29th ANNUAL MEETING OF THE AMERICAN AGING ASSOCIATION
14th ANNUAL MEETING OF THE AMERICAN COLLEGE OF CLINICAL GERONTOLOGY
13th ANNUAL GRANTEE CONFERENCE OF THE AMERICAN FEDERATION FOR AGING RESEARCH

June 2-5, 2000

Tufts University Human Nutritional Research Center on Aging
and
Tremont Boston Hotel
Boston, Massachusetts

STRESS IN AGING: MODELS, MECHANISMS, AND INTERVENTIONS

Preconference Workshop:
DNA Microarrays in the Study of Aging
Chair: Tomas Prolla

1. Tom Johnson: **THE EFFECT OF GERONTOGENES ON DIFFERENTIAL GENE FUNCTION MEDIATING INCREASED HEALTH AND LONGEVITY IN C. ELEGANS.**
2. Tomas Prolla: **THE GENE EXPRESSION PROFILE OF THE AGING PROCESS WAS ANALYZED IN SKELETAL MUSCLE, NEOCORTEX AND CEREBELLUM OF MICE.**
3. James Nelson: **USING cDNA EXPRESSION ARRAYS AND TARGETED MUTATIONS TO PROBE THE ANTI-AGING ACTIONS OF CALORIE RESTRCTION.**
4. Rich Miller: **STATISTICAL METHODS FOR ANALYSIS OF MICROARRAY DATA, WITH APPLICATION TO LONG-LIVED MUTANT MICE.**

Session 1:
Mechanisms of Mitochondrial Involvement in Oxidative Stress and Aging
Chair: Rajindar Sohal

5. Rajindar Sohal: **THE MECHANISMS OF GENRATION OF REACTIVE OXYGEN SPECIES (ROS) IN MITOCHONDRIA.**
6. Enrique Cadenas: **THE MODULATION OF MITOCHONDRIAL PRODUCTION OF OXYGEN RADICALS BY NITRIC OXIDE AND ITS IMPACT ON CELL SIGNALING MECHANISMS.**
7. Dean Jones: **MITOCHONDRIAL REDOX SIGNALLING DURING APOPTOSIS.**
8. Jose Vina: **DAMAGE TO MITOCHONDRIAL DNA IN AGING AND APOPTOSIS.**

Session 2:
Cellular Impact of Oxidative Stress
Chairs: Judd Aiken and Giuseppe Attardi

9. Judd Aiken: **AGE-ASSOCIATED MITOCHONDRIAL GENETIC AND ENZYMATIC ALTERATIONS: STUDIES IN RODENTS AND PRIMATES.**
10. Giuseppe Attardi: **ROLE OF MITOCHONDRIAL DNA IN AGING PROCESS.**
11. Konstantin Khrapko: **A MAJORITY OF MYOCYTES IN THE AGED HUMAN HEART CONTAIN CLONALLY EXPANDED SOMATIC MUTATIONS IN MITOCHONDRIAL DNA.**
12. Gino Cortopassi: **NEURONAL MODELS FOR STUDYING mtDNA MUTATION AND PATHOPHYSIOLOGY.**

Session 3:
Oxidative Stress and Neurodegeneration
Chair: Douglace Wallace

13. Moussa B.H.: **cDNA MICROARRAY FOR STUDYING NEURODEGENERATION AND NEUROPROTECTION IN PARKINSON'S DISEASE.**

SUBMITTED PAPERS

14. Robert A. Floyd: **REACTION PRODUCTS OF NITRIC OXIDE IN ALZHEIMER'S BRAIN AND A TRANSGENIC MOUSE MODEL INDICATE NEUROINFLAMMATORY**
15. Vijaya B. Kumara: **IDENTIFICATION OF AGE DEPENDENT CHANGES IN EXPRESSION OF SENESCENCE ACCELERATED MOUSE (SAMPS) HIPPOCAMPAL PROTEINS BY EXPRESSION ARRAY ANALYSIS.**

Session 4:
Anti-Oxidants and Interventions
Chair: Jim Joseph, Tufts University

16. Bruce Ames: **PREVENTING CANCER AND DELAYING AGING WITH MICRONUTRIENTS.**
17. Jim Joseph: **OXIDATIVE STRESS VULNERABILITY AND INTERVENTIONS IN BRAIN AGING.**
18. Maret Traber: **VITAMIN E BIOKINETICS, OXIDATIVE STRESS AND AGING.**
19. Abraham Reznick: **CIGARETTE SMOKE DAMAGE TO SALIVARY PROTEINS AND ENZYMES AND THE EFFECTS OF ANTIOXIDANTS: IMPLICATIONS FOR SALIVA OF AGED PEOPLE.**

Session 5:
Integrated Responses to Physiological Stress
Chair: Kevin Kregel

20. Douglas Seals: **IS PRIMARY HUMAN AGING ASSOCIATED WITH AUGMENTED SYMPATHETIC-CARDIOVASCULAR RESPONSIVENESS TO ACUTE STRESS?**
21. Gregory Cartee: **DOES AGE ALTER SKELETAL MUSCLE ADAPTABILITY TO THE PHYSIOLOGIC STRESS OF EXERCISE OR CALORIC RESTRICTION?**
22. Wendy Kohrt: **MECHANICAL LOADING STRESS: BENEFICIAL OR DETRIMENTAL TO THE AGED SKELETON**
23. Kevin Kregel: **DOES AGE ALTER THE SYSTEMATIC AND CELLULAR RESPONSES TO PHYSIOLOGICAL STRESS?**

Session 6:
Molecular Characterization of the Stress Response
Chair: Nikki Holbrook

24. Terry Oberly: **MORPHOLOGIC ASSESSMENT OF OXIDATIVE DAMAGE.**
25. Ahmad Heydari: **AGE- RELATED ALTERATIONS IN THE ACTIVATION OF HEAT SHOCK TRANSCRIPTION FACTOR 1 IN RAT HEPATOCYTES.**
26. Nikki Holbrook: **ALTERATIONS IN PROLIFERATION- AND STRESS-ACTIVATED SIGNALING PATHWAYS IN AGING HEPATOCYTES.**
27. Paul Milbury: **BALANCING MARKER OF OXIDATIVE DAMAGE AND REDOX STATUS IN INTERVENTION STUDIES.**

SUBMITTED PAPERS

28. H. Van Remmen: **INCREASED SENSITIVITY TO OXIDATIVE STRESS-INDUCED APOPTOSIS IN FIBROBLASTS AND CARDIOMYOCYTES FROM MNSOD AND GPX1 KNOCKOUT MICE.**
29. A.D.N.J. de Grey: **REVERSING AGE-RELATED MITOCHONDRIAL DNA DECLINE: ALTERNATIVES TO MITOCHONDRIAL GENE THERAPY.**
30. Andrzej Bartke: **REDUCED BODY TEMPERATURE AND PLASMA GLUCOSE LEVELS IN LONG-LIVING GH-R-KO MICE.**
31. John C. Guerin: **CENTENARIAN ROCK FISH PROJECT-THE BIOCHEMICAL STUDY OF SLOW OR NEGLIGIBLE AGING IN ROCKFISH PROVEN TO LIVE AT LEAST 140 YEARS.**
32. Lorenzo M. Refolo: **ALTERED CHOLESTEROL METABOLISM MODULATES BETA-AMYLOID DEPOSITION IN A TRANSGENIC MOUSE MODEL FOR ALZHEIMER'S DISEASE.**

DIETARY INTERVENTION IN AGING AND AGE-ASSOCIATED DISEASES

Chairs: Mohsen Meydani and Robert M. Russell

33. D.M. Snodderly: **LUTEIN AND AGE- RELATED EYE DISEASE.**
34. B. Dowson-Hughes: **DIETARY CALCIUM AND VITAMIN D IN BONE AGING.**
35. Christopher Heward: **CLINICAL SETTINGS FOR DIETARY AND OXIDATIVE STRESS INTERVENTION.**
36. S.N. Meydani: **CAN FUNCTIONAL FOOD IMPROVE THE IMMUNE RESPONSE IN ELDERLY?**
37. Mary Sano, Columbia: **ANTI-OXIDANT FOR TREATMENT AND PREVENTION OF ALZHEIMER'S DISEASE.**
38. X.D. Wang: **DIETARY ANTIOXIDANTS AND LUNG CANCER PREVENTION.**

ANNUAL LUNCHEON AND AWARDS:

2000 Nicolai Awardee – **Tanya Jonassen**
"Coenzyme Q and Aging in the Nematode Caenorhabditis Elegans." from the Department of Chemistry and Biochemistry, University of Los Angeles.

2000 Nicolai Awardee – **Daniel E. Kolker**
"Circadian Gene Expression in Young and Old Hamsters." from Northwestern University, Evanston, IL.

2000 Glenn Award – **Kate J. Claycombe**
"Age-Associated Increase in Macrophage (MO) Cyclo-oxygenase-2 (Cox-2) Expression is Mediated Through Increased Ceramide Levels and ERK Activity." From JM USDA/HNRCA at Tufts University, Boston, MA 02111.

2000 Harman Lecturer – **Rich Weindruch, Ph.D.**
"Caloric Intake, Oxidative Stress and Aging." From the University of Madison-Wisconsin, VA Hospital (GRECC-5D), 2500 Overlook Terrace, Madison, WI 53705-2286.

2000 Irving Wright Award Lecturer – **Edward Lakatta, M.D.**
"The Old Heart: Operating on the Edge." from the Gerontology Research Center, NIH/NIA, Baltimore, MD.

1

THE EFFECT OF GERONTOGENES ON DIFFERENTIAL GENE FUNCTION MEDIATING INCREASED HEALTH AND LONGEVITY IN *C. ELEGANS*. Thomas E. Johnson [1,2]*, James Cypser[1], Sam Henderson[2], Stuart K. Kim[3], Chris Link[1], Jim Lund[3], Shin Murakami[1], and Pat Tedesco[1]; [1]Institute for Behavioral Genetics, University of Colorado at Boulder, CB447, Boulder, Colorado, 80309, USA. [2]GenoPlex Inc., 6840 Broadway, Denver, Colorado, 80221, USA., [3]Department of Developmental Biology, 279 Campus Drive, Stanford University, Stanford CA 94305-5329.

Caenorhabditis elegans, a simple nematode worm, has been the focus of many aging projects. More than 40 single-gene mutants in *C. elegans* have been demonstrated to lead to increased life span (a rigorous, operational test for being a gerontogene) of 20% or more; these are referred to collectively as "Age" mutants. These Age mutants must contain changes in key functions that are rate-limiting determinants of longevity; moreover, important genes can be identified independent of prior hypotheses as to actual mode of gene action in extending longevity and/or "slowing" aging. These Age mutants define as many as seven (possibly) distinct pathways and/or modes of action, as defined by primary phenotype. Moreover, the three of the best-studied mutants (*age-1, clk-1, spe-26*) alter age-specific mortality rates in a characteristic fashion, unique to each gene. We have developed a whole-genome microarray that we are using to discover those changes in transcript abundance that are associated with normal aging and with the differential effect of each gerontogenes. Since all Age mutants (so far without exception) increase the ability of the worm to respond to a variety of stresses, including heat, UV and reactive oxidants, we anticipate that many of the outputs of these gerontogenes will affect transcripts involved in stress response; preliminary results seem to bear these predictions out. This is a work in progress with the whole-genome chips carrying all 19,099 predicted genes just coming on line recently. We suggest that there may be important, evolutionarily conserved components of the longevity/stress-response pathways that have been conserved between nematodes and mammals and that effects of these Age genes at the transcript level may be one way to uncover such genes.

2

THE GENE EXPRESSION PROFILE OF THE AGING PROCESS WAS ANALYZED IN SKELETAL MUSCLE, NEOCORTEX AND CEREBELLUM OF MICE. Thomas A. Prolla*, University of Wisconsin-Madison, Department of Genetics & Medical Genetics, 445 Henry Mall, Madison, WI 53706.

The gene expression profile of the aging process was analyzed in skeletal muscle, neocortex and cerebellum of mice. Use of high-density oligonucleotide arrays representing 6347 genes revealed that aging resulted in a differential gene expression pattern indicative of a marked stress response in all tissues. Most alterations were either completely or partially prevented by caloric restriction, the only intervention known to retard aging in mammals. Transcriptional patterns of calorie-restricted animals suggest that caloric restriction retards the aging process in skeletal muscle by causing a metabolic shift toward increased protein turnover and decreased macromolecular damage, whereas caloric restriction may retard aging in the brain by reducing inflammatory responses.

3

USING cDNA EXPRESSION ARRAYS AND TARGETED MUTATIONS TO PROBE THE ANTI-AGING ACTIONS OF CALORIE RESTRICTION. James F. Nelson*, Department of Physiology, MC 7756, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900

Recent work using gene expression profiling indicates that calorie restriction (CR) has profound effects on the expression of many genes. The challenge is to determine how those changes are regulated and their significance to the anti-aging actions of calorie restriction. We have begun to use expression profiling to address these questions. Our aim is to determine the significance, if any, of the moderate hyperadrenocorticism associated with CR on the anti-aging action of CR. We are using the glucocorticoid-deficient CRH knockout mouse to assess whether selective elimination of the hyperadrenocortical state from the CR animal attenuates any of the actions of CR. We now report, using expression array profiling, that a significant fraction of changes in gene expression normally induced by CR are attenuated, either completely or partly, in calorie restricted CRH knockout mice. These data are largely verified by independent assessment of gene changes using rt-PCR or independent array technologies. Assuming that the elimination of the hyperadrenocortical state from the CR animal accounts for these changes, this result indicates that glucocorticoids contribute the to altered gene expression and underlying shifts in metabolic processes associated with chronic calorie restriction.

4

STATISTICAL METHODS FOR ANALYSIS OF MICROARRAY DATA, WITH APPLICATION TO LONG-LIVED MUTANT MICE. Richard A. Miller*, University of Michigan, Ann Arbor, MI.

We present a series of procedures for practical analysis of data on mRNA expression levels obtained from cDNA array methods. The approach features use of a normal distribution of values from non-expressed background genes to adjust for technical variations between analyses of individual samples from nominally replicate biological samples, such as individual mice or cell cultures, to allow discrimination of expressed genes at any preferred level of statistical significance. The algorithm discounts data from cDNAs whose position, on the array, is too close to targets complementary to high-abundance mRNAs to permit accurate quantitation. The procedure develops a linear regression equation that is essentially undistorted by expressed genes that vary greatly between replicate specimens, as a prelude to assessment (by principal components) of variation among replicate specimens or between experimental and control specimens. Two applications will be illustrated: (a) tabulation of genes whose variation among individual replicate mice is exceptionally high or low; and (b) tabulation of genes whose expression differs significantly between normal mice and those of the long-lived mutant Ames dwarf mouse (Prop1df/df).

5

THE MECHANISMS OF GENERATION OF REACTIVE OXYGEN SPECIES (ROS) IN MITOCHONDRIA. Raj Sohal*, Department of Biological Sciences, Southern Methodist University, 220 Fondren Science Building, Dallas, Texas 75275.

Pointing out the variations among different sites in the electron transport chain among mitochondria from different tissues. Comparisons of rates of ROS generation will be made between: (i) animals of different ages, (ii) different species of animals, and (iii) under regimens which cause variations in life spans, to propose that rate of ROS generation is inversely associated with life span and directly correlated with oxidative molecular damage. Evidence will be presented to demonstrate that protein oxidation during aging leads to specific losses of enzyme activities. In general, the argument will be proffered that ROS generation is a likely cause of mitochondrial damage during aging.

6

THE MODULATION OF MITOCHONDRIAL PRODUCTION OF OXYGEN RADICALS BY NITRIC OXIDE AND ITS IMPACT ON CELL SIGNALING MECHANISMS. Enrique Cadenas*, Department of Molecular Pharmacology & Toxicology, School of Pharmacy, University of Southern California, Los Angeles, CA 90089-9121.

This subject addresses the role of nitric oxide -a free radical involved in a wide array of physiological and pathological phenomena- in mitochondrial function and integrity, production of oxygen radicals, and cell < signaling mechanisms. The pathways of nitric oxide utilization in mitochondria will be discussed in terms of the steady-state levels of nitric oxide and oxyradicals and subsequent formation and fate of peroxynitrite, a molecule which appears to play a key role in damage of mitochondrial protein and informational molecules. This approach is relevant for the formulation of a quantitative and physiological approach to the molecular mechanism(s) underlying the deficits in mitochondrial and cellular functions related to aging.

*** Presenter**
<G> Post Doctoral Candidate for Glenn Award
<N> Pre Doctoral Candidate for Nicolai Award
<A> AFAR grantee participant

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MITOCHONDRIAL REDOX SIGNALING DURING APOPTOSIS. Dean P. Jones*. Department of Biochemistry, Emory University, Atlanta, GA 30322

Considerable evidence is available to show that cells become oxidized during the process of apoptosis, that oxidants induce apoptosis, and that thiol antioxidants delay or inhibit apoptosis. Thus, there is a very clear role for redox signaling in activation of apoptosis. In studies of mitochondria-mediated apoptosis, we found that cytochrome *c* release resulted in an activation of mitochondrial superoxide generation. This generation of reactive oxygen species resulted in a dramatic oxidation of the cellular glutathione pool. In cells deficient in respiration because they lack a mitochondrial genome (rho zero), we found that release of cytochrome *c* during apoptosis did not result in oxidation of the glutathione pool. Thus, the major oxidation that occurs during apoptosis is a function of mitochondrial superoxide generation following cytochrome *c* release. In a human retinal pigment epithelial cell model for study of oxidant-induced apoptosis in age-related macular degeneration (ARMD), we found that cytochrome *c* release occurred in association with a loss of the mitochondrial membrane potential. This release of cytochrome *c* resulted in cleavage and activation of procaspase 3, with resulting cleavage of PARP and other caspase substrates, DNA fragmentation and externalization of phosphatidylserine. These results suggest that oxidant-induced apoptosis in these cells is mediated by activation of the mitochondrial permeability transition. However, analysis of Fas and Fas ligand showed increased expression on the cell surface and in mRNA and protein with time courses that preceded loss of mitochondrial membrane potential. A Fas-blocking antibody partially inhibited oxidant-induced apoptosis. Thus, the results indicate that a death receptor-mediated pathway as well as a mitochondria-mediated pathway may occur concomitantly in response to oxidative stress.

8

DAMAGE TO MITOCHONDRIAL DNA IN AGING AND APOPTOSIS. J Viña*, F V Pallardó, J Sastre, Departamento de Fisiología. Universidad de Valencia, SPAIN

Mitochondria are involved in aging. We showed that imitochondria inside cells are affected in aging (1). The levels of 8 hydroxy-2'deoxyguanosine (oxodG) are increased in mitochondrial DNA (mtDNA) in normal aging. Glutathione is oxidized in aging. The redox ratio (GSSG/GSH) is much higher in mitocondria that in the cytosol. There is a relationship between oxidation of glutathione and oxidative damage to mtDNA(2). We have also found that there is an inverse relationship between age associated oxidative damage to mtDNA and motor coordination in old mice (3). Oxidative stress occurs in apotosis too. We found that there are mechanisms common to aging and to apoptosis. Thus, we found that mtDNA is damaged oxidatively in apoptosis. An oxidation of glutathione was also observed both in fibroblasts and in the mammary gland during apoptosis. This is due to an increase in peroxide production by mitochondria of apoptotic cells. Like in aging, in apoptosis there is a relationship between oxidation of glutathione and oxidative damage to mtDNA (4). These changes are prevented by oral administration of antioxidant vitamins (2) and by other antioxidants like poliphenols contained in extracts of plants such as Gingko Biloba (5). Late onset administration of antioxidants is effective in preventing some of the age associated oxidative damage to cells (3). Dietary antioxidants are also effective in the prevention of disease states that are caused by an increased oxidant production by mitochondria (6,7) References. 1.- Sastre et al (1996) Hepatology 24,1199-1205 2.-Garcia et al. (1996) FASEB J 10, 333-338. 3.-Viña et al Free Rad Res (1999), 29,617-623 4.- Pallardó et al (1999) FASEB J (1999) 13, 1055 - 1064 5.- Sastre et al. (1998) FRBM 24, 298-304 6.-García et al (1998) J. Clin Invest 101,1-6 7.-García et al (1999) Hepatology 29, 985- 987

9

AGE-ASSOCIATED MITOCHONDRIAL GENETIC AND ENZYMATIC ALTERATIONS: STUDIES IN RODENTS AND PRIMATES. Jonathan Wanagat and Judd Aiken*. Department of Animal Health and Biomedical Sciences. University of Wisconsin, Madison, WI.

We will discuss studies that implicate age-related mtDNA deletions and mitochondrial enzymatic abnormalities in skeletal muscle aging. Previous homogenate studies of age-related mitochondrial changes in skeletal muscle have not provided a clear picture of the impact of mtDNA deletions and associated enzymatic abnormalities on individual cells or fibers. Our laboratory, using *in situ* histological studies of mitochondrial genotypic and phenotypic alterations in skeletal muscle from aging rhesus monkeys and rats has shown that: (1) the number and length of mitochondrial abnormalities increase with age and are associated with sarcopenic changes; (2) the mitochondrial genotypic and enzymatic abnormalities often co-localize with regions of intra-fiber atrophy; and (3) the fiber type (i.e., I, IIa, IIb) affects the phenotype of the resulting mitochondrial enzymatic abnormality. Together, these findings suggest a biologically relevant role for mitochondrial genotypic and phenotypic abnormalities in skeletal muscle aging.

10

ROLE OF MITOCHONDRIAL DNA IN AGING PROCESSES. G. Attardi+, Y. Michikawa+, F. Mazzucchelli*, N. Bresolin*, G. Scarlato*, M. Greco+, G. Villani+, Y. Bai+, +Division of Biology, California Institute of Technology, Pasadena, CA 91125, USA. *Institute of Clinical Neurology, University of Milan, 20122 Milan, Italy.

The idea that during mammalian cell aging there is an increasing incidence of mitochondrial DNA (mtDNA) mutations has received, in the last ten years, support from direct experimental observations of an aging-related occurrence in this DNA of oxidative and alkylation derivatives of nucleotides, of small deletions and insertions and, especially, of large deletions. However, the low frequency of these mutations has raised questions about their functional significance. Furthermore, in contrast to the large deletions, the search for aging-dependent point mutations in human mtDNA has given inconclusive and often discordant results. This is in part due to the lack of a reliable method for detecting mutations which occur in heteroplasmic form, i.e., together with wild-type mtDNA, as expected for aging-related mutations, and to the search having been largely limited to the protein- and RNA-coding regions of mtDNA. It is known that the main control region of mtDNA, that includes the D-loop and adjacent transcription promoters, is the most variable portion of the human mitochondrial genome, in which heteroplasmic point mutations have also been reported, and for which there may be a higher probability of aging-related damage. In the present work, for the reasons stated above, the main mtDNA control region and, in particular, the segment which contains the critical control sequences for mtDNA replication was chosen for a detailed analysis of aging-dependent mtDNA damage. For this purpose, a novel approach for the specific detection of heteroplasmic mtDNA mutations, in particular, one which also excluded any interference by nuclear mtDNA pseudogenes, was developed. The use of this approach has surprisingly revealed high copy point mutations at specific positions in the control region of human fibroblast mtDNA from normal old, but not young individuals. Furthermore, longitudinal studies showed the appearance of the mutations in a given individual only at advanced age. Most dramatically, some mutations appeared in more than one individual. Thus, a T to G transversion at position 414 was found, in a generally high proportion (up to 50%) of mtDNA molecules, in eight of 14 genetically unrelated individuals above 65 years of age (57%), while it was absent in all 13 individuals younger than 65. These observations clearly point to a novel phenomenon, and provide the first evidence of a large accumulation of aging-dependent point mutations in mtDNA. Furthermore the occurrence of these mutations at sites critical for mtDNA replication and their abundance strongly argue for their having a functional effect.

11

A MAJORITY OF MYOCYTES IN THE AGED HUMAN HEART CONTAIN CLONALLY EXPANDED SOMATIC MUTATIONS IN MITOCHONDRIAL DNA. **K. Khrapko***, N. B. Bodyak*, S. McGrath#, N. Van Orsouw#, J. Vijg# and J.Y. Wei*; *Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA #Cancer Therapy and Research Center, San Antonio, TX.

*** Presenter**
<G> Post Doctoral Candidate for Glenn Award
<N> Pre Doctoral Candidate for Nicolai Award
<A> AFAR grantee participant

Quantitative information on the cell-to-cell distribution of all possible mtDNA mutations in young and aged tissues is needed to assess the relevance of these mutations to the aging process. In the present study, we used PCR amplification of full-length mitochondrial genomes from single cells and 2-D denaturing gradient gel electrophoresis to scan human cardiomyocytes for all possible large deletions and most functionally relevant point mutations in mtDNA. Our results indicate that both deletions and point mutations are not evenly distributed across the tissue. Instead, mutants of the same type appear to concentrate in individual cells suggesting clonal expansion as a mechanism of their accumulation. Different cells contained different mutations, which indicates that these mutations are somatic in origin. The frequency of such clonal expansions is very high; in fact, almost any cardiomyocyte in aged but not in young tissue is expected to contain at least one clonally expanded mutation, which accounts for up to 100% of the mtDNA in the cell. Since most of mutations we detect have the potential to disrupt mitochondrial function, our observations indicate that mitochondrial mutations have the potential to play an important role in human myocardial aging.

12

NEURONAL MODELS FOR STUDYING mtDNA MUTATION AND PATHOPHYSIOLOGY. A. Wong[1], L. Cavelier[1], H Collins[2], MF Seldin[2], ML Savontaus[3], M McGrogan[4], GA Cortopassi[1]*; [1]Dept. Molecular Biosciences, 1311 Haring Hall, UC Davis, CA 95616, [2]Rowe Program in Genetics, UC Davis, CA 95616, [3]Department of Medical Genetics, University of Turku, Finland , [4]Layton Biosciences, Gilroy, CA

Mitochondrial deletion mutations accumulate with age, preferentially in tissues rich in postmitotic neurons and myofibers. In an attempt to generate a more relevant cellular model system for the study of the potential contribution of mtDNA mutations to age-related deficits of neuronal function, we have demonstrated the transfer of mutant mtDNAs from patient lymphoblasts to a pre-neuronal cell line N tera2 (Nt2). Restriction digests were consistent with transfer of patient mtDNA, and homoplasmic lines were identified. A potential issue was the contamination of transmitochondrial cell lines with patient nuclear DNA, but assay of >50 variable microsatellite loci was inconsistent with nuclear contamination by donor cells. mtDNA and nuclear DNA copy number were similar in control and transmitochondrial cell lines. Nt2 cells bearing mutant mitochondria were differentiable with retinoic acid into postmitotic cells with a neuronal morphology. Such cells could represent a useful model in which to study the effects of cellular context on mtDNA mutation accumulation and pathophysiology.

13

cDNA MICROARRAY FOR STUDYING NEURODEGENERATION AND NEUROPROTECTION IN PARKINSON'S DISEASE. Moussa B.H. Youdim* , Edna Grunblatt, Gila Maor, Yona Royak and Silvia Mandel; Eve Topf and National Parkinson Fouindation (US) Centers of Neurodegenerative Diseases, Technion Faculty of Medicine and Department of Pharmacology, Haifa, Israel.

Parkinson's Disease (PD) is an aging disorder associated with progressive degeneration of melanin containing dopamine neurons of substantia nigra pars compacta (SNPC) originating in raphe nucleus . Although its etiology is not known biochemical evidence support the notion for pivotal roles of iron, oxidative stress and inflammatory processes in cascade of events leading to neurodegeneration. Since its 6-hydroxydopamine (6-OHDA) and MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyrdine) animal models initiate dopaminergic neurodgeneration by similar mechanisms, we have investigated the alteration in gene expression in brains from PD and MPTP-treated mice using the human and mice cDNA expression array membranes, followed by RT-PCR and in-situ hybridization methods for confirmation. The expression pattern of genes provide indirect information about function and dysfunction. It will also give an over view of known and unknown mechanisms in the process of neurodegeneration and will provide new directions for development of effective neuroprotective drugs, not possible with conventional biochemical methods. Chronic MPTP treatment in mice induced alterations of some 49 different genes involved in iron metabolism, oxidative stress, inflammatory processes, neurotorphic factors, glutamate, nitric oxides synthase, heat shock proteins and cell cycle. Pretreatment of mice with neuroprotective drugs eg. R-apomorphine prevented the increase or decrease of most, but not all those genes and induced neuroprotection of dopamine neurons. Our in vitro cell culture and in vivo studies have indicated a pivotal role for iron in for activation and translocation of the inflammatory, redox-sensitive transcription factor, NF-kappa B, determined by western analysis,, EMSA and immunofluoresencwe assays, as an initial step in the process of neurodegenration. We are currently examining the expression of genes in SNPC from parkinsonina brains and the neuroprotective action of several compounds individually or in combination.

14

REACTION PRODUCTS OF NITRIC OXIDE IN ALZHEIMER'S BRAIN AND A TRANSGENIC MOUSE MODEL INDICATE NEUROINFLAMMATORY PROCESSES OCCUR. Kenneth L. Hensley[1], David G. Morgan[2], Marcia Gordon[2], William Markesbery[3] and Robert A. Floyd*[1] [1]Oklahoma Medical Research Foundation, Oklahoma City, OK 73104; [2]University of South Florida, Tampa, FL 33612; [3]University of Kentucky, Lexington, KY 40536.

Oxidative stress and quasi-inflammatory processes recently have been recognized as contributing factors in the pathogenesis of Alzheimer's disease (AD). Reactive nitrating species have specifically been implicated in AD based on immunochemical and instrumental detection of nitrotyrosine in AD brain protein. Using high performance liquid chromatography with electrochemical array detection (HPLC-ECD), we have previously demonstrated 2-7 fold increases in protein nitrotyrosine in affected regions of the AD brain, while the cerebellum is relatively spared from protein nitration. The ratio of 3-nitrotyrosine / tyrosine was found to be $1/10^4 - 1/10^3$. Building on this previous work, a study was undertaken to investigate the significance of lipid-phase nitration in AD. The current report documents a significant increase in the lipid nitration product 5-nitro-γ-tocopherol in affected regions of the AD brain as determined by HPLC-ECD analysis. In the superior and middle temporal gyri and the inferior parietal lobule of AD brains, the 5-nitro-γ-tocopherol / γ-tocopherol ratio is increased 2-3 fold above that found in corresponding regions of normal brains. Up to 30% of the γ-tocopherol pool is nitrated in the AD cortex, compared with approximately 10% in normal cortex. In contrast, nitrotocopherol is not increased in the cerebellum, which is relatively spared from classical AD-associated histopathology. Similar increases in 5-nitro-γ-tocopherol were found in the cortex of 10-12 month old transgenic mice expressing both mutant amyloid precursor protein and mutant presenilin-1 (APP/PS1). These data indicate that nitric oxide-derived species are significant contributors to lipid oxidation in the AD brain. The findings are discussed in reference to the neuroinflammatory hypothesis of AD, and the possible role of γ-tocopherol as a major lipid-phase scavenger of reactive nitrogen species.

15

IDENTIFICATION OF AGE DEPENDENT CHANGES IN EXPRESSION OF SENESCENCE ACCELERATED MOUSE (SAMP8) HIPPOCAMPAL PROTEINS BY EXPRESSION ARRAY ANALYSIS. Vijaya B. Kumar*[4], Mark W. Franko, Susan A. Farr, John E. Morley and H. James Armbrecht. Geriatric Research, Education and Clinical Center, St. Louis VA Medical Center, MO 63125 and Division of Geriatrics, St. Louis University Health Science Center, St. Louis, MO 63104.

Aging is associated with extensive cognitive impairment, though the biochemical and physiological basis of these deficits are unknown. As the hippocampus plays a vital role in cognitive functions, we have selected this tissue to analyze changes in gene expression at two different ages. Array technology is utilized to explore how gene expression in hippocampus is affected by accelerated cognitive impairment in Senescence-Accelerated Mouse (SAM P8) strain. We show that the expression of genes associated with stress response and xenobiotic metabolism are strongly affected at a time when cognitive impairment occurs. Affected genes include those involved both in signaling and chaperone function. Effector and regulator family of chaperones, which play an important role in protein folding, and also the xenoitic metabolizing enzymes that play crucial role in anti-oxidant systems show significant changes in gene expression between 4 and 12 months.

*** Presenter**
<G> Post Doctoral Candidate for Glenn Award
<N> Pre Doctoral Candidate for Nicolai Award
<A> AFAR grantee participant

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PREVENTING CANCER AND DELAYING AGING WITH MICRONUTRIENTS. Bruce N. Ames*. University of California, Berkeley; CHORI 5700 MLK Jr.Way, Oakland CA 94609 (510) 450-7625; bnames@uclink4.berkeley.edu

Approximately 40 micronutrients are required in the human diet. Deficiency of any of the micronutrients: folic acid, vitamin B12, vitamin B6, niacin, vitamin C, vitamin E, iron, or zinc, appears to mimic radiation in damaging DNA by causing single- and double-strand breaks, oxidative lesions, or both [1]. The percentage of the U.S. population that has a low intake (<50% of the RDA) for each of these eight micronutrients ranges from 2% to 20+%; half of the population may be deficient in at least one of these micronutrients, particularly the poor [1]. Folate deficiency occurs in approximately 10% of the U.S. population, and in a much higher percentage of the poor. Folate deficiency causes extensive incorporation of uracil into human DNA (4 million/cell), leading to chromosomal breaks [2]. This mechanism is the likely cause of the increased cancer risk [4], and perhaps the cognitive defects, associated with low folate intake. Our new evidence, and mechanistic considerations, suggest that vitamin B12 (14% of the U.S.elderly) and B6 (10% of the U.S.) deficiencies also cause high uracil and chromosome breaks. Micronutrient deficiency may explain, in good part, why the quarter of the population that eats the fewest fruits and vegetables (5 portions a day is advised) has about double the cancer rate for most types of cancer when compared to the quarter with the highest intake. 80% of American children and adolescents and 68% of adults do not eat 5 portions a day. Common micronutrient deficiencies are likely to damage DNA by the same mechanism as radiation and many chemicals, appear to be orders of magnitude more important, and should be compared for perspective. Remedying micronutrient deficiencies is likely to lead to a major improvement in health and an increase in longevity at low cost.

Aging appears to be in good part due to the oxidants produced as by-products of normal metabolism by mitochondria [3,5]. In old rats mitochondrial membrane potential, cardiolipin levels, respiratory control ratio, and overall cellular O2 consumption are lower than in young rats and the level of oxidants (per unit O2) and mutagenic aldehydes from lipid peroxidation is higher [3]. Ambulatory activity declines markedly in old rats. Feeding old rats the normal mitochondrial metabolites acetyl carnitine and lipoic acid for a few weeks, restores mitochondrial function, lowers oxidants to the level of a young rat, and increases ambulatory activity [6,7,8]. Thus, these two metabolites can be considered necessary for health in old age and are therefore conditional micronutrients. This restoration suggests a plausible mechanism: with age increased oxidative damage to proteins and lipid membranes causes a deformation of structure of key enzymes, with a consequent lessening of affinity (Km) for the enzyme substrate; an increased level of the substrate restores the velocity of the reaction, and thus restores function. [1=Tox.Letters 102, 5-18, 1998; 2=PNAS 94, 3290, 1997; 3=PNAS 94, 3064, 1997; 4= PNAS 96,12216, 1999; 5=PNAS 95, 288, 1998; 6=PNAS 95, 9562, 1998; 7=FASEB J. 12, 1183-1189, 1998; 8=FASEB J. 13, 411, 1999]

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OXIDATIVE STRESS VULNERABILITY AND INTERVENTIONS IN BRAIN AGING. J. A. Joseph*, N. Denisova, B. Shukitt-Hale, K. Youdim and D. Fisher, USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111, U.S.A.

There is an overwhelming amount of data which suggest that in aging most if not all of the components are present which would provide the necessary environment for the development of neurodegenerative diseases such as AD in individuals so pre-disposed. These include: a) increased sensitivity to OS that may be the result of alterations in membrane constituencies, reductions in the synthesis of glutamine synthetase, increases in membrane lipid peroxidation, and redox active iron. b) decrements in receptor-G protein uncoupling such that receptor sensitivity is reduced c) increases in inflammation (see above) possibly mitigating the effects of anti-inflammatory agents, d) regional increases in membrane lipids such as cholesterol or sphingomyelin which would alter the biophysical properties of membranes (e.g., fluidity), and e) an exaggerated response of the aged neurons to hyper- phosphorylation and the formation of paired helical filaments f) decreases in motor and cognitive function. Thus, the deleterious effects of aging appear to be multifactorial involving oxidative stress, inflammation and membrane changes. If this is the case, then it would be important to utilize agents which have a multiplicity of actions and which can be effective in reducing or forestalling the age-changes in the factors involved in the behavioral deficits that are observed in aging. One method which has been employed to reverse or forestall the deleterious effects of brain aging on behavior in our laboratory has been to utilize nutritional supplementation using fruits or vegetables high in polyphenolic compounds which have multiple actions [e.g., antioxidiant, anti-inflammatory. For example in one recent study we maintained 19 mo Fischer 344 rats for 2 months on a spinach, strawberry or blueberry (BB)-supplemented diet. The supplemented rats showed reversals in deficits in neuronal signaling (e.g., carbachol-stimulated GTPase activity from striatal slices) as well as in cognitive behaviors. The BB supplementation was particularly effective in this regard, since it was the only supplement to effect reversals in motor behavioral decrements. In an attempt to determine the possible mechanisms involved in these reversals, additional experiments were conducted which showed that the BB extract had significant anti-inflammatory activities against TNF in a cell model. Moreover, senescent animals maintained on the BB supplementation for two months showed considerable protease activity increases in several brain areas, selective increases in hippocampal neurogenesis, and decreases in markers of inflammation in the quadraceps and gastrocnemius, along with increases in blood vessel diameter in these muscles. Finally, preliminary data indicate that there may be selective increases in neurogenesis in the BB-supplemented animals. These findings suggest that, in addition to their known beneficial effects on cancer and heart disease, phytochemicals present in antioxidant rich foods may be beneficial in reversing the course of neuronal and behavioral aging.

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VITAMIN E BIOKINETICS, OXIDATIVE STRESS AND AGING. Maret G. Traber*, Linus Pauling Institute, Oregon State University, Corvallis, OR 97331-6512, USA. maret.traber@orst.edu

Vitamin requirements are traditionally established by determining the deficiency symptom and the minimum amount of the nutrient needed to reverse it. Vitamin E does not fit this pattern because its deficiency symptom in humans is a peripheral neuropathy that can take 50 years to become apparent. However, neuropathy may not be the most sensitive marker for a suboptimal vitamin E intake. Epidemiologic and intervention studies have suggested that pharmacologic vitamin E intakes are associated with a decreased risk of some chronic diseases, notably heart disease. Does a chronic low intake of vitamin E result in inadequate cellular defense against lipid peroxidation? Are there are specific cellular functions accomplished only by *RRR*-alpha-tocopherol, the biologically most active form of vitamin E, that are compromised by suboptimal intakes. Although the various vitamin E forms have nearly similar antioxidant activities, they are quite different in their abilities to prevent vitamin E deficiency symptoms in experimental animals. Studies in humans using vitamin E forms labeled with deuterium have demonstrated that a- and γ-tocopherols, and different stereoisomers of α-tocopherol (e.g. *RRR*- and *SRR*-) are equally well absorbed, but only *RRR*-α-tocopherol is maintained in the plasma. The critical factor appears to be the hepatic α-tocopherol transfer protein (TTP) because it is required to maintain normal plasma concentrations of α-tocopherol; humans with a genetic defect in TTP become vitamin E-deficient. Metabolism of vitamin E may also be important; non-α-tocopherol forms of vitamin E are readily metabolized and excreted into the urine. Thus, the requirement for vitamin E in humans seems to depend on the function of TTP, oxidative stress and on vitamin E metabolism all of which could be affected by aging.

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CIGARETTE SMOKE DAMAGE TO SALIVARY PROTEINS AND ENZYMES AND THE EFFECTS OF ANTIOXIDANTS: IMPLICATIONS FOR SALIVA OF AGED PEOPLE. A.Z. Reznick,[1]* R. Kohen,[2] S. Lischinsky,[3] E. Diamant,[3] I. Klein,[1] and R. Nagler.[3] [1]Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, [2]Hebrew University-Hadassah Medical School, Jerusalem, and [3]Rambam Medical Center, Haifa, Israel.

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Using cyclic voltammetry it has been shown that the reducing ability of human saliva decreases considerably with donor age [R. Kohen et al., *Exp. Gerontol.* 27, 161 (1992)]. We recently embarked on a project studying the effect of cigarette smoke (CS) on salivary enzyme activities, salivary antioxidant defense systems, and the capacity of several exogenous antioxidants to curtail the CS-associated damages.

Saliva is the first body fluid to confront the inhaled CS. *In vitro* exposure of saliva to nine puffs of CS showed a 400% increase in salivary protein carbonyls, and using immunoblot assay to dinitrophenylhydrazine it was shown that certain salivary proteins are more highly carbonylated than others. Ascorbate and desferri oxamine (DES) had little effect on protein carbonyl accumulation, while glutathione (GSH) and N-acetylcysteine (NAC) considerably inhibited this accumulation. These results resemble those obtained previously with plasma, indicating that aldehydes, present in CS, are the major contributors to the formation of protein carbonyls in saliva.

Following the exposure to CS, the activities of several salivary enzymes—amylase, lactic dehydrogenase (LDH), and acid phosphatase (ACP)—were found to be significantly reduced (83, 57, and 77%, respectively). However, CS had no effect on the activities of aspartate aminotransferase and alkaline phosphatase. Addition of 1 mM of GSH and NAC considerably protected LDH and amylase activities, suggesting that –SH groups are affected in LDH and amylase. On the other hand, addition of 1 mM ascorbate caused a further loss of LDH and amylase activities, which could be partially prevented by the addition of DES, implicating metal-catalyzed oxidation processes. However, loss of ACP activity was completely unaffected by any of the above antioxidants. It is concluded that the loss of salivary enzyme activities may be due to various agents in the CS that affect the enzyme activities via different mechanisms. Similar studies on the effect of CS on salivary peroxidase activity and level of uric acid, as major antioxidants of saliva, showed inactivation of peroxidase and loss of uric acid. However, none of the above exogenous antioxidants could efficiently protect peroxidase from CS-associated loss of activity. Finally, recent studies on saliva from aged people showed significant changes caused by CS that were even less protected by the antioxidant defense systems.

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IS PRIMARY HUMAN AGING ASSOCIATED WITH AUGMENTED SYMPATHETIC-CARDIOVASCULAR RESPONSIVENESS TO ACUTE STRESS? Douglas Seals*, University of Colorado-Boulder, Boulder, CO.

It is generally assumed that in adult humans, advancing age is associated with exaggerated sympathetic nervous system and cardiovascular reactivity to acute stress. However, much of the evidence underlying this concept stems from experiments employing indirect and potentially imprecise measurements of sympathetic nervous system activity (SNA). Over the past decade we have systematically tested this general hypothesis using state-of-the-art techniques to measure regional and net whole-body SNA under resting conditions and in response to acute stressors that activate SNA via different physiological pathways. Our results demonstrate that in contrast to the current belief, in general, sympathetic-cardiovascular responsiveness to stress is not augmented with healthy aging in adult humans, although some region- and stress-specific differences are observed with age.

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DOES AGE ALTER SKELETAL MUSCLE ADAPTABILITY TO THE PHYSIOLOGIC STRESSES OF EXERCISE OR CALORIE RESTRICTION? Gregory D. Cartee*, Ph.D., University of Wisconsin-Madison.

Our research focuses on adaptive responses of glucose metabolism by skeletal muscle with various types of physiologic stress. We have evaluated the conditions of increased energy expenditure (exercise) and reduced energy intake (calorie restriction) in young and old animals. Many studies have demonstrated that, during youth, skeletal muscle is a highly malleable tissue: interventions can modify the amounts of energy stores and induce altered expression of proteins and activity of enzymes. The purpose of this lecture will be to describe, across the life span, how skeletal muscle responds and adapts to exercise (acute and chronic) and calorie restriction (brief and prolonged).

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MECHANICAL LOADING STRESS: BENEFICIAL OR DETRIMENTAL TO THE AGED SKELETON? Wendy M Kohrt*, Division of Geriatric Medicine University of Colorado Health Sciences Center 4200 E Ninth Ave, Campus Box B-179 Denver, CO 80262

It has been suggested that mechanical loading forces are not as effective in inducing bone mineral accretion in aged bones as in young bones. This raises the possibility that certain types of exercise may actually be harmful for older osteopenic individuals. However, it is not clear whether it is aging, per se, that differentiates the bone (re)modeling response to loading, or possibly secondary factors of aging (e.g., deficiency of osteogenic hormones, growth factors, micronutrients etc). The effects of different types of exercise on bone mineral density in older women and men will be presented. Some potential mediators of the osteogenic response to mechanical loading will also be discussed.

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DOES AGE ALTER THE SYSTEMIC AND CELLULAR RESPONSES TO PHYSIOLOGICAL STRESS? Kevin C. Kregel*, The University of Iowa.

Aging is associated with a loss of ability to modulate the stress response. Following exposure to a physiological stress such as environmental heating, older organisms show increased morbidity and mortality. Although mechanisms underlying the age-related alterations in stress responses are currently unclear, experimental evidence implicates age-related oxidative stress in the loss of functional capacity with age. We have been utilizing an integrated approach that includes whole animal, cellular, and molecular techniques to test this hypothesis. The purpose of this presentation will be to address potential mechanisms for the altered physiological responses to stress with aging. Specific issues to be discussed include the age-related changes in free radical formation, gene regulation, stress protein responses, and cellular injury that have been observed in response to physiologically relevant levels of environmental stress.

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MORPHOLOGIC ASSESMENT OF OXIDATIVE DAMAGE. Terry Oberley* VA Hospital, Room A35, 2500 Overlook Terrace, Madison, WI 53705.

Biochemical studies have indicated changes in antioxidant enzyme activities and increased oxidative damage products in many disease states, particularly aging and diseases associated with aging, such as neurodegenerative diseases and cancer. To try to determine cellular and subcellular localization of oxidative damage, our laboratory has developed quantitative light and electron microscopy immunogold techniques using specific antibodies to oxidative damage products. Results from studies of different pathologic processes, including aging, will be presented to illustrate that both localization and quantitation of oxidative damage products is possible. These analyses give important insights into the nature of various pathologic processes.

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AGE-RELATED ALTERATIONS IN THE ACTIVATION OF HEAT SHOCK TRANSCRIPTION FACTOR 1 IN RAT HEPATOCYTES. Ahmad R. Heydari*, Department of Nutrition & Food Science, Wayne State University, Detroit, MI 48202 Ahmad.Heydari@wayne.edu).

A characteristic feature of senescence is the progressive decline in the ability of an organism to respond to stress. Because heat shock proteins protect cells from a variety of stresses, we studied the expression of hsp70 by hepatocytes isolated from young (4 to 6 months of age) and old (24 to 28 months of age) male Fischer F344 rats. The induction of hsp70 transcription by heat shock is significantly reduced in hepatocytes isolated from old rats compared to hepatocytes isolated from young/adult rats, and the decline in hsp70 transcription is correlated to a decrease in the induction of heat shock transcription factor 1 (HSF1) binding to the heat shock element (HSE). Interestingly, the reduction of calorie intake by 40% (caloric restriction) reverses the age-related alterations in the induction of HSF1 binding activity in hepatocytes obtained from these animals. However, the age-related decrease in HSF1 binding activity to DNA is not due to reduced levels of HSF1 that are available for activation by heat shock. In fact, the levels of HSF1 are 2- to 3-fold higher in hepatocytes from old rats. The age-related

increase in HSF1 protein levels in hepatocytes appears to arise from a decrease in the degradation of the HSF1, i.e., no age-related alterations in HSF1 mRNA were observed, while the HSF1synthesis decreased approximately 50% with age. In addition, no evidence was found for an impairment in HSF1 oligomerization in hepatocytes from old rats, e.g., the level of HSF1 trimers, the nuclear translocation of HSF1, and the phosphorylation of HSF1 after heat shock are similar in hepatocytes isolated from young/adult and old rats. However, the thermostability of the DNA binding activity of HSF1 was significantly reduced with age in a cell free system as well as in isolated hepatocytes. These data are consistent with the proposition that an age-related decrease in the turnover of HSF1 gives rise to an accumulation of post-translationally modified HSF1 molecules that show decreased thermostability and reduced DNA binding activity.

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ALTERATIONS IN PROLIFERATION- AND STRESS- ACTIVATED SIGNALING PATHWAYS IN AGING HEPATOCYTES. Nikki Holbrook*, Gerontology Research Center, NIH/NIA Baltimore, MD 21224.

Multiple signal transduction pathways are activated in response to environmental stress, several of which overlap significantly with those mediating proliferation responses. Some of those are death promoting, while others provide pro-survival functions. Thus, cell fate is determined by the balance between the relative activities of the various pathways activated. There is increasing evidence for the involvement of such pathways in influencing longevity in lower organisms, and we and a number of other laboratories have provided evidence for alterations in some stress-response pathways with aging in mammalian systems. This talk will provide a general summary of some of the key pathways shown to undergo changes with aging. In addition, I will describe findings from our own studies using a rat hepatocytes model in which we have observed alterations in several distinct pathways involved in regulating cellular responses to proliferative signals as well as stressful stimuli. Finally, I will discuss recent studies attempting to identify common mechanisms leading to deficient stress responsiveness with aging and enhance or restore responsiveness in aged cells to that of their younger counterparts.

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BALANCING MARKER OF OXIDATIVE DAMAGE AND REDOX STATUS IN INTERVENTION STUDIES. Paul Milbury*, Antioxidant Research Laboratory, Jean Mayer USDA Human Nutritional Research Center on Aging, Tufts University, Boston, MA 02111.

During the last decade the National Cancer Institute and the National Institute of Environmental Health Sciences (NIEHS) have held two workshops to determine the best method and/or indicator for assessing oxidative stress status in human studies. Consensus was not reached at either of these meetings. The NIEHS Biomarkers of Oxidative Stress Study (BOSS) was initiated to comparing different markers of oxidative stress damage measured within the same biological samples. The BOSS and other studies are revealing inconsistencies in the responses of the putative biomarkers to oxidant challenges. It appears that no single biomarker can adequately assess the complex dynamics of oxidative stress. Selection of the most appropriate analyte indicators for a particular disorder may depend on the nature of the specific pro-oxidant challenge, the differential generation of reactive oxygen intermediates, and the cell and tissue targets involved or studied. Further, it appears inappropriate to consider oxidative stress status outside the context of the antioxidant defense mechanisms operating within the same milieu. Marked inter-individual differences in the production of biomarkers of oxidative stress may result from the array of genetic and environmental factors present even in the most well controlled intervention studies. Therefore, careful assessment of baseline measures of redox status should be an essential component of eligibility criteria for clinical trails of antioxidants. Analysis of suites of biomarkers for antioxidants and oxidative stress utilizing pattern recognition algorithms presents a new approach for understanding the nature of a pro-oxidant challenge and determining the safety, efficacy and potential mechanism(s) of antioxidant interventions.

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INCREASED SENSITIVITY TO OXIDATIVE STRESS-INDUCED APOPTOSIS IN FIBROBLASTS AND CARDIOMYOCYTES FROM MNSOD AND GPX1 KNOCKOUT MICE. H. Van Remmen*, L. Estlack, and Z.M. Guo, Dept. of Physiology University of Texas Health Science Center and GRECC, South Texas Veterans Health Care System, San Antonio, Texas, 78229

We have studied whether reduced activity of the mitochondrial antioxidant enzymes MnSOD and glutathione peroxidase 1 (Gpx1) leads to increased sensitivity to apoptosis through pathways involving the mitochondria and the permeability transition. Primary cardiomyocytes isolated from neonate mice (wildtype, *Sod2*$^{+/+}$, *Sod2*$^{+/-}$) were treated with t-butyl hydroperoxide (t-BuOOH) to induce oxidative stress and cell death was assessed by cell viability. Cell death was less than 10% in cardiomyocytes from wildtype mice following treatment with t-BuOOH and approximately 20 and 40% in cardiomyocytes from *Sod2*$^{+/-}$ and *Sod2*$^{-/-}$ mice, respectively. In cells pretreated with cyclosporin A (Csa) to inhibit the permeability transition, cell death was less than 4% in all three lines. Cells from the *Sod2*$^{+/-}$ and *Sod2*$^{-/-}$ mice treated with the superoxide dismutase mimetic, MnTBAP had significantly lower levels of cell death than cells not exposed to MnTBAP demonstrating a direct role for reduced MnSOD in inducing cell death. Similar results were obtained in primary cultures of neonatal skin fibroblasts from these mice. Oxidative stress resulting from treatment with t-BuOOH led to a reduction of mitochondrial membrane potential as measured by retention of $DiOC_6(3)$ that was found to be reversed by inhibition of the permeability transition with Csa. The induction of cell death in response to t-BuOOH was also measured in fibroblasts from Gpx1$^{-/-}$ knockout mice and a Gpx1$^{-/-}$/ *Sod2*$^{-/-}$ cross. Cell death was approximately 20% in Gpx1$^{-/-}$ and over 50% in Gpx1$^{-/-}$/ *Sod2*$^{-/-}$ fibroblasts following treatment with t-BuOOH and was inhibited by Csa. The increase in cell death in response to t-BuOOH was due to apoptosis as demonstrated by DNA fragmentation measured by the COMET assay and partial reversal of cell death by caspase inhibition. Cell death induced by treatment of the fibroblasts with 25mM C_2 ceramide which causes production of reactive oxygen species by mitochondria resulted in increased cell death in fibroblasts from the *Sod2*$^{+/-}$, *Sod2*$^{-/-}$, Gpx1$^{-/-}$ and Gpx1$^{-/-}$/ *Sod2*$^{-/-}$ mice compared to cells from the wildtype mice that was inhibited by treatment with Csa to block the permeability transition. These data indicate that cells from mice with reduced levels of MnSOD and/or glutathione peroxidase 1 activity are more sensitive to oxidative stress-induced apoptosis through a mechanism that involves the mitochondria and the permeability transition.

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REVERSING AGE-RELATED MITOCHONDRIAL DNA DECLINE: ALTERNATIVES TO MITOCHONDRIAL GENE THERAPY. A.D.N.J. de Grey*, University of Cambridge, Cambridge CB2 3EH, UK.

The age-related accumulation of mutant mitochondrial DNA (mtDNA) in non-dividing cells remains a promising candidate for a primary determinant of the rate of aging of homeotherms, even though absolute levels of mutant mtDNA even in the very elderly may be extremely low [de Grey ADNJ, 2000, Arch Biochem Biophys 373:295-301]. Consequently there is a need to identify potential interventions that might retard, or ideally reverse, this accumulation. Such reversal might be "simulated" by introducing suitably modified transgenic copies of the mtDNA's protein-coding genes into the nucleus; their products would enter mitochondria by the same pathways as used by naturally nuclear-coded mitochondrial proteins. However, this approach faces two serious obstacles: delivery of engineered DNA to non-dividing cells is still very difficult, and the mitochondrially-encoded proteins are too hydrophobic to be importable across the mitochondrial membranes (though ways around this have been proposed [de Grey ADNJ, 2000, Trends Biotechnol, submitted]). Genuine reversal of mutant mtDNA accumulation could be achieved by direct reintroduction of transgenic copies, or by expansion of the residual wild-type mtDNA. The latter might not require gene therapy, so is potentially attractive. Cells often become completely taken over by mutant mtDNA, however, so this would entail ablating such cells and triggering division of their mitochondrially wild-

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type neighbours. In skeletal muscle, however, only ~1mm segments of a fibre become mutant. Affected fibres generally survive indefinitely, possibly by means that are highly toxic to their extracellular environment and thence to the whole body. Inhibiting the cell membrane redox activity that is proposed to mediate such toxicity [de Grey ADNJ, 1998, J Anti-Aging Med 1:53-66], faces the problem that that enzyme's physiological function may be indispensable. Transient exposure to such inhibitors might be non-toxic and might still ablate the OXPHOSless segments, but it is unclear whether they would then be efficiently repaired. A recent advance in cybrid technology suggests a better-targeted alternative. Rhodamine 6G kills mitochondria rapidly but is harmless to the rest of the cell, allowing the treated cell then to be fused with an enucleated cell containing mitochondria of a chosen genotype. If a similar agent could be selectively targeted to OXPHOSless fibre segments, the mitochondria in those segments would be ablated; they might then be repopulated with wild-type mitochondria from the neighbouring regions of the fibre. Targeting of such an agent may be feasible, since such segments have been hypothesised to express very high levels of plasma membrane proteins involved in transmembrane electron transport, which may be a sufficiently distinctive surface character to mediate targeting. The ability of neighbouring regions to repopulate a de-mitochondrionated segment can now be tested in vitro, using cybrid myofibres cocultured with neurons, which survive for many months. The system would also be expected to work on other cell types, since destruction of any cell's mitochondria leads to very rapid cell death; tumour cells appear to overexpress the same membrane proteins, so the approach may also have anti-cancer potential.

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REDUCED BODY TEMPERATURE AND PLASMA GLUCOSE LEVELS IN LONG-LIVING GH-R-KO MICE. A. Bartke*, W. Hunter, S. Hauck, N. Danilovich[2], K. Coschigano[3], and J. Kopchick[3]. Department of Physiology, Southern Illinois University School of Medicine, Carbondale, IL 62901-6512; [2]Clinical Research Institute of Montreal, Montreal, PQ H2W 1R7, Canada; [3]Edison Biotechnology Institute, Ohio University, Athens, OH 45701.

We have recently reported that growth hormone receptor/growth hormone binding protein knock out (GH-R-KO) mice live considerably longer than normal animals from the same line (Kopchick & Laron, Molec. Genetics Metab. 68:232-236, 1999). Due to the absence of GH receptors, GH-R-KO mice are GH resistant and diminutive in size. Preliminary studies of learning and memory suggest that in addition to increased life span, GH-R-KO mice experience delayed aging. Our objective was to identify mechanisms linking GH resistance with prolonged longevity in these animals. In the present study, we have examined body core temperature and plasma glucose and thyroid hormone levels in GH-R-KO and normal (N) mice because alterations in these characteristics are associated with prolonged longevity in GH, prolactin, and thyrotropin-deficient Ames dwarf mice. Body core temperature (Tco) was measured by telemetry using miniature transmitters implanted in the peritoneal cavity. Tco was recorded every 15 min for 24 h in 6 GH-R-KO and 6 N mice and the results were averaged for each consecutive 60 min period for analysis. Tco was consistently numerically lower in GH-R-KO than in N mice (in 22 of 24 comparisons) and these apparent differences were statistically significant around lights off and during a three hour period preceding lights on when the differences approached 1°C. These reductions in Tco may be related to GH resistance because plasma levels of thyroxine and 3-iodo-thyronine were not altered in GH-R-KO vs. N animals. Glucose levels were measured 1.5 hrs after lights on and 1.5 hrs before lights off using 8 females and 8 males of each phenotype and were found to be significantly reduced in GH-R-KO vs. N mice (AM: 77 ± 3.2 vs. 96 ± 3.4 mg/dl; $P<0.001$; PM: 66 ± 3.6 vs. 92 ± 4.7 mg/dl; $P<0.0001$). Glucose levels were significantly higher in the morning than in the afternoon in GH-R-KO ($P<0.05$) but not in normal mice. Plasma insulin levels were greatly reduced in GH-R-KO vs. N mice (<4.0 vs. 32.0 ± 10.9 ? IU/ml) while plasma corticosterone levels were not significantly different except for an elevation of corticosterone levels in GH-R-KO vs. N males in the afternoon. From these and previous findings, we conclude that GH resistant GH-R-KO mice have reduced Tco, greatly increased sensitivity to insulin, and reduced plasma glucose levels. Further studies will be necessary to determine whether these characteristics of GH-R-KO mice may be causally related to their prolonged longevity and to evaluate other potential mechanisms of delayed aging in these animals. Comparisons of endocrine characteristics and longevity in GH-R-KO and Ames dwarf mice suggest that a congenital primary defect in GH signaling is associated with prolonged longevity regardless of thyroid status or plasma PRL levels.

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CENTENARIAN ROCKFISH PROJECT — THE BIOCHEMICAL STUDY OF SLOW OR NEGLIGIBLE AGING IN ROCKFISH PROVEN TO LIVE AT LEAST 140 YEARS. John C. Guerin (*), Project Director, ba182@LAFN.ORG, 4424 N.E. 83rd Ave., Portland, OR 97220; Researchers Guido Krupp, Christian-Albrechts University, Kiel, Germany, Giel Bosman, University of Nijmegen, The Netherlands, David E. Williams, Oregon State University, Corvallis, OR, Jerry D. Hendricks, also of Oregon State University.

We propose it is useful for Gerontology to study extreme longevity in animals. Rockfish, turtles and sturgeon have all been demonstrated to live 140 or more years. But these animals are not only long-lived, they don't appear to grow old. Instead, they exhibit negligible senescence — chronological lifespans without increased mortality. This project intends to uncover the mechanism(s) that allow these animals to live so long without aging, and to apply this knowledge for human benefit. Currently four lines of research have been started, with samples available for further proposed research. Guido Krupp is analyzing telomerase expression in brain, heart and liver tissues from rockfish up to 93 years old (age measured by otolith). According to the telomere hypothesis, DNA replication leads to telomere shortening, resulting in a cellular mitotic clock. Telomerase resets it by telomere synthesis. Since rockfish grow throughout their life with little senescence, this requires continuous cell proliferation and cell turnover. For maintaining this cell proliferation capacity, telomerase should be active in cells of all somatic tissues, irrespective of fish age. Preliminary results have confirmed this expectation, and even in brain tissue, significant telomerase activity was detected, at levels comparable to human tumor cell lines. Giel Bosman is researching Anion Exchange proteins in rockfish brain. These proteins are known in humans to increase with aging, especially with degeneration in Alzheimer's disease-affected brain areas. Preliminary results have shown that antisera against various domains of human erthrocyte band 3 (AE1) react with rockfish brain tissue as judged by immunochemical analysis. Immunoblots show several reactive protein bands that have approximately the same molecular weights as those observed in human brain tissue. In this blind study clear differences were observed between samples, but it is not known yet if the differences are age-related. David E. Williams has exposed both Rougheye and Yelloweye liver samples up to 101 years old to oxidative damage. He found the generation of TBARS (in relation to lipid peroxidation) was dramatically reduced compared to rat or monkey liver microsomes. Parodoxically, the polyunsaturated fatty acid (PUFA) content of rockfish is relatively high compared to trout, for example, indicating the rockfish had additional protection from oxidative reactions. Attempts to duplicate this research a year later with samples that had been stored at -80 degrees C. were unsuccessful. Dr. Williams suspects the protection must be thermal labile, possibly a protein or peptide. Jerry D. Hendricks performed histological examinations of old-, young- and medium aged rockfish spleen, liver and kidney organs. He found an increase of melanomacrophage centers in older specimens, although the physiological consequences of these centers does not seem to affect survival. No other cellular indicator existed to differentiate between the cells of young and old rockfish, which ranged over 80 years between youngest and oldest samples. Proposed lines of research include the following: Biochemical profiling and comparison of numerous parameters such as oxidative stress and antioxidants CoQ10 and Vitamin E, hormones, enzymes, vitamins, minerals, etc.; Comparative biochemical study between long-lived and short-lived species of rockfish; Mitochondria membrane integrity and DNA lesions; Establishing a non-lethal method to determine rockfish ages.

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ALTERED CHOLESTEROL METABOLISM MODULATES BETA-AMYLOID DEPOSITION IN A TRANSGENIC MOUSE MODEL FOR ALZHEIMER'S DISEASE. L.M. Refolo*[1], M.A. Pappolla[2], B. Malester[1], J. LaFrancois[1], T. Thomas-Bryant[2], R. Wang[3], G.S. Tint[4], K.E.Duff[1]; [1]Nathan S. Kline Institute for Psychiatric Research, Orangeburg, NY, 10962, [2]University of South Alabama Medical School, Mobile, AL, 36617, [3]The Rockefeller University, New York, NY, 10018, [4]Dept. of Veterans Affairs, New Jersey Health Care System, E. Orange, NJ, 07018.

Recent data suggest that cholesterol metabolism is linked to susceptibility to Alzheimer's disease (AD). However, no direct evidence has been reported linking cholesterol metabolism and the pathogenesis of AD. Using a transgenic mouse model for AD amyloidosis, we tested the hypothesis that amyloid b-peptide (Aß) deposition can be modulated by cholesterol metabolism. To test our hypothesis we examined the effects of a high fat/high cholesterol diet and a cholesterol-lowering drug on CNS Aß accumulation. Our data showed that diet induced hypercholesterolemia resulted in significantly increased levels of formic acid extractable Aß peptides in the CNS. Furthermore, the levels of total Aß were strongly correlated with the levels of both plasma and CNS total cholesterol. Biochemical analysis of amyloid precusor protein (APP) metabolites revealed that, compared to control, the hypercholesterolemic mice had significantly decreased levels of sAPPα and increased levels of ß-CTF; suggesting alterations in APP processing in response to hypercholesterolemia. Neuropathological analysis indicated that the hypercholesterolemic diet significantly, increased ß-amyloid deposit number and deposit size. Treatment of mice with the cholesterol-lowering drug BM15.766 significantly reduced both plasma cholesterol levels and CNS Aß. These data demonstrate that high dietary cholesterol increases Aß deposition and accelerates the AD-related pathology while lowering cholesterol levels reduces Aß accumulation in this animal model. Thus, lowering cholesterol, by dietary or pharmacological means, may be a valid approach for reducing the risk of developing AD.

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LUTEIN AND AGE-RELATED EYE DISEASE. Max Snodderly*, Schepens Eye Research Institute, 20 Staniford Street, Boston, MA 02114.

With the inclusion of lutein in Centrum multivitamin formulations, and nationwide television advertising to promote it, there is increasing public interest in the science behind publicity. There is much correlative evidence to support a role of lutein and/or zeaxanthin, the retinal carotenoids, in protecting the retina and the lens from age-related loss of visual function. Unfortunately, the only intervention trials that have been completed to date are based on small numbers of subjects without placebo controls. This lack of information is due in part to earlier difficulties in assessing the ability of dietary interventions to actually alter the retinal carotenoid concentrations as opposed to merely altering serum levels. However, new instruments are capable of measuring the retinal carotenoids as macular pigment density in human retinas in vivo. Therefore the methodology is available to monitor the incorporation of these nutrients into the retina and carry out studies on large populations. However, the time course of ocular aging is slow, and the most insightful approach to evaluate dietary intervention would be longitudinal studies that are difficult to organize and to support within the framework of short-term grants. The American Aging Association would benefit the vision research community greatly if it helped to develop a context that would facilitate longitudinal studies of visual aging.

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DIETARY CALCIUM AND VITAMIN D IN BONE AGING. B. Dawson-Hughes*, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, Ma 02111.

Calcium and vitamin D nutrition have a recognized role in preserving bone health in the elderly. Loss of estrogen at menopause induces an increase in the bone remodeling rate and accelerated bone loss. Although calcium and vitamin D will not prevent estrogen-deficiency related bone loss, they will mitigate this loss. A recent meta-analysis addressed the issue of whether calcium supplementation matters in women taking hormone replacement therapy and found that bone density gains at the spine, hip, and forearm were greater in the estrogen users who also took added calcium than they were in the women taking estrogen only. Calcium may also amplify the effects of other antiresorptive therapies for osteoporosis. The role of calcium and vitamin D in reducing fragility fractures has been studied most extensively in elderly women. Combined supplementation of very elderly French nursing home residents significantly reduced their rates of hip fracture. These women reported dietary calcium intakes of about 500 mg/day and had low-normal hydroxyvitamin D levels. More recently, combined calcium and vitamin D supplementation reduced non-vertebral fracture rates in younger, home-dwelling men and women, mean age 71 years, who consumed an average of 700 mg of calcium and 200 IU of vitamin D in their diets each day. Supplementation with calcium or vitamin D alone has generally produced less consistent benefit than combined supplementation. Based on substantial scientific evidence, the National Academy of Sciences recently increased the recommended intakes to 1,200 mg of calcium and 400 IU (age 51-70) or 600 IU (age 71 and older) of vitamin D per day for men and women. A recent study examined the effect of discontinuing supplemental calcium and vitamin D on rates of bone remodeling and bone loss in men and women age 68 an older. A preliminary report indicates that the skeletal changes induced by the supplements had largely reversed by two years off the calcium and vitamin D. Thus, older adults should meet the recommended intakes of calcium and vitamin D on a continuous basis in order to achieve and retain the maximal benefit.

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CLINICAL SETTINGS FOR DIETARY AND OXIDATIVE STRESS INTERVENTION. Christopher B. Heward*, Chief Scientist, Director of Research & Development, The Kronos Group, 4455 East Camelback Road, Suite B-200, Phoenix, AZ 90278, Senior Research Fellow, Program on Medicine, Technology, and Society, Department of Neuropsychiatry and Biobehavior, School of Medicine, University of California, Los Angeles.

This presentation will provide a general overview of the Kronos approach to "Clinical Gerontology" with special emphasis on dietary intervention. Other than caloric restriction, no single diet has been particularly associated with increased longevity. Still, the negative health consequences of poor eating habits are obvious to anyone in a clinical environment. We have found that intake of readily available dietary supplements can produce significant changes in certain indicators of oxidative stress in our patients. In addition, relatively simple changes in diet can dramatically reduce known risk factors for a variety of age-related diseases. Clinical data from our laboratory will be presented and a number of case studies will be discussed in detail.

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CAN FUNCTIONAL FOOD IMPROVE THE IMMUNE RESPONSE IN THE ELDERLY? Simin Nikbin Meydani *, Nutritional Immunology Laboratory, JM USDA Human Nutritional Research Center on Aging at Tufts University, Boston, MA.

Many studies have shown that food components play an important role in the maintenance of the immune response and resistance to immune- related diseases. The incidence of neoplastic and infectious diseases increases with age, as does their consequent morbidity and mortality. The well- documented age- associated dysregulation of the immune system is an important contributor to the increased incidence and mortality from these diseases. The age- related changes of the immune response have mostly been reported for cell- mediated functions such as delayed- type hypersensitivity skin response, antibody response to T- cell- dependent antigens, the ability to proliferate or produce cytokines in response to antigenic or mitogenic stimulation and the ability to fight viral and parasitic infections.

Since marginal deficiency of several nutrients has been reported in the elderly, it has been proposed that repletion of the elderly with recommended or above recommended levels of a single nutrient or a mixture of nutrients would enhance the immune response in the elderly and thus would improve their ability to defend against invading patho-

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gens. Various strategies have been used with mixed results. Supplementation with a nutrient mixture 1-4 times recommended levels has been shown to improve the immune response and in one study was shown to decrease antibiotic use and days lost from work due to infectious diseases. Other studies investigating the effect of different mixtures of vitamins and minerals on the incidence of infectious diseases have produced mixed results. Some of the discrepancy in results is due to flaws in experimental designs and/or in the methods used to document incidence of infections.

Several investigators have used single nutrient (vitamins C, E, ß-carotene, selenium, zinc and lipids) intervention to improve the immune response in the elderly. Among the nutrients tested, vitamin E seems to be most effective in improving the immune response. Moreover, vitamin E has been shown to decrease viral titer in old mice inflected with influenza virus. These studies will be discussed in more detail. The effectiveness of vitamin E or any other single intervention in improving resistance to infectious diseases in human needs to be demonstrated. Interventions with lipids containing fatty acids that reduce prostaglandin production, such as fish oil and black currant seed oil, have also been shown to modify the immune response in the elderly.

Very few studies have evaluated the effect of non- essential components of food on the immune response of the elderly. Many of these components, in particular those with antioxidant property or those classified as pre- and pro- biotic, could potentially be beneficial, for improving not only the peripheral immune response, but also the gut-associated immunity, which exhibits age- related changes as well. Along these lines it is interesting to note that the elderly have higher incidence of gastrointestinal infection and exhibit higher morbidity and mortality from food- born pathogens.

In summary, while further research is needed, studies to date indicate that improvement of the immune response and resistance to infectious diseases in the elderly through intervention with food components is feasible. Since infectious diseases are among the leading causes of morbidity and mortality, nutrient- induced improvement in immune response would have a significant impact on the quality of life of the elderly.

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ANTI-OXIDANT FOR TREATMENT AND PREVENTION OF ALZHEIMER'S DISEASE. Mary Sano*, Columbia University College of Physicians and Surgeons, New York, NY.

Several mechanisms have been proposed by which anti-oxidants may have a benefit in treating and preventing Alzheimer's Disease. A double blind placebo controlled study of vitamin E and selegline was conducted in patients with Alzheimer's Disease of moderate severity. This study demonstrated that both agents delayed clinical progression of the disease by about 6 months over a 2-year trial. In addition vitamin E delayed nursing home placement and reduced the rates of functional decline by 25 percent. However neither treatment was associated with a benefit in cognition. This trial has raised optimism for the use of anti-oxidant agents in the treatment of earlier stages of disease and for disease prevention. Randomized clinical trials are currently underway to determine if vitamin E may be useful in secondary prevention of Alzheimer's Disease.

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DIETARY ANTIOXIDANTS AND LUNG CANCER PREVENTION. Xiang-Dong Wang*, USDA-HNRCA 711 Washington street, Boston, MA 02111.

Tobacco smoking contain both carcinogen and free radicals which cause a variety of DNA damage, including mutations in oncogenes and tumor suppressor genes. If smoking induced DNA damage can not be repaired, this will lead to aberrant cell with transformation and ultimately, to lung cancer. The best protection against lung cancer is the avoidance of tobacco smoke. However, the number of current smokers remains alarmingly high. Therefore, nutritional intervention is an appropriate way to rationally and realistically modify cancer risk.

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A large body of observational epidemiologic studies has consistentlydemonstrated that individuals eating more fruits and vegetables, which are rich in antioxidants, and people having higher serum ß-carotene levels have a lower risk of cancer. The consistency of the results from observational studies is particularly strong for lung cancer. In contrast to these observations, two human intervention studies using high dose ß-carotene supplements have reported an increase in the risk of lung cancer among smokers. Understanding the mechanism(s) of the carcinogenic response to high dose ß-carotene supplementation reported in the human intervention trials is of importance due to continuing interest of both research scientists and the public in ß-carotene's potential as a chemopreventive agent.

However, it is highly unlikely that another human intervention study will be conducted in smokers receiving ß-carotene supplements to address the key mechanistic questions of the promoting or inhibiting actions of ß-carotene in lung carcinogenesis. Therefore, the use of appropriate in vitro experiments and/or an animal model are the most justifiable approaches to resolve these mechanistic issues. Recent reports including ours from both in vitro and in vivo studies have provided useful information on the controversy regarding the chemopreventive activity of ß-carotene: the carcinogenic response to high dose ß-carotene supplementation reported in the human intervention trials is related to the instability of the ß-carotene molecule in the free-radical-rich yet antioxidant-poor environment of the lungs of cigarette smokers. The presentation of high doses of ß-carotene via supplements to the highly oxidative environment of the lung will result in increased levels of oxidative metabolites of ß-carotene. The increased ß-carotene oxidative metabolites may promote carcinogenesis by 1) inducing carcinogen-bioactivating enzymes and bioactivating tobacco-smoke procarcinogens; 2) facilitating the binding of metabolites of benzo[a]pyrene to DNA; 3) enhancing retinoic acid catabolism and down-regulating RARß; and/or 4) acting as a pro-oxidant, causing damage to DNA.

Since 1) the stability of ß-carotene is dependent on other antioxidants, particularly vitamin C and vitamin E; 2) intact ß-carotene protects against oxidative DNA damage at relatively low concentrations but loses this capacity at higher doses, and 3) combination of ß-carotene, vitamin C and a-tocopherol may provide synergistic protection against oxidative damage, investigations on the effectiveness of a combination of antioxidants (ß-carotene, vitamin C and α-tocopherol) as an effective chemopreventive strategy against lung cancer should be studied.

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39. VITAMIN E ENHANCES THE FUNCTION OF PURIFIED T CELLS FROM OLD MICE. O. Adolfsson*, L. S. Leka, S. N. Meydani, JM-USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111.

Aging is associated with a decline in T cell-mediated function as demonstrated by a decrease in *in vitro* T cell proliferation and interleukin-2 (IL-2) production. Vitamin E (E) has been shown to increase IL-2 production and proliferation of T cells from aged animals and humans. This was shown to be, in part, due to E-induced reduction in T-cell suppressive factor prostaglandin (PG) E_2, the production of which by macrophages (MØ) is increased with age. Also, a direct effect of E on T cell function, not mediated through its PGE_2 lowering effect on MØ, has been suggested. To evaluate this, T cells purified from the spleens of young (4 mo) and old (24 mo) C57BL/6 mice were incubated with E (46 µM) and activated with immobilized anti-CD3 and soluble anti-CD28 monoclonal antibodies (mAb). At 48 hr, cell supernatants were collected and IL-2 was measured using an enzyme-linked immunosorbent assay (ELISA) as well as intracellular staining of IL-2 protein and flow cytometry. Cells were harvested at 72 hr following an 8 hr pulse with [^{3}H]-thymidine, and proliferation was quantified by liquid scintillation counting. IL-2 production and proliferation of T cells from old mice were both significantly lower whether stimulated by anti-CD3 alone or in combination with anti-CD28 mAb compared to that of young. E significantly enhanced both IL-2 production and proliferation of T cells from young and old mice. However, the percent increase was significantly greater by T cells from old mice compared to that of young. Vitamin E restored IL-2 production by T cells from old mice to the level produced by young. Thus, in addition to its lowering of MØ PGE_2 production, E enhances T cell function directly. The mechanism of this effect of E on T cells is currently under investigation.

40 Abstract Not Presented

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TISSUE SPECIFIC EFFECTS OF CALORIC RESTRICTION ON EARLY EVENTS IN THE INSULIN SIGNALING PATHWAY. R. Michael Anson*, George S. Roth, and Mark A. Lane, Nutritional and Molecular Physiology Section, Laboratory of Neurosciences, National Institute of Aging, Baltimore, MD 21224-6825

Calorie restriction (CR) without malnutrition is the only known non-genetic method for altering both the average and maximum lifespan of a species. The mechanism is not known. However, several lines of evidence suggest an important role for the insulin-signaling pathway. For example, in nematodes, ablation of certain genes homologous to genes in the mammalian insulin pathway increases longevity and enhances stress resistance. Furthermore, mice lacking one of the proteins which undergo serine phosphorylation in response to insulin, $p66^{Shc}$, have also been reported to live longer than wild type. These findings suggest an important role for the insulin response pathway in the control of lifespan. The regulation of the insulin response by nutrient intake also supports involvement of the pathway as a mediator in the response to CR. For example, one of the earliest changes caused by CR is an increase in insulin sensitivity. This has been reported to occur prior to a significant loss in bodyweight and even in the presence of high circulating free fatty acids. The "disposable soma" theory of aging has been extended to CR (R. Holliday, *Bioessays* 1989 Apr;10(4):125-7), and predicts that the biological mechanism of CR will involve a shift in resource allocation away from growth and reproduction toward enhanced life maintenance and stress response. We believe that it is possible such a shift may be reflected at the molecular level by alterations in the levels or activities of the major effector systems activated by insulin, including the IRS-1 pathway and the Shc pathway. As a first step in testing this hypothesis, the current work examined the levels of expression of these early effectors of the insulin response pathway. CR reduced the level of IRS-1 in adipose tissue, and a non-significant trend was seen in the same direction in liver and muscle. In contrast, three major isoforms of the Shc adapter protein were unaltered. These findings suggest that CR has a tissue specific effect on the insulin-signaling pathway, and offer some support for the notion that a molecular shift occurs during CR that differentially alters these major components of the insulin/growth factor pathway.

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IMMUNE REGULATION OF ATHEROGENESIS IN THE ELDERLY THROUGH MODULATION OF CHOLESTEROL METABOLISM. Allison B. Reiss, Nahel W. Awadallah*, David L. Delano, Edwin S.L. Chan, M. Carmen Montesinos, and Bruce N. Cronstein; New York University School of Medicine, New York, NY 10016.

Circulating levels of autoantibodies, which have recently been implicated in the activation of endothelial cells, are known to increase with age, and it has also been demonstrated that levels of circulating immune complexes are increased in elderly as compared to younger individuals. Our most recent data provides a direct physiologic link between the exposure of THP-1 monocytoid cells, a model for human monocytes, to immune complexes and the ability of the cells to defend against cholesterol overload. The transformation of macrophages into foam cells due to cholesterol overload is a major step towards the development of atheroma. In a series of experiments, we have demonstrated that foam cell formation from THP-1 cells in the presence of acetylated LDL increases dramatically upon exposure to immune complexes. THP-1 cells are first treated with phorbol 12,13-dibutyrate (300 nM, six days, 37°C, 5% CO_2) to induce macrophage transformation and adherence. THP-1 differentiated macrophages are then incubated (37°C, 5% CO_2, 24 hrs, 50% human serum/50% RPMI medium) with 50 µg/ml acetylated LDL with or without immune complexes (0.48 mg/ml). Immune complex-mediated increase in lipid uptake in the presence of acetylated LDL is blocked by pre-incubation of the THP-1 cells with an anti-C1q receptor antibody, but not by antibody against ICAM-1, an adhesion molecule found on the surface of monocytes (conditions as described above with or without a 1 hour pre-incubation with 10 µg/ml R139 or 0.005 mg/ml anti-ICAM-1 for each condition). These results are consistent with our finding that in THP-1 cells, as well as human arterial endothelial cells (HAEC) and human peripheral blood monocytes, expression of the cholesterol-metabolizing enzyme cholesterol 27-hydroxylase is downregulated in the presence of immune complex via the C1q receptor. (HAEC cholesterol 27-hydroxylase mRNA decreased by 60% ± 2.3%, n=4; $p<0.001$, THP-1 cholesterol 27-hydroxylase mRNA decreased by 49.3% ± 5.1%, n=3; $p<0.002$, human peripheral blood monocytes 80% decrease in cholesterol 27-hydroxylase mRNA). Cholesterol 27-hydroxylase is known to exert an anti-atherogenic effect on the vasculature both as a consequence of cholesterol removal and by virtue of its major metabolite, 27-hydroxycholesterol, which, among other anti-atherogenic functions, enhances reverse cholesterol transport. Plasma levels of oxidatively-modified LDL have been shown to be increased in the elderly as compared to younger individuals, and scavenger receptor-mediated uptake of oxidatively-modified LDL by activated macrophages has been implicated in the pathogenesis of atherosclerosis. The present study supports the hypothesis that immune-mediated downregulation of cholesterol 27-hydroxylase functions synergistically with oxidative stress in the development of coronary artery disease in the elderly.

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MUSCARINIC ACTIVATION OF MAPK IN PC12 CELLS AND HIPPOCAMPAL SLICES. Jennifer L. Berkeley* and Allan I. Levey, Department of Neurology, Emory University.

Stimulation of muscarinic acetylcholine receptors (mAChR) activates a variety of downstream pathways, several of which lead to MAPK phosphorylation and activation. Activation of the MAPK signaling pathways are important for many cellular functions, including cell growth, differentiation and synaptic plasticity. In addition, MAPK pathways have been shown to be important in regulating amyloid precursor protein (APP) processing. As mAChR have also been shown to be important in many similar functions, including learning and memory and APP processing, we examined the activation of MAPK by endogenous mAChR in both PC12 cells and in hippocampal slices.

In PC12 cells, we determined by immunoprecipitation and RT-PCR that the m1, m4 and m5 mAChR subtypes are endogenously expressed, and that m4 accounts for ~95% of the receptors with m1 and m5 comprising the remaining ~5%. By western blot analysis with an antibody to phosphorylated MAPK, we observed a dose dependent increase in MAPK phosphorylation in cells stimulated with 0.1 µM to 1 mM carbachol (CCh) that was abolished by atropine pretreatment. The maximal response occurred after a 5 minute stimulation and was reduced to baseline by 30 minutes of continuous stimulation. To determine the mAChR subtype responsible for MAPK activation, we used a highly specific m1 toxin derived from Eastern green mamba venom. This toxin completely abolished mAChR induced MAPK activation, indicating that m1 is the principle mAChR subtype responsible for MAPK activation in PC12 cells though it represents only a small proportion the total mAChR population.

In hippocampal slices, CCh treatment also caused an increase in MAPK phosphorylation that was inhibited by atropine as shown by immunocytochemistry with the same phospho-MAPK antibody. This response was most dramatic in the soma and dendrites of pyramidal neurons of CA1 and granule cells of dentate gyrus. However, it was completely absent from CA3. This distribution suggests that m1 is also responsible for MAPK activation in the hippocampus.

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45. CATALASE EXPRESSION IN DELAYED AND PREMATURE AGING MOUSE MODELS. Holly M. Brown-Borg* University of North Dakota-School of Medicine, Grand Forks, ND 58203-2817.

The physiological decline that occurs with aging is thought to result, in part, from accumulation of oxidative damage produced by reactive oxygen species (ROS) generated during normal metabolism. Two genetic mouse models of aging, the Ames dwarf and growth hormone (GH) transgenic, suggest that hormone levels may play a role in antioxidative defense and aging. To explore this possibility, catalase (CAT), an enzyme involved in elimination of ROS, was evaluated in long-lived dwarf and short-lived transgenic mice. Catalase activity and/ or protein was significantly elevated in livers from dwarf mice at 3, 6, 13-15 and 24 months of age when compared to age-matched wild type mice. In contrast, a 50 and 38% reduction ($P<0.05$) in CAT protein was observed in 3 and 10-12 month old GH transgenics respectively, when compared to wild type mice. Kidneys from old dwarf mice exhibited significantly increased CAT activity (22%), protein (16%) and mRNA expression (59%) compared to wild type mice. Conversely, kidneys from GH transgenic mice showed reductions in CAT activity. In heart tissues, CAT activities were 54, 65 and 59% higher in dwarfs compared to wild types. A dramatic 81% decrease ($P<0.05$) in CAT activity was observed in the old 10-12 month old GH transgenic group while CAT protein was reduced 24% when compared to age-matched wild type animals. While only small differences in CAT were observed in brain tissues from dwarf mice compared to normal siblings, a 26% decrease in activity was found in GH transgenic mice. The results of this study suggest that hormonal status modulates antioxidative mechanisms and that CAT is important in overall defense capacity with respect to lifespan in both decelerated (dwarf) and accelerated (transgenic) mammalian models of aging.

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SKIN DERMAL FIBROBLASTS FROM OLDER DONORS PROLIFERATE SLOWER AND EXPRESS LESS GROWTH FACTOR RECEPTOR LEVELS THAN THOSE FROM YOUNGER DONORS; POSSIBLY BECAUSE OF INCREASED RECEPTOR PHOSPHORYLATION. Reenstra WR[1], Orlow DL[1], and Buras JA*[2] [1]Department of Pathology, Boston University School of Medicine, Boston MA 02118; [2]Department of Emergency Medicine, Brigham and Women's Hospital, Boston MA 02115.

Our laboratory has the long-term goals of understanding and manipulating cellular aging processes. It has been observed previously that cells from older donors grow slower and respond to extracellular signals at a decreased rate than cells from younger individuals. This is particularly important in wound healing where there is an observed decrease in wound healing rates in older individuals. The cells in the dermis receive signals to migrate, divide and produce matrix from external sources that are transmitted through transmural receptors. The focus of this project is on age-associated decreases in the epidermal growth factor (EGF) receptor (EGF-R) signal transduction. We specifically set out to determine the mechanism of the observed decreases with age in EGF-R number and ligand affinity, and decreased and delayed EGF-R tyrosine phosphorylation. Utilizing our in vitro model system of aging, where dermal fibroblasts are taken from donors of different ages and studied below passage level 5, we determined via, western blot, 3-D gel analysis and confocal microscopy that EGF-R phosphorylation on threonine occurs to a greater degree in old donor than in young donor fibroblasts and further determined that the increased phosphorylation occurs specifically on the 654 residue. This threonine has previously been reported to produce all the changes in EGF signal transduction that we have observed with aging. These studies suggest that skin fibroblasts from older donors have a decreased proliferation and responsiveness to growth factors because of an increase in growth factor receptor regulation. Ultimately this information will provide the basis for understanding the role of aging in dermal fibroblast response and migration in wound healing.

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ETS ABNORMALITIES AND SARCOPENIA IN RAT SKELETAL MUSCLE. Entela Bua* and Judd M. Aiken. Department of Animal Health and Biomedical Sciences, University of Wisconsin-Madison, Madison, WI 53706

The hypothesis that electron transport abnormalities (ETS) and age-related muscle mass loss (sarcopenia) are linked was investigated. Three muscles (soleus, adductor longus and vastus lateralis) that exhibit varied degrees of sarcopenia were studied in 5, 18, and 36-month-old male Fisher 344 X Brown Norway F_1 ($F344XBNF_1$) hybrid rats. The muscle mass and fiber number was determined for all three muscles to characterize sarcopenia. Significant decreases in soleus and vastus lateralis muscle mass was observed with age. The level of sarcopenia in vastus lateralis muscle was greater than in soleus muscle. Adductor longus did not show any age-associated decrease in muscle mass. Multiple serial sections were analyzed for the activities of two mitochondrial enzymes, cytochrome c oxidase (COX) and succinate dehydrogenase (SDH). The number of COX negative and SDH hyper-reactive fibers increased with age in both vastus lateralis and soleus muscles. No ETS abnormal fibers were identified in adductor longus muscle in any age group. Cross sectional area of individual fibers, was measured along the length of 1000 mm. ETS abnormal fibers in vastus lateralis muscle showed a decrease in cross sectional area in the abnormal region while ETS abnormal fibers in soleus and normal fibers did not show significant changes in cross sectional area. These results suggest that different muscles accumulate different levels of ETS abnormalities during normal aging. Vastus lateralis, which undergoes more sarcopenia, exhibits more ETS abnormalities and associated atrophy than soleus and adductor longus muscles, which are more resistant to sarcopenia suggesting a direct association between sarcopenia and ETS abnormalities.

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AGE-INDUCED CHANGES IN THE EXPRESSION OF CARDIAC EXTRACELLULAR MATRIX COLLAGEN, FIBRONECTIN AND INTEGRINS. Maria Lonnett Burgess*, Timothy R. Whitehead, Jennifer C. McCrea and Heather, L. Hedrick; Dept. of Health Sciences, Boston University, and University ofIllinois @ Urbana-Champaign.

The progressive shift from young age to senescence is characterized by structural and functional changes in the cardiac extracellular matrix (ECM), which supports and aligns myocytes and blood vessels, and maintains myocardial mass and structure. ECM collagen and fibronectin modulate diastolic stiffness and protect myocytes from overstretch. ECM binding to membrane-bound receptors, integrins, directly links ECM to cardiac muscle and fibroblast cells. This study tested the hypothesis that the expression of these ECM proteins and integrins would be altered in advanced age. Old Balb-C mice (20 mo.) exhibited cardiac hypertrophy, and greater LV collagen, fibronectin, alpha 1 and alpha 5 integrin protein expression than middle-aged (12 mo.) or young (2 mo.) hearts ($p<.05$). Beta 1 integrin expression was lower in old hearts ($p<.05$). These data show that advancing age is associated with higher collagen, fibronectin, alpha 1 and alpha 5 integrin expression, suggesting that ECM ligands and integrins undergo consistent regulation in the aging heart.

49 Abstract Not Presented

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MITOCHONDRIAL DNA DELETIONS ARE PRESENT IN INDIVIDUAL AGE-ASSOCIATED RAGGED RED FIBERS: ANALYSIS BY LASER CAPTURE MICRODISSECTION AND PCR. Zhengjin Cao*, Jonathan Wanagat and Judd M. Aiken. Department of Animal Health and Biomedical Science, University of Wisconsin-Madison, Madison, WI 53706

Both deleted mitochondrial DNA (mtDNA) genomes and mitochondrial electron transfer system (ETS) abnormalities are associated with aging. We hypothesized that ETS abnormal fibers (e.g. ragged red fiber) in rat skeletal muscle would be directly linked to deletions of the mitochondrial genome. In this model, age-associated damage (e.g. oxidative stress) occurs to mtDNA resulting in the partial deletion of mitochondrial genome. Due to the replicative advantage of the small,

truncated genome, the abnormal mtDNA genomes eventually predominate over wild-type genomes within a segment of the fiber. The energy deficiency in the affected area stimulates the replication of mitochondrial genome and the expression of nuclear-encoded ETS enzyme activity resulting in an ETS abnormal phenotype (ragged red). We used laser capture microdissection (LCM) to isolate ten micron thick sections of individual fibers from 38-month-old Fisher 344 x Brown Norway F1 hybrid rat skeletal muscle. Total DNA, isolated from the lysate from individual normal and ragged red fiber sections, was used as template for PCR-based deletion analysis. We found mtDNA deletions in 28 of the 29 ragged red fibers analyzed. Deletions ranged in size from 5 to 9 kilobase pairs and precise deletion breakpoints were determined by cloning of deletion products and automated fluorescent sequencing.

The segmental nature of the ragged red region of the fibers required the analysis of mitochondrial genotype along the length of abnormal fibers. Within individual abnormal fibers, deleted mtDNA could only be detected in the fiber segment that concomitantly displayed the ragged red phenotype and not in adjacent phenotypically normal portions of the same fiber. The identical deletion was detected at different points within a segmental ragged red region supporting the clonal origin of these deletions. These findings demonstrate the precise nature of the mutation associated with the ragged red phenotype in aging rat muscle and the ability of laser capture microdissection to facilitate the study of age-related changes in skeletal muscle at the cellular level.

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GENERAL ENERGY MECHANISMS OF ORGANISM AGING AND BASIC FUNCTIONAL MODEL OF LIVING CREATURES. Valery Chuprin, M.S.*; William Mihajlovic, PhD[1]; Association of Engineers and Scientists of New Americans, New York Institute of Technology, New York, NY, U.S.A. [1]

Energy Concept.

We consider that any living organism is a natural formation with a dissipative structure created by continuous energy processes. One of the better ways to describe this process employs an energy concept based on the Second Law of Thermodynamics, which states that all kinds of natural energy is completely expended or falls below levels necessary for the processes to continue functioning. The primary evidence of energy dissipation, and the resulting structure degeneration, in a living organism appears in its external layer, the border between living and non-organic matter, where the environment's influence is felt full-force.

Functional Model.

In accordance with the natural law of similarity of matter organization in nature, the Energy Concept may be applied in a functional model using structure and behavior of single-celled organisms as a prototype for destructive processes in more complex organisms developed from single cells. The correspondence lies in the parallel between the cell's three-part structure (nucleus, cytoplasm, and outer membrane) and the human body (central organs, body fluids along with connective tissues, and external membranes).

This similarity manifests itself not only visually, but also in identical responses of single-cell and multi-cell developed organisms (the human body) to physiological stimuli (through stress response). This similarity is corroborated by membrane theory for living organisms.

Application of Model.

According to the energy nature of living matter and the proposed functional model, decreased integrity in the external membrane of a human body (20% of its weight) is a first cause of structure degeneration, aging, for the whole organism. The aging process then progresses from the outside to the inside, as in single-cell organisms. So, much of our efforts toward the restoration and maintenance of mechanisms responsible for structural development should be focused accordingly, directly on the membrane, the skin. It is well known from medicine that all parts of the human body- bones, blood, muscle, skin, and so on- have some ability to restore themselves. Therefore, actual revival of not only aging tissues of the human body's membrane, but also the entire body, may be hoped for.

Contained herein is an analysis of current aging theories, further explanation of this proposed model, and suggestions for ways to activate the body's own anti-aging mechanisms to insure longevity. That is the path to actual rejuvenation and victory over currently unavoidable aging.

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AGE-ASSOCIATED INCREASE IN MACROPHAGE (MØ) CYCLOOXYGENASE-2 (COX-2) EXPRESSION IS MEDIATED THROUGH INCREASED CERAMIDE LEVELS AND ERK ACTIVITY. K.J. Claycombe*, D. Wu, H. Palmer, A. Beharka, M. Nikolova-Karakashian, A. H. Merrill Jr., E. Paulson & S. Nikbin Meydani. *JM USDA/HNRCA at Tufts University, Boston, MA 02111.*

Cyclooxygenase-2 (COX-2) catalyzes the rate-limiting step in synthesis of prostaglandin E2 (PGE2) which has been shown to contribute to the age-related decline in T cell immune function and pathogenesis of age-associated diseases such as arthritis, cancer, and atherosclerosis. Previously, we have shown that MØ from old mice have a significantly higher level of lipopolysaccharide (LPS)-induced PGE2 production and COX activity than young, due to increased expression of COX-2 protein and COX-2 mRNA levels. Ceramide, a sphingolipid second messenger, has been shown to upregulate transcription of COX-2 gene via activation of members of the mitogen-activated protein kinase (MAPK) family (erk, jnk, and p38 kinases). The molecular mechanism of age-related upregulation of COX-2 expression has not been determined. We hypothesized that age-associated increase in MØ COX-2 expression is due to increases in ceramide levels and its subsequent activation of MAPKs. To test this hypothesis, we stimulated peritoneal MØ from young (4 mo) and old (24 mo) C57BL/6NIA mice with LPS and compared the cellular ceramide and sphingosine levels. Results showed that LPS-induced intracellular ceramide levels were significantly higher in peritoneal MØ of old mice than those of young mice while the sphingosine levels showed no difference between the two age groups. To further determine the role of ceramide in upregulation of COX-2 expression and PGE2 production, peritoneal MØ from young mice were treated with exogenous ceramide. Results showed that addition of ceramide increased PGE2 production in a dose dependent manner (up to 5- fold) by increasing COX activity. Furthermore, ceramide-induced increase in COX activity and PGE2 production was due to increase COX-2 mRNA as well as COX-2 hnRNA levels. To distinguish the effect of ceramide on PGE2 production from that of other down-stream sphingolipid metabolites such as sphingosine, various inhibitors of enzymes involved in sphingolipid metabolism were added. Results showed that inhibition of ceramidase, which catalyzes ceramide conversion to sphingosine, caused no significant increase in PGE2 production. To determine signaling intermediates that are involved in ceramide-induced upregulation of COX-2 gene expression, we treated peritoneal MØ of old and young mice with LPS and measured erk and jnk kinase activities. Results indicated that LPS-induced erk kinase activities were higher in old animals than young, while LPS-induced jnk kinase activities were not different between the two age groups. Furthermore, treatment of peritoneal MØ with PD98059, erk inhibitor, abolished LPS-induced erk activity and PGE2 production. Collectively, these data suggest that the increase in ceramide level with age contribute to the age-associated increase in COX-2 gene expression and PGE2 production. This effect of ceramide might be mediated through upregulation of erk kinase activation.

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HYPOXIA-REOXYGENATION INJURY CONTRIBUTES TO AGING OF MOUSE LIVER. *Alessandra Colantoni,[3] Lisa A. Duffner,[2,4] Ramazan Idilman,[1] Kristy A. Grabowsky,[2] David H. Van Thiel,[1] Pamela L. Witte,[2,3] Elizabeth J. Kovacs[2,3,4,5]. [1]Division of Gastroenterology, [2]Cell Biology, Neurobiology and Anatomy, [3]Immunology and Aging Program, [4]Burn and Shock Trauma Institute, [5]Department of Surgery, Loyola University Chicago, Maywood, IL, 60153.

The reduced hepatic clearance of drugs and the increased frequency of adverse drug reactions associated with aging reflect a decline not only in liver functional mass but also in blood flow. Hypoxia is inevitably

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associated with oxidative stress and hypoxia/reoxygenation injury. Lipid peroxidation, protein oxidation and apoptosis characterize hypoxia/reoxygenation injury in the liver. The aim of the study was to investigate whether oxidative stress contributes to aging of the liver in a mouse model. Liver was obtained from young (3 month old) and aged (18 month old) BALB/c mice. Formalin fixed, paraffin embedded tissue was used for histological evaluation. No significant differences in liver morphology between young and aged mouse livers were seen. Apoptosis was measured by immunohistochemical identification of apoptotic nuclei by fragment end labeling of DNA (FragEL, Oncogene). The total apoptotic cells represented 1.5% and 9.7% of the total cells in young and aged mouse livers, respectively ($p = 0.001$). Among the apoptotic cells in the aged livers, 15% were hepatocytes, 40% sinusoidal endothelial cells, and 45% bile duct cells. Lipid peroxidation, expressed as malonaldehyde (MDA) tissue levels, and protein oxidation, measured by protein carbonyl content (PCC), were significantly higher in aged than young mouse livers. MDA liver tissue levels were 0.27±0.05 uM/mg protein in the young and 0.45±0.08 uM/mg protein in the aged mice ($p=0.02$), while PCC increased with age from 1.1±0.2 uM/mg protein to 3.4±0.1 uM/mg protein ($p=0.01$). These results suggest that aging of the liver is characterized by: 1) increased oxidative injury and 2) increase programmed cell death. Oxidative stress contributes to the initiation of apoptosis. Since the apoptotic cells in the aged livers are almost exclusively sinusoidal endothelial cells and bile duct cells, the cells most sensitive to oxidative stress injury, we can hypothesize that reactive oxygen species induced apoptosis contribute to the aging of the liver. The absence of a significant age-induced hepatocyte injury can explain why liver function is preserved with aging.

54 Abstract Not Presented

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AGE-RELATED DECREASE IN CHAPERONE-MEDIATED AUTOPHAGY. Ana Maria Cuervo* and J. Fred Dice. Department of Physiology. Tufts University School of Medicine. Boston, MA 02111.

Lysosomal degradation of cytosolic proteins by a selective mechanism known as chaperone-mediated autophagy decreases in senescent cells and old animals. The objective of this study is to identify the defect(s) that lead to the decreased lysosomal activity with age. The transport of cytosolic proteins into lysosomesl for their degradation by this pathway is mediated by cytosolic and lysosomal chaperones and requires the binding of the substrate to a receptor protein at the lysosomal membrane. This autophagic pathway shows tissue-dependent activity and it is maximally activated in conditions of stress such as nutrient deprivation or after exposure to some toxin compounds.

We have used lysosomes isolated from livers of 3-, 9- and 22-months old rats to analyze their ability to bind, take up, and degrade previously identified substrate proteins for this pathway. We have also analyzed levels and activity of the main components of this lysosomal pathway in different subcellular fractions. We have found that: 1) binding of substrate proteins to the lysosomal membrane is reduced by age, but rates of substrate degradation once inside the lysosomal membrane remain constant 2) levels of the cytosolic chaperone and its ability to bind substrate proteins or ATP do not change with age 3) levels of the receptor protein at the lysosomal membrane progressively decrease with age but its activity is not impaired 4) the content of intralysosomal chaperone increases in old animals probably as a compensatory mechanism for the reduced receptor levels.

In conclusion a decrease in the levels of a receptor protein in the lysosomal membrane with age is the cause of reduced chaperone-mediated autophagy. Age-related changes in the normal turnover of the receptor and its dynamic distribution between the lysosomal membrane and matrix explain, at least in part the decrease in receptor levels with age. The decrease in protein degradation might contribute to the abnormal accumulation of proteins in the cytosol of senescent cells.

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56 Abstract Not Presented

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CELLULAR AGING AND LYSOSOMAL EXPRESSION DURING CELL DEATH. Vimaris DeJesus[1]*, Claudette Davis[2], Yengsi Chen[1], David Calhoun[1], Zara Zakeri[2], Karen Hubbard[1]. [1]The City College of New York and Graduate School and University Center of CUNY. [2]Queens College and Graduate School and University Center of CUNY.

Replicative senescence is characterized by an irreversible inhibition of cell division. The occurrence of cellular senescence has a direct correlation with donor age, and as such is used extensively as a cell model system for aging. The relevance of apoptosis during cellular aging is still in question and has not been studied in detail. Cell death induces a wide variety of physiological changes in the cell. During organismal aging, the capability to respond to cell death signals is of utmost importance for cell/tissue turnover and may have an impact on age related pathologies. Our studies compare the differences in response to cell death-inducing stimuli in young fibroblasts and old fibroblasts. The level of cell death was quantified using cell counts based on trypan blue exclusion and *in situ* with TUNEL assays. We also measured the levels of four lysosomal enzymes. HS74 cells (fetal bone marrow fibroblast strain) were used as our model cell system. We have chosen to initially study four lysosomal enzymes as markers for induction of lysosomal activity as a consequence of induction of cell death. These enzymes are ß-galactosidase, acid phosphatase, α-galactosidase and ß-glucoronidase. Ceramide, okadaic acid, TNF-α, which are known cell death inducers, were implemented in these studies. We found using trypan blue exclusion assays that young cells to be less sensitive to all cell death whereas old cells are dramatically more sensitive to okadaic acid are highly TUNEL positive. Senescent cells exposed to TNF-α were TUNEL positive but not to the same extent as cells treated with okadaic acid. Ceramide did not induce positive TUNEL reactivity in senescent cells. When examining lysosomal activity, our results indicate that young cells treated with all cell death inducers resulted in an induction of ß-galactosidase activity. In those cells treated with okadaic acid there is higher level of ß-galactosidase activity. In senescent HS74 cells, which already have an induction of ß-galactosidase activity, there was no difference between control senescent cells and those treated with the three inducers chosen. These results suggest that there is deregulation on ß-galactosidase induction by cell death during senescence. Acid phosphatase activity was induced in young cells treated with okadaic acid. However, TNF-α induced both acid phosphatase and α-galactosidase activities in senescent HS74 cells. Our results suggest that apoptosis can be induced during senescent in highly specific manner and that there is concurrently a selective induction of the lysosomal enzymes during cellular aging.

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AGE-RELATED AND BRAIN-REGION SPECIFIC SENSITIVITY TO OXIDATIVE STRESS. THE ROLE OF MEMBRANE MOLECULAR STRUCTURE. N.A. Denisova*; D. Bielinski, and J.A. Joseph. USDA Human Nutrition Research Center on Aging at Tufts University, Boston MA, 02111.

"...old men have grey beards...their faces are wrinkled,
Their eyes purging thick amber and plumtree gum,
and they have a plentiful lack of wit, together with
Most weak hams..." (Shakespeare, Hamlet, 2.2)

Aging is a mystery and many researchers are trying to discover the secret of this process. Several hypotheses are currently existing; however, the free radical hypothesis of aging is the most accepted. The damage caused by oxidants may result in cell death. Although many studies implicated calcium as an important factor in cell vulnerability to oxidative stress and as the main cause of neuronal degeneration, the basic mechanisms responsible for this process remain unclear. Previous research has suggested that the age-related decline in physiological functions may be the result of substantial alterations in membrane molecular structure. In a model recently developed to study the parameters altering vulnerability to oxidative stress, we showed that modification of the membrane molecular structure (e.g., by modifying levels of cholesterol or sphingomyelin, phospholipid asymmetry, etc.) may be crucial for the regulation of calcium activity and cells' sensitivity to oxidative stress (Denisova et al., 1998; 1999). The purpose of the

present study was to elucidate the mechanism of interactions between age-related and brain-region specific modifications in the membrane molecular structure and vulnerability to oxidative stress.

Several brain regions: striatum, hippocampus and frontal cortex, responsible for the different functional activities, were isolated from young (6mo.) and old (22 mo.) Fischer rats. To analyze whether there is an area- or age-specific response to oxidative stress we analyzed Ca^{45} fluxes in different fractions (e.g., homogenate, crude membrane fraction, and synaptosomes) isolated from individual brain regions and exposed to low (5mM) and high (300mM) H_2O_2 at 37°C. The increases in vulnerability were determined by assessing deficits in the ability of these fractions to extrude and/or sequester Ca^{2+} following 30 mM KCl-induced depolarization (*recovery*).

Although our results demonstrate a significant decline in *recovery* with age in all analyzed fractions, synaptosomes showed the highest deficit. In addition, striatum was the most sensitive to oxidative stress compared to other brain regions. The specific activity of sphingomyelin-specific phospholipase C and, consequently, the level of sphingomyelin metabolites, was significantly higher in striatum as well. In addition, our results showed a differential response of brain membranes to low and high levels of oxidative stress as a function of age.

Thus, the present study demonstrates a significant interaction between brain-region specific alteration in the membrane levels of sphingolipids and their metabolites and vulnerability to oxidative stress in aging.

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APOE GENOTYPE, VITAMIN E AND COGNITIVE STATUS IN OLDER WOMEN: A PILOT STUDY. JE Dunn, Ph.D.*[1]; S Weintraub, Ph.D.[2]; K. Kleinman, Ph.D.[1]; [1] New England Research Institutes, Watertown MA20472; [2]Center for Cognitive Neurology & Alzheimer's Disease Center, Northwestern University Medical School, Chicago IL 60611.

The ε4 allele of apolipoprotein E (ε4) is a risk factor for Alzheimer's disease (AD) and vascular dementia, and may increase susceptibility to neurologic effects of physiologic stress (Tardiff et al 1997) and injury (Mayeux et al 1995). Vitamin E is a neuroprotective antioxidant, presently under study as a means to delay progression of AD (Grundman, 2000) and mild cognitive impairment, but studies of Vitamin E as a means to prevent these conditions are fewer, and inconclusive. This cross-sectional study examines combined effects of ε4 (presence of 1 or 2 alleles) and Vitamin E supplement use (VitE) in women age 60 and over, without dementia diagnosis, and enrolled in a pilot study of estrogen, VitE, ε4 and cognitive function (CF). Of 159 women, 128 had complete data. CF was assessed by a neuropsychological test battery. Adjusting for age, education, depression score and race, VitE was associated with higher mean scores only on two of the individual tests: Immediate visual memory (WMS-R Visual Reproduction I (2.4 points; p=.04) and CERAD Constructions (.55 points; p=.02). However, VitE was associated with scoring below age and education-specific cutpoints on 2 or more tests in the battery ("score below cutpoint", or SBC): Unadjusted OR= 0.47; 95%CI 0.22-0.97). ε4 was not associated with lower mean scores on any individual test but was associated with SBC (unadjusted OR= 2.6; 1.2-5.8). Logistic regression was used to examine combined effects of ε4 and VitE on SBC, adjusting for depression and race. Compared to those without ε4 or VitE (Noε4/ NoE), relative odds of SBC were 1.25 (0.40-3.92) for ε4/ NoE, 0.32 (0.12-0.88) for Noε4/VitE, and 2.46 (0.7-8.65) for ε4/VitE. When individual test score data were stratified by ε4, the association of VitE with higher mean scores was seen exclusively in those without ε4 for immediate visual memory (3.1 points; p=.03) and CERAD Constructions (0.78 points; p=.003), adjusting for age, education, depression, and race. Larger studies are needed to determine whether ε4, with or without VitE, is actually associated with below-norm performance on cognitive tests. Still, these preliminary data suggest that vitamin E may be protective, but only in persons without the ε4 allele. This may help to explain inconsistent results of previous studies.

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60 Abstract Not Presented

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DETECTING EARLY GLAUCOMATOUS VISUAL FIELD LOSS WITH ARTIFICIAL NEURAL NETWORKS. Shawn P. Gallagher, M.A.[1]*, David P.M. Northmore, D. Phil[1], Donna Leonardo, D.O.[2]; Program for Neuroscience, University of Delaware, Newark, DE[1], Eye Specialists of Lancaster, Lancaster, PA[2].

Purpose: To develop an artificial neural network (ANN) that can accurately diagnose early glaucomatous loss based on the results of a static threshold test. This study also addresses the ANNs ability to diagnose test results that are correctly and incorrectly categorized as Borderline glaucomatous by the Glaucoma Hemifield Test (GHT).

Methods: Backpropagation ANNs were programmed to discriminate between normal and glaucomatous visual field results from the Humphrey Instruments HFA-II system. ANN architecture was varied in an attempt to optimize diagnostic accuracy as measured by receiver operating characteristics. The ANNs were then trained using normal and mildly affected fields, normal and moderately affected fields, or normal and severely affected fields in order to determine the effect of training data on diagnostic accuracy.

Results: Diagnostic accuracy did not improve with ANN architectural complexity; a simple, single-layer ANN diagnosed normal and glaucomatous fields with a sensitivity of 78 percent and a specificity of 78 percent. The same network diagnosed Borderline normal and glaucomatous fields with a sensitivity of 58 percent and a specificity of 74 percent. These results were no worse than those from larger two-layer ANNs ($P > 0.10$). The quality of the training data affected diagnostic accuracy; the single-layer ANN was best at diagnosing advanced and Borderline glaucoma when it was trained on a wide variety of affected fields, instead of only mildly, moderately, or severely affected fields.

Conclusion: A relatively simple ANN is effective for detecting glaucomatous visual field loss and may improve the diagnostic specificity of the GHT in Borderline cases. The performance of such a network will be optimized if it is trained on a wide range of glaucomatous fields.

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ANALYSIS OF GENE EXPRESSION USING cDNA ARRAYS IN ZEBRAFISH (*DANIO RERIO*) SUBJECT TO DIETARY RESTRICTION. Glenn S. Gerhard[1,3]*, Elizabeth J. Kauffman[1], Xujun Wang[1] and John Quackenbush[2]. Department of Pathology[1], Penn State College of Medicine, Hershey, PA 17033 and The Institute for Genomic Research[2], Rockville, MD 20850. Current address[3]: Department of Pathology, Dartmouth Medical School, White River Junction, VT, 05009.

The zebrafish is an increasingly important model in the study of vertebrate development, yet its utility as a model for aging research has not yet been fully explored. We have been characterizing aging in the zebrafish in an effort to exploit its inherent gerontological advantages that include a maximum life span of greater than 5 years, characterisitics of mammalian-like "gradual" senescence, the ability to modulate the rate of aging with either calories or temperature, as well as expanding genetic and biological resources.

We have conducted a pilot study using cDNA arrays to analyze gene expression in zebrafish tail muscle in fish subject to dietary restriction (DR). All fish were fed a brine shrimp and flake food "ad libitum" (AL) regimen for two months, in which 3 feedings per week are brine shrimp with the remainder of the diet flake food. DR was then gradually implemented by reducing the amount of flake food by 10% per week until the DR fish were consuming 60% of the amount of flake food as the AL fish on a weekly basis. This partial pair-fed strategy resulted in a 15% ($p<0.004$) reduction in body weight after two months of restriction. Despite the reduction in dietary consumption and body weight, metabolic rate was not statistically different between AL and DR fish.

For gene expression analysis, microarrays were prepared with approximately 300 zebrafish expressed sequence tag (EST) clones. Fluorescently labeled total RNA prepared from AL and DR caudal peduncle tail segments were combined and hybridized in small volumes to the arrayed DNA. Hybridization was assayed by scanning with a custom confocal laser scanning microscope. Hybridization signals were detected from approximately 12% of the arrayed clones with duplicate hybridizations using the same RNA resulting in a correlation coefficient of 0.77. Four ESTs exhibited a greater than 50% reduction

in expression with DR, while one was up-regulated by more than 50%. A general down-regulation was detected in genes involved in oxidative metabolism and several that serve as structural components. These pilot data indicate that cDNA arrays are a powerful tool for the analysis of gene expression in the zebrafish, a species that may fill an important biological gap that currently exists between invertebrate and vertebrate models of aging.

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GENE EXPRESSION AND REGULATION DURING CELLULAR SENESCENCE. Shanaz Adi Ghandhi *, Deguang Zhu , Karen Hubbard, City College of New York New York, NY 10031.

Cellular senescence is the terminal growth phase of normal cells in culture and is typified by morphological and cellular changes.

The purpose of this study is to analyse macro-molecular changes at the level of RNA processing and regulation. It is known that post-transcriptional modifications of pre-mRNA significantly controls gene expression in eukaryotic cells. Senescent human fibroblasts have numerous alterations in gene expression one of which may be post-transcriptional regulation. Our model proposes RNA splicing and transport proteins, heterogeneous nuclear ribonucleoproteins A1 and A2 as candidates for these processes in old cells. Both hnRNPs A1 and A2 are functional in the biogenesis of mature RNA. We have previously identified altered activities and proteins levels of hnRNP A1 and A2 in senescent fibroblasts. These alterations could result in the modulations of expression of target mRNAs by mechanisms such as splice site switching, cleavage, polyadenylation and RNA stability. We have found that by overexpressing hnRNP A1 and A2 in transient transfection studies that the expression of the cell cycle regulatory protein p16 INK4A and it's alternatively spliced isoform p14ARF is altered. There is also an apparent change in splicing activity of young and senescent cells in vitro. This was done using a b-globin pre-mRNA transcript as substrate. Further characterisation of the exact nature of splicing extract activity and differences due to age is the next step in this study. I also propose substrates such as Ich-1 (caspase 2) and Bcl-X that both encode positive and negative regulators of cells growth as possible substrates for in vivo studies of differential splicing effects due to age.

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CANDIDATE MICROBIAL SEQUENCES FROM GIANT CELL ARTERITIS (GCA) LESIONS. Lee Goodglick*, Melissa Goldman, Hallie L. Sandusky, Nurit Ziv, Prim Kanchanastit, T. Goodglick, L.K. Gordon, Department of Pathology & Laboratory Medicine and the Jules Stein Eye Institute, UCLA, Los Angeles, CA 90095.

GCA is a granulomatous inflammatory lesion of medium and large arteries which is prevalent in the elderly population. The etiology of GCA is unknown, although the immunologic features potentially suggest the presence of a microorganism. Our group has specifically examined whether a microbial agent is present at GCA lesions and whether such an agent could be responsible for the initiation and/or progression of the disease. We have started to do this by testing whether microbial sequences are specifically present in GCA lesions. To do this, we have utilized genomic representational difference analysis (RDA). Laser dissecting microscopy was used to isolate cells from GCA lesions and adjacent uninvolved temporal artery. Using genomic RDA we isolated 10 gene fragments; 4 of these sequences had high homology with prokaryotic genes and therefore were considered high-priority candidates for further study. PCR analysis revealed that 2 of these clones were present in DNA isolated from archived paraffin-embedded GCA lesions. Moreover, an examination of serum from GCA+ individuals (in contrast to healthy age-matched controls) showed the presence of IgG which recognized in vitro translated proteins from these clones. Currently, we are ascertaining the identity of these microorganisms as well as their potential causative role in GCA.

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***IN VITRO* ACTIVATION OF CASPASES IN NERVE TERMINALS.** K.H. Gylys*, J.A. Fein*, and G.M. Cole§,; *UCLA School of Nursing, Box 956919, L.A., CA; §Sepulveda VAMC GRECC, Sepulveda CA 91343 and UCLA Depts. Med. And Neurol., L.A., CA 90095.

In a project aimed at examination of early events in synapse loss in Alzheimer's disease, we have measured caspase activation and annexin-V fluorescence in a rat brain crude synaptosomal preparation. Both staurosporine and buffer treatment increased caspase-3 enzyme activity approximately four-fold (from 0.17 to 0.59 and 0.69 relative fluorescence units (RFU)/ g protein, respectively) over a 3 hour period. Caspase-1 activity showed a 50% increase over the same time period (from 0.30 to 0.42 and 0.47 RFU/?g protein for staurosporine and buffer treatment, respectively). In order to definitively identify early apoptotic events in the intact neuronal particles in this heterogenous preparation, we have also examined annexin-V fluorescence in viable (calcein AM positive) particles by quantitative measurement of double labeled particles with flow cytometry techniques. Flow cytometry, or fluorescence-activated cell sorting (FACS) allows measurement of size, granularity, and simultaneous analysis for up to four fluorescence parameters; using this methodology we have developed techniques for analysis of the neuronal element of the crude synaptosomal preparation (Gylys et al., *in press*). Annexin fluorescence in this preparation is increased from 48 to 63 RFU by staurosporine and to 64 RFU by treatment with buffer alone. In a freshly prepared homogenate, 25% of the viable particles are 'apoptotic,' or positive for annexin-V. This proportion increases to 40% after 5 min treatment with either staurosporine or buffer (32°C), and to a maximum of 50% after 30 min. After 3 hours, the proportion of double labeled particles is 36% in synaptosomes treated with buffer , and 25% in those treated with staurosporine. These results support the hypothesis that caspase activation in distal neuronal terminals may precede conventional apoptotic changes observed in the cell body, and suggest the utility of this preparation as a model system for the study of early apoptotic events.

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RESTORATION OF DIASTOLIC FUNCTION IN SENESCENT RAT HEARTS BY ADENOVIRALGENE TRANSFER OF SARCOPLASMIC RETICULUM CA2+ ATPASE. Ulrich Schmidt MD, PhD, Roger J. Hajjar MD*. Harvard Medical School, Massachusetts General Hospital, Charlestown, MA 02129.

Senescent hearts are characterized by diastolic dysfunction and a decrease in sarcoplasmic reticulum (SR) Ca2+ ATPase protein (SERCA2a). To test the hypothesis that an increase SERCA2a could improve cardiac function in senescent rats (aged 26 months), we used a catheter-based technique of adenoviral gene transfer to achieve global myocardial transduction of SERCA2a in vivo. Adult rat hearts aged 6 months and senescent rat hearts infected with Ad.bgal were used as controls. Two days after infection, parameters of systolic and diastolic function were measured in open-chested rats. Cardiac SERCA2a protein and ATPase activity were significantly decreased in senescent hearts compared to adult rats (D:-30±4% and -49±5%) and were restored to adult levels after infection with Ad.SERCA2a. At baseline, LVSP and +dP/dt were unaltered in senescent hearts, however, diastolic parameters were adversely affected with an increase in the left ventricular time constant of isovolumic relaxation (t) and diastolic pressure (D:+29±9%, +38±12%) and a decrease in -dP/dt (D:-26±11%). Overexpression of SERCA2a did not significantly affect LVSP but did increase +dP/dt (D:+28±10%) in the senescent heart. Overexpression of SERCA2a restored t and -dP/dt to adult levels. Infection of senescent hearts with Ad.SERCA2a markedly improved rate-dependent contractility and diastolic function in senescent hearts. These studies support the hypothesis that decreased Ca2+-ATPase activity contributes to the functional abnormalities observed in senescent hearts and demonstrates that Ca2+ cycling proteins can be targeted in the senescent heart to improve cardiac function.

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MEASURING THE LEVELS OF 8-HYDROXY- 2-DEOXYGUANOSINE IN NUCLEAR AND MITOCHONDRIAL DNA IN TISSUES FROM F344 RATS AND B6D2F1 MICE WITH AGE AND DIETARY RESTRICTION USING A NEWLY DEVELOPED NaI DNA ISOLATION METHOD. Michelle L. Hamilton*, Zhong Mau Guo and Arlan Richardson; GRECC, South Texas Veterans Health Care System, University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284.

DNA is one of the cellular targets for oxidative damage, and it has been suggested that the accumulation of oxidative damage in DNA with age could be physiologically important to an organism. One of the major oxidative lesions in DNA is 8-hydroxy-2-deoxyguanosine (oxo^{8}dG). Recent studies showing that oxidative damage occurs during the isolation of DNA have brought into question the reliability of the previous studies in which oxo^{8}dG levels were measured in tissues of animals as a function of age. In this study, the levels of oxo^{8}dG in both nuclear (nDNA) and mitochondrial (mtDNA) DNA were measured using the newly developed NaI DNA isolation method. The levels of oxo^{8}dG and 2-deoxyguanosine (2dG) were measured using high performance liquid chromatography coupled to an electrochemical detector system (HPLC-EC) and reported as a ratio of oxo^{8}dG/10^5 2dG. The levels of oxo^{8}dG in nDNA were reduced almost 100-fold with the NaI compared to the classical phenol DNA isolation method. No significant increase in the formation of oxo^{8}dG was found when DNA was re-extracted by the NaI method while the phenol method gave significantly increased the levels of oxo^{8}dG. Therefore, it appears that the levels of oxo^{8}dG in DNA isolated by the NaI method accurately reflects the levels of oxo^{8}dG found DNA in the cells. Using the NaI method, it was found that the oxo^{8}dG levels in mtDNA were 4-fold greater than in nDNA.

The levels of oxo^{8}dG were measured in nDNA and mtDNA isolated by the NaI method from various tissues of 6- 18- and 24-month-old F344 rats and 6- and 25-month-old B6D2F1 mice fed *ad libitum* and calorie restricted diets. There was a significant increase in the level of oxo^{8}dG with age in nDNA in the liver (84%), kidney (362%), brain (346%) and heart (371%) of the F344 rat and the liver (39%), kidney (21%), brain (180%) and heart (240%) of the B6D2F1 mice fed *ad libitum*. Dietary restriction significantly reduced the levels of oxo^{8}dG in nuclear DNA in all tissues of the B6D2F1 mice studied and in heart and brain of the F344 rats. The levels of oxo^{8}dG in mtDNA from liver in F344 rats increased approximately 74% with age, while in the B6D2F1 a 49% increase with age was observed. Dietary restriction was found to significantly reduce levels of oxo^{8}dG in mtDNA from liver in both rodent strains. Thus, using a method that minimizes the generation of DNA oxidation during DNA isolation, I have shown that oxidative damage (oxo^{8}dG) to DNA increases with age in tissues from both the F344 rats and the B6D2F1 mice and that dietary restriction reduces the levels of DNA oxidation in most tissues.

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CALORIE RESTRICTION (CR) LOWERS SERUM LEVELS OF TRIIODOTHYRONINE (T3), A PRINCIPAL METABOLIC REGULATOR. [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 [2]National Institute on Aging, Intramural Research Program, NIH, Baltimore, MD.

Calorie Restriction (CR) is widely recognized as an intervention which extends mean and maximal lifespan as well as prevents or slows the onset of many age-related disease in short-lived species. Evidence to suggest that many of these benefits can also be seen in longer-lived species is increasing. The unanswered question remains, however, of the biological mechanism which underlies these anti-aging effects. Although changes in whole animal metabolic rate have been discounted as one of the possible CR mechanisms, other adaptations in energy metabolism may play a role. There are two principal hormones, thyroxine (T4) and triiodothyronine (T3), which are secreted from the thyroid gland that are involved in maintaining normal metabolism.

This study was designed to assess changes in these thyroid hormones during the initial adaptation to CR in young (n=12, 4 yr at start) and old (n=12, 20 yr at start) male rhesus monkeys (*Macaca mulatta*). Each age group was divided into six control (ad libitum fed) and six experimental (fed ad libitum for one month after which intake was reduced 10% per month to a final restriction level of 30%). At each level of restriction (ad lib, 10%, 20%, 30%) as well as 6 months at 30% and 1 year at 30%, serum samples were obtained and analyzed for total T3, total T4 and free T4.

There were lower levels of total T3, total T4 and free T4 in older animals compared to younger animals. There was a decrease in total T3 in young animals on CR but no change in total T4 or free T4. Results for the old animals tended to be similar but the decrease in total T3 was not significant. The results of this study are in agreement with those conducted in rodents. Further, our findings suggest that the decrease in total T3, the active metabolic regulator, may lower the metabolic rate of animals on CR.

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SEGMENTAL MITOCHONDRIAL ABNORMALITIES CONCURRENT WITH SKELETAL MUSCLE FIBER OXIDATIVE DAMAGE, ATROPHY AND SPLITTING. Allen Herbst*, Pranali Pathare, Jonathan Wanagat and Judd Aiken. Department of Animal Health and Biomedical Sciences, University of Wisconsin-Madison, Madison, WI 53706.

The molecular mechanisms behind age-related skeletal muscle mass and fiber loss have not been elucidated. We hypothesize that age-associated mitochondrial abnormalities are correlated to histological changes in aging rat skeletal muscle. Rectus femoris muscle from 5, 18 and 36-month-old rats was examined by exhaustive serial cryosectioning and subsequent histochemical staining for cytochrome c oxidase, succinate dehydrogenase and 8-hydroxydeoxyguanosine through 1,000 microns. Between 5 and 38 months of age, rectus femoris muscle in the Fisher 344 x Brown Norway F1 hybrid rat demonstrated a 33% decrease in mass concomitant with a 30% decrease in total fiber number at muscle mid-belly. As animals aged, we observed significant increases in the number of mitochondrial abnormalities with 289 ± 8 in the entire 5 month old rectus femoris compared to 1094 ± 126 in 38 month old as calculated from the volume density of these abnormalities. The majority of electron transport system abnormal regions (86.1%) stained positive for oxidatively damaged nucleic acids. Segmental age-associated electron transport abnormalities were frequently concomitant with fiber atrophy and were associated with fiber splitting. These data suggest a causal role for age-associated mitochondrial abnormalities in the development of sarcopenia.

70 Abstract Not Presented

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COENZYME Q AND AGING IN THE NEMATODE *Caenorhabditis elegans*. Tanya Jonassen,*‡ Pamela L. Larsen† and Catherine F. Clarke‡, ‡Department of Chemistry and Biochemistry, University of California, Los Angeles, and †The Andrus Gerontology Center, University of Southern California.

The nematode *Caenorhabditis elegans* has been used as a model for the genetic studies of aging. Mutations in the *clk-1* gene of *C. elegans* result in slowed development and rhythmic behaviors, and an extended life span. The *C. elegans clk-1* gene is a homologue of *COQ7*, a gene in *Saccharomyces cerevisiae* required for the biosynthesis of ubiquinone (coenzyme Q). Coenzyme Q is a redox active lipid that functions in the electron transport chains of mitochondria and plasma membranes, and plays an important role as an antioxidant. The nematode, rat and human homologues of *clk-1/COQ7* all function to restore coenzyme Q biosynthesis in the yeast *coq7* null mutant. Given the functional conservation of yeast rat, human and *C. elegans* CLK-1/Coq7 polypeptides, it is crucial to test whether changes in the level of coenzyme Q may be responsible for the slowed development, behavior and rate of aging in the nematode model. We have examined whether mutations in the *clk-1* gene effect the level of coenzyme Q in *C. elegans*. We have found conditions where the level of coenzyme Q affects developmental timing, behavior and lifespan. The studies to be presented show that the *clk-1* mutations do impact the level of coenzyme Q in the nematode system. This system provides a model that is ideal for evaluating the relationship between coenzyme Q and aging. These studies also indicate that *C. elegans* provides a metazoan model uniquely suited to address questions regarding Q uptake, metabolism and redistribution.

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72 Abstract Not Presented

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AN AGING-RELATED MITOCHONDRIAL DNA BREAK SITE IS A TARGET OF REACTIVE OXYGEN SPECIES-INDUCED DNA DAMAGE. David W. Killilea[1*], Valentina Grishko[2], Pavel Babal[2], Susan P. LeDoux[3], Glenn L. Wilson[3], and Mark N. Gillespie[2]: [1]Department of Molecular and Cellular Biology, University of California, Berkeley, CA; [2]Department of Pharmacology, University of South Alabama, Mobile, Alabama; [3]Department of Cellular and Molecular Biology, University of South Alabama, Mobile, Alabama.

Among the many biochemical and biophysical abnormalities demonstrated by aging cells is the accumulation of mitochondrial genomes with deleted genetic material. Since reactive oxygen species (ROS) are believed to play a role in aging-associated phenomena, we tested the hypothesis that ROS can induce DNA damage at a known fragile site in the mitochondrial DNA (mtDNA). Pulmonary artery smooth muscle cells (PASMC) exposed to exogenous xanthine oxidase (XO) or hypoxia demonstrated increased production of ROS using 2,7-dichlorofluorescein as a fluorescent marker. Ligation-mediated PCR was then used to determine the location of DNA damage at single-nucleotide resolution within a region of the mitochondrial genome containing the break point for the 5 kb common deletion fragment often found in aging mammalian cells. Exposure to hypoxia or XO caused DNA damage throughout the sequenced region. Numerous bases were identified as hot spots, i.e. multiple DNA damage events at the same bases in independent experiments. The majority of these hot spots clustered within the break site for the 5 kb common deletion fragment in mtDNA for both treatments. However, hypoxia, unlike XO, caused no detectable increase in lipid peroxidation or loss of cellular viability. This study indicates that both exogenous and physiologic ROS-generators can induce DNA damage relevant to specific genomic abnormalities associated with cellular aging.

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THREE DIFFERENT PROPARGYLAMINES SHARE PROPERTIES OF INCREASING ANTIOXIDANT ENZYME ACTIVITIES IN DOPAMINERGIC BRAIN REGIONS AS WELL AS EXTRA-BRAIN TISSUES IN THE RAT. C.Minami[1], K. Kitani[*1], W.Maruyama[1], T. Yamamoto[1], C.M.Carrillo[1,2], G.O.Ivy[1,3] ; [1]National Institute for Longevity Sciences, 36-3, Gengo, Morioka-cho, Obu-shi, Aichi 474-8522, [2]National Univ. Rosario, Suipacha, Rosario, Argentina, [3]Life Sciences Division, Univ. Toronto, Ont. Canada.

Several groups including our group[1)] have reported that (-) deprenyl can extend the survival of animals of different species[2,3)]. The underlying mechanism(s), however, remains unelucidated. We have suggested that another property of the drug, that of enhancing superoxide dismutase (SOD) and catalase (CAT) activities in brain dopaminergic regions, may be at least a partial cause[2,3)]. We have recently reported that another propargylamine, rasagiline (N-propargyl-1(R)-aminoindan) also increases these enzyme activities not only in brain dopaminergic regions as was shown for (-)deprenyl but also in extra-brain dopaminergic tissues such as the heart and kidneys[4]. These observations prompted us to further examine whether other propargylamines including (-)deprenyl also share these interesting properties.

Saline solutions of R-2-HMP (R-2heptyl-N-demethyl propargylamine, kindly provided by Dr. A.A.Boulton at Saskatchewan Univ. in Canada) or (-)deprenyl or saline alone were continuously infused s.c. for 3.5 wks in male F-344 rats (7.5-8.5-month-old). Selected brain regions as well as extra-brain tissues were excised and SOD and CAT activities determined.

Both R-2-HMP and (-)deprenyl infusions significantly increased SOD and CAT activities in brain dopaminergic regions as well as in the heart and kidneys. Among the 4 doses of R-2-HMP tested (0.1, 0.25, 0.5, 1.0 mg/kg/day), 0.25 mg/kg/day was most effective for SOD and 0.1 mg/kg/day for CAT. (-)Deprenyl was more effective at a dose of 2.0 mg/kg/day than at a dose of 1.0 mg/kg/day. Relatively speaking, (-)deprenyl tended to be more effective than R-2-HMP in increasing enzyme activities. Interestingly, (-)deprenyl also enhanced activities in the spleen.

These observations may help to explain more directly the mechanisms for the reported effects of (-)deprenyl such as an anti-tumor effect and/or immunomodulatory effect and may possibly be causally related to the life prolonging effect of (-)deprenyl.

1) Kitani et al. Life Sci 52:281-288, 1993. 2) Kitani et al. Ann N Y Acad Sci 786:391-409, 1996. 3) Kitani et al. Ann N Y Acad Sci 854:291-306, 1998. 4) Carrillo et al. Life Sci (in press) 2000.

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CIRCADIAN GENE EXPRESSION IN YOUNG AND OLD HAMSTERS. D.E. Kolker[1*], T.H. Horton[1], J. Dutton[2], E.P. Wallen[2], and F.W. Turek[1]. [1]Northwestern University, Evanston, IL and [2]University of Wisconsin - Parkside, Kenosha, WI.

Golden hamsters show age-related changes in circadian physiology and behavior similar to those seen in humans. Recent discoveries of the genes that comprise the molecular machinery of the circadian pacemaker provide us with the opportunity to investigate both the molecular basis for the aging of the circadian clock, and interventions that may attenuate or reverse some of these changes. Thus, we performed two sets of experiments to investigate the role of one gene, *BMAL1*, in the aging of the circadian timing system. In the first experiment, we examined the 24-hr expression pattern of *BMAL1* mRNA in the suprachiasmatic nuclei (SCN) of young and old hamsters by in situ hybridization. We found that in both groups *BMAL1* mRNA levels oscillated across the day (ANOVA: $p << 0.0001$), but that in old animals the mean level of mRNA was lower (ANOVA: $p << 0.01$). However, there was not a statistically significant difference in the amplitude of *BMAL1* mRNA (ANOVA: age x time interaction effect $p > 0.05$).

We performed a second experiment to determine whether increasing the amplitude of a time-giving cue (*zeitgeber*) could reverse one of the effects of age in golden hamsters. As hamsters age, they fail to show a phase-shifting response to triazolam. Previous research has indicated that increasing the amplitude of an entraining stimulus might have beneficial effects on old rodents' circadian organization. Young and old hamsters received either a control diet or one supplemented with melatonin (600 ng per gram of food). At circadian time (CT) 8 they were given either triazolam (2.5 mg in DMSO) or vehicle in a crossover design. Melatonin feeding restored the phase-shifting effects of triazolam to old hamsters (ANOVA: age x food x drug interaction $p << 0.0001$). However, in situ hybridization revealed that this effect was not correlated with an increase in *BMAL1* mRNA levels in the SCN. These data suggest that this effect of exogenous melatonin is on either a different part of the known circadian feedback loop or on a different pathway altogether.

77 Abstract Not Presented

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IMPAIRED IMMUNITY IN AGED MICE AFTER BURN TRAUMA: RESTORATION BY ANTI-INTERLEUKIN-6 ANTIBODY TREATMENT. *Elizabeth J. Kovacs,[1,2,3,4] Lisa A. Duffner,[1,3] Meredith S. Gregory,[1,3] and Kristy A. Grabowski[1]; [1]Department of Cell Biology, Neurobiology, and Anatomy, [2]Immunology and Aging Program, [3]Burn and Shock Trauma Institute, [4]Department of Surgery, Loyola University Medical Center, Maywood, IL 60153.

An overwhelming amount of evidence suggests that the elderly are less able to survive burn injury than are young healthy individuals. Since burn victims of all ages often succumb to secondary infectious complications rather than the primary injury, and the integrity of the immune system diminishes with age, it is likely that aged individuals are predisposed to a poor outcome by virtue of their weak immune system. Elevated production of macrophage-derived proinflammatory media-

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tors, such as interleukin-6 (IL-6), is thought to trigger post injury immunosuppression in young adults. Since healthy aged individuals produce high circulating levels of this and other mediators, the combination of the age-dependent and burn-induced elevation in the production of these mediators could further suppress immune responses and contribute to the rapid demise of aged burn patients. We observed that aged (18-24 month old) mice had a 70% mortality rate after burn trauma compared to 14% in young (3-4 month old) mice. Young mice were able to mount a robust delayed-type hypersensitivity (DTH) response regardless of injury. In contrast, the response in aged sham-injured mice was diminished ($p<0.05$) when compared to young animals and completely absent 24 hours after thermally injury ($p<0.01$). IL-6 was undetectable in the serum of sham-injured young mice, but elevated significantly at 24 hr after injury ($p<0.01$). In the absence of injury, aged mice had circulating IL-6 levels which were significantly greater than that of young mice ($p<0.05$). After burn trauma, the level of this cytokine in aged mice was increased 4-fold over that of sham injured aged mice ($p<0.01$). To test whether IL-6 played a role in the post trauma immunosuppression, mice were given anti-IL-6 antibody or control IgG 30 min after burn. While anti-IL-6 antibody had no effect on the DTH response in sham-injured young mice, the treatment suppressed the response by over 40% in thermally injured young mice ($p<0.05$). In contrast, treatment of aged mice with anti-IL-6 restored immunity in both sham and burn injured groups ($p<0.05$). The DTH response in sham injured aged mice given anti-IL-6 was restored to 88% that of young mice and in thermally injured aged mice to 56%. These data show that IL-6 is overproduced in aged mice after injury and that blocking this cytokine improves immune status. Since giving anti-IL-6 antibody suppressed immunity in young mice, it suggests that age-specific therapies should be considered for treatment of injured patients.

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USE OF FLUORESCENT PROTEINS TO STUDY DIMINISHED V(D)J RECOMBINATION DURING B CELL DEVELOPMENT IN AGED MICE. Joseph E. Labrie III* and Rachel M. Gerstein, Univ. of Massachusetts Medical Center, Worcester, MA 01655.

The continuous generation of B lymphocytes is required for an adaptive immune system capable of responding to new pathogenic challenges throughout life. The adaptive immune response is diminished in aged mice and humans. A potential contributing factor may be age-related alterations in B cell development. In aged mice, B cell development is attenuated at two phases, the transition from the pro- to pre-B cell stage, and the transition from the immature to mature stage. While in the pro-B cell stage of development the immunoglobulin heavy chain is generated through the process of V(D)J recombination. Signaling through the heavy chain is required for further developmental progression into the pre-B cell stage. The process of V(D)J recombination is dependent upon expression of Recombinase Activating Gene 1 (RAG1) and RAG2. Expression of these two genes in the bone marrow declines with age in mice. We hypothesis that with age, decreased expression of RAG1 and RAG2 leads to reduced V(D)J recombination in pro-B cells and contributes to the diminished production of pre-B cells. We have characterized RAG2 gene expression in young and aged mice using a transgenic mouse in which Green Fluorescent Protein is expressed under the regulation of the RAG2 locus. We have also developed a FACS (Fluorescent Activated Cell Sorter) reporter of recombinase activity, and experimental systems to study V(D)J recombination in pro-B cells. With these techniques we can directly measure, at the single cell level, both RAG2 gene expression and recombinase activity, in conjunction with surface markers that identify stages of B cell development. This work introduces new techniques to study the affect of aging on the immune system and may identify factors responsible for diminished B cell production in aged mice and humans. This may ultimately lead to therapeutics that can restore B cell development and adaptive immunity in aged individuals.

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SIMULTANEOUS- CUE OLFACTORY DISCRIMINATION LEARNING IN MICE. J. Larson,* D. Sieprawska, & S. Tobey. Psychiatric Institute, Department of Psychiatry, College of Medicine, University of Illinois, Chicago, IL 60612.

The rodent olfactory system has a number of advantages as a model system for understanding the neurophysiological causes for sensory and cognitive consequences of aging. The development of behavioral methods to test olfactory learning and memory in mice would allow investigations of the physiological basis of learning and memory deficits that occur with aging and also allow assessments of the role of genetic factors and genetic manipulations that influence aging or serve as models for age-related neurodegenerative diseases. The present study demonstrates that mice can be trained to discriminate simultaneously-presented odors and exhibit long-term memory for these discriminations. Male C57BL/6J mice (three months old) were trained to criterion performance (90% correct responses in a block of 20 trials) on a series of 16 simultaneous-cue, two-odor discriminations. All of the mice tested (n=6) reached criterion performance on each of the discrimination problems; the number of errors made before reaching criterion was highest for the first 1-3 problems and decreased substantially thereafter. The mice were then trained on four additional problems and tested for memory of these discriminations at retention intervals of 1, 2, 4, and 8 weeks. The mice showed good long-term retention of odors even at the longest memory delay. These results demonstrate the feasibility of automated olfactory learning and memory assessment procedures in mice. Future studies will test for age-related changes in olfactory learning and memory performance in normal mice and in mice genetically engineered to model aspects of age-related neurodegenerative processes.

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DECREASED EXPRESSION OF THE GENES INVOLVED IN GLUTATHIONE SYNTHESIS IN OLD RATS. Rui-Ming Liu*, Department of Environmental Health Sciences, University of Alabama at Birmingham School of Public Health, Birmingham, AL 35294-0022.

Although glutathione (GSH) concentration has been reported to diminish with age, the mechanism underlying such age-associated decline in the GSH content is not well understood. In this study, we examined the gene expression of three enzymes involved in de novo GSH synthesis in young, adult, and old Fisher 344 rats. It was found that the activity of ?-glutamylcysteine synthetase (GCS), the rate-limiting enzyme in de novo GSH synthesis, was significantly decreased with increased age in liver, kidney, lung, and red blood cells (RBC). Parallel with the decreased enzyme activity, the protein and mRNA contents of both GCS subunits also changed inversely with age in liver, kidney, and lung, implying a decreased GCS gene expression during aging. The activity of glutathione synthetase (GS), the enzyme catalyzing the second step in de novo GSH synthesis, was decreased only in lung and kidney, accompanied with a decrease in GS mRNA content. On the other hand, there was no significant age-associated decrease in the activity of ?-glutamyl transpeptidase (GGT), a membrane-bounded enzyme which provides cells with substrates for de novo GSH synthesis by breaking down extracellular GSH. Such a reduced GCS gene expression was accompanied by a decline in total GSH content without any change in cysteine concentration. Furthermore, the concentrations of plasma insulin and cortisol were not declined with age. This study showed, for the first time, that the expression of both GCS subunit genes as well as GS gene was decreased in some organs of old rats. Such a decline in GCS and GS gene expression would result in a reduced rate of GSH biosynthesis, which may underlie the decrease in GSH content and the increase in oxidative damage observed in aged tissues. The results from this study also confirm a major role of GCS in maintaining GSH homeostasis.

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THE EFFECT OF NOVEL SELECTIVE BUTYRYLCHOLINESTERASE INHIBITORS ON THE PERFORMANCE OF AGED RATS IN A 14-UNIT T-MAZE. J.M Long*; N.H. Greig; Q.S. Yu; E.L. Spangler; H.W. Holloway; D.K. Ingram. Laboratory of Neurosciences, National Institute on Aging, National Institutes of Health, Baltimore, MD 21224.

Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) are two forms of cholinesterases that are highly homologous and coexist throughout the body. In brain, AChE and BChE primarily localize to neurons and glia respectively. One function of AChE is to terminate the action of the neurotransmitter acetlycholine in the synapse. In the human hippocampus many cholinergic neurons are colocalized for both enzymes and the levels of each are changed in Alzheimer's disease (AD). Thus, inhibition of these enzymes may be of clinical value in AD and normal aging. We have previously shown that phenserine, a long-acting and selective inhibitor of AChE, significantly improves the performance of aged rats in a 14-unit T-maze. The present study examined the ability of three different novel BChE inhibitors to improve performance in the same task.

Aged (23-25 month) male Fischer-344 rats were randomly assigned to 3 different drug treatment groups; cymserine, bisnorcymserine and phenethylcymserine (15, 110 and 5000 fold selective for BChE vs. AChE respectively) or to a vehicle control group. Three doses were administered for each compound: 0.25, 1.0 and 2.5 mg/kg. All rats received i.p. injections (one in a.m. and one in p.m.) over 4 consecutive days. On Day 1, rats were injected and returned to their cages. On Day 2, rats were trained to avoid shock in a straight runway. On Days 3-4, rats received training in a 14-unit T-maze (4 trials in a.m. and 4 trials in p.m.). Preliminary data suggests that phenethylcymserine improves the performance of aged rats in this complex maze task. This suggests that selective modulation of BchE, similar to AChE, may represent a promising strategy for the amelioration of cognitive deficits associated with aging and AD.

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AGE EFFECTS OF POSTURAL CONTROL MECHANISMS: THE UPPER EXTREMITIES. Carl W. Luchies*, Yongseok Won, Jeff Schiffman, and Antonis Stylianou, Mechanical Engineering, The University of Kansas, Lawrence, KS, 66045.

Falling down is common, dangerous, and contributes significantly to mobility impairments in older adults. Even though the arms may play an important role in avoiding a fall, how they are used and how effective they are in avoiding a fall has not been studied. The objective of this study is to determine the effect of disturbance expectation, arm constraints, and age on the postural control response used to restore balance. Our first hypothesis is that unexpected, compared to expected balance disturbances, invoke earlier postural control responses, as measured by anterior deltoid and tibialis anterior muscle latency times and utilize greater upper extremity arm responses, as measured by the shoulder excursion angles. Our second hypothesis is that unconstrained, compared to constrained arm movement, reduces the number of steps used to regain balance and decreases the time required to arrest body movement in the direction of the disturbance. Our third hypothesis is that elderly, compared to young, more frequently use an upper extremity arm response. To test the hypotheses, this study is evaluating postural control responses of 12 healthy female Young Adult (YA) and 12 healthy female Elderly Adult (EA) subjects when each responds to unexpected and expected posterior waist pulls with either their arm movement constrained (crossed in front) or unconstrained. We are quantifying the postural control response in terms of the tibialis anterior and anterior deltoid muscle latency times, weight shift times, step duration times, arm and leg kinematics, and step strategy.

Statistical analyses are being used to determine the effects of age, disturbance expectation, and arm constraints on the postural control response to the disturbance. Preliminary results suggest that the expectation of the postural disturbance affects the balance response in the YA differently than in the EA. For example, an unexpected, compared to an expected postural disturbance, decreased liftoff and landing time in the YA, but increased liftoff and landing time in the EA. The change in the liftoff time corresponded to a change in the weight shift time in both groups.

Results from the proposed study should benefit clinical fall prevention programs. Two important issues are addressed in this study: the methods used to evaluate balance and the role of the upper extremities in balance recovery. We recently demonstrated that balance should be challenged to accurately assess balance in the older adults. The current study may suggest that balance should be assessed using an unexpected balance disturbance and the utilization of the upper extremities during balance recovery may be an important marker of instability. This result would suggest that clinical evaluation of balance should involve an assessment of the upper and lower extremity response to unexpected balance disturbances. This would be a new insight that therapists would utilize in developing clinical evaluation tests used to identify those at risk of falling and the design of effective fall intervention programs.

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VITAMIN C REDUCES H_2O_2, HEAT SHOCK PROTEIN70, AND ACTIVATION OF PROTEIN KINASE FOLLOWING EXPOSURE TO TUMOR NECROSIS FACTOR. Antonio Martin[2], Eduardo Casado, Kuresh Youdim, and James A. Joseph. USDA, Human Nutrition Research Center on Aging at Tufts Univ., Boston, MA 02111.

Inflammation is a highly complex biochemical protective response to cellular insult which is accompanied by increased expression of injury mediators such as Tumor Necrosis Factor (TNFα), and production of reactive oxygen species (ROS), which has been suggested to play an important role in neurodegenerative disease. Increased production of TNFα by astrocytes has been observed in several brain pathologies. Cultured human astrocytes (HA) were employed to investigate the effect of vitamin C (C) and polyphenols upon exposure to TNFα. When incubated in C-enriched medium (0-300 mM) human brain astrocytes increased their C content in a concentration- and time - dependent manner with maximum incorporation after 7 h. TNF-α increased the formation of ROS, decreased cell viability, increased the expression of heat shock protein (Hsp70), and evoked a series of phosphorylation events, such as activation of protein kinases (MAPKs), including JNK/SAPK and ERK. Incubation of HA with TNFa (0-20 ng/mL) in Neurobasal medium caused a dose dependent activation of JNK by 700% as assessed by immunocomplex kinase assay employing GST-N-terminal-c-Jun as a substrate. When HA were pre- supplemented with C (200 mM) for 7 h before removal of extracellular C and addition of TNFα (1 ng/mL), reduced Hsp70 by 63%, and JNK and ERK activation were inhibited by 36, and 46% respectively, after 15 m of incubation with TNFa *vs.* non-C loaded HA. During the incubation with TNF?, C was significantly reduced compared to control. We conclude that enrichment of HA with vitamin C increases cell viability, reduces cell stress as Hsp70 indicates, and prevents the activation of a divergent MAPK cascade, contributing to reduction of the inflammatory response thus preserving cellular/tissue function.

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EXAMINATION OF THE CEREBRAL VASCULATURE IN FRONTOTEMPORAL DEMENTIA. Janet A. Martin[1]*, Jytte O. Larsen[2], and Carl W. Cotman[1]; [1]Institute for Brain Aging & Dementia and Department of Neurobiology & Behavior, University of California, Irvine, USA, [2]Stereological Research Laboratory, Denmark.

Frontotemporal dementia (FTD) is likely the second most common cause of progressive dementia in the population under 70 years of age. It is characterized by personality and behavioral changes, and neuropathologically involves neuron loss, gliosis and microvacuolation. Because relatively little is known about the pathophysiological mechanisms underlying this debilitating syndrome, we have endeavored to explore the possible etiological role of the vasculature in the pathogenesis. Based on previous findings of astrocyte degeneration and reduced cerebral perfusion in FTD, we examined 1) whether the degeneration of astrocytes led to breakdown of the blood-brain barrier and 2) whether this astrodegeneration correlated with vascular density. Immunohistochemical analysis of serum protein extravasation for blood-brain barrier compromise indicated minimal compromise in FTD compared to Controls. Quantitative vascular length density estimations did not vary significantly in FTD versus Controls. These findings raise important questions about the nature of the blood-brain barrier and the relationship between vasculature and perfusion.

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ALZHEIMER'S DISEASE HOTSPOTS IN NEW YORK AND NEW ENGLAND. Rolf Martin*, H R Herbs; Sherman, CT 06784, DrRJMartin@Netscape.net.

The 24-fold increase in US mortality due to Alzheimer's disease, called the "epidemic of the century" by Lerner (J Clin Endocrinol Metab 1999 Jun;84(6):1830-4), was investigated to determine whether and to what extent the increase is real or simply reflects changing cause-of-death assignment, as Hoyert & Rosenberg suggest (Natl Vital Stat Rep 1999 Jun 30;47(20):1-8). New England and northwest US hotspots were identified in Centers for Disease Control data (wonder.cdc.gov). New England and NY were chosen for further investigation because they contain multi-county hotspots and coldspots with an 8- to 10-fold range of risk. County maps were prepared (www.bettermemories.com/alzheimersmaps) and correlations with other diseases were examined. Stepwise increases in Vermont, New Hampshire and Maine counties were simulated to determine whether increases in these states include sudden jumps, perhaps when new health facilities opened and cause-of-death assignments changed. These states were chosen for simulation because they contain many small counties with only several cases per year and are likely to show rate changes if one health center changed patterns of cause-of-death assignments. Compared to New York City counties, New England and northwest hotspots have a proportionate mortality ratio (PMR) of 10.17 (95% CL: 16. 15, 6.95). Hotspots in VT, NH and ME have PMRs of 9.96 (14.54, 7.50), 8.44 (13.17, 5.90) and 9.41 (15.51, 6.13). To check whether these local maximums are simply random hotspots, geographic and epidemiologic trends were analyzed. Increases from NYC along the (i) southwest NY border, (ii) eastern NY border, (iii) CT border through VT, (iv) through central NH, (v) through central = ME, (vi) up the coast through ME, and (vii) east on Long Island, all proved significant at the 0.05 level. The CT Dept of Public Health conducted a separate town-by-town evaluation of a related Connecticut hotspot and found no trivial explanation for the increase. In NY, Alzheimer's very strongly correlated with Parkinson's disease (r =3D 0.87, p < 0.000001), senile psychotic conditions (0.74, 0.0001), cerebrovascular disease (0.57, 0.001), nonhereditary-nondegenerative nervous system disease (0.76, 0.000001), nutritional (protein-calorie) deficiency (0.69, 0.005), brain cancer (0.5= 6, 0.003), all cancer between ages 55 and 85+ (0.54, 0.002), cancer of the l= ung and thorax (0.70, 0.00001) and emphysema (0.56, 0.002). Rates were weakly correlated with multiple myeloma (0.31, 0.12) and cancer of the large intestine (0.22, 0.26) but not with breast cancer, Hodgkin's disease, non-Hodgkin's lymphoma, prostate cancer, all cancer between ages 0 and 24= , myocardial infarction or diabetes mellitus. Simulation results indicated no unusual incidence-rate jumps in either small or large counties. The simulation, geographic patterns, high PMRs and tight correlation with nervous system diseases and certain cancers raise the possibility that the "epidemic of the century" is real and may be due in part to the kind of nerve-specific, carcinogenic pesticides elsewhere linked with Parkinson's disease. Since care for Alzheimer's patients costs approximately $100 billion annually in the US, a comprehensive investigation into these hotspots appears justified.

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AN INTERNET TEST BATTERY FOR MEASUREMENT OF AGE-ASSOCIATED COGNITIVE DECLINE AND OPTIMUM SUPPLEMENT COMBINATIONS. Rolf Martin*, HR Herbs; Sherman, CT 06784 DrRJMartin@Netscape.net

The Connecticut High Performance ("CHIP") Test Battery at www.bettermemories.com was developed two years ago to enable Internet measurement of decision and memory skills that all too frequently decline with age, and to enable investigators and individuals to determine which foods, medicines, vitamins and herbs most effectively delay cognitive decline and restore diminished skills. Calibration results from studies of short-term benefits of vitamin E, ginkgo, ginseng and other supplements suggest that use of the Internet allows more precise measurement of decision speed and short-term memory than is generally reported, simply because individuals obtain results day after day, for weeks or months, rather than from isolated visits to a laboratory test center. Split-half data evaluations performed to date indicate that median reaction time measurement error is 1.3% —significantly less than the apparent 2% annual decline in the 70- to 80-year age group observed by Salthouse (Dev Psychol 30(2):240-259, 1994). The test battery includes six types of subtests: simple and choice reaction time (with 2-choice, 3-choice and 4-choice options), forward and reverse digit-span (number recall), word-list and word-pair recall, simple arithmetic, Sternberg memory scanning, and a measure of planning and response execution from the AGARD STRES battery (Wetherell, Environ Health Perspect 104 Suppl 2:247-73, 1994). Test-retest correlations for forward digit-span were 0.91 for the ginseng study and 0.94 for the ginkgo study. The average length of numbers during forward digit-span measurements increased by 13% during the ginseng study and by more than 25% during the ginkgo study, suggesting that this memory test is sensitive to improvements provided by ginkgo and agents that act by similar mechanisms. Four-choice reaction times improved by 5 to 7% when individuals began consuming vitamin E, ginseng or green tea, and by 8% or more when combinations of vitamin E, ginkgo, ginseng, rosemary, green tea, coenzyme Q10, alpha-lipoic acid and acetyl-l-carnitine were consumed. The test web site includes software for automatic measurement-accuracy calibration of each computer, for graphing cumulative data sets and for evaluating whether observed changes are statistically significant. Cumulative results can be e-mailed directly to any address, for individuals to discuss performance changes with health care providers and for development of databases supporting studies of cognitive decline and improvement. The site appears suitable for monitoring adverse drug reactions and for determining best amounts and combinations of health supplements for individual site visitors. Over thirty years ago, Dr. Alex Comfort developed a plan for long-term evaluation antioxidant supplements based in major part on neuropsychological measurement (Lancet 2(7635):1411-4, 1969). This web site is intended to enable the studies he described so eloquently to be conducted without unnecessary expense. With baby boomers approaching retirement and Alzheimer's disease mortality at historically high levels, there appears to be no better time than now to begin such studies.

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EFFECT OF LONG-TERM CALORIE RESTRICTION ON BODY COMPOSITION AND LEPTIN IN RHESUS MONKEYS. J. A. Mattison[1*], A. M. Handy[2], E. M. Tilmont[1], A. Black[1], D. K. Ingram[1], G. S. Roth[1], and M. A. Lane[1]; [1]National Institute on Aging, Intramural Research Program, NIH, Baltimore, MD [2]R.O.W. Sciences, Inc., Gaithersburg, MD.

Caloric restriction (CR) is the only means known to consistently extend average and maximal lifespan and to slow the rate of aging in rodents and recent evidence suggests similar findings in nonhuman primates. Monkeys experience similar physiological responses to a 30% reduction in food intake as do rodent models, namely lower body weight, plasma glucose, and insulin, factors which may contribute to the reduction in disease onset in CR animal models. As part of a long-term study of the effects of a 30% reduction in food intake in rhesus monkeys (*Macaca mulatta*), the present study examined the changes in body composition and plasma leptin after 10 years on the study.

Body composition measurements have been assessed quarterly by dual-energy x-ray absorptiometry (DXA). Calorie restricted monkeys, both male and female, weighed significantly less, have reduced fat mass and lean body mass (LBM) compared to control-fed counterparts. The distribution of body fat is also altered in restricted animals such that trunk:leg fat ratio and waist:hip measurements are reduced in restricted animals.

Plasma leptin concentration following long-term restriction was lower in restricted monkeys compared to control-fed, however, the difference was not significant. Although leptin has been reported to function in the brain to suppress appetite, plasma levels were not correlated to food intake in either restricted or control-fed monkeys. Leptin in males and females in both diet groups was positively correlated with body weight, fat mass, LBM, trunk:leg fat ratio, and waist:hip measurements ($p<0.0001$). These data are in agreement with previous findings that leptin is a reflection of body composition rather than its predictor.

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CONSERVATION OF NEURON NUMBER AND SIZE IN ENTORHINAL CORTEX LAYERS II, III, AND V/VI OF AGED PRIMATES. David A. Merrill[1]*, Jeffrey A. Roberts[2] and Mark H. Tuszynski1[3]; [1]Department of Neurosciences, University of California-San Diego, La Jolla, CA. 92093-0626, [2]California Regional Primate Research Center, Davis, CA., 95616-8542, and [3]Veterans Affairs Medical Center, San Diego, CA. 92161.

Past dogma asserted that extensive loss of cortical neurons accompanies normal aging. However, recent stereological studies in humans, monkeys and rodents have found little evidence of age-related neuronal loss in several cortical regions, including the neocortex and hippocampus. Yet to date a complete investigation of age-related neuronal loss or size change has not been undertaken in the entorhinal cortex, a retrohippocampal structure essential for learning and memory. The aged rhesus macaque monkey (Macaca mulatta), a species which develops ß-amyloid plaques and exhibits cognitive deficits with age, is considered the best commonly available model of aging in humans. In the present study, we examined changes in total neuron number and size in layers II, III and V/VI of the intermediate division of the entorhinal cortex in aged vs. non-aged rhesus monkeys using unbiased stereological methods. Total neuron number was conserved in aged primates when compared to non-aged adults in entorhinal cortex layer II (aged = 56,500 ± 12,100, non-aged adult = 48,500 ± 10,900; p = 0.37), layer III (aged = 205,600 ± 50,700, non-aged adult = 187,600 ± 60,300; p = 0.66), and layers V/VI (aged = 246,400 ± 76,700, non-aged adult = 236,800 ± 69,600; p = 0.87). In each of the layers examined, neuronal area and volume were also conserved with aging. This lack of morphologically evident neurodegeneration in primate entorhinal cortex with aging further supports the concept that fundamental differences exist between the processes of normal "healthy" aging and pathological age-related neurodegenerative disorders such as Alzheimer's disease.

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ENERGY EXPENDITURE AND LONG TERM DIETARY RESTRICTION IN ADULT RHESUS MONKEYS. Jon Ramsey [1*], Kelly Wink [1], Richard Weindruch [1,2,4], and Joseph Kemnitz [1,3]. [1]Wisconsin Regional Primate Research Center, Departments of [2]Medicine and [3]Physiology, University of Wisconsin, Madison, WI 53715; [4]Veterans Administration Hospital, Geriatric Research, Education and Clinical Center, Madison, WI 53705.

The purpose of this study was to determine the effect of long-term dietary restriction on energy expenditure in adult rhesus monkeys. Energy expenditure was measured by indirect respiration calorimetry in three groups of adult monkeys. Group 1 consisted of males on dietary restriction for 10 years, while groups 2 (female) and 3 (males) were on dietary restriction for 4 years. All animals were fed a purified diet, with the restricted animals receiving approximately 20-30% of the intakes of the control monkeys. In the Group 1 animals, total energy expenditure was lower in the restricted compared to the control animals (2100 vs. 2526 kJ/d, P=0.018). This was entirely the result of a lower resting energy expenditure (P<0.01) in the restricted animals while daytime energy expenditure was not different (P=0.13) between groups. Similarly, differences in total energy expenditure between restricted and control animals in groups 2 and 3 were entirely the result of differences in resting energy expenditure while daytime energy expenditure was not different between groups. In all three groups, when energy expenditure was adjusted for differences in total mass or lean body mass using analysis of covariances, resting and total energy expenditure were not different (P>0.10) between restricted and control animals. Energy expenditure divided by metabolic body size ($kg^{0.75}$) or body surface area ($kg^{0.67}$) was also not different between restricted and control groups. In all three groups, the restricted animals showed a trend towards a higher ratio of daytime to resting energy expenditure (an indicator of physical activity). Respiratory quotient was not different between control and restricted animals in any of the three groups. The results of this study suggest that dietary restriction does not result in a sustained decrease in mass-adjusted energy expenditure. The ratio of daytime to resting energy expenditure, however, did tend to be higher in restricted compared to control animals suggesting that dietary restriction maintains or increases activity associated energy expenditure in adult rhesus monkeys.

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AGE-ASSOCIATED CHANGES IN RECEPTOR CELL FUNCTION CONTRIBUTE TO OLFACTORY DYSFUNCTION. *N. Rawson[1], G. Gomez[1], F. Lischka[1], C. Hahn[2], D. Lowry[3], E. Pribitkin[3], M. Pelchat[1] and B. Cowart[1]. [1]Monell Chemical Senses Center, [2]University of Pennsylvania and [3]Thomas Jefferson University, Philadelphia PA 19104.

Age-associated olfactory dysfunction may include deficits in sensitivity, discrimination and identification, and enhanced adaptation with slower resensitization. Anatomical studies suggest that age-associated olfactory loss may relate to a decrease in the area of the olfactory epithelium. However, animal studies suggest that even a large loss in the area of sensory epithelium does not significantly influence olfactory performance. Age-associated changes in calcium regulatory mechanisms have been observed in the CNS, and altered calcium homeostasis in olfactory receptor neurons (ORNs) could contribute to some of these age-associated olfactory deficits. To determine whether age-associated functional changes may occur at the level of the ORN, we used calcium imaging techniques to evaluate the functional characteristics of over 300 ORNs obtained from surgery or via biopsy from human subjects ranging in age from 12 – 84, who are also given psychophysical tests to evaluate olfactory performance. Odorants used include those known to stimulate adenylate cyclase or phospholipase C and to elicit an increase in intracellular calcium (ICa) in rat olfactory neurons. Human ORNs respond to these odorants with either increases or decreases in ICa, and these changes can be linked to distinct transduction pathways.

To determine whether age-associated functional changes occur at the level of the receptor cell, we have used biophysical techniques to evaluate the functional characteristics of neurons obtained from surgery tissue or via biopsy from over 300 human subjects ranging in age from 12 – 84. Our data demonstrate that functioning ORNs are as plentiful in olfactory tissue biopsies from older subjects (> 65) as in younger subjects. Further, we have found that, rather than being less sensitive, they are more likely to respond to odorant stimuli, but are less selective than ORNs from younger subjects, often responding to more than one odorant. Loss of selectivity at the receptor cell level would be predicted to cause impaired discrimination/identification and increased likelihood of cross-adaptation. We have begun to test subjects whose ORNs have also been studied using a cross—adaptation test with two odors included in the ORN assay. Preliminary data suggest that subjects whose ORNs showed lack of selectivity also cross-adapted to the two odors, while younger subjects whose ORNs were selective did not. These data may explain in part the finding that older subjects often experience greater difficulty in odor discrimination and identification tasks, and might also contribute to reduced perceptual sensitivity in real-world situations. Studies of these neuronal biopsies thus provide a valuable tool to test specific hypotheses about the effects of age or disease on neuronal mechanisms involved in calcium homeostasis and signal transduction. Findings may also provide new insight into age- and disease-associated functional or molecular changes that may be occurring elsewhere in the central nervous system.

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VISUAL ATTENTION TRAINING INTERVENTION FOR OLDER DRIVERS. Richardson, E.D.* Becker, J., Marottoli, R.A., & Inouye, S.K., University of Colorado.

INTRODUCTION: Older drivers have a higher incidence of crashes per mile driven, and these crashes are associated with greater morbidity and mortality. However, cessation of driving represents not only a major loss of independence but is associated with substantial decline in activity levels and increase in depressive symptoms. Thus, interventions that can decrease the risk for crashes and postpone or prevent loss of independence related to driving cessation would contribute to the public health in general, as well as quality of life for many older Americans.

Evidence from our own research suggests that visual attention, involving visual search, selective attention, and switching attention, is a cognitive ability critical to driving. We conducted a pilot study of a visual attention treatment to evaluate its (1) feasibility with and acceptability to older drivers, and (2) preliminary efficacy in improving visual attention among older drivers. This study represents an initial step in developing and refining an intervention to improve driving among at-risk older individuals. The intervention incorporated cognitive rehabilitation principles of remediation (restoring the ability), compensation (circumventing the disability), and generalization (transferring learning to multiple situations), and included computerized cognitive tasks, instructional activities, and home-based behavioral assignments.

METHOD: Study Participants: Twenty active drivers at least 65 years old were recruited from the community. Individuals with self-reported history of dementia, memory loss, Alzheimer's disease or Parkinson's disease were excluded. Individuals were excluded if they obtained a score less than 20/30 on the Mini-Mental State Examination (MMSE; Folstein et al., 1975) or if their near visual acuity was worse than 20/40 bilaterally. Baseline Assessment: Eligible participants were administered a brief battery of visual attention tests, including digit, letter, and symbol cancellation, and they were administered computerized visual reaction time tests. A trained research assistant conducted assessments in the participants' homes, in our lab, or at facilities provided by local senior centers. Intervention: The components of the pilot intervention included *remediation training* using computerized tasks, *compensation training* of adaptive behaviors using didactic sessions, and *generalization training* using home-based assignments. The interventions were administered by a trained research assistant, who saw each participant for eight sessions over a one month period (two sessions per week). Follow-up Assessment and Ratings of Acceptability: The research assistant administered the same battery of cognitive tests that had been administered at baseline. Using 7-point Likert scaling, participants were asked to rate the difficulty, enjoyment, anxiety and frustration experienced using the computer programs, and completing the classroom and home-based exercises. Information on whether participants performed the home-based activities were obtained.

RESULTS: Acceptability Ratings: Each intervention session took 65 minutes to complete. Overall acceptability composite scores were calculated for the intervention. High mean acceptability scores were obtained, with computer training mean rating of 5.0 (SD 0.6) and classroom training mean rating of 6.0 (SD 0.7). Individuals in general needed frequent reminders to engage in homework exercises, with "having forgotten" being the major reason for not completing tasks. Effectiveness of Intervention: Mean scores on non-computerized scanning tasks (number correct) suggested modest gains between baseline and followup assessment. Reaction time on computerized tasks indicated a modest decrease between baseline and followup assessment, suggesting improved (faster) performance.

CONCLUSIONS: Results of this pilot study indicate that it is feasible to conduct a combined computerized and didactic-based intervention to target visual attention among older drivers. We were able to test out the logistics of performing the intervention in various locations (homes, lab, senior centers). We also found that the separate components of the intervention were acceptable to older drivers, and these individuals enjoyed the interventions despite some initial concerns about using a computer. The home-based assignments appeared to be less successful with our participants as most of them required interim reminders to perform them. Initial data from this pilot also suggest modest gains in performance on visual attention tasks after the intervention. Subsequent research will be necessary to determine whether these gains represent a real effect of treatment vs. a practice effect.

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REGULATION OF PHOSPHORYLATION OF HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEINS A1/A2 DURING CELLULAR SENESCENCE. I. Rios*, K. Hubbard; Department of Biology, The city College of NY, New York, NY 10031.

Heterogeneous nuclear ribonucleoproteins (hnRNP's) are several classes of RNA binding proteins, which are important in the biogenesis of mature messenger RNA (mRNA). We have previously found that two hnRNP A1 and A2 have diminished protein levels in senescent fibroblasts. These two RNA binding proteins modulate splice site usage, general-splicing factors, polyadenylation and cleavage efficiency. They have also been implemented in mRNA stability and transport from nucleus. Thus the alteration in activity of the hnRNP A1 and A2 proteins may affect the level of expression of mature mRNA's and contribute to the senescent phenotype. In addition hnRNP A1 and A2 bind to single-stranded telomeric DNA in a sequence specific manner. However in senescent fibroblasts the RNA binding activity of hnRNP A1 and A2 to a model RNA substrate diminishes as opposed to telomeric DNA binding activity which increases.

In this study we have examined the phosphorylation status of hnRNP A1 and A2 and have found that in senescent fibroblasts metabolically labeled with ^{32}P-ortho-phosphate there are increased levels of phosphorylated A1 and A2 proteins. We are currently investigating the mechanism for phosphorylation of the hnRNP A1/A2 proteins and have initiated studies examining phosphorylation by p38 MAP kinase. Alteration of nucleic acid binding activity due to post-translational modification may have a significant impact on the function of these RNA binding proteins with ultimate consequences for gene expression during senescence.

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AMYLOID PRECURSOR PROTEIN GENE REGULATION AT THE LEVEL OF TRANSLATION BY INTERLEUKIN-1 AND IRON THROUGH 5'UNTRANSLATED REGION SEQUENCES, A NEW THERAPEUTIC TARGET FOR ALZHEIMER'S DISEASE. Jack Rogers[1]*, Nigel Greig[5], Catherine Cahill[6], Hunt Potter[3], Lars Nilsson[3], Rudolph Tanzi[1], Ashley Bush[1], Steve Gullans[4], Tony Giordano[2] [1,6]Genetics and Aging Unit and Diabetes Research, Massachusetts General Hospital, Boston, MA; [3] Department of Biochemistry and Molecular Biology, University of South Florida College of Medicine, Tampa FL; Message Pharmaceuticals, Malvern, PA; [4]Renal Division, Department of Medicine, Brigham and Women's Hospital, Boston, MA; [5] Drug Design and Development, N.I.A., Baltimore , MD.

In astrocytes, and some neuronal derived cells, Interleukin-1 is the primary inflammatory cytokine that regulates APP gene expression at the level of message translation, and not at the level of transcription. We found that the APP gene 5' untranslated region (5'UTR) is structurally very similar to sequences in the 5'UTRs of mRNAs coding for the light (L) and heavy (H) subunits of the iron storage protein, ferritin. Like ferritin, the APP gene is regulated by up to 15-fold at the translational level in response to IL-1 without changing the steady-state levels of APP-mRNA. An important APP-mRNA 5'UTR translational enhancer maps between nucleotide (nt) position + 55 and + 144 from the 5' cap site. The APP sequence is homologous to related translational control elements in the 5'UTRs of the light (L) and heavy (H) ferritin genes. Sequences further upstream in the APP gene 5'UTR (between positions +40 nt to +100 nt from 5' cap site of APP-mRNA) confer iron responsive regulation to a CAT reporter gene in astrocytoma cells, consistent with a homology with the Iron Regulatory Element (IRE) in ferritin mRNAs.

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Labeled transcripts for 5'UTR sequences in APP-mRNA interact specifically with a protein related to the Iron Regulatory Protein.

Knowledge of how APP gene regulation is integrated into iron metabolism will provide information about the, as yet, unknown function of APP. However, our data will also provide new therapeutic approaches for developing RNA-directed drugs that can slow down the progression of Alzheimer's disease. This strategy is exemplified by the fact that Phenserine, a known cognitive enhancer and acetyl-choline esterase inhibitor, was shown to also suppress APP translation through APP-mRNA 5'UTR sequences. We are now developing high throughput screens to assay for improved medicinal compounds capable of suppressing APP-mRNA translation via the APP-mRNA 5'UTR stem-loop. Lead compounds will be further characterized for their capacity to suppress secretion of Ab, the pathogenic cleavage product of APP. Our long term goal is to develop RNA-directed antibiotics that are therapeutic to Alzheimer's disease.

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SKELETAL MUSCLE MITOCHONDRIAL MORPHOMETRY IN HEART FAILURE PATIENTS: DOES RESISTANCE EXERCISE ALTER SIZE AND SHAPE? Cheryl J. Santoro*[1], Lynn Bairosr, Sebrina Levesque[2], Arthur Cosmas[1], Thomas Manfredi[2], Danial Forman[3], [1]School of Allied Hlth.,Univ.of Connecticut,Storrs,CT, [2]Exercise Science, Univ.of Rhode Island,Kingston,RI, [3]Division of Cardiology, Boston Med. Ctr, Boston Univ., Boston, MA.

We determined the effect of a strength-training program on the *skeletal muscle mitochondrial morphometry* in chronic heart failure patients. Past research has demonstrated that exercise intolerance in heart failure patients is more related to alterations in skeletal muscle ultrastructure and oxidative metabolism than to cardiac output. Six heart failure patients participated in a 16-week supervised strength training program involving the upper and lower extremities. At baseline and at 16 weeks, percutaneous needle biopsy samples of the vastus lateralis were taken from each subject and prepared for electron microscopic examination of the mitochondria. The pre and post training skeletal muscle was analyzed for mitochondrial size distributions and morphology. Baseline biopsy specimens indicated that heart failure patients have smaller mitochondria than normal adults and many mitochondria showed evidence of swollen membranes and irregular shaped cristae. Resistance training increased the size of the mitochondria toward sizes more typical of age-matched adults free of disease. Mitochondrial structural abnormalities were less frequent following resistance training. We conclude that resistance training in patients with heart failure can improve the skeletal muscle ultrastructural morphology, implying an improvement in muscle oxidative metabolism. This finding explains in part the improved tolerance without compromised cardiac function found in these patients after resistance training.

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PHOSPHORYLATION OF S6K1 AND THE RIBOSOMAL S6 PROTEIN ARE ALTERED DURING REPLICATIVE SENESCENCE OF HUMAN FIBROBLASTS. Christian Sell*, Henry Hoff, Hong Zhang, Lankenau Medical Research Center, Wynnewood, PA.

The p70 ribosomal S6 kinase (S6K1) is rapidly activated following growth factor stimulation of quiescent fibroblasts and inhibition of this enzyme results in a G1 arrest. A6K1 activity regulates the synthesis of *both ribosomal proteins and initiation factors*, leading to an increase in protein synthesis.

We have examined the activation of S6K1 in human fibroblasts following mitogen stimulation. In early passage fibroblasts S6K1 is activated following serum stimulation as evidenced by increased kinase activity and site-specific phosphorylation. In contrast, site-specific phosphorylation of S6K1 at Thr421/Ser424 is diminished in senescent fibroblast cultures. A second phosphorylation site within S6K1 (Ser411) is phosphorylated even in the absence of serum stimulation and the enzyme shows increased phosphorylation as judged by decreased electrophoretic mobility. Inhibitor studies indicate that this phosphorylation is dependent upon the mammalian Target of Rapamycin (mTOR), PI 3-kinase and the MAPK pathway.

In order to understand the consequences of the altered phosphorylation of the S6K1, we examined the phosphorylation state of the ribosomal S6 protein. In early passage fibroblasts the ribosomal S6 protein is phosphorylated upon serum stimulation while the phosphorylation of the ribosomal S6 protein is drastically reduced in senescent fibroblasts. These results suggest that the intracellular regulators of S6K1 are altered during replicative senescence leading to a deregulation of the enzyme and a loss of ribosomal S6 phosphorylation.

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THE TESTIS CONTRIBUTES TO THE ACCUMULATION OF DNA DAMAGE IN THE AGING MAMMALIAN BRAIN. Shuren Shen*, Carol Oteham, Dawn M. Cooley, Lawrence T. Glickman, David J. Waters; From the Departments of Veterinary Clinical Sciences and Veterinary Pathobiology, Purdue University, West Lafayette, IN 47907, USA

The disparity in life expectancy between men and women is a well recognized phenomenon in most countries of the world. The theory of antagonistic pleiotropy, proposed over 40 years ago[1], predicts certain genes that are beneficial early in life exert detrimental effects during the post-reproductive senescent period. We hypothesized that the testis, which is essential early in life for reproductive success, may diminish life expectancy through detrimental effects on cellular processes in essential organs, such as the brain. To test this hypothesis, we studied dogs to determine if castration reduces the extent of DNA damage within the brain. Eight, sexually-intact male beagle dogs 9 to 10.5 years of age (62 to 69 years in human age equivalents[2]) were randomly assigned to receive either no treatment or surgical castration. Six months later, the extent of nuclear DNA damage (single strand breaks, alkali-labile sites, and sites of base excision repair) was measured in brain tissue and peripheral blood lymphocytes using the alkaline comet assay[3]. All experiments used freshly harvested brain tissue (cerebral cortex) or peripheral blood lymphocytes, 20 minutes alkaline unwinding, and 30 minutes of electrophoresis (25V, 300mA) at pH>13. For each dog, extent of DNA damage was scored per 100 cells using a modification of Collins' method[4]. Each cell was scored as follows: Type 0 = no damage; Type 1 & 2 = mild to moderate damage; Type 3 & 4 = extensive DNA damage. Mean (± standard deviation) percentage of extensively damaged cells (Type 3 & 4) were compared between treatment groups using an ANOVA. Thirty-seven ± 18 percent of cells in the cerebral *cortex of castrated dogs had extensively* damaged DNA, compared with 83 ± 8 percent of cells in the control group (p=0.0005). The castrated group also had a two-fold reduction in the percentage of peripheral blood lymphocytes with extensive DNA damage (mean ± SD = 12 ± 2 compared to 24 ± 2 in the control group; p<0.0001). These data provide the first evidence that the testis contributes to the accumulation of DNA damage within the aging mammalian brain. Accumulation of DNA damage has been implicated in age-related functional decline in the human brain[5,6]. It is widely accepted that testicular androgens have undesirable effects late in life by stimulating the development of prostatic disease. However, our findings suggest that the aging brain, a previously unrecognized target, may also be vulnerable to the potentially adverse effects of the testis. The expanding arsenal of endocrine manipulations, initially developed for the treatment of prostate cancer, might have the capacity to decelerate age-related degeneration within essential organs, such as the brain. If so, interventions to antagonize the testis might have broader clinical application than previously expected.

REFERENCES

1. Williams, G.C. *Evolution* 11, 398-411 (1957).
2. Patronek, G.J., Waters, D.J., & Glickman, L.T. *J Gerontology* 52A, B171-178 (1997).
3. Rojas, E., Lopez, M.C. & Valverde, M. *J Chromatogr B Biomed Sci Appl* 722, 225-254 (1999).
4. Collins, A.R., Ai-guo, M. & Duthie, S.J. *Mutation Res* 336, 69-77 (1995).
5. Mecocci, P., MacGarvey, U., Kaufman, A.E., Koontz, D., Schoffner, J.M., Wallace, D.C & Beal, M.F. *Ann Neurol* 34, 609-616 (1993).
6. Randerath, K., Putman, K.L., Osterburg, H.H., Johnson, S.A., Morgan, D.G., & Finch C.E. *Mutation Res* 295, 11-18 (1993).

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EFFECT OF AGE ON OBJECT EXPLORATION, HABITUATION, AND RESPONSE TO SPATIAL AND NON-SPATIAL CHANGE. Barbara Shukitt-Hale*, Gemma Casadesus, Ippolita Cantuti-Castelvetri and James A. Joseph. USDA, Human Nutrition Research Center on Aging at Tufts Univ., Boston, MA 02111.

Aged rats show decrements in performance on cognitive tasks that require the use of spatial learning and memory, that is, the ability to acquire a cognitive representation of location in space and effectively navigate the environment. Previous studies to assess spatial and object recognition, as well as locomotor activity and habituation, which are also impaired in aging, have used separate apparatuses to measure these parameters, and therefore the data are not always directly comparative. This study utilized a behavioral test which has fully comparable spatial and non-spatial components, thereby enabling both of these parameters to be measured under identical conditions as a function of age.

Two age groups of male F344 rats [11 young (6 month) and 9 old (22-24 month)] were repeatedly exposed to a given spatial configuration of objects contained in an open field (8 successive 6-minute trials, separated by 3 minutes) to identify the different strategies, used by the subjects, to detect novel arrangements in a given environment. After habituation to the novel environment had occurred (1 trial with no objects, followed by 3 trials with 4 salient objects placed in a square arrangement and a fifth one in the center of the field), the spatial arrangement of the objects was modified (2 trials). A reaction to the displacement of objects was measured by the renewal of exploratory activity of the subjects, a measure of response to spatial change. Object novelty was tested in the same situation (2 trials) by examining how the subject reacted to the substitution of a familiar object by a new one at the same location, a measure of response to non-spatial alteration.

For trials 2-8, old animals had less frequency and duration of contact with old objects than young animals, particularly on trial 2, and old object contact decreased from trial 2 to 8, in both groups. On trial 5, the first trial with displaced objects, the old animals had less contact and spent less time with the displaced objects when compared to the young rats. For trials 7 and 8, the two trials with a new object in the field, there were no age differences seen with respect to frequency or duration of new object exploration, and both groups had more occurrences of contact and spent more time with the new objects on trial 7 compared to the old objects. Overall, the old animals were inactive for longer periods of time than the young animals, particularly on trials 1 and 2, and they reared less, although there were no differences in grooming among the age groups.

Therefore, older rats have trouble building a spatial representation of the environment and using it to detect changes, although object recognition is not impaired with age. This test is a reliable measure of object exploration, habituation, and response to spatial and non-spatial change, and the differential effect of aging seen in this task cannot be attributed to the testing conditions, but rather a selective impairment in the aged animals.

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DIMINISHED SURVIVAL OF MESENCEPHALIC DOPAMINE NEURONS TO AGED HOSTS OCCURS DURING THE IMMEDIATE POST-GRAFTING INTERVAL. C.E. Sortwell*, M. R. Pitzer, M.D. Camargo and T.J. Collier; Dept. of Neurolog. Sciences, Rush-Presby.-St. Luke's Med. Center, Chicago, IL 60612.

The survival rate of dopamine (DA) neurons in grafts to young adult rats is extremely poor, 5-20%. Our laboratory has recently demonstrated that this survival is even poorer in grafts to the aged striatum. Previous studies, including results from our own laboratory, have demonstrated that massive cell loss following grafting to young adult hosts occurs during the cell suspension procedure and during the first few days following grafting. To examine the time course of cell loss in grafts to aged hosts we compared survival rates of DA neurons in grafts to young and aged hosts at varying intervals after transplantation. Male, Fischer 344 rats with unilateral 6-OHDA lesions were grafted to the striatum with approximately 300,000 embryonic mesencephalic cells and analyzed at 4 and 14 days after grafting using tyrosine hydroxylase (TH) immunohistochemistry and TUNEL labeling. Preliminary results reveal that by 4 days following transplantation a significant reduction in grafted TH+ neurons was evident (decrease of 77%) compared to TH+ neuron survival rates in young adult rats. Analysis of differences in TUNEL+ cells is ongoing and will be presented at the meeting. Given the massive cell loss occurring during the immediate post-grafting interval, we suggest that this time period should be the target interval for interventions leading to substantive increases in grafted DA neuron survival. Investigations into the mechanisms of diminished cell survival in grafts to aged hosts is an ongoing pursuit of our laboratory.

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INHIBITION OF VE-CADHERIN BY GREEN TEA CATECHINS SUPPRESSES ANGIOGENESIS. F. Tang* and M. Meydani, Vascular Biology Program, JM USDA-Human Nutrition Research Center on Aging at Tufts University, Boston, MA, 02111.

Cancer is a leading cause of mortality in middle age and older adults. Tumor growth is dependent on angiogenesis, the formation of new blood vessels from pre-existing ones, to provide oxygen and nutrients to the fast growing cancerous tissue. Inhibition of angiogenesis, thus, may suppress cancer development. Vascular endothelial cadherin (VE-Cad), an adhesive transmembrane protein localized at areas of intercellular contact, is involved in endothelial cell-cell recognition during vascular morphogenesis. Modulation of this molecule may alter angiogenesis. Although green tea catechins have been shown to inhibit tumor growth in animal models, the mechanism of cancer inhibition is not well understood. We found that antibody (20 ?g/mL) directed against VE-cadherin significantly inhibited VEGF (50 ng/mL) – and H_2O_2 (30 ?M)– induced angiogenesis (80% and 100% respectively, $p<0.01$) as assessed by measuring total length of tube formation in 3-dimensional collagen gel (3-D gel) in vitro. We also found that incubation of HMVEC with major green tea catechins dose- dependently (0.5, 2, 5 and 20 ?M) inhibited VEGF– and H_2O_2 – induced angiogenesis. Among the green tea catechins, epigallocatechin gallate (EGCG) and epicatechin gallate (ECG) at a dose of 5 ?M were the most effective catechins to inhibit angiogenesis (74 and 88% respectively, $p<0.05$). Expression of VE-cadherin as measured by laser scanning cytometry was dose-dependently reduced by EGCG (7, 20 and 30 % decreased at doses of 0.5, 2 and 20 ?M respectively, $p<0.05$) in VEGF-stimulated HMVEC. This study demonstrates that VE-cadherin molecule is important in angiogenesis and the inhibitory mechanism of green tea catechins on angiogenesis is in part through the suppression of the VE-cadherin derived pathway.

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RATES OF ENDOGENOUS MITOCHONDRIAL DNA DAMAGE AND HUMAN AGING. MM Vilenchik*, AK Balin; The Sally Balin Medical Center, 110 Chesley Drive, Media, PA 19063

The purpose of this work to ascertain if spontaneously produced mitochondrial DNA (mtDNA) lesions could be responsible for the formation of the unrepairable common 5 kb and 7.4 kb mitochondrial DNA (mtDNA) deletions that accumulate with aging. As we noted 5 years ago, these deletions accumulate exponentially with increasing chronological age in certain tissues of Americans and Japanese. This increase parallels the human death rate of the United States and Japan respectively. However, molecular mechanisms that cause the mtDNA deletions to accumulate remain to be elucidated. Using published data describing the specific endogenous DNA damage rate per human or rodent cell, we estimated the rates of damages per mitochondrial part of the human genome based on the calculation of the total size of the mtDNA (per cell). The damage rates in the mtDNA we estimate are minimal because they assume that all DNA accumulates damage at the same rate whereas it is likely that mtDNA actually undergoes more damage than nuclear DNA. To evaluate the biological relevance of these rates we estimated (a) the minimal amount of mtDNA damage that would occur before the mtDNA replicates and (b) the dose of ionizing radiation that induces an equivalent number of the same or similar DNA lesions in mammalian cells. Every nucleated human cell contains about

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1000 molecules of mtDNA, each with a length of 16.5 kb (200-1000 mitochondria per cell with 2-10 mtDNAs per mitochondrion). Thus, one cell contains in its mitochondrial part of the genome at least 2 x 10^7 bp. We estimate that endogenous DNA lesions such as single strand breaks, apurinic sites (alkali-labile sites) and oxidative DNA lesions of certain kinds are all produced spontaneously at a rate about 1 damage per hour. The mtDNA half-life reported for mammalian tissues is about 10 days during which at least 200 DNA lesions, such as an oxidative DNA damage and single strand breaks, would be produced in the mitochondrial part of the cell's genome. An equivalent level of the same or similar DNA damage is induced by about 50 Gy (1 Gy = 100 rads) of low-LET ionizing radiation such as gamma rays (the equivalence has been estimated using published data of the DNA damage induced per cell per Gy of the low-LET ionizing radiation). Thus, one conclusion that can be derived from this study is that there must be mechanisms of the mtDNA repair that constantly function to remove the endogenously produced damage to the mtDNA. It has been well documented recently, that mtDNA contains enzymes for different (but not all) DNA repair pathways including those that remove certain oxidative DNA lesions. A novel result of our study is that it provides an approximate quantitative prediction of not only the rates of production of the most common mtDNA lesions but also of the rate of their repair necessary to prevent disintegration of the mitochondrial part of the genome before the mtDNA can be replicated.

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COMPARISON OF THE SPONTANEOUS AND UV LIGHT-INDUCED MITOCHONDRIAL DNA ALTERATION RATES AND LEVELS IN THE SUN EXPOSED AND NON EXPOSED HUMAN TISSUES. AK Balin, MM Vilenchik*; The Sally Balin Medical Center, 110 Chesley Drive, Media, PA 19063

We have determined that the rate of spontaneous production of mitochondrial DNA (mtDNA) lesions, such as certain oxidative DNA lesions or abasic sites are at a minimum of 1 damage per mtDNAs in each cell per hour and that this damage could contribute to mechanisms of accumulation of common mtDNA deletions with chronological age. However, these determinations were made by assuming that mtDNA is protected from exogenous sources of DNA damage. The purpose of this work is to compare, using data from the literature, the endogenous DNA damage rate and levels of common mtDNA deletions reported for sun -protected tissue with that measured by others in photoaged skin. The rate of production of pyrimidine dimers, which is the most common type of DNA damage induced by UV light has been determined to be many-fold higher than 1 damaged site per hour. However, the nucleotide excision repair pathway, a major mechanism for the removal of ultraviolet-induced DNA damage, has not been found in the organelle as yet. This paradox immediately raises questions about mechanisms for removing pyrimidine dimers from mtDNA in sunlight exposed human skin and role of the mtDNA damage in photoaging. To answer to some of these questions, we compared reported levels of common mtDNA deletions in human sun -protected tissues and in sun -exposed skin. More than 10 % of all the mtDNA was found to be deleted in photoaged skin. For comparison, the amount of mtDNA deletions measured in human muscles ranged from about 0.005 to 0.14 % depending on chronological age. We conclude: (1) that accumulation of DNA damage in the organelle is a plausible mechanism of accumulation of common mtDNA deletions in both postmitotic and replicating aging human cells; and (2), that common mtDNA deletions could be a molecular marker of both physiological aging and in particular of accelerated aging such as photoaging of the human skin.

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EFFECT OF DIETARY SUPPLEMENTATION WITH FISH OIL IN COMBINATION WITH DIFFERENT LEVELS OF VITAMIN E ON IMMUNE RESPONSE IN HEALTHY ELDERLY HUMAN SUBJECTS. Wu, D.*, Meydani, M., Han, S-N., Leka, L.S. and Meydani, S.N. JM USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111.

Fish oil (FO) or n-3 polyunsaturated fatty acids have been shown to be beneficial in preventing cardiovascular and inflammatory diseases. However, a suppressive effect on cell-mediated immune response has also been reported in a number of studies. We previously showed that this undesirable effect could be reversed by addition of vitamin E (E) in non-human primates. The current study was designed to determine E level that will result in enhancement of T cell-mediated function while reducing pro-inflammatory cytokines in healthy elderly when used in combination with FO. Forty male and female healthy volunteers (>65 y) were supplemented with 5 capsules of Omeaga-500 FO/d (providing 1.5 g EPA, 1 g DHA and 5 IU E/d) in combination with 0, 100, 200 or 400 IU E for 3 mo. FO supplementation significantly increased plasma EPA and DHA, and decreased AA/EPA ratio to the same extent in all groups. FO supplementation without E had no effect on delayed type hypersensitivity skin test (DTH), lymphocyte proliferation (LP), interleukin (IL)-2 and IL-1? production. However, the groups receiving FO + 100 or 200 IU/d E had significantly higher ($p<0.05$) DTH response (maximal diameter) while the group receiving FO + 400 IU/d E tended to have higher ($p<0.06$) DTH when compared with their own baselines. A significant correlation between plasma E level and DTH response was observed. In addition, the group receiving FO + 200 IU/d E also showed an increased lymphocyte proliferation induced by concanavalin A (T cell mitogen) as well as a decreased production of inflammatory cytokine IL-1??($p<0.05$) compared to the baseline. Thus consumption of 2.5 g EPA + DHA with 200 IU/d E enhances T cell-mediated response in elderly while decreasing IL-1??production.

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INCORPORATION OF POLYPEHNOLICS BY ENDOTHELIAL CELLS INCREASES THEIR RESISTANCE TO OXIDATIVE STRESS INSULT. Kuresh A. Youdim*, Antonio Martin, Willy Kalt[1], Jane McDonald[1] and James A. Joseph. Human Nutrition Research Center on Aging at Tufts University, United States Department of Agriculture, 711 Washington Street, Boston, MA02111, USA. [1] Agriculture & Agri-Food Canada, Atlantic Food & Horticulture Research Centre, 32 Main Street, Nova Scotia, B4N 1J5.

BACKGROUND: There is a continued increase in age-associated diseases such as cancer, cardiovascular disease and neurological disorders such as Alzheimer's and Parkinson's disease. An interaction between an oxidative stress (OS) component and vascular cells has been suggested to play a role in these disorders. As such, if OS is involved in either manifesting or propagating these deleterious actions, then these alterations should be prevented or retarded by antioxidants. In this regard, we have reported that rats fed diets supplemented with strawberry, spinach or blueberry extracts exhibited less deficits in neuronal signal transduction (e.g., striatal dopamine release and GTPase activity, and calcium clearance from hippocampal synaptosomes) and cognitive behavior impairment compared to untreated aged animals. Amongst these extracts examined, blueberry appeared most effective.

PURPOSE: Examine biological fate following interactions between dietary polyphenolics from blueberries, namely anthocyanins, and phenolic acids, with vascular endothelial cells and their subsequent protection against OS insult in an attempt to elucidate which family may be eliciting the most protective impact against OS.

METHODS: Bovine aortic endothelial cells (EC) were maintained in plated cultures at 37°C in M-199 and DMEM (1:1v/v) containing 5% FBS, 100U/antiiotics, and 1.25 µg/ml amphotericin B, and grown to confluence in a humidified atmosphere of 95% air, 5% CO_2. The culture medium was replaced every 2 days until the cells attained confluence, then subcultured every 4 days. Pure polyphenolic families were incubated with EC for 2hr (37°C) at 0.5 and 0.05mg/mL. Subsequently cellular and intracellular localization was determined using acidic and organic extractions. Putative antioxidant effects against hydrogen peroxide (H_2O_2); 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH);

and 3-morpholinosydnonimine hydrochloride (SIN-1) at the intracellular and membrane levels were investigated using the dichlorofluorescein (DCF) and cis parinaric acid (cPnA) assays respectively.

RESULTS: Anthocyanins, and phenolic acids, were incorporated into EC with mixed efficacy. In general, localization was predominantly within the cell plasma membrane. Nonetheless, results indicated that increased protection against free radical induced damage by H_2O_2, (100μM), AAPH (200μM) and SIN-1 (10μM) at the intracellular and membrane level was observed following supplementation, and that based on supplementation at equal weights, anthocyanins provided the greatest overall protection. In addition, polyphenolics appeared to ameliorate OS insult from H_2O_2 > SIN-1 > AAPH.

CONCLUSIONS: Endothelial dysfunction has been proposed to play an important role in the initiation and development of vascular disease, with studies having shown that administration of antioxidants improves endothelial function. The findings from the current study provide new information regarding the possible biological fate of dietary polyphenolics, isolated from blueberries, and the differences in their putative antioxidant effects in vitro. It is unclear from these initial findings why protection against H_2O_2 was most efficacious but one can speculate that enhanced protection resulted from more efficient quenching of H_2O_2 and its down steam metabolites as compared with those of the other inducers. Thus, the enhanced protection of blueberry extract at reducing declines in neurological parameters may in part be due to its anthocyanin component.