofuscin granules, their consistent sudanophilia, acid-fastness, and positiveness to PAS (resistant to diastase digestion), as well as their lectin affinities denoting the presence of diverse saccharide residues have been generally described in neurons and other postmitotic cells of old humans and animals. The purpose of this sequential study was to explore possible age-related variations of these properties in cortical cerebral neurons of the left temporo-parietal areas from male and female individuals dying from the first to the ninth decade of life of causes which would not ordinarily affected these cells. Autofluorescence was studied with a Zeiss microscope equipped with a halogen lamp and epi-fluorescent condenser with a 390-440 nm BP filter and a barrier 475 nm filter. Sudanophilia was visualized by oil red O staining, acid-fastness by the long Ziehl-Neelsen reagent, PAS reactivity by the periodic Schiff-reagent, and the saccharides moities by seven different biotinylated lectins (Kit LK-2000, from Vector, Lab. Burlingame, CA). Few granules of lipofuscin randomly scattered in the cytoplasm of less than 10% cortical neurons were detected in one specimen from a 5-year old boy, but all showed typical autofluorescence, sudanophilia, acid-fastness and PAS reactivity. In specimens from the second to the ninth decade of life all these characteristic properties were also detected in the lipofuscin granules which at these stages accumulated preferentially in the perikarion of the majority of cortical neurons. However, and totally unexpectedly, no saccharide moities were detected by lectin histochemistry in lipofuscin granules until the fifth decade of life. Specimens from the fifth decade only showed mannose residues, and those from the sixth decade only showed mannose and sialic acid. At the eight and ninth decades the lipofuscin granules showed the presence of mannose, sialic acid, acetyl glucosamine and galactosamine, lactose and fucose. These new findings indicated that very little saccharides are present in lipofuscin of cerebral cortical neurons during early and middle years of life, and suggested that the possitiveness to PAS present from the first decade of life may be due to the presence of vicinal glycols in unsaturated lipids rather than in those of saccharides.

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DIETARY RESTRICTION AND 2-DEOXY-D-GLUCOSE PROTECT NEURONS IN THE BRAIN AGAINST EXCITOTOXIC, OXIDATIVE AND ISCHEMIC INJURY: EVIDENCE FOR THE INVOLVEMENT OF STRESS PROTEINS. J.Lee*, Z.F. Yu, A.J. Bruce-Keller, Y. Kruman, S.L. Chan, & M.P. Mattson. Department of Anatomy & Neurobiology, and Sanders-Brown Research Center on Aging, University of Kentucky, Lexington, KY 40536.

Although dietary restriction (DR) can extend life span in rodents, its impact on age-related neurodegenerative conditions is unknown. We recently reported that rats maintained on DR exhibit increased resistance of hippocampal and striatal neurons to excitotoxic and metabolic insults (*Ann. Neurol.* 45:8-15 (1999)). We now report that DR in adult rats' results in reduced brain damage and improved behavioral outcome in a middle cerebral artery occlusion-reperfusion (MCAO-R) stroke model. In principle, administration to ad libitum fed rodents of an agent that reduces glucose availability to cells should mimic certain aspects of DR. Administration of 2-deoxy-D-glucose (2DG), a non-metabolized analog of glucose, to adult rats resulted in a highly significant reduction in seizure-induced spatial memory deficits and hippocampal neuron loss. As was the case with DR, 2DG greatly increased the resistance of rats to kainate-induced deficits in performance in water maze learning and memory tasks. 2DG administration also reduced ischemic brain damage and improved behavioral outcome following MCAO-R. Pre-treatment of rat hippocampal cell cultures with 2DG decreased the vulnerability of neurons to excitotoxicity (glutamate), an oxidative insult (Fe^2+) and chemical hypoxia. The protective action of 2DG was associated with decreased levels of cellular oxidative stress and enhanced calcium homeostasis. 2DG treatment increased levels of the stress-responsive proteins GRP-78 and HSP-70 in cultured hippocampal neurons, without affecting levels of Bcl-2 or GRP-75, suggesting that mild reductions in glucose availability can increase neuronal resistance to oxidative and metabolic insults by a mechanism involving induction of stress proteins. DR and 2-DG administration resulted in an increase in the level of the stress protein HSP-70 in striatal cells in vivo. The neuroprotective effects of DR and 2-DG in the excitotoxicity and ischemia models documented in our studies suggest that DR may reduce risk for age-related neurodegenerative disorders, and may improve outcome following stroke. In addition, our findings establish cell culture and in vivo models of "chemical food restriction" which may prove useful in elucidating mechanisms of neuroprotection, and in developing preventative approaches for neurodegenerative disorders that involve oxidative stress and excitotoxicity.

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DIETARY RESTRICTION ATTENUATES AGE-RELATED DECREASE IN REPAIR OF SPECIFIC GENES. ZhongMao Guo* and Arlan Richardson, Department of Physiology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78284.

Restriction of total calories in rodents has been shown to increase the life span and maintain important physiological processes a youthful state for a longer period of life. In this study, we have focused on the effect of dietary restriction on the ability of cells to repair DNA damage specific to genes. The repair of UV-induced DNA damage in specific genes was measured in cultured hepatocytes isolated from young (6-month-old) and old (24-month-old) rats fed ad libitum or a calorie-restricted diet. Our results demonstrated that cultured hepatocytes

preferentially removed cyclobutane pyrimidine dimers (CPDs) from DNA fragments containing transcriptionally active genes, e.g., the albumin and phosphoenolpyruvate carboxykinase (PEPCK) genes, as compared to the genome overall or the DNA fragment containing the transcriptionally inactive embryonic myosin heavy chain (MHCemb) gene. The rate of removal of CPDs from DNA fragments containing the albumin gene and the cAMP-induced PEPCK gene was reduced about 40% in the hepatocytes isolated from old rats compared to young rats. This is due to a decrease in the repair of the transcribed strand of these fragments. This age-related decrease in repair the transcribed strand of these active genes may be caused by an age-related decrease in the level of the DNA repair-transcription coupling proteins, e.g., CSA and CSB proteins but does not appear to be caused by an age-related decrease in the transcription of these genes. In contrast, the extent of repair (24 hours after UV-irradiation) of the genome overall and the DNA fragment containing the MHC gene in hepatocytes was reduced about 44% with age. Dietary restriction attenuated the age-related decrease *in the rate* or the extent of repair for the DNA fragments and the genome overall.

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TELOMERIC SHORTENING IN AGING RAT LENS AND DELAY BY CALORIC RESTRICTION. Jin Li*, William R. Pendergrass, Norman S. Wolf., University of Washington, Pathology Department, Seattle WA. 98195.

The purpose of this study is to determine if telomeric shortening can be detected in the lens epithelia of aging rats and whether it is delayed by chronic caloric restriction. The relative telomeric lengths were compared in interphase nuclei in the lenses of Brown Norway Rats maintained on an ad libitum diet (AL) or on chronic caloric restriction CR. The relative telomeric content of nuclei were quantitated using Fluorescent In Situ Hybridization (FISH). Lenses were removed from sacrificed animals and ~ 15 micron frozen sections cut for analyses. Sections were cut from both the Central region of the lens epithelia and the Pre-equatorial region. The sections were then dried 60 min, fixed with 1% paraformaldehyde 10 min, and hybridized with an FITC labeled peptide nucleic acid probe 18 bases long and complementary to the telomeric regions, using the method described by Lansdorp et al (Human Molecular Genetics: v. 5, p. 685, 1996). The hybridized sections were washed free of unbound probe and stained with a fluorescent DNA counter stain, 7AAD (7-amino-actinomycin D). The ratio of telomeric fluorescence / DNA fluorescence was determined per nucleus using computer assisted confocal microscopy. Only the sections from the central region of the lenses demonstrated significant changes with age or diet using a paired t-test. Preliminary results from central region sections cut from 5 sets of young, old Al, and old CR animals indicate that age related telomeric shortening may be occurring during aging of the AL rats, (26-33 months) compared to young AL controls (5-7 months) with $p < .06$. CR old rats showed retardation of age related telomere shortening ($p < .05$ vs old AL), correlating with a delay in cataract formation also seen with CR.

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CATARACT AS A BIOMARKER OF AGING; CALORIC RESTRICTION DELAYS BOTH. N.S. Wolf*, Yi Li, W.R. Pendergrass; C. Schmeider, A. Turturro. Dept. Of Pathology, University of Washington, Seattle, WA, 98195; National Center for Toxicological Research, Jefferson, AR, 72079

Cataract has been proposed to be the result of accumulated oxidative free radical damage to lens epithelial cells. We looked at its incidence in rodents (1) as a biomarker of aging and (2) for the effect of CR on its time of occurrence and the degree of oxidative resistance by lens epithelial cells. A combined total of 500 animals in 5 common laboratory strains of mice and rats in specific age groupings were examined by slit lamp for the incidence of age-related cataract. All individuals in all strains developed cataract in advanced form by late life. In comparing 2 rat strains with long and short lifespans, respectively, the time of development of age-related cataract was correlated to their lifespans, developing later in the long-lived animals. This finding is compared to several publications indicating that early cataract occurrence in humans correlates with shorter lifespans. In addition, mice and rats maintained on long term caloric restriction (CR) developed cataracts significantly later in life, correlating with the extension of their maximum lifespans produced by CR, and remaining centered on the last quarter of lifespan. Our studies also have shown a decreased resistance to oxidative damage in vitro by lens epithelial cells from old versus young mice and a highly significant protective effect by CR, as measured by lens cell clonal growth after H202 exposure. It is concluded that mice and rats of standard laboratory strains provide excellent models for the study of cataract and that the progressive development of this pathology is suitable as a biomarker of aging in which the mechanism can be studied.

49 ***NICOLAI AWARD***

AGE AND FIBER TYPE AFFECT THE PHENOTYPE OF AGE-ASSOCIATED ELECTRON TRANSPORT SYSTEM ABNORMALITIES IN RHESUS MONKEY SKELETAL MUSCLE. Marisol E. Lopez[1]* Nathan L. Van Zeeland[1] Richard Weindruch[2], and Judd M. Aiken[1]. Departments of [1]Animal Health and Biomedical Sciences and [2]Medicine and VA Geriatric Research, Education and Clinical Center, University of Wisconsin-Madison, Madison WI 53706 USA

Vastus lateralis muscle of rhesus monkeys of different ages was histologically examined for age-associated electron transport system (ETS) abnormalities. An average of 180 serial cross-sections (totaling 1440 microns in length per muscle) were obtained from muscle biopsies of nine animals (ages 11 to 34 years old). Multiple cross-sections of the muscle were analyzed for cytochrome c oxidase (COX) and succinate dehydrogenase (SDH) activities. Fibers were identified that were devoid of COX activity (COX^-) and/or exhibited increased SDH activity (SDH^{++}). We found that the abundance of skeletal muscle fibers displaying ETS abnormal regions increased with the age of the animal and that the ETS phenotype was affected by the fiber type. The ETS abnormal phenotype also changed with age with middle-aged animals primarily exhibiting the COX^- phenotype while COX^-/SDH^{++} abnormalities becoming more common in the muscle of old animals. The use of a longitudinal analysis allowed us to determine the length of ETS abnormal regions within affected fibers. Finally, the measurement of cross-sectional area along the length of these ETS abnormal fibers determined that some of these fibers have a tendency to atrophy (adjacent ETS normal fibers did not show major changes in cross-sectional area). This study stresses the importance of longitudinal analysis, as well as, fiber type analysis to determine the abundance of fibers harboring ETS abnormal regions. Furthermore, fibers displaying ETS abnormal regions may contribute to the age-associated loss of muscle mass.

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THE INSULIN RESISTANCE SYNDROME AND ITS DELAY AND MITIGATION BY LONG-TERM CALORIE RESTRICTION: IMPLICATIONS FOR CALORIE RESTRICTION-MIMETIC THERAPY. B.C. Hansen, H.K. Ortmeyer, and N.L. Bodkin*, Obesity and Diabetes Research Center, Dept. of Physiology, School of Medicine, University of Maryland, Baltimore, MD 21201, USA.

The anti-aging paradigm of long-term calorie restriction (CR) *in non-human primates* is a very powerful technique for slowing and/or preventing the development of obesity (Hansen et al, Obesity Research, 1995), the development of peripheral insulin resistance (Bodkin et al, J. Gerontology, BS, 1995) and the develoment of Type 2 diabetes mellitus (Hansen and Bodkin, Diabetes, 1993). In the present study we examined the effectiveness of calorie restriction in rhesus monkeys (Macaca mulatta) to prevent or delay the development of the Insulin Resistance Syndrome, also known as the metabolic syndrome X. This age-related syndrome of metabolic abnormalities has been defined to include the following features: central obesity, hyperinsulinemia, dyslipidemia, impaired glucose tolerance, and/or hypertension. The development of the Insulin Resistance Syndrome in 30 ad libitum-fed non-diabetic monkeys was compared longitudinally to its development in 8 long-term calorie restricted monkeys. In the ad libitum fed animals we sought to determine the sequence of appearance of the metabolic disturbances, to assess their degree of linkage across time, and to examine differences between those monkeys manifesting all components of the syndrome and those monkeys manifesting only one or two of these five key features.

The expression of Syndrome features was: hypertriglyceridemia ~70%, impaired glucose tolerance ~50%, and hypertension ~33%. The presence of hypertension seemed to play a minimal role in the rate of progression. In contrast, advanced age and peripheral insulin resistance was a characteristic of those monkeys exhibiting all components of the Syndrome. In a parallel group of 8 monkeys, calorie restriction (at levels titrated to maintain normal adult lean body mass) was extraordinarily effective in delaying or preventing all features of the Syndrome. We suggest that calorie restriction-mimetic agents thus pose significant potential for mono therapy for this Syndrome and warrant further investigation.

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BODY COMPOSITION AND BONE MINERAL DENSITY DURING INITIATION OF CALORIC RESTRICTION IN YOUNG AND AGED RHESUS MACAQUES. A. Black*, E.M. Tilmont, A.M. Handy, D.K. Ingram, G.S. Roth and M.A. Lane. Intramural Research Program, Gerontology Research Center, NIA, NIH Baltimore, MD 21224

Human body composition changes with advancing age in that lean mass (LM) decreases and fat mass (FM) increases. Age-related bone loss is also recognized and has been shown to be influenced by changes in both fat and lean mass. Caloric restriction (CR), undernutrition without malnutrition, is known to extend mean and maximal lifespan and retard physiologic aging in many systems. In rodents and nonhuman primates, moderate CR reduces body weight (BW), FM and LM. Long term CR delays skeletal maturation in young animals and results in slightly decreased bone mineral density (BMD) at selected sites in adults. The strong association between BMD and body composition during long-term CR, as well as the absence of evidence of altered bone metabolism, suggests that reduced biomechanical forces may the drive the changes in BMD. This study investigates the temporal relationship between changes in body composition and bone mass during the initiation of CR in rhesus monkeys. Two groups of young [12 males (YM):4 yrs, 12 females (YF): 3-4 yrs] and one group of old [male (OM): 18-24 yrs] rhesus macaques *(Maccaca mulatta)* were included. Six animals from each group were gradually restricted to 30% of ad libitum feed consumption while the rest of the animals continued to eat *ad libitum*. Dual energy x-ray absorptiometry (DEXA) was used to measure bone mineral density and body composition at baseline, after one month at 10, 20 and 30% restriction and again after 6 months and 1 year on 30% restriction. Total body, mid and distal radial BMD measurements were made. Blood samples were collected at these same time points for measurement of biochemical markers of bone turnover. Young males were heavier and had more lean mass than young females, while old males were heavier and had greater percent body fat than young males. CR lowered BW in all three age groups, although significance was achieved at different time points. Gains in lean mass were greater in *ad lib* YMs and YFs than CR animals, while LM declined in all OMs over time. CR reduced FM in old males after reaching 30% restriction, a change that temporally mirrored changes in BW. BMD increased over time at all sites in both young groups, with no significant differences between *ad lib* and CR animals. Similarly, BMD was not affected by diet in OMs, although mid radius BMD decreased over time in this group. In summary, CR was strongly associated with reduced gains in LM and BW in restricted YMs and YFs while the primary effect in OMs was reduced FM and BW. Body composition parameters were altered during the initiation of CR in young and old rhesus macaques without the accompanying changes in BMD previously reported in chronically restricted animals.

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A NONHUMAN PRIMATE MODEL OF MENOPAUSE: EVALUATION OF REPRODUCTIVE CYCLING AND HORMONES. [1,2]A.M. Handy*, [2]A. Black, [2]D.K. Ingram, [2]G.S. Roth, [2]E.M. Tilmont and [2]M.A. Lane. [1]R.O.W Sciences, Inc., Gaithersburg, MD 20878 [2]Intramural Research Program, Gerontology Research Center, National Institute on Aging, NIH, Baltimore, MD 21224.

Menopause, defined as the gradual decline and eventual cessation of female reproductive function, is associated with an increased risk of several age-related health problems among older women. Because of their genetic proximity and physiological similarity, nonhuman primates are often used as models of human disease. Several studies of surgically-induced menopause in monkeys suggest their potential utility as a model for reproductive aging, however, few studies have reported on naturally-occurring changes in reproductive indices with aging. Our study was designed to assess the potential of female rhesus monkeys as models of human reproductive aging using 21 animals in three age groups [J (3-5yr: n=8), A (8-15yr: n=9), O (>18yr: n=5)]. Daily observations of menstrual bleeding were performed over a two-year period. All animals continued to cycle during the study and therefore, none could be considered postmenopausal. Blood samples were collected once a year during the follicular phase (day 5) of the menstrual cycle and analyzed for estradiol, progesterone, LH and FSH. Estradiol decreased with age, whereas progesterone and LH showed no significant change. FSH was elevated and correlated with decreased estradiol in some, but not all older monkeys and the correlation with age was significant. The older animals (23-28yr) show signs of oligormenorrhea such as increased number of abnormal cycle lengths and acyclicity. The results of this study suggest that rhesus monkeys exhibit many of the same hormonal and menstrual cycling changes observed during human aging. Further, our findings support the use of nonhuman primates as models of human female reproductive aging.

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DEVELOPMENT AND UTILITY OF A MULTICENTER, LONGITUDINAL DATABASE OF AGING IN NONHUMAN PRIMATES [1]K. Poydence*, [2]J. Robinson, [3]J. Kemnitz, [4]S. Kohama, [5]M. Hayek, [6]D. Ingram, [6]G. Roth and [6]M. Lane. [1]R.O.W. Sciences, Gaithersburg, MD 20878, [2]National Center for Research Resources, NIH, Bethesda, MD 20892, [3]Wisconsin Regional Primate Research Center, University of Wisconsin, Madison, WI 53715, [4]Oregon Regional Primate Research Center, Beaverton, OR 97006, [5]The Iams Company, Lewisburg, OH 45338, [6]Intramural Research Program, Gerontology Research Center, NIA, NIH, Baltimore, MD 21224.

Nonhuman primates are often used as models of disease. In recent years, their use in biogerontological studies has increased. However, studies of aging in primates are often limited to very small numbers of animals due to reduced availability of older monkeys and the high costs of maintaining and conducting studies using nonhuman primates. Another major difficulty is the lack of comparative aging data in various primate species. In an attempt to address these problems, we are developing a multicenter, longitudinal database of aging in nonhuman primates. This proposed database will serve three major purposes: to identify and evaluate candidate biomarkers of aging, to develop a normative aging data repository and to be used as an aid in clinical veterinary practice. Pooled data from various species can be utilized to identify and evaluate candidate biomarkers of aging which may be used in intervention studies and may ultimately be applicable to humans. In the present study we examined the utility of the database to establish serum albumin as a potential biomarker of aging. Albumin met all selection criteria. It exhibited cross-sectional and longitudinal correlations with chronological age. Furthermore, individual differences in albumin values remained stable over time and the rate of decline in serum albumin was inversely proportional to species lifespan in primates and canine and feline models. The database also has the capability of providing a practical way to determine age-specific normal values for blood chemistry parameters. Establishing these norms would aid researchers and veterinarians as animal lifespans exceed those for which data are currently available. In addition to serum albumin, we identified several blood chemistry parameters that appeared to change with age. Establishing normal age-related changes in blood chemistry parameters will provide a valuable reference for veterinarians treating the increasing number of geriatric primates being used in research facilities.

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AGE-RELATED DECLINE IN FOOD INTAKE AND MOTIVATION FOR FOOD IN RHESUS MONKEYS. T. Moscrip*, M. Lane, G. Roth, D. Ingram. *R.O.W. Sciences, Gaithersburg, MD 20878, Intramural Research Program, Gerontology Research Center, NIA, NIH Baltimore, MD 21224.

Studies in humans have indicated an age-related decline in dietary intake that is particularly pronounced at advanced ages (Silver et al., J. Am. Geriatr. Soc., 41, 211, 1993). In the most severe cases, this

condition is known as cachexia, and can result in severe loss of body mass because of insufficient nutritional intake. We examined data on dietary intake from a longitudinal study of aging in rhesus monkeys *(Macaca mulatta)* collected for over 10 years. Monkeys entered the study at three different age ranges: (1-3 years, 4-8 years, and ³18 years). The monkeys are provided a highly standardized, nutritionally fortified diet (biscuit form) during two daily meals, morning and early afternoon. Food intake studies have been conducted quarterly. These involve daily weighing of food provided and calculating weekly intake for each monkey adjusting for food spillage. When examining monkeys over 5 years of age, we have noted a significant age-related decline in food intake that becomes pronounced after age 20 years in both male and female monkeys. Food intake was significantly correlated with body weight, and body weight also declined with age. To assess motivation for food, we used a tray-type apparatus containing evenly spaced recessed wells. This tray was attached to the monkey's home cage, and a food biscuit was placed in the well closest to the monkey's cage. By reaching out its arm through an opening in the cage, the monkey could retrieve the biscuit from the well. For additional trials, the biscuit was placed into wells progressively farther from the monkey's reach until it was unable to retrieve the biscuit. The time that the monkey spent attempting to retrieve the unreachable biscuit was recorded as a measure of hunger motivation. The measure also showed an age-related decline that appeared generally earlier in adult life (<15 years). It appears that age-related decline in food intake and hunger motivation observed in humans is a phenomenon that generalizes to other primate species.

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ENDOTOXIN-INDUCED CYTOKINE RESPONSES IN RHESUS MONKEYS: EFFECTS OF AGE AND CALORIE RESTRICTION. P. Mascarucci[1*], D. Taub[2], A. Handy[1], H. De Angelis[1], C. Weininger[1], G. Roth[1], M. Lane[1], D.K. Ingram[1], [1] LCMB/MPCS and [2] LI/CIS

Many studies representing a variety of species report age-related disregulation in cytokine production. In human studies, however, the picture of aging regarding this question is unclear. There are highly inconsistent results regarding age effects on Type 2 cytokines (e.g. IL-6, IL-10) as well as proinflammatory cytokines (e.g. IL1• and TNF•). Elevated levels of IL-6 and IL10 have been observed both in stimulated cultures of peripheral blood mononuclear cells (PBMCs) as well as greater levels of IL-6 in the sera of elderly people. However, these results are not always consistent, and the quality of the samples regarding freedom from disease can be questioned. Results for stimulated IL1 production in PBMC are even more varied with some studies showing age-related increases and others, age-related declines that appear greatly dependent upon the type of stimulation. A similar conclusion exists for age changes in TNF production.

It appears that much of the inconsistency in results can be attributed to different techniques (cellular populations, type of stimulus), but more likely to the quality of the subject samples, regarding, primarily their health status. Therefore, to provide better experimental control than has been achieved in many past human studies, we have undertaken an aging study in a nonhuman primate, the rhesus monkey *(Macaca mulatta)*. In addition, we can evaluate the effects an intervention that has been successful in retarding some parameters of immunological aging in rodents, specifically dietary calorie restriction (CR). It is well documented that CR without malnutrition retards a great variety of aging processes, extends median and maximum life span, and decreases the incidence of cancer and other age-associated diseases in different animal models. Results emerging from the NIA study of CR in rhesus monkeys have indicated many parallels to findings in rodent studies, regarding potential retardation of many age-sensitive parameters.

To investigate the pattern of cytokine production in aging and possible effects of CR, we obtained blood cells from control and CR monkeys of different ages (12-28 years). We evaluated production of different cytokines, IL-6, IL-10, IL-1, TNFα, in whole blood, and PBMC, following *in vitro* exposure to lipopolysaccharide (LPS 25 g/ml) *(ex-vivo)*.

The current preliminary data suggest an age-related disregulation in LPS stimulated IL-6 and IL-1 production in whole blood samples from male rhesus monkeys in the control group. No significant age differences were observed in IL-10 and TNFa production. Long-term CR appears to attenuate the age-related difference in LPS stimulated IL-6 and IL1 production, but these results were not statistically significant given the small sample of aged monkeys examined thus far. Therefore, a number of well described, age-related conditions, some of which could contributing to the pathologic phenotype of old age, may actually represent secondary effects to this age-associated change in cytokines production.

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PET DOGS AS A MAMMALIAN MODEL TO STUDY THE ASSOCIATION BETWEEN AGING AND SPONTANEOUS CANCER DEVELOPMENT DJ. Waters *. Department of Veterinary Clinical Sciences, Purdue University, West Lafayette, IN 47907

In order to further understand the influence of organismal aging on cancer development, we have studied pet dogs with spontaneous tumors. Pet dogs and humans share a 25-40% lifetime risk for cancer-related mortality. In humans, the histologic spectrum of cancers that develop early in life (e.g. childhood cancers) differs significantly from the cancers that affect elderly adults. We determined the histologic spectrum of 1068 neoplasms that developed in young dogs (≤ 2 years old). Similar to children, the vast majority of young dogs with malignant tumors have non-epithelial cancers.

The profound difference in maximum life span between small and large breed dogs provides a unique opportunity to identify biomarkers that could be used to predict different rates of aging in individuals within the same species. In addition, these breed-specific differences in life span have the potential to seriously confound any analysis of the association between chronologic age and risk for age-related diseases. To address this concern, we developed an algorithm based on mortality data from over 23,000 pet dogs to convert the chronologic age of dogs to physiologic age in human year equivalents (Patronek et al, J Gerontol 1997; 52A: B171-178). Using this algorithm, we have shown that age at prostate cancer diagnosis is similar between pet dogs and humans (Waters et al, J Natl Cancer Inst 1996; 88: 1686-87).

Observations from the human oncology clinic and data from experimental tumor models suggest young hosts that develop tumors may have a poorer prognosis than elderly tumor-bearing hosts. To test this hypothesis, we studied 69 dogs with spontaneous breast cancer that underwent surgical excision of their cancers. Analysis of the association between postoperative outcome and physiologic age showed that the youngest dogs had significantly poorer prognosis (p=0.02). Importantly, there was no significant association between chronologic age and outcome. These results demonstrate the utility of our physiologic age algorithm to overcome the confounding effects of life span differences across different breeds.

The influence of organismal aging on the biological behavior of cancers may be different for epithelial malignancies and non-epithelial malignancies. In order to test this hypothesis, we compared the relative frequency of benign versus malignant breast (epithelial) and vascular (non-epithelial) tumors in the youngest (≤ 2 years old) and oldest (> 10 years old) subsets of pet dogs. Review of pathology records of over 4000 tumor-bearing dogs showed no significant difference in the ratio of benign to malignant breast tumors in the youngest and oldest dogs. In contrast, vascular tumors that developed in young dogs were 4X more likely to be benign than the vascular tumors of elderly dogs (p=0.006). These data are intriguing because they suggest that the influence of host age on the development of malignant tumors may be tissue specific.

In summary, pet dogs provide a mammalian model to determine how organismal aging influences the development of lethal cancers. Ultimately, we hope to design interventions using pet dogs to guide human interventional trials that will decrease age-related functional decline and decrease cancer-related mortality.

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EFFECTS OF ADVANCED AGING ON PLASMA CATECHOLAMINE RESPONSES TO THERMAL STRESS

Eric C. Petrie (*)[1,2], Marcella Pascualy[1,2], Kayla Brodkin[3], Elaine R. Peskind[1,2], Murray A. Raskind[1,2]. VA Puget Sound Health Care System, [1]Mental Illness Research, Education and Clinical Center, [3]Geriatric Research, Education and Clinical Center and [2]Dept. of Psychiatry and Behavioral Sciences, University of Washington, Seattle, Washington 98195.

Increased basal norepinephrine (NE) concentrations have been demonstrated repeatedly in studies of "early aging" (EA; under age 75) human subjects. We asked if "advanced aging" (AA; over age 80) enhanced the effects of early aging on plasma NE and epinephrine (EPI) concentrations at rest and in response to a thermal stress elicited by the cold pressor test (CPT). Eight medically well, cognitively intact AA subjects (84.4 ± 0.86 [mean ± SEM] years), 28 medically well cognitively intact EA subjects (70.3 ± 1.28 years), and 19 medically well young subjects (Y; 25.4 ± 0.92 years), were studied.

Both basal NE (AA = 469 ± 100.1, EA = 242.7 ± 13.6, Y = 178.4 ± 16.9 pg/mL; F[2,44]=14.55, p<0.005) and the acute (5 min) NE increase following CPT (AA = 259.4 ± 110.1, EA = 81.2 ± 23.9, Y = 83.7 ± 10.9 pg/mL; F[2,44]=4.89, p<0.05) were significantly higher in AA than in either EA or young subjects (p's<0.05 by Neuman-Keuls). Basal plasma EPI concentrations were higher in the AA group than in the other groups (AA = 90.0 ± 9.1, EA = 56.7 ± 3.4, Y = 72.4 ± 10.1 pg/mL) and an acute (5 min) plasma EPI increase following CPT occurred only in the AA group (AA = 32.50 ± 18.1, EA = -0.25 ± 3.72, Y = 0.26 ± 5.1 pg/mL; F[2,44]=4.72, p<0.05).

These results suggest specific effects of advanced vs early aging on basal and stress-induced activation of both the sympathoneural and sympathoadrenomedullary components of the sympathetic nervous system in man.

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FAMILY CARING FOR ELDERS IN HOSPITAL: A MODEL DERIVED FROM MULTIPLE PERSPECTIVES. Hong Li*[1], Barbara J. Stewart[2], Margaret A. Imle[2] [1]School of Nursing, University of Rochester, Rochester, NY 14642, [2]School of Nursing, Oregon Health Sciences University Portland, OR 97201.

More than 11 million persons aged 65 and over are hospitalized each year in the United States (Graves & Owings, 1998, Advanced Data from Vital and Health Statistics; No.301. National Center for Health Statistics). Although family caregiving is likely to be continued during hospitalization of elderly relatives, limited research has been conducted regarding the processes by which families provide such care. The purpose of this qualitative study was to develop a framework of family care, including the kinds of caregiving activities performed and antecedent, mediating, and outcome variables associated with the care process.

Altogether 25 interviews were done with 16 participants (6 family members, 6 patients, and 4 nurses); 7 participants were interviewed once and 9 were twice. Qualitative analysis was based on Lofland and Lofland (1984) approach.

In the derived model, characteristics of patients, family caregivers, family-patient relationship, hospital and nurses influence family care actions both directly and indirectly as mediated through family worry and patient preferences. The processes of family care actions include carrying on, modifying, starting new, sharing and arranging care for the older patient. These actions lead to outcomes of continuity of life patterns for patients (e.g., feeling cared for, preventing dysfunctional syndromes) and for caregivers (e.g., increased preparedness, increased rewards). Outcomes for nurses include decreased direct workload and increased rewards. This model explicates family care for hospitalized elders from perspectives of family members, patients and nurses.

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SYSTEMIC GROWTH HORMONE DOES NOT PREVENT MUSCLE ATROPHY IN DENERVATED HINDLIMBS OF AGED MICE. Anna Weiss*, Eyeleth Bronosar, Abraham Reznick. The Department of Anatomy and Cell Biology. The Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa 31096, Israel.

Dissuse, whether caused by bedrest, casting or paralysis, causes a rapid muscle atrophy. Various studies indicated beneficial effects of growth hormone (GH) on muscle mass and activity. The present study tested the ability of GH to prevent muscle wasting caused by denervation. Male ICR mice aged 14 months were subjected to unilateral hindlimb denervation by resecting the right sciatic nerve, while the left sham-operated legs served as controls. Animals were treated by daily s.c. injections of either 1 mg/kg body weight of rat recombinant growth hormone (rGH, kindly given by BioTechnology General, Rehovot, Israel) or by injecting an equivalent volume of the vehicle. Animals were sacrificed one month following denervation and the triceps surae muscle, which is comprised of the soleus, the gastrocnemius and the plantaris muscles, was excised in toto.

In the vehicle-treated group, denervation caused a 52% decrease in the weight of triceps surae muscle in denervated legs in comparison to the control legs (p<0.01). The muscle weight increased following rGH-treatment by 12% and 15% in the denervated and control limbs respectively (p<0.05 in comparison to vehicle-treated animals).

The activity of creatine phosphokinase (CPK) in triceps surae muscle in the denervated limbs of the vehicle-treated animals decreased to 10% of its activity in the control leg . CPK activity increased by 150% and 250% in the denervated and control leg respectively in rGH treated animals, so it still was less than 10% of the control leg value. In conclusion, the present findings show that in aged mice, systemic GH treatment could not prevent muscle wasting in limbs paralyzed by denervation. In addition, our findings indicate clearly that GH exerts beneficial effects on the non-paralyzed muscles of the control, apparently over-loaded limb.

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ANTI-AGING ACTIONS OF NOVEL NITRONES. M. J. Forster*, Y. Wang, L. Nguyen and J. Kelleher-Andersson. Department of Pharmacology, University of North Texas Health Science Center, Fort Worth, TX and Centaur Pharmaceuticals, Sunnyvale, CA.

Aging involves an oxidative stress imbalance along with an immune function decline. NFkB appears to play a pivotal role in regulating both these functions. Specifically, an increased NFkB binding to consensus sequences in brain tissue (Helenius et al., Biochem. J., 318: 603-608, 1996; Toliver-Kinsky, J. *et al., Neurosci.* Res. 48:580-587, 1997) and a decreased induction of NFkB activity in T cells (Trebilcock and Ponnappan, Mech. Ageing Dev., 102:149-163, 1998) are observed in the elderly. Historically, the free radical "trapping" ability of nitrones, e.g. phenylbutylnitrone (PBN), was thought to be the primary factor accounting for their ability to improve age-related cognitive impairments in aged animal models. Further links between oxidative stress regulation and inflammation has necessitated a closer examination of nitrone's effects on NFkB function and ROS generation. We measured NFkB activity in liver or neuronal cells stimulated by H2O2 and/or Fe2+. Though nitrones appeared more potent than the water soluble vitamin E (Trolox) at inhibiting NFkB, inhibition between nitrones varied markedly. In contrast, Trolox inhibited ROS generation to a greater extent than nitrones could trap, with PBN being one of the most potent nitrones. We then determined if the ability to regulate NFkB activity in culture by novel nitrones, would equate to efficacy in an aging mouse model.

In vivo studies tested efficacy of novel nitrones to prevent or ameliorate cognitive deficits associated with aging in C57BL/6 mice. Old (23.5 months) and young (4.5 months) C57BL/6 mice received daily oral treatment with novel nitrone or the vehicle for a period of up to 29 weeks. Following 2 weeks of treatment, the mice began testing on a discriminated-avoidance, recent memory task. Previous studies using this task had indicated that aged C57BL/6 mice showed slower acquisition of memory performance, as well as faster time-dependent decay of recently acquired memory (Forster, M. J. and Lal, H., Behav. Pharmacol. 3, 337-349,1992). The old mice receiving 0.1 or 10 mg/kg/day of a novel nitrone showed more rapid learning of the recent memory task when compared with the old mice receiving treatment with the vehicle. The rate of learning by the old mice treated with novel nitrone was comparable to or superior to that of the young mice receiving chronic treatment with the vehicle. During the retention phase, when mice were tested for memory under conditions of high demand (after a delay of 90 minutes), performance was more accurate in old mice treated with 0.1 or 10 mg/kg novel nitrone than in the old vehicle controls. Analysis of body weight data over the study period failed to indicate significant differences attributable to treatment with novel nitrone. However, mortality was significantly lower among the old mice treated with novel nitrone when compared with old vehicle controls. The results indicate that novel nitrone may be efficacious in the treatment of memory dysfunction associated with normal aging or degeneration, and may delay the progress of memory decline associated with those conditions. Additionally, it would appear that a novel nitrone is able to promote longevity when initiated relatively late in life.

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PHARMACEUTICAL TREATMENTS TO PREVENT AND REVERSE GLYCATION PRODUCTS. Anthony Cerami* and Malcolm Meistrell III. The Kenneth S. Warren Laboratories, Tarrytown, NY 10591.

Recent studies reveal that reducing sugars, such as glucose, react with proteins through non-enzymatic glycosylation to form irreversible, covalently cross-linked proteins known as Advanced Glycation Endproducts (AGEs). Furthermore, it has been demonstrated that this naturally occurring process, accelerated in diabetics due to hyperglycemia, impairs biological functions leading to cardiovascular disorders, as well as, diabetic and age-related complications. Pharmaceutical intervention to prevent or reverse these complications have focused on inhibiting the formation of AGEs by compounds such as Pimagedine or breaking the glucose derived cross-links by selective cleavage. Pimagedine, an AGE inhibitor compound, recently finished Phase III clinical trials in Type I diabetics. Pimagedine treatment significantly reduced urinary protein, LDL cholesterol, triglycerides, and diastolic blood pressure. In addition, Pimagedine exhibited improvements in estimated creatinine clearance and glomerular filtration rate. 3-[2'-phenyl-2'-oxoethyl]-4,5-dimethylthiazolium chloride, also known as ALT-711, is a novel class of stable, orally active, catalytic compounds designed to specifically break protein cross-links thus attenuating the pathological conditions associated with AGEs. *In vitro* experiments reveal that ALT-711 breaks approximately 80% of BSA-AGE-collagen cross-links. ALT-711 also demonstrates beneficial effects in animal models of diabetic and age-related complications. Streptozotocin induced diabetic rats receiving 1 mg/kg, i.p., daily for three weeks exhibited a significant increase in cardiac output, cardiac index, and systemic arterial compliance when compared to diabetic animals. Additional studies indicate that ALT-711 improves the cardiovascular function of aged dogs and primates. Mongrel dogs, aged 10-14 years, receiving 1 mg/kg, p.o., daily for four weeks demonstrated a 66 % reduction in left ventricular chamber stiffness, 31 % increase in end diastolic volume, and a 26 % reduction in end diastolic pressure. Similarly, aged monkeys (18-25 years) treated with 1 mg/kg for 22 days exhibited a statistically significant reduction in pulse wave velocity beginning at 1 week post-treatment and lasting through week 8. Clinical trials with ALT-711 are now being conducted.

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ABSTRACT NOT RECEIVED

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EMERGING PARADIGMS AND NEW PERSPECTIVES FOR HUMAN GENETICISTS INTERESTED IN THE BIOLOGY OF AGING. George M. Martin*. Department of Pathology, University of Washington, Seattle, Wa, 98195.

Most human geneticists interested in aging have been preoccupied with rare constitutional mutations that lead to the premature onsets and/or accelerated rates of development of diseases that typically escape the force of natural selection. The frequencies of these idiosyncratic "private" mutations are ~10^{-6} and their prevalence in any population is largely a matter of genetic founder effects and genetic drift. These can prove to be very powerful entering points for basic biochemical mechanisms of aging as they occur in most of us. On the other hand, they can reflect rather special and rare mechanisms of decline in structure and function. Increasing attention is therefore being given to the roles of common polymorphisms (frequencies>10^{-2}). These may include many alleles that have been selected for enhanced early fitness. Accordingly, they may lead to knowledge of more "public" mechanisms of aging, in the sense that they may reflect relevant gene actions common to many populations of humans, many species, or, conceivably, all species. As a test of the degree to which both "private" and "public" genetic modulations can converge on a common major mechanisms for the genesis of a major late life disorder, our laboratory and other laboratories have used knowledge of gene action revealed by the rare autosomal dominant forms of dementia of the

Alzheimer type (DAT) as a guide for research on polymorphisms that impact upon common late onset "sporadic" forms of DAT. The results continue to support variations in the metabolism of the beta amyloid precursor protein as a central mechanism. We have also begun a series of investigations on the role of various polymorphisms at the Werner locus in the susceptibility of specific age groups to atherosclerosis. These studies could potentially lead to the discovery of alleles that lead to enhanced structure and function of the cardiovascular system during the latter part of the life course. There is currently great interest in determining associations between polymorphisms and unusual longevities ("healthy" centenarians). Interestingly loci might thus be identified in certain well-defined populations, but there are major conceptual and technical difficulties. Here I shall argue that we can make more rapid progress by a genetic analysis of "elite" aging for a host of very specific phenotypes, as these are more likely to be under the control of a much smaller number of major loci.

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AGE-RELATED CHANGES IN DOLICHOL (D) LEVELS AND AUTOPHAGIC-PROTEOLYTIC (A/P) ACTIVITY IN RAT LIVER. G. Cavallini, C. Dolfi, A. Donati *, C. Paradiso and E. Bergamini Istituto di Patologia Generale, Università di Pisa Via Roma 55, 56126 PISA - Italy

Aging may be the consequence of a failure of somatic maintenance leading to accumulation of abnormal components (1). Surprisingly, little attention was given to age-changes in membrane and organelles maintenance and to accumulation of abnormal components in membranes. D is a polisoprenoid uncatabolized compound excreted by liver cells into bile, perhaps together with undigested contents of autophagyc vacuoles, that exhibits a remarkable age-related increase in tissues (2). A/P is a major pathway for the degradation of liver protein, cytomembranes and organelles; that is active during fasting under the control of plasma aminoacids and of pancreatic hormones (3). In this study we explored the age-related changes in D level (assayed by the HPLC procedure:2) and in the aminoacidic control of A/P (assessed as described by 4) in the liver of Sprague-Dawley male rats fed ad libitum or submitted to caloric restriction (every-other-day feeding ad libitum, CR) beginning at ages 2-months, CR_2, 6-months, CR_6, 12-months, CR_{12}, 18-months, CR_{18}.

Table 1: Age-related changes in hepatic dolichol levels and in control of A/P activity (*p<.01) and prevention by CR ($).

	2 mo. AL	24 mo. AL	24 mo CR_2	24 mo CR_6	24 mo CR_{12}	24 mo CR_{18}
D	37.3 ± 8.60	140.5 ± 13.47*	38.18 ± 8.51$	65.3 ± 16.40$	118.5 ± 40.40	169.0 ± 26.27
A/P	0.270 ± .0233	0.142 ± .0109*	0.301 ± .0234$	0.292 ± .0077$	0.185 ± .0256	0.203 ± .0315

D is given as mg/g wet tissue. A/P activity is given as the difference between maximum (no aminoacids in the medium) and minimum (4 fold higher than the physiological aminoacid concentration) proteolytic activity. Results are given as nmol/val/10 mg cell wet weight. Means ± SEM are given.

Caloric restriction beginning at 2 and 6 months of age only, preserves the juvenile level of D while the same intervention beginning at older age (12 and 18 months) has no significant effect. A similar time-dependency of the effect of CR was found with A/P activity. In conclusion, dolichol accumulation and impaired degradation of membranes by A/P follow similar temporal patterns. Hence, results are compatible with the hypothesys that the anti-aging effects of CR primarily depend on a better maintenance of liver cell membrane and organelles by the process of A/P, perhaps due to a more intense stimulation of A/P during the longer interval between feeding.

References

1. Bergamini, E., Gori, Z. (1995). Aging Clin. Exp. Res. 7 n. 6, 473-475.Marino, M., Dolfi, C., Paradiso, C., Cavallini, G., Masini, M., Gori, Z., Pollera M., Trentalance, A., Bergamini, E. (1998) J. Gerontol. 53A, B87-B93.

Mortimore, G.E. (1992) in Advances in Molecular and Cell Biology (JAI Press Inc.) 4, 201-228.

Miotto, G., Venerando, R., Marin, O., Siliprandi, N., Mortimore, G.E. (1994) J. Biol. Chem. 269 n. 41, *25348-25353.*

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THE NITRONE-SPIN TRAP PBN ALTERS THE CELLULAR RESPONSE TO H2O2: CALCIUM-DEPENDENT ACTIVATION OF THE EGF RECEPTOR/ ERK PATHWAY. Waleed N. Hassan[1], Ippolita Cantuti-Castelevetri[2], Natalia A. Denisova[2], James A. Joseph[2], & K. Eric Paulson[1,3*]. [1]The Department of Biochemistry, Tufts University School Of Medicine, 136 Harrison Ave., Boston, MA 02111. [2]USDA-ARS, 7111 Washington St., Aging at Tufts University, 711 Washington St., Boston, MA 02111.

The nitrone spin-trap PBN has been shown to protect neuronal cells from reactive oxygen species both in culture and in vivo. As an approach to understanding the molecular mechanisms by which PBN may function to protect cells, wee examined whether PBN alters the cellular response to reactive oxygen species. H_2O_2 stimulation of PC-12 cells results in weak activation of both the ERK and JNK signal transduction pathways. PBN pretreatment of PC-12 cells, followed by H_2O_2 stimulation results in strong and selective activation of the pro-survival ERK pathway. H_2O_2 induction of the ERK activity in PBN pretreated cells was shown to be dependent on extracellular Ca^{+2} influx via voltage-sensitive calcium channels. Further analysis of the ERK pathway showed that in PBN pretreated cells, EGF receptor and the adapter protein SHC were phosphorylated in a Ca^{+2}-dependent, ligand-independent manner following H_2O_2 stimulation. Interestingly, H_2O_2 stimulation of PBN pretreated cells results in only 30% of the increase in intracellular Ca^{+2} as compared to untreated cells following H_2O_2 stimulation. These data suggest a model in which PBN acts to selectively sensitize the EGF receptor/ERK pathway to respond to H_2O_2 induced increases in intracellular Ca^{+2}.

66 ***GLENN AWARD***

DIETARY RESTRICTION AND 2-DEOXYGLUCOSE ADMINISTRATION IMPROVE BEHAVIORAL OUTCOME AND REDUCE DEGENERATION OF DOPAMINERGIC NEURONS IN MODELS OF PARKINSON'S DISEASE. Wenzhen Duan*, Mark P. Mattson, Sanders-Brown Research Center on Aging and Department of Anatomy & Neurobiology, University of Kentucky, Lexington, KY 40536.

Parkinson's disease (PD) is an age-related disorder characterized by progressive degeneration of dopaminergic neurons in the substantia nigra (SN) and a clinical profile of akinesia, tremor and rigidity. Oxidative stress and mitochondrial dysfunction are implicated in the neurodegenerative process in PD. Dietary restriction (DR) can extend lifespan and reduce levels of cellular oxidative stress and age-related disease in several different organ systems, but the impact of DR on the brain is largely unkown. We now report that DR (alternate day feeding regimen for 3 months) in adult mice results in resistance of dopaminergic neurons in the substantia nigra to MPTP toxicity. MPTP-induced loss of tyrosine hydroxylase immunoreactive neurons and deficits in motor function were ameliorated in DR rats. In an effort to mimick the beneficial effect of DR on dopaminergic neurons, we administered 2-deoxyglucose (2DG, a non-metabolizable analog of glucose) to mice fed ad libitum. Mice receiving 2DG (200 mg/kg body weight, 7 daily injections) exhibited reduced damage to dopaminergic neurons in the SN and improved behavioral outcome following MPTP treatment. 2DG treatment suppressed oxidative stress, preserved mitochondrial function and attenuated cell death in cultured dopaminergic cells exposed to the complex I inhibitor rotenone or Fe^{2+}. 2DG induced expression of the stress proteins HSP-70 and grp78 in dopaminergic cells suggesting involvement of these cytoprotective proteins in the neuroprotective action of 2DG. Our findings demonstrate striking beneficial effects of DR and 2DG in an animal model of PD and, when considered in light of recent epidemiological data, suggest that DR may prove beneficial in reducing the incidence of PD in human populations.

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CALORIC RESTRICTION MIMETICS: GREAT TASTE, LESS FILLING, LONGER LIFE. George S. Roth*, Donald K. Ingram and Mark A. Lane, National Institute on Aging, Baltimore, MD 21224.

Dietary caloric restriction (CR) is the only intervention conclusively shown to slow aging and extend life span and health span in mammals. The beneficial "anti-aging" effects of CR appear to be closely linked with several metabolic hallmarks, including lower plasma insulin, greater insulin sensitivity, lower body temperature, and anti-tumorigenicity. We have been evaluating 2-deoxyglucose, an inhibitor of the early stages of glycolysis, as a potential CR mimetic. This sugar elicits many of the same metabolic effects as CR, without the necessity of reducing food intake. Unfortunately, at higher dosages, 2-deoxyglucose exerts toxicity in rodents. However, other classes of naturally occurring compounds, which are found in plants and fruits, also appear to exert some of the same metabolic effects as CR. These include gymnemic acid and gymnemosides, which block glucose adsorption into the blood, and hydroxycitrate, which inhibits lipogenesis. It, thus, appears feasible to develop nutriceuticals which may serve as "CR mimetics", eliciting the beneficial "anti-aging" effects, without toxicity or reduced food consumption.

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ANTIOXIDANTS AND INFLUENZA INFECTION IN THE AGED. Simin Nikibin Meydani,*; Sung Nim Han , USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111.

The incidence of infectious disease increases with age and is associated with higher morbidity and mortality in the aged compared to the young. Of all infections, respiratory infections are most common; they are one of the leading causes of mortality in the elderly. We have previously shown that vitamin E supplementation enhances immune response in the elderly. A series of experiments using murine model of influenza infection was conducted to determine the clinical significance of immuno-enhancement by vitamin E in the aged. Vitamin E supplementation (500ppm) was shown to significantly reduce lung viral titer in young and old animals infected with influenza virus. This effect of vitamin E, however, was more dramatic in old than in young animals. Furthermore, vitamin E supplementation significantly reduced appetite and weight loss in the aged mice; a clinical symptom normally observed following influenza infection. Other dietary and non-dietary antioxidants such as glutathione and melatonin did not influence resistance to influenza infection in aged mice. This latter implies that the beneficial effect of vitamin E might not be due solely to its antioxidant property. To determine the mechanism of vitamin E-induced reduction of influenza viral titer, several immune parameters important in controlling influenza infection were determined. These studies indicate that vitamin E exerts its effect mainly through enhancement of Th1 cytokines (INF-y and IL-2) and in part by increasing the natural killer cell activity. Further studies, using transgenic mice or in vivo neutralization methods, are needed to confirm the involvement of Th1 cytokines in vitamin E-induced reduction of influenza viral titer.

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DIETS HIGH IN ANTIOXIDANTS CAN REVERSE AGE-RELATED DECLINES IN CEREBELLAR b -ADRENERGIC RECEPTOR FUNCTION AND MOTOR LEARNING. Paula C. Bickford*[1], Lori Breiderick[1], Amber Pollack[1], Barbara Shukitt-Hale[2], and James Joseph[2]., [1]Dept. Pharmacology, Neurosciences Training Program, UCHSC, and Dept Veterans Affairs Medical Center, Denver, CO 80262 and [2]USDA and Human Nutrition Research Center on Aging, Boston, MA

Aging is a complex phenomenon which effects many biological systems including the central nervous system (CNS). Changes in the CNS occur at many levels during aging that may underlie the observed behavioral changes in motor balance and coordination, motor or procedural learning and declarative learning. One theory of aging, the free radical theory proposes that the accumulation of free radical damage with time results in the eventual decline of the biological system and leads to death of the organism. The free radical theory of aging has gained significant support with the demonstration that life span of fruit flies can be increased by the over-expression of SOD and catalase. A decline in the capacity of normal antioxidant defense mechanisms has been postulated as a causative factor in aging related declines in the function of biological systems.

A model system that we have used to examine the effects of oxidative stress and aging is the cerebellar b-adrenergic receptor system and its correlation with motor learning. Aging is associated with a decline in the ability of isoproterenol to augment the actions of GABA on cerebellar Purkinje neurons. This loss of NE action is accompanied by a loss in motor learning measured by the ability of rats to learn to negotiate a runway that consists of aluminum pegs arranged in an irregular pattern. We have examined the effects of nutritional supplementation of aged rats with diets high in antioxidant capacity. Foods such as blueberries and spinach can prevent and/or reverse age-related declines in cerebellar noradrenergic receptor function and the accompanied loss of motor learning.

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PREVENTION AND REVERSAL OF AGE-RELATED MOTOR AND COGNITIVE BEHAVIORAL DEFICITS WITH ANTIOXIDANT DIETS. [1]B. Shukitt-Hale*, [2]P.C. Bickford, and [1]J.A. Joseph. [1]USDA, Human Nutrition Research Center on Aging at Tufts Univ., Boston, MA 02111, and [2]Dept. Of Veterans Affairs Medical Center, Denver, CO 80262.

Increased vulnerability to oxidative stress (OS) is thought to be a causative factor in age-related CNS functional declines. Decrements in motor function and memory are two main behavioral parameters that are altered in senescence in both humans and animals. If the generation and accumulation of oxygen free radicals are important factors in causing these age-related behavioral decrements, chronic enhancement of antioxidant defenses could slow or retard this process by reducing OS, resulting in improved performance. Therefore, antioxidants added to the diet may be one defense strategy to prevent, intercept, or reverse age-induced OS; fruits and vegetables are the main source of dietary antioxidants. It was recently found that certain fruits and vegetables (e.g., strawberries, blueberries, and spinach) have a high total antioxidant capacity, possibly due to their flavonoid content. Therefore, we assessed psychomotor and cognitive performance in aging, male, Fischer 344 rats that were provided fruit and vegetable diets high in antioxidants to determine if they could prevent, or even reverse, the behavioral changes seen with age. In the prevention study, diets included a control, or ones supplemented with vitamin E (500 IU/kg), or strawberry or spinach dried aqueous extract (1.36 mmol Trolox ORAC equivalent per kg diet), fed to the rats from age 6 to 15 months of age. In the reversal study, diets included strawberry, spinach, or blueberry, fed to the rats from 19 to 21 months of age. Complex motor performance was assessed with several tests: rod walk, wire suspension, plank walk, accelerating rotarod, and inclined screen. Cognitive spatial learning and memory performance was measured by the working memory version of the Morris water maze (MWM). We found that chronic treatment with spinach prevented age-related declines in cognitive learning; this group had a shorter latency to find the platform in the reference memory trial of the MWM compared to the control group. Furthermore, both the spinach and vitamin E groups showed a shorter distance to the platform on Trial 1 compared to the control group. Additionally, in the reversal study, the blueberry-fed group displayed improved performance on two motor tests, which rely on balance and coordination, rod walking and the accelerating rotarod. All diet groups, but not the control group, showed differences between Trial 1 and Trial 2 latencies and distances to the platform (e.g., Trial 2 latencies were less than Trial 1), demonstrating one-trial learning, even with the 10 min retention interval. MWM changes were not due to swim speed. Therefore, age-related changes in behavior may result from an inability to cope with OS that occurs throughout the life-span, and enhancement of antioxidant defenses through dietary means, particularly phytochemicals present in antioxidant rich foods, could reverse these functional age-related CNS deficits.

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MITOCHONDRIAL DECAY INCREASES OXIDATIVE STRESS IN THE AGING RAT HEART: REVERSAL BY DIETARY SUPPLEMENTATION WITH (R)-α-LIPOIC ACID. *Eric T. Shigeno*, Jung H. Suh, Alma E. Rocha, and Tory M. Hagen. Linus Pauling Institute, 571 Weniger Hall, Oregon State University, Corvallis, OR 97331.*

Mitochondrial function was determined in freshly isolated cardiac myocytes from young (2 mo) and old (28 mo) Fischer 344 (F344) rats. Cellular oxygen consumption in cells from old rats declined nearly 60% (718 ± 12 μmol O_2/min per 10^6 cells versus 299 ± 43 μmol O_2/min per 10^6 cells, respectively) compared to cells from young rats, indicating a significant decline in general metabolic rate. To determine whether this decline was due to mitochondrial decay, average mitochondrial membrane potential (the driving force for ATP synthesis) was assessed by incubating cardiac myocytes with Rhodamine 123 (R123) followed by flow cytometry. R123 staining in cardiac myocytes from old rats was 53% lower than that for cells from young rats, suggesting a significant decline in the ability of mitochondria to meet cellular energy demands. To understand whether mitochondrial decay resulted in increased oxidant production, cells were incubated with 2,7-dichlorofluorescin diacetate, a compound that readily enters cells and fluoresces upon encountering reactive oxygen species. Results show that oxidant production, mainly from mitochondria, increased nearly 3-fold in cardiac myocytes from old rats compared to that of cells from young rats. In order to determine whether increased mitochondrial oxidant production resulted in heightened myocardial oxidative stress, cellular antioxidant status, as measured by ascorbic acid and GSH/GSSG ratios were quantified in rats of increasing age. Tissue ascorbate levels declined markedly (2-fold) between 2 and 12 mo of age and the rate of loss was significant but less pronounced in myocardia from rats between 12 and 28 mo of age. Myocardial GSH/GSSG ratios of 28 mo old rats decreased by 1.5-fold compared to that of 2 mo old rats. These results, coupled with increased mitochondrial oxidant production, suggests that the aging rat heart is under a significantly heightened oxidative stress.

We previously showed that dietary supplementation with (R)-α-lipoic acid (LA), a co-factor for α-keto acid dehydrogenases and a potent antioxidant, reverses the decline in hepatocellular antioxidant levels and lowers the heightened oxidative stress seen with age (Hagen et al, FASEB J. 13, 411-418, 1999). To assess whether LA supplementation could also reverse the decline in myocardial antioxidant levels, 2, 12, 24, and 28 mo old F344 rats were fed AIN-93M diet with or without 0.2% (w/w) LA for 2 weeks prior to sacrifice. Results show that LA supplementation restores myocardial ascorbic acid levels in all age groups to that of unsupplemented 2 mo old rats. LA supplementation's restorative effect on glutathione (GSH) levels was apparent but less significant with age. α-Tocopherol levels did not change with age nor did LA supplementation further affect tocopherol concentrations. Overall, these results suggest that dietary lipoic acid supplementation may significantly attenuate the mitochondrial-induced age-related increased oxidative stress evident in cardiac myocytes.