TWENTY-SIXTH ANNUAL MEETING — American Aging Association
ELEVENTH ANNUAL MEETING — American College of Clinical Gerontology
Friday through Tuesday
October 4-8, 1996
The Sir Francis Drake Hotel
San Francisco, California

MINISYMPOSIUM:

"Nutritional Modification of Age-Related Changes in Neuronal Sensitivity to Oxidative Stress"

Organized and Chaired by: James A. Joseph

1. Pellmar, T.: **Free radical actions on neural transmission**
2. Mason*, R.P., Walter, M.F., Mason, P.E.: **Changes in membrane molecular structure with oxidative stress: X-ray diffraction analysis**
3. Wood*, W.G., Igbavboa, U., Avdulov, N.A., Chochina, S.V., and Schroeder, F. : **Transbilayer cholesterol domains and membrane structure and function**
4. Werrbach-Perez, K, Tong, L, Toliver, T, Taglialetela, G., Maroto, C., Wortwein, G., Yu, J., and Perez-Polo*, R.: **Altered signal transduction in the aged rat**
5. Bickford*, P.C., Chadman, K., Taglialatela, G., Shukitt-Hale, B., Prior, R., Cao, G., and Joseph J.A.: **Antioxidant diet protects against**
6. Vatassery* G.T., Lai, J.C.K., Smith, W.E., and Quach, H.T.: **The effect of peroxynitrite on respiration and vitamin E content of rat brain mitochondria**
7. Joseph*, J. A., Denisova. N., Strain, J., and Erat, S.: **Membrane factors involved in increased vulnerability to oxidative stress in aging**

MINISYMPOSIUM:

"Vision - Relationships Between Oxidation, Nutrition and Aging"

Organized and Chaired by: Allen Taylor

8. Taylor, A: **Associations between nutrition, cataract risk and age-related lens insult**
9. Kleiman N.: **Sequelae of oxidative damage to the genome in the lens**
10. Hyman, L.,: **Nutritional and other risk factors for age-related macular degeneration**
11. Snodderly, M.: **Evidence for protection against age-related macular degeneration by the carotenoids of the macular pigment**
12. Mosoni,* L., Berlett, B.S., Levine, R.L., and Stadman, E.R.: **Susceptibility of oxidatively modified synthetase to proteolysis by the 20S proteasome**
13. Yin*, D., Nilsson, E., Brunk, U.: **A rapid technique to create lipofuscin-loaded cells**
14. Paradiso, C., Rosellini, V., Zambelli, A., Masini, M., Pollera, Z., Bergamini*, E.: **Age-related changes in the regulation of autophagy (A) and proteolysis in rat isolated hepatocytes**

MINISYMPOSIUM:

"Dietary Modulation of the Aging Immune System"

Organized and Chaired by: Simin Nikbin Meydani

15. Murasko, D.M.: **Heterogeneity of immune response in elderly subjects: Influence of diet, genetics, and psychometric status**
16. Chandra, R. K.: **Nutrition, immunity and infection in the elderly**
17. Meydani, S. N.: **Antioxidant nutrients and immune response in aged: Clinical implications and mechanisms of action**
18. Fernandes, G.: **Modulation of programmed cell death and auto-immunity by ω-3 fatty acids**
19. Beharka*, A. A.,Wu, D., Han, S.N., and Meydani, S.N.: **Vitamin E reduces macrophage- mediated supression of T cell immune fuction**
20. Roy,* M. Kiremidjian-Schumacher, L, Wishe, H.I., Cohen, M.W., and Stotzky, G.: **Effect of Selenium on IL-2 production and IL-2 receptor expression in aged mice**
21. Pahlevani*, M, Harris, M.D., and Richardson, A.: **Effect of caloric restriction and aging on transcriptional regulation of IL-2 gene in rat lymphocytes**
22. Greeley*, E.H., Kealy, R.D., Ballam, J.M., Lawler, D.E., and Segre, M.: **The effect of diet restriction on the canine immune system**

MINISYMPOSIUM:

"Effect of Aging on Nutrient Metabolism in the Gastrointestinal Tract"

Organized and Chaired by : Robert M. Russell

23. Russell, R.M.: **Aging and the gastrointestinal tract**
24. Holt, P.R.: **Intestinal cell turnover with aging**
25. Majumdar,* A.P.N., Fligiel, S.E.G. and Tureaud, J.: **Aging: Increased sensitivity of the gastric mucosa to EGF and TGF-α**
26. Saltzman, J. R.: **The significance of gut bacteria in the aging gastrointestinal tract**
27. Armbrecht*, H.J., Boltz, M.A., Hodam, T.L. and Kumar, B.V.: **Age-related changes in intestinal absorption of dietary calcium**
28. Schmucker, D. L.: **Effects of aging on liver structure and function**
29. Seitz, H.K.: **Alcohol metabolism in aging**

Submitted Papers - Poster Session

Competition for The Walter Nicolai Prize and The Glenn Award

30. Adachi*, H. and Ishii, N.: **Mutants altering life span in *caenorhabditis elegans* and oxidative stress**
31. Bains, J.S.: **Differential effect of vitamin E on catalase activity and glutathione content in aging *zaprionus paravittinger (Diptera)***
32. Binkley*, H., Doerr, E., Finestone, A., Pina, I., and Kendrick, Z.V.: **Effect of water exercise on development of muscular strength and endurance in elderly inner city women**
33. Chen*, L.H., Saxon-Kelley, D., and Snyder, D.: **Effects of age and dietary restriction on hepatic endogenous antioxidant defenses in male Lobund-Wistar rats**
34. Choi*, S.J., Cho, K.H., Song, J.S., Kim,Y.B.., Kang, D.R., Yu, J.K., and Han, S.S.: **Effects of crude drug, *ganoderma lucidum*, on the improvement of learning, memory and antioxidant system in SAMR1 and SAMP8**
35. Chung*, S.S., Eimon, P.M., Weindruch, R., and Aiken, J.M.: **Analysis of the breakpoint regions of age-associated mitochondrial DNA deletions from skeletal muscle and brain tissues of C57BL/6 mice**
36. Denisova*, N.A., Arnio, E.B., Cantuti-Castelvetri, I and Joseph, J.A.: **Two pools of sphingomyelin may differentially affect vulnerability to oxidative stress**
37. Fang,* N., Lu, H., Zheng, D., Zhang, S., Liu, J.: **An experimental study on the effect of captopril on the proliferation of vascular wall after balloon angioplasty**
38. Fang*, N., Lu, H., Wang, H., and Wu, Y. : **Cardiovascular effects of cilazapril in elder subjects with hypertension**
39. Gardner*, E.M. and Murasko, D.M.: **Effects of caloric restriction on immune function in Fischer 344x Brown Norway (F1) rats**
40. Greenberg, R.C.: **The assessment of oxidative stress and possible therapeutic intervention**
41. Haywood, L.J.: **Influence of chest pain patterns among African American, Latino and white patients from two socioeconomic strata**

42. Mielke, J.G., Murphy, M.P., Maritz, J., Bengualid, K.M. and Ivy*, G.O.: **Chronic chloroquine administration in aged mice attenuates excitotoxic blood-brain barrier dysfunction**
43. Jahngen-Hodge*, J., Obin, M.S., Nowell, T., Gong, J., Abasi, H., Blumberg, J. B., and Taylor, A.: **Glutathione links oxidation to protein degradation**
44. Jurma., O.P., Hom, D.G., and Andersen*, J.K.: **Antisense reduction of glutathione levels results in dopaminergic cell death via increased calcium influx**
45. Khalsa, D.S.: **New directions in the reversal of memory loss**
46. Khodeir*, M., Conte, E., Morris, J., and Volicer, L. : **Effect of decreased mobility on body composition in patients with Alzheimer's disease**
47. Khokhlov*, A.N. and Shvedova, O.V.: **Once again on the cholesterol theory of aging and death: An attempt at reappraisal**
48. Koltover, V: **Age-changes of ESR signals and free-radical production in mitochondria**
49. Kuzmenko, A.: **Nutritional and dietary antioxidants on lipid free-radical oxidation in age-related disease**
50. Hayek*, M.G., Han, S.N., Taylor, S.F., Bender, B.S., Meydani, M., Smith, D.E., Eghtesada, S., and Meydani, S.N.: **Dietary vitamin E supplementation decreases lung viral titer and H_2O_2 production in old C57bl/6NIA mice infected with influenza**
51. Yanase, S. and Ishii*, N.: **Molecular cloning of oxygen-inducible genes in *caenorhabditis elegans***
52. Ordman, R.: **Urinary excretion of calcium in students and mature women taking supplements**
53. Park*, J.Y., Youngman, L. and Ames, B.N.: **The effect of iron overload on malondialdehyde (MDA) and 0X08deoxyguanosine levels in liver, kidney, lung, heart, brain, and plasma in young and old rats**
54. Pestova, M.I., Gmoshinski, I.V., Terekhov*, A.I., and Mazo, V.K.,: **Milk and different dietary lipid compositions: Its influence on sensitization to formaldehyde and formaldehyde-detoxifying system in experimental animals**
55. Reddy*, K.K., Ramamurthy, R., Papa Rao, A., Kumar Reddy, T.P.: **Are CHD risk factors explained by obesity and body fat distribution? Effects of age, sex and life styles**
56. Reddy*, B.N. and Sharma, S.P.: **Effect of estrogen and progesterone on cholinergic and gabaergic enzymes of aging Wistar rats**
57. Salehil*, M., Hodgkins, M.A., Merry, B.J., and Goyns, M.H.: **Investigation of changes in gene expression during aging of the rat brain by differential display**
58. Shimokawa*, I., Higami, Y., Horiuchi,S., Iwasaki, M., Ikeda,T., and Tomita, M.: **Immunohistochemical survey of Ne-carboxymethyliysine-related glycoxidation products in rat tissues: Effects of aging and dietary restriction**
59. Shukitt-Hale*, B. and Joseph, J.A.: **Deficits in psychomotor and cognitive performance produced by dopamine administration under reduced glutathione conditions**
60. Taylor*, J. and Witkowski, J.: **Are telomeres used to gauge the integrity of genomic DNA?**
61. Wu*, D., Beharka, A.A., Meydani, A., Adolfsson, O., Smith, D.E., Han, S.N., Meydani, M., and Meydani, S.N.: **Long-term dietary supplementation with antioxidants modulates cytokine production by peritoneal macrophages from old mice**

MINISYMPOSIUM:

" New Research Insights Into Sarcopenia and Osteopenia in the Elderly"

Organized and Chaired by: Richard Wood

62. Lukaski, H.: **Muscle function and aging: Nutritional interventions**
63. Cannon, J. G.: **Molecular aspects of muscle damage and repair**
64. Lee*, C.M., Weindruch, R., Roecker, E.B., Aspnes, L.E., and Aiken, J.M.: **Dietary restriction attenuates age-associated histochemical alterations in rat skeletal muscle fibers**
65. Manfredi*, T. G., Fielding, R A., Ding, W., Fiatarone, M.A., Evans, W. J., Cosmas, A., and Cannon, J. G. : **Ultrastructural features of exercise-induced skeletal muscle injury in young and older adults**
66. Reznick, A.Z.: **Muscle degeneration with aging, response to trauma, and capacity for recovery**
67. Wood, R.: **Nutrition, genetic markers and bone loss in the elderly**
68. Marcus, R.: **The role of exercise in preventing bone loss and building bone mass**
69. Horska*, A., Ingram, D.K., Roth, G.S., and Spencer, R.G.S.: **Effect of long-term caloric restriction and exercise on peripheral muscle bioenergetics in rats**
70. Cosmas*, A.C., Edington, D.W., McCafferty, and Manfredi, T.G.: **Exercise and longevity: Further evidence for a threshold age**

Annual Luncheon and Awards:

Excellence in Journalism Award —
Judy Foreman

"Judy Foreman is awarded the 1996 Excellence in Journalism Award in recognition of her outstanding contributions, through journalism, to the general public's knowledge and understanding of biomedical aging research and the potential benefit of such research to all Americans."

Research Award —
Robert A. Floyd, Ph.D.

"Robert A. Floyd, Ph.D., is the 1996 recipient of the Research Award. This award is presented to Dr. Floyd in recognition of his extensive studies on the role of oxidative damage in the pathogenesis of neurodegenerative disorders."

Distinguished Achievement Award —
Charles M. Schulz

"Established to call attention to the fact that chronological age is not a barrier to a full and productive life, the Distinguished Achievement Award of the American Aging Association for 1996 is presented to Charles M. Schulz in recognition of the pleasure his comic strip 'Peanuts' has brought to innumerable people throughout the world during the past 46 years. Everyone knows Snoopy and Charlie Brown!"

Hayflick Lectureship —
Edward J. Masoro, Ph.D.

"Edward J. Masoro, Ph.D., Professor Emeritus of Physiology, University of Texas Health Science Center at San Antonio, was invited to present this lecture in recognition of his outstanding career in biomedical gerontology."

MINISYMPOSIUM:

" Functional Modulation of Food Restriction During Aging"

Organized and Chaired by: Pamela Starke-Reed

Starke-Reed, P.: **Introduction: Modulation of aging by dietary restriction**

71. Roth *,G., Ingram, D.K., Lane, M.A., Kowatch, M.A., Eastman, H.B., and Holbrook, N.J.: **In vitro approaches to examining "anti-aging" effects of caloric restriction**

72. Richardson*, A., McCarter, R, and Nelson, J.F.: **Recent advances in molecular mechanism of food restriction in rodent models**

73. Aspnes*, L.E., Chung, S.S., Havighurst, T, Weindruch, R., and Aiken, J.M.: **Caloric restriction attenuates age-associated mitochondrial DNA deletions in skeletal muscle groups of rats and mice**

74. Lane*, M. A., Ingram, D.K., and Roth, G.S.: **Calorie restriction in Rhesus monkeys: Potential for retardation of aging in long-lived species**

75. Verdery*, R, and Walford, R.: **Caloric restriction in Bbiosphere II: Effects of energy restriction on lipid and lipoprotein levels and HDL subfractions**

MINISYMPOSIUM:

"Dietary Antioxidants and Other Novel Compounds in Aging and Age-Related Diseases"

Organized and Chaired by : Mohsen Meydani

76. Meydani, M.: **Introduction: Antioxidant modulation of aging and age-related diseases**

77. Block, G.: **Dietary antioxidants and degenerative diseases of aging: Population studies**

78. Petot*, G.J., Cole, R., Conaway, C., Debanne, S.M., Esteban-Santillan, C., Koss, E., Lerner, A.J., and Rowland, D.Y.: **Adult lifetime dietary patterns of antioxidant vitamin and carotenoid consumption in a case- control study of risk factors for Alzheimer's disease**

79. Reiter*, R.J.: **Melatonin: A novel antioxidant and its relation to aging**

80. Packer, L.: **The antioxidant network and aging**

81. Kitani,*K., Kanai, K, Miyasaka, K., Ivy, G.O., Carrillo, M.C.: **Is the extension of life span of rats by deprenyl casually related to its effect on antioxidant enzyme activities?**

82. Bagchi,*, M., Bagchi, D, Patterson, E.B., Tang, L, and Stohs, S.J.: **Age- related changes in lipid peroxidation and antioxidant defense in Fischer 344 rats**

Submitted Papers - Oral Presentations

Chairperson: Gwen O. Ivy

84. Ingram*, D.K. and Roth, G.S.: **Survival of rats on standard laboratory diet fed ad libitum or under 30% restriction compared to a high oat fiber diet fed ad libitum**

85. Schwarze*, S.R, Feuers, R.J., and Aiken, J.M.: **Age-associated decline of electron transport system activity and RNA levels in *Drosophila melanogaster***

86. Kim*, M.J., Aiken, J.M., Ershler, W.B., and Weindruch, R.: **Effects of oxidative stress on expression of cytokines by peripheral blood mononuclear cells in Rhesus monkeys**

87. Hirsch*, H.R., Liu, X., and Witten, M.: **Mortality rate crossovers when suicide is genetically programmed**

88. Postaire*, E., Bejot,M., and Reiter, R.J.: **A randomized open trial to assess the influence of an alpha-lactabumin concentrate whey protein supplementation on endogeneous melatonin synthesis**

89. McClaran*, J., Franco, E., and Berglas, R.: **The quantitative impact of one-year increments of age on hospital resource utilization: Bias or service?**

90. Delmonico, M., Manfredi*, T.G., DiPietro, L., Cosmas, A., Dain, J., Riebel, D., and Lamont,L.: **The correlation of physical activity, fibrinogen, and glycated hemoglobin in post-menopausal women**

91. Kendrick*, Z.V., McGettigan, J.C., Paolone, R., and Ruoti, R.: **Effect of water exercise on knee flexor and extensor peak torque, resting metabolic rate, and body composition in elderly adults**

92. Gupta, S.K.: **Protein malnourished Wistar rats: A model to trace probable mechanisms of lipofuscin formation**

93. Jarvik*, L.F., Matsuyama, S.S., Fairbanks, L., LaRue, A., Small, G., and Stoddard, M.: **Microtubules and cognition in Alzheimer's disease**

1

FREE RADICAL ACTIONS ON NEURAL TRANSMISSION. Pellmar, T.C. Radiation Pathophys-iology and Toxicology Department, AFRII, Bethesda, MD 20889-5603.

Free radicals are implicated in the etiology of many nervous system pathologies such as Alzheimer's Disease and may contribute to normal aging. To understand the neurological consequences of reactive oxygen species, we have investigated their actions in *in vitro* preparations such as the hippocampal brain slice, cortical synaptosomes and tissue culture. In these systems, the concentrations of reactive oxygen species can be experimentally controlled and the many different cellular processes subject to free radical attack can be defined. We generate free radicals through a variety of methods. Hydrogen peroxide, which reacts with tissue iron, produces hydroxyl radicals through the Fenton reaction. The auto-oxidation of dihydroxyfumarate generates superoxide. Ionizing radiation interacts with the aqueous environment producing a variety of radical species.

Using intracellular and field potential recording techniques in field CA1 of guinea pig hippocampal slices, free radical exposure was found to decrease synaptic potentials and to disrupt the generation of action potentials. Long-term potentiation, a long-lasting enhancement of synaptic efficacy, is also impaired, probably as a consequence of the oxidation of the NMDA receptor. Presynaptic mechanisms are implicated in the free radical-induced decrease of inhibitory and excitatory postsynaptic potentials. Synaptosomal release studies demonstrate a decrease in calcium-dependent evokedl release of GABA and of glutamate. In contrast, basal release of the neurotransmitters increases in the presence of free radicals. This could be a consequence of reduced reuptake of the neurotransmitters as seen with glutamate in glial cell cultures. Protein oxidation is the likely target for changes in synaptic efficacy while lipid peroxidation is associated with impaired action potential generation following free radical exposure.

Antioxidants and free radical scavengers can provide protection against these neurophysiological actions of ree radicals. Dimethyl sulfoxide, thiourea, Trolox C all limit the damage produced by reactive oxygen species. Protection from superoxide is provided by the combination of enzymes, superoxide dismutase (SOD) and catalase, but not by SOD alone. Repair mechanisms appear to limit the free radical damage in neuronal systems. Depletion of glutahhione does not enhance the actions of free radicals but prevents recovery suggesting this pathway as an intrinsic repai: process.

An increased understanding of the cellular actions associated with reactive oxygen species will provide a logical approach to the development of treatments and preventive measures for free-radical damage to neural tissue.

2

CHANGES IN MEMBRANE MOLECULAR STRUCTURE WITH OXIDATIVE STRESS: X-RAY DIFFRACTION ANALYSIS. Mason*, R.P., Walter, M.F., and Mason, P.E. MCPwHahnemann School of Medicine, Allegheny University of the Health Sciences, Allegheny Campus, Pittsburgh, PA 15212

During the course of aging, cellular membranes are subjected to oxidative stress from both endogenous and exogenous sources of free radicals. The effect of oxidative stress on cell membrane structure and function is currently an area of intensive scientific investigation. The objective of this study was to directly examine changes in the molecular structure of membranes following exposure to free radicals for various time periods. The molecular structure of the membrane samples was then directly examined at high resolution with the use of small-angle x-ray diffraction technology. The results of this study demonstrated that exposure of membrane vesicles to free radicals produce significant and dose-dependent alterations in the basic architecture of the lipid bilayer. One-dimensional electron density profiles generated from the diffraction data showed a marked reduction in the membrane bilayer hydrocarbon core width from 36Å to 30 Å while the overall membrane width, including surface hydration, decreased from 48.7Å to 44.6 Å. In addition, a broad increase in molecular volume was observed 0-11 Å from the center of the membrane bilayer. These pronounced changes in membrane structure were observed in membranes reconstituted from both synthetic and native lipid sources, including cardiac membranes. Biochemical analyses also demon-strated that lipid peroxidation could be inhibited with pharmacological levels of certain lipophilic agents, including the calcium channel blocker amiodipine. These data provide direct evidence for changes in membrane structure following oxidative stress that may be attenuated by certain lipophilic pharmacological agents. These alterations in membrane physical properties following oxidative stress may underlie alterations in membrane function with aging, including signal transduction and ion transport.

3

TRANSBILAYER CHOLESTEROL DOMAINS AND MEMBRANE STRUCTURE AND FUNCTION. Wood*, W.G., Igbavboa, U., Avdulov, N.A., Chochina, S.V., and Schroeder, F. GRECC, VA Medical Center and Dept. of Pharmacology, University of Minnesota, School of Medicine, Minneapolis, MN 55417.

Previous studies examining age differences in membrane fluidity and cholesterol content have reported on the average or total change in membranes, respectively. However, plasma mem-branes consist of two leaflets that are asymmetric in fluidity, lipid distribution and function. Cholesterol is a major lipid of plasma membranes accounting for over 40 mol% of total membrane lipid, and it is asymmetrically distributed in the two leaflets. The exofacial leaflet of synaptic plasma membranes (SPM) contains approximately 12-14% of the total SPM cholesterol, and the exofacial leaflet is more fluid than the cytofacial leaflet. We have shown that changes in cholesterol distribution are associated with animal models of alcoholism and models of cardiovascular disease. Increasing age can be added to conditions that alter leaflet structure. Fluidity and cholesterol distribtuion were determined in the SPM exofacial and cytofacial leaflets of 3-4, 14-15, and 24-25 mo old C57BL/6NNIA male mice using trinitrobenzenesulfonic acid quenching procedures and fluorescence spectroscopy. The exofacial leaflet of SPM from young mice was significantly ($p<0.03$) more fluid compared with the cytofacial leaflet. This large difference in fluidity between the two leaflets was abolished in SPM of the oldest age group. Total SPM cholesterol and the cholesterol-to-phospholipid molar ratio did not differ among the three different age groups of mice. However, the transbilayer distribution of cholesterol was significantly ($p<0.001$) modified with increasing age. There was an approximately twofold increase in exofacial leaflet cholesterol in the oldest group compared with the youngest age group. Age differences in SPM leaflet structure may be an important factor regulating activity of certain membrane proteins. For example, Ca2++Mg2+-ATPase activity in SPM was significantly inhibited by low levels of cholesterol oxidation induced by cholesterol oxidase treatment of the exofacial leaflet. Activity of NA++K+-ATPase activity, on the other hand, was unaffected by cholesterol oxidation even when 40% of SPM cholesterol had been oxidized. SPM of aged mice may be more susceptible to oxidative damage as a result of the changes in the transbilayer distribution of cholesterol with increasing age.

4

ALTERED SIGNAL TRANSDUCTION IN AGED RAT. Werrbach-Perez, K., Tong, L, Toliver, T., Taglialatela, G., Maroto, C., Wortwein, G., Yu, J., and Perez-Polo*, R. University of Texas Medical Branch at Galveston Texas.

Nerve growth factor **(NGF)** is a neurotrophin **(NT)** that determines neuronal commitment to apoptosis and maintenance of cholinergic neurotransmitter functions in part by maintaining glutathione **(GSH)** and acetylcholine **(ACh)** levels respectively. We have shown that age-associated changes in the activities of the transcription factors **AP-1 and NFkB** reflect protective cellular attempts to counteract oxidative stress, and that exogenous NGF treatments synergisticly augment stress responses via activation of NFkB and AP-1. Since intraventricular **(icv)** NGF treatment can reverse age-associated cognitive and cholinergic deficits, and increase antioxidant levels, we have used two *in vivo* paradigms that are compatible with mechanistic studies on the role of signal transduction in: **1)** impairment of oxidative stress responses and **2)** cholinergic function in aged brain. Thus, we will characterize AP-1 and NFkB responses to: **1)** hyperoxia- and GSH depletion-induced oxidative stress and **2)** a partial cholinergic immunolesion that mimics age-associated intrinsic cholinergic deficits. Age-associated deficits result in part from altered activities of NFkB and AP-1 in the basal forebrain and hippocampus of 30 month old (aged) rats, regions affected in aged CNS. In the hippocampus and basal forebrain of aged rats the NT, cholinergic, and transcription factor responses to partial immunolesions of cholinergic neurons are altered. The IgG 192-saporin icv treatment (PIL) paradigm used mimics age-associated cognitive and cholinergic deficits and their reversal by NT treatment. We propose that PIL exacerbates age-associated shifts in NFkB activity levels and causes age-specific transient perturbations in AP-1 activity, while NGF-

mediated responses in PIL-treated aged rats are attenuated. NFkB and AP-1 activity in the hippocampus and basal forebrain of aged rats are more responsive to perturbations in GSH homeostasis as compared to 3 month old counterparts. We hypothesize that prooxidant insults will perturb NFkB function, while stabilization of GSH levels will quench the NFkB stimulation observed in the aged rat CNS.

5

ANTIOXIDANT DIET PROTECTS AGAINST OXIDATIVE STRESS, INDUCED CHANGES IN CNS FUNCTION. Bickford*, P.C., Chadman, K., Taglialatela, G., Shukitt-Hale, B., Prior, R., Cao, G., and Joseph, J.A. Department of Veterans Affairs Medical Center, Denver, CO, Department of Human Biology Chemistry and Genetics, University of Texas, Medical Branch, Galveston, TX; USDA RAS HNRCA at Tufts University, Boston, MA.

Oxidative stress and reactive oxygen species have been proposed as a major contributor to the aging process and to many neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. A decline in the capacity of normal antioxidant defense mechanism has been postulated as a causative factor in aging related declines in the function of biological systems. However, a causative link between increased oxidative stress and aging has not been demonstrated. To further investigate this link we exposed 8 month old F344 rats to normobaric 100% 0_2 for 48 hours and then examined 3 parameters of CNS function that show age-related declines: cerebellar b-adrenergic receptor physiology, striatal dopamine (DA) release and NGF levels. All three parameters of CNS function show declines after exposure to this oxidative stress. The protective effect of diets high in antioxidants was studied to determine if these diets could protect against the toxic effects of the high oxygen environment. A diet supplemented with strawberry extract (9.4g/kg) or Vitamin E (500 IU/kg) was fed to 6 month old F344 rats for 8 weeks prior to oxygen exposures. Rats on the strawberry diet demonstrated significant protection from the oxygen induced deficit in b-adrenergic receptor function in the cerebellum. In oxygen exposed rats only 20% of cells show isoproterenol induced augmentation of GABA responses. However, in the rats fed the strawberry diet 78% of cells show this normal response to isoproterenol, a number equivalent to non-oxygen exposed rats. The effects of the vitamin E diet will also be discussed.

6

THE EFFECT OF PEROXYNITRITE ON RESPIRATION AND VITAMIN E CONTENT OF RAT BRAIN MITOCHONDRIA. Vatassery*, G.T., Lai, J.C.K, Smith, W.E., and Quach, H.T. Veterans Administration Medical Center and the University of Minnesota, Minneapolis, MN, 55417; and Idaho State University, Pocatello, ID 83209.

Aerobic cells form superoxide as a byproduct of normal respiration or from specific enzymatic and nonenzymatic reactions. Similarly, a variety of cells including neurons, endothelial cells, neutrophils and macrophages produce nitric oxide which has been shown to be a neurotransmitter in the nervous system. Nitric oxide and superoxide can combine to form peroxynitrite, a powerful oxidizing agent with a half life of only one or two seconds. Therefore, the biochemical reactions of peroxynitrite are important in understanding oxidative stress in the brain. Here we have studied the effect of incubation of mitochondria with peroxynitrite upon respiration and concentrations of the membrane antioxidant vitamin E.

Samples of brain hemispheres were obtained from 4 month old Fischer 344 rats. Mitochondria were isolated by a centrifugation procedure involving Ficoll gradients. The peroxynitrite was prepared as follows: a mixture of sodium nitrite and hydrogen peroxide was acidified with hydrochloric acid and almost immediately sodium hydroxide was added. The excess hydrogen peroxide was destroyed by passing the solution through manganese dioxide. The mitochondria were suspended in a medium containing 300 mM sucrose, 5 mM HEPES, 5 mM KH2P04 and 1 mM EGTA. The oxygen consumption was determined using a Clark type electrode in a micro cell (0.6 ml). A mixture of glutamate (10 mM) and malate (2.5 mM) was used as substrate and ADP concentration used was 386 µM. After determinations of the respiratory control ratios the mitochondrial samples were removed and analyzed for vitamin E and its quinone by HPLC with electrochemical detection. The respiratory control ratios and the concentrations of tocopherol compounds were also determined after treatment of the mitochondria with different concentrations of peroxynitrite. The respiratory control ratios declined as a function of the concentration of peroxynitrite added to the mitochondrial suspension. Simultaneously, alpha tocopherol in the mitochondria was consumed and an equivalent amount of alphatocopherolquinone (the oxidation product) was produced. When the concentration of peroxynitrite was increased to 750 µM the mitochondrial respiration was uncoupled. In contrast, literature reports indicate that such uncoupling of respiration and phosphorylation did not occur with heart mitochondria. Thus, brain mitochondria tend to be more sensitive to oxidative stress induced by peroxynitrite and antioxidants such as vitamin E may play an important role in protecting brain mitochondria from oxidative stress induced by peroxynitrite. These results may have implications for the pathogenesis and treatment of neurodegenerative diseases where oxidative stress is postulated to play a role.

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MEMBRANE FACTORS INVOLVED IN INCREASED VULNERABILITY TO OXIDATIVE STRESS IN AGING. Joseph*, J.A., Denisova, N., Strain, J., Erat, S. USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA.

Very little is known about the mechanisms involved in declines in memory and motor functions that occur during normal aging. One contributing factor may be decreases in neurotransmitter receptor sensitivity in dopaminergic and cholinergic receptor systems that occur in neuronal signal transduction (ST) and cell death that may be the result of age-related decreased calcium regulation induced by increased vulnerability to oxidative stress. Subsequent research has suggested that this increased vulnerability may be the result of a) alterations in membrane structure b) receptor type and subtype. Recent work has indicated that the vulnerability to OS (generated by *in vitro* exposure of striatal slices from young (6 mos) and old (24 mos) animals to H_20_2 increases significantly as a function of age that is prevented by prior exposure of the tissue to the vitamin E analog, Trolox prevented the H_20_2 induced deficits in oxotremorine-enhancement of K^+-evoked dopamine release (K^+-ERDA). Interestingly, pre-exposure of the slices from young and old animals in cholesterol (CHL) also prevented these NO- or H_20_2-induced alterations in K^+-ERDA, suggesting that membrane constituency may affect OS sensitivity. Additional examinations indicated that the incorporation of the membrane lipid, sphingomyelin into PC-12 cells to levels seen in aging altered the ability of PC-12 cells to regulate calcium (i.e., decreased the ability of cells to extrude or sequester calcium) and increased the sensitivity to H_20_2 exposure, as assessed via calcium clearance. This latter paradigm was utilized, since it has been shown that long-lasting increases in cytosolic Ca^{2+} may contribute to cell death by generating enzymes (e.g., NO synthase) which further increase the free radical production in the cell and lead to cell death.

Subsequent examinations have indicated that both receptors and receptor subtypes show differential sensitivity to oxidative stress. Thus, low K_M GTPase (an indicator of receptor-G protein coupling/uncoupling) analyses were carried out in striatal slices from young and old animals that had been exposed to dopamine (to induce oxidative stress), membranes prepared and stimulated with carbachol, quinelorane, SKF38393, or 8OH-DPAT-HBr to stimulate muscarinic, D_2,D_1, or 5HT1 receptors respectively. Age differences in striatal DA uptake were also addressed using [^{3}H] incorporation. Selective, age-related decreases in carbachol-stimulated low K_M GTPase activity were induced (33%) only in the striatal slices from the young animals. Similar results were observed with 5HT1 stimulation. However, with the D_1 and D_2 receptor stimulation the results were reversed. There were greater deficits following DA incubation in low K_M GTPase activity stimulated by the D_1 and D_2 agonists in the striatal membranes from the old animals (D_1 35% vs 55%, D_2 30% vs 66% young-old), even though DA uptake was reduced as a function of age. These findings indicate that DA receptors, especially the D_2 subtype, which show considerable loss as a function of age, are extremely sensitive in the old animals to OS induced by dopamine. In this regard, it has been suggested for a number of years that OS induced by DA auto-oxidation may be involved in the nitrostriatal neurodegeneration seen in Parkinson's disease and aging.

Additional experiments examining the vulnerability of COS-7 cells transfected with one of 5 muscarinic receptor subtypes to H_20_2 and examining calcium regulation indicated that the degree of alteration of calcium regulation was highly dependent upon the muscarinic subtype with cells transfected with the M1 receptors showing significant increases in vulnerability. Taken together, these rather diverse findings indicate that alterations in membrane microenvironments as well as receptors can determine sensitivity to oxidative stress during aging.

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ASSOCIATIONS BETWEEN NUTRITION, CATARACT RISK AND AGE-RELATED LENS INSULT. Taylor, A. Director of the Laboratory for Nutrition and Vision Research, Jean Mayer, USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111.

There are a dozen studies which correlate photo-oxidative stress, smoking, risk for cataract, and intake of various foods and/or nutrients. Most of these have associated a single measure of intake with cataract risk. However, a recent study correlated long-term nutrient intake with cataract risk. The overall impression created by the data is that oxidative stress is associated with a greater risk for cataract. Elevated intake of antioxidant nutrients in foods or supplements can diminish some of these risks. Among the nutrients which have been shown to have beneficial effects are ascorbate, tocopherol, and some carotenoids. These data have been reviewed in the Am. J. Clin. Nutr. (1995) 62(suppl):1439-475 and will be reconsidered. For ascorbate, I will also describe intake-tissue correlations.

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SEQUELAE OF OXIDATIVE DAMAGE TO THE GENOME IN THE LENS. Kleiman, N.J. Department of Ophthalmology, Columbia University, New York, NY 10032.

The eye lens is an avascular organ consisting of a single layer of epithelial cells on its anterior surface which divide and differentiate into anuclear, amitochondrial fiber cells, making up the bulk of the lens, throughout life. The primary pathology of the eye lens is opacification or cataract. The majority of human cataract is termed "maturity onset" or age related, because of its typical development between 50 and 80 years of age. While the initiating event(s) in cataractogenesis is unknown, it is likely that a combination of environment, aging and genetics contributes to cataract development. Much evidence suggests that oxidative mechanisms are involved at a very early stage. Oxidative stress induced damage to lens proteins and membranes has been extensively reported. Only relatively recently, however, has the exquisite sensitivity of the genome to oxidative stress induced damage been considered as an important factor in cataract development. A variety of approaches and methodologies, including alkaline elution, single cell gel assay, nick translation, thymidine incorporation, ELISA, sucrose gradient sedimentation, confocal microscopy and RNase protection assay, indicate that under very mild oxidizing conditions, significant levels of DNA single strand breaks, various types of base damage and significant effects on gene expression and RNA and DNA synthesis can be detected in lens organ and epithelial cell culture. Additional experiments suggest lens cells previously exposed to H_2O_2 appear less sensitive to subsequent DNA damaging insult and that expression of certain DNA repair and peroxide scavenging enzymes may be increased. *In vitro* studies indicate that lens epithelial cells are well primed to deal with oxidative insult by both detoxifying insulting agents and rapidly repairing DNA damage by endogenous repair enzymes. Examination of lens epithelial cells from human patients with cataract, however, suggests an increased level of various types of DNA damage as well as increased levels of micronuclei and apoptotic cells as compared to aged matched non-cataractous controls. While it is unclear whether such findings indicate a causal or derivative relationship with cataract development, it is hoped that by understanding the ways in which lens epithelial cells respond to DNA damaging stresses future directions for research in therapeutic treatment for lens opacities will be obtained.

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NUTRITIONAL AND OTHER RISK FACTORS FOR AGE-RELATED MACULAR DEGENERATION. Hyman*, L. University at Stony Brook, Stony Brook, NY, 11794-8036

Age-related macular degeneration (AMD) is the leading cause of severe irreversible visual loss in adults in the United States and is, by far, the most frequent cause of new cases of blindness in persons 65 years and older. Although epidemiologic studies have evaluated a number of nutritional and other risk factors for the two major types of AMD, neovascular and non-neovascular, only increasing age has been consistently identified across all studies. A possible role for antioxidant nutrients, e.g., vitamins C, E, carotenoids, in AMD has been suggested by several recent studies. Results from N-HANES, a cross-sectional study, suggested a protective effect for AMD with frequent consumption of foods rich in vitamin A, considered an indicator of carotenoid intake. Another cross-sectional study that evaluated plasma levels of vitamins and supplement use, found a protective effect for AMD with high plasma levels of vitamin E and an antioxidant index. One case-control study identified a protective association between neovascular AMD and a number of individual carotenoids as well as total carotenoids using both dietary and biochemical methods. In addition, this study found an association between neovascular AMD and a biochemical antioxidant index at a level of decreased risk of about two-thirds. A protective association of comparable magnitude was also identified between neovascular AMD and a similar biochemical antioxidant index in another case-control study. Although the findings across the studies varied according to the type of antioxidant identified and antioxidant index used, all of these studies suggest a protective association between AMD and some antioxidant. These observations are consistent with the hypothesis, supported by laboratory data, that an antioxidant mechanism may play a role in AMD.

Other risk factors that have been positively associated with AMD in one or more studies include hypertension, other cardiovascular diseases, total serum cholesterol, high intake of saturated fat and cholesterol, cigarette smoking, light or medium eye color, hyperopia, lens opacities and a family history of AMD. Results vary by type of AMD suggesting that certain risk factors may be specific for neovascular AMD; and, factors associated with both AMD types are more strongly associated with the neovascular form.

In sum, findings from epidemiologic studies suggest that: 1) there is a multifactorial etiology for AMD that includes other systemic conditions, genetic and environmental factors; and, 2) certain of the suggested risk factors, e.g., antioxidant nutrients, cigarette smoking, are potentially modifiable, thus raising the possibility of mechanisms to decrease the risk of AMD. Further study is necessary to confirm these observations and to evaluate the role of these potentially modifiable factors, particularly antioxidants, in the prevention and progression of AMD. Longitudinal and clinical trial data such as those to be provided by the National Eye Institute sponsored Age-related Eye Disease Study, a longitudinal study and clinical trial of vitamin supplements for AMD and lens opacities should contribute towards clarifying the relationships of AMD with these nutritional and other risk factors.

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EVIDENCE FOR PROTECTION AGAINST AGE-RELATED MACULAR DEGENERATION BY THE CAROTENOIDS OF THE MACULAR PIGMENT. Snodderly, D.M. Schepens Eye Research Institute and Harvard Medical School, Boston, MA 02114.

The central region of primate retinas accumulates the carotenoid pigments lutein and zeaxanthin from the diet to form a yellow spot called the macula lutea. The site of most acute vision, the fovea, is at the center of the macula lutea; when age-related degeneration of the macula invades the fovea, severe visual loss occurs. We have applied a psycho-physical (perceptual) technique to measure the density of the yellow macular pigment *in vivo*. With this method we have studied how risk factors for age-related macular degeneration (AMD) are related to the density (thus concentration) of carotenoids in the retina. We have found that macular pigment density is lower in women, smokers, and persons with light-colored irises. Each of these factors has been identified in at least two major epidemiologic studies as a risk factor for AMD. This consistent pattern suggests that macular pigment is protective against AMD. The largest case-control studies relating nutritional variables to AMD have found dietary intake and higher blood concentrations of L and Z to protect against the neovascular form of AMD. Because macular pigment density is positively correlated with dietary intake and blood concentrations of lutein (L) and zeaxanthin (Z), this suggests a protective role for macular pigment. However, correlations between blood and retinal levels of carotenoids are weaker for women than for men. The fact that most study populations have a majority of females means that blood and dietary measures may be imprecise predictors of the state of the retina. The lack of knowledge about tissue carotenoid concentrations may have contributed to the null outcomes in some studies of carotenoids and AMD.

The fact that nutritional factors can lower the risk for AMD offers the hope of preventing many cases of this increasingly common disease. An effective preventive approach, however, faces several challenges. First, people who are at risk must be identified before severe visual loss occurs. Second, any intervention must increase tissue levels of the appropriate nutrients without unwanted side effects. Finally, the prescribed approach has to be simple and pleasant enough so that people will follow it. To achieve these goals, the most effective approach would

take into account the personal characteristics of each individual. Instead of blanket recommend-ations for dietary intake, the prevention of AMD may require sensitive visual tests in conjunction with individualized nutritional counseling.

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SUSCEPTIBILITY OF OXIDATIVELY MODIFIED GLUTAMINE SYNTHETASE TO PROTEOLYSIS BY THE 20S PROTEASOME. Mosoni*, L., Berlett, B.S., Levine, R.L., and Stadman, E.R. Laboratory of Biochemistry, National Heart Lung and Blood Institute, National Institute of Health, Bethesda, MD, 20892; Laboratoire d'Etude du Metabolisme Azote, INRA Clermont-Ferrand-Theix, 63122 Saint Genes Champanelle, France.

How the oxidation of proteins can influence their susceptibility for proteolysis is still poorly understood. Several studies indicate that oxidized proteins are targeted for degradation, in particular by the proteasome. Failure to remove oxidized proteins causes accumulation of inactive enzymes, and this has been shown to occur during aging. In this study, we produced a range of oxidized glutamine synthetase preparations by incubation in 0 to 160 mM H_2O_2. Under these conditions, methionine residues are progressively oxidized to methionine sulfoxide, reaching a maximum of 12 among the 16 methionine residues per subunit. We measured the rate of degradation of oxidized glutamine synthetase by the 20S proteasome purified from rat liver. After a 5 to 50 min incubation at 37° C with proteasome, TCA soluble peptides and amino acids produced by the reaction were detected using fluorescamine. Surface hydrophobicity of oxidized glutamine synthetase was also evaluated by ANSA binding assay. Oxidation of the first four methionine residues did not increase the proteolytic susceptibility of glutamine synthetase. Oxidation of the next 8 methionines led to a linear increase in both proteolytic susceptibility and surface hydrophobicity. Thus, glutamine synthetase can be substantially oxidized without affecting its susceptibility for proteolysis, leaving open a window during which repair may occur. When the extent of oxidation increases, degradation of the protein is likely to be targeted through a change in its hydrophobicity. A decrease in repair mechanisms could be part of the reasons for the accumulation of oxidized proteins observed during aging.

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A RAPID TECHNIQUE TO CREATE LIPOFUSCIN-LOADED CELLS. Yin*, D., Nilsson, E., and Brunk, U. Department of Pathology, Linköping University, S-581 85 Linköping Sweden.

Although lipofuscin (age pigment) that accumulates by time within secondary lysosomes of post-mitotic cells is considered as a hallmark of aging, the general opinion seems to be that lipofuscin is an innocent residual material without much influence on cellular functions. Even if degenerative cellular affects have occasionally been suggested, convincing reports about influence by lipofuscin on metabolism are still missing. A rapid way of creating lipofuscin-loaded cells is thus urgently needed to allow relevant comparative experiments. Such a method has recently been developed in our laboratory.

Artificial ceroid/lipofuscin is produced by exposing different biomaterials to UV light within a laminar air hood overnight. Thiobarbituric acid reactive sub-stances (TBARS) form in increasing amounts during the early stages of the UV light exposure, while they later decrease. The curve reflects peroxidation, fragmentation and eventual polymerization. The artificial age pigments exhibit lipofuscin-like greenish-yellowish autofluorescence when studied by LM fluorometry. In the TEM it has the fine structure of lipofuscin with pronounced osmiophilia. By fluorometry, the fluorescence maximum is consistently found at 430 nm when excited at 350 nm. Similar fluorescent maxima were observed using different fractions of rat liver as the origin of lipofuscin, e.g., nuclei, light mitochondria, heavy mitochondria and microsomes. Extracts with chloroform/methanol and sodium dodecylsulphate show identical fluorescent characteristics. Five fluorescent bands were obtained after a thin-layer chromatographic separation, when the pigments were extracted by chloroform/methanol.

After sonification, this artificial lipofuscin may be fed to cells in culture, both to post-mitotic cells, such as cardiamyocytes, and to mitotic cells, e.g., fibroblasts, macrophages and retinal pigment epithelial cells. The cells endocytose the material and transfer it to their secondary lysosomes which consequently, are transformed to residual bodies containing condensed lipofuscin. Models of lipofuscin-loaded cells are now employed by us to study their effect on various cellular functions and the aging process.

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AGE-RELATED CHANGES IN THE REGULATION OF AUTOPHAGY (A) AND PROTEOLYSIS IN RAT ISOLATED HEPATOCYTES. Paradiso, C., Rosellini, V., Zambelli, A., Masini, M., Pollera, M., Gori, Z., Bergamini*, E. Institute of General Pathology, University of Pisa, 56122 Pisa, Italy

The process of autophagy/proteolysis is a major pathway for protein degradation in rat hepatocytes, during fasting, under the control of nine physiological amino-acids (AA) (Gln, Leu, Tyr, Phe, Pro, Met, His, Trp and Ala) and pancreatic hormones (insulin and glucagon) (1,2). Gerontological interest in this process stems from the fact that indigested membrane remnants (containing unrecyclable lipids) in liver lysosomes are eventually discharged into bile (3), and from recent hypothesis that depressed A in *ad libitum* fed animals might help (and active A in food restricted animals might prevent) overload of cell membrane with lipophylic waste, that change membrane fluidity and may affect signal transduction (4).

In this research, the age-related changes in the regulation of A were explored in isolated liver cells from 2 and 24 months old Sprague-Dawley male rats fed *ad libitum* (AL) or subjected to two different types of anti-aging diet restriction (40% restriction of daily food intake, DR, or every other day feeding *ad libitum*, EOD, from age 2 months. Isolated hepatocytes prepared as described by Seglen (5) were incubated in Krebs-Ringer bicarbonate buffer at 37°C, gas O_2/CO_2 (95/5). Mixtures of plasma AA were added as multiples of a standard reference mixture that simulated normal concentration of AA in rat plasma. When present, glucagon was 10^{-7} M. The sequestration of LDH into vacuoles was measured in the sedimentable corpes of electrodisrupted cells (6). Protein degradation was assessed from the release of valine in the incubation medium by the HPLC procedure (7).

Results show that in younger liver cells LDH sequestration and proteolysis are significantly inhibited by the AA mixture to a similar extent, and that glucagon antagonizes inhibitions. In older cells 1x AA mixture was ineffective both on sequestration and proteolysis and glucagon was not effective. The juvenile response to AA and hormone was fully restored both by DR and by EOD.

In conclusion, the processes of A and proteolysis undergo important, significant changes during aging, that are fully prevented by anti-aging diet restrictions.

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HETEROGENEITY OF IMMUNE RESPONSE IN ELDERLY SUBJECTS: INFLUENCE OF DIET, GENETICS, AND PSYCHOMETRIC STATUS. Murasko, D.M. Allegheny University of the Health Sciences, Dept. of Microbiology and Immunology, 2900 Queen Lane, Philadelphia, PA 19129.

Decreases in the ability of T cells to proliferate in response to mitogenic and antigenic stimuli are among the most consistent changes observed with increased age. However, this decrease is most apparent when the mean response of a population is examined; the response of individual elderly subjects demonstrates considerable heterogeneity. Our studies have explored possible explanations for this heterogeneity including genetics, diet, and psychometric parameters. Using inbred mice and rats, it is apparent that the absolute level of T cell proliferation is controlled by the genetic background of the rodents. However, cross-sectional studies in rats have indicated that, regardless of genetic background, a significant decrease in T cell proliferation is seen at the median lifespan of each strain. Forty percent caloric restriction in rodents extends lifespan and postpones the onset of many of the age-associated changes in T cell response. However, longitudinal assessment of immune response suggests that, regardless of the strain or the level of caloric restriction, decreases in mitogen-induced T cell proliferation are not precisely timed to the maximum lifespan of the individual. Using ambulatory, healthy humans, we examined whether or not micronutrient status correlates with level of response. In cross-sectional studies, there is no apparent association between any of a panel of micronutrients and either mitogen-induced T proliferation or response to influenza immunization. Finally, the influence of cognitive impairment

and depression was examined. Although there is a trend for decreasing T cell proliferation with increasing level of depression and/or dementia, psychometric status is not a good correlative of decreases in T cell response. In summary, while large differences in diet and psychometric parameters can result in changes in T cell response, neither is a major factor influencing the heterogeneity of immune responses of healthy elderly subjects we have examined.

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[Abstract Withdrawn]

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ANTIOXIDANT NUTRIENTS AND IMMUNE RESPONSE IN AGED: CLINICAL IMPLICATIONS AND MECHANISMS OF ACTION. Meydani, S.N., Nutritional Immunology Laboratory, Jean Mayer, USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111

Aging is associated with decline in immune responsiveness which contributes to the increased incidence of infectious and neoplastic diseases in the elderly. These age-associated changes are mostly observed in T cell-mediated functions, possibly due to intrinsic changes in T cells and increased production of suppressive factors from macrophages (Mf). We have shown that spleens from old mice and peripheral blood mononuclear cells (PBMC) from old subjects synthesize significantly more prostaglandin (PG) E2 than their young counterparts. Vitamin E supple-mentation of old mice or healthy older subjects significantly improved T cell mediated functions. This effect of vitamin E was associated with a decrease in splenocyte and PBMC *ex vivo* PGE2 production. Recently completed human studies showed that supplementation with vitamin E significantly improves *in vivo* indices of T cell-mediated function, including delayed-type hypersensitivity (DTH) skin response and antibody production in response to vaccine. This effect of vitamin E was also associated with decreased incidence of self-reported infection. In addition, vitamin E supplementation in an animal model of influenza reduced lung viral titers in old mice. Animal and *in vitro* studies indicate that the immunostimulatory effect of vitamin E is mostly mediated through its inhibition of PGE2 production by Mf. It is, therefore, concluded that vitamin E supplementation is effective in enhancing immune response in the aged and that this immunostimulatory effect may reduce the incidence of infectious diseases in the aged.

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MODULATION OF PROGRAMMED CELL DEATH AND AUTOIMMUNITY BY ω-3 FATTY ACIDS. Fernandez, G. Department of Medicine, University of Texas Health Science Center at San Antonio, Texas.

Programmed cell death (PCD) or apoptosis plays an important role in maintaining optimum physiological functions. However, both markedly increased or decreased rate of PCD may facilitate in developing life-shortening diseases such as AIDS or autoimmune disorders respectively. Thus, maintenance of physio-logically active PCD may be important in maintaining disease-free lifespan. Recently, slightly increased PCD in rodents fed calorie restriction (CR) is linked to reduced malignancy and increased lifespan. Although CR is also known to prolong lifespan of short-lived autoimmune-prone mice (e.g., NZBxNZWF$_1$, MLR/LPR), yet increased lifespan of short-lived strains of mice is also obtained by feeding *ad libitum* with ω-3 lipid enriched diet.

We recently observed a significantly increased lifespan of B/W female mice by feeding AL diet containing both 5% or 20% fish oil versus 5% or 20% corn oil (CO). Higher ω-3 levels in the diet significantly increased the lifespan of lupus disease prone B/W mice. Although CO is beneficial for lowering cholesterol, yet increased consumption of ω-6 is highly proinflammatory against autoimmmune disorders and found to accelerate renal disease. A combination of ω-3 and CR is found far more effective in delaying the renal disease than a diet with ω-6 and CR. Our recent studies reveal fish oil, however, maintains decreased fibronectin-1 and ICAM-1 mRNA expression in kidney tissues when compared to CO fed 6-7 mo old female B/W mice.

Increased lifespan by ω-3 lipids when compared to ω-6 fatty acids is primarily found due to decreased pro-inflammatory cytokines and eicosanoids and increased antioxidant enzymes such as glutathione peroxidase and catalase mRNA levels. Further, elevated PCD was also observed in ω-3 AL and CR fed animals when compared to mice fed ω-6 fatty acid diets. Increased PCD was also correlated with higher Fas mRNA levels in spleen cells. Increased PCD, both in FO and CR fed mice, appears to be due to the decreased change in membrane fluidity which may facilitate PCD by increasing expression of glucocorticoid receptors. Both FO and CR fed mice showed elevated glucocorticoid levels. In summary, use of ω-3 fatty acids supplement, which is known in humans to reduce autoimmune and cardiovascular disease is possibly due to changes in cytokines and endocrine hormones and maintenance of physiologically active PCD which may reduce autoimmune disease by decreasing autoreactive immune cells during aging.

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VITAMIN E REDUCES MACROPHAGE MEDIATED SUPPRESSION OF T CELL IMMUNE FUNCTION. Beharka*, A.A., Wu, D., Han, S.N., Meydani, S.N., Nutritional Immunology Laboratory JM USDA HNRCA at Tufts University, Boston, MA 02111

In old mice and humans vitamin E (E) supplementation increases splenocytes mitogenic responses, IL-2 production, and delayed-type hypersensitivity skin response while decreasing prostaglandin (PG) E_2 production. We hypothesized that the immunostimulatory effect of E is mediated through decreased Mf-mediated suppression particularly that of PGE_2 production. To test this hypothesis, first experiments were conducted in which combinations of purified Mf and T cells from young or old mice were cultured together. Co-cultures containing T cells and Mf from old mice fed laboratory chow had reduced ConA-stimulated proliferation and IL-2 production ($p<0.05$). Addition of PGE_2 decreased and indomethacin, a PGE_2 inhibitor, increased proliferation and IL-2 production. *In vitro* addition of antioxidants known to decrease PGE_2 production (E and glutathione), but not one that has no effect on PGE_2 production (DPPD) improved T cell proliferation and IL-2 production ($p<0.05$). Addition of E to T cells from old mice before culturing with Mf increased their responsiveness. Secondly, old mice were fed a semi-purified diet containing 30 or 500 ppm dl-a-tocopherol acetate (E). Co-culture experiments were conducted in which combinations of purified Mf and T cells from these mice were cultured together. Mf from 500 ppm E mice produced less PGE_2 than 30 ppm E mice ($p<0.05$). Co-cultures containing T cells and Mf from mice fed 30 ppm E had reduced ConA-stimulated proliferation and IL-2 secretion ($p<0.05$) than those consisting of T cells and Mf from mice fed 500 ppm E. Addition of Mf from mice fed 500 ppm E increased proliferation and IL-2 secretion ($p<0.05$) by T cells from mice fed 30 ppm E. However, responses of T cells from mice fed 500 ppm E were not effected by addition of Mf from mice fed 30 ppm E. Addition of indomethacin increased ConA-induced proliferation and IL-2 production ($p<0.05$) by cultures from mice fed 30 ppm E but not from mice fed 500 ppm E cultures. These experiments provide evidence that decreased production of PGE_2 by Mf contributes to the immunostimulatory effect of E; however, E appears to have a small direct effect on T cells independent of its effects on Mf.

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EFFECT OF SELENIUM ON IL-2 PRODUCTION AND IL-2 RECEPTOR EXPRESSION IN AGED MICE. Roy*, M., Kiremidjian-Schumacher, L., Wishe, H.I., Cohen, M.W., and Stotzky, G. New York University Dental Center and Biology Department, New York, NY 10010.

Previous studies by our laboratory have demonstrated that selenium (Se), an essential nutrient required for optimal growth of mammalian cells, enhances the capacity of a host to generate cytotoxic lymphocytes (CTL) and macrophages and to destroy tumor cells in young adult mice and humans. This study examined the effect of dietary supplementation (2.00 ppm for 8 weeks) with Se (as sodium selenite) on the ability of lymphocytes from aged (24-month old) male C57BL/6JNIA mice to respond to: 1) stimulation with mitogen (phytohemagglutinin) or alloantigen; 2) develop into cytotoxic effector cells and destroy tumor cells and 3) produce interleukin 2 (IL-2). Supplementation with Se resulted in a significant increase in the ability of spleen lymphocytes from aged animals to undergo blastogenesis, as indicated by significantly higher amounts of nuclear incorporation of ^{3}H-thymidine after stimulation with mitogen. The dietary regimen restored the age-related deficiency of the cells to respond to stimulation by nuclear DNA synthesis and cell proliferation, at least, to the level of cells from unsupplemented young adult animals. Furthermore, populations of *in vivo*, alloantigen-activated lymphocytes from Se-supplemented aged animals contained significantly higher numbers of cytotoxic lymphocytes than those from Se-normal aged animals, which resulted in an enhanced capacity to destroy tumor cells.

The significant increase in the number of cytotoxic effector cells within these activated T-lymphocyte populations was probably the result of an enhanced clonal proliferation of cytotoxic precursors cells,

followed by the differentiation of greater numbers of cytotoxic effector cells. This effect occurred in the absence of changes in the ability of the cells to produce IL-2, which has been demonstrated to be depressed in aged individuals. This observation confirmed our earlier finding that dietary supplementation with Se does not affect the production of IL-2. The data suggested that Se restores the age-related defect in cell proliferation through an increase in the number of high affinity IL-2 receptors (IL-2R). The effect was related to the ability of Se to enhance the expression of both the a (p55) and b (p70/75) subunits of the IL-2R, which resulted in a greater number of biologically active high affinity IL-2R/cell. The high affinity IL-2R on cells from Se-supplemented animals functioned normally in terms of ligand binding and kinetics of IL-2 internalization but their greater numbers/cell resulted in the internalization of significantly larger amounts of IL-2/cell. As Se supplementation results in an earlier expression of greater numbers of high affinity IL-2R, the presence of Se in the cell environment can result in an accelerated clonal expansion of activated lymphocytes.

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EFFECT OF CALORIC RESTRICTION AND AGING ON TRANSCRIPTIONAL REGULATION OF IL-2 GENE IN RAT LYMPHOCYTES. Pahlavani*, M.A., Harris, M.D. and Richardson, A. Geriatric Research, Education, and Clinical Center, Audie L. Murphy Memorial Veterans Hospital, and Department of Physiology, University of Texas Health Science Center, San Antonio, TX 78284.

The objective of this study was to determine if the increase in the induction of interleukin-2 (IL-2) expression with dietary restriction correlates with changes in binding activity of the IL-2 specific transcription factor NFAT (nuclear factor of activated T cells) in splenic T cells from male Fischer 344 rats. Splenic T cells were isolated from young (6 month) and old (24 month) rats that had free access to food (control group) and from dietary restricted old (24 month) rats that, beginning at 6 weeks of age, were fed 60% (40% food restriction) of the diet consumed by the control rats. T cells were stimulated with concanavalin-A (conA) and the IL-2 activity, IL-2 mRNA levels, and NFAT binding activity were measured in these rats. In addition, the induction of AP-1 binding activity and c-fos and c-jun mRNA levels were also measured in these rats. We found that the induction of IL-2 activity and IL-2 mRNA levels in T cells decreased with age. However, the levels of IL-2 activity and IL-2 mRNA levels in splenic T cells isolated from dietary restricted old rats were higher ($p<0.05$) than those in control old rats. The ability of nuclear extracts isolated from control and restricted old rats to bind to the NFAT oligonucleotide or AP-1 oligonucleotide decreased significantly ($p<0.001$) with age. The induction of NFAT binding activity was significantly ($p<0.05$) higher in dietary restricted old rats than control old rats. In contrast to NFAT binding activity, AP-1 binding activity from dietary restricted old rats was similar to old rats fed *ad libitum*. The induction of c-fos but not c-jun decreased with age, and the induction of c-fos mRNA levels was significantly ($p<0.05$) increased in dietary restricted old rats than in age-matched control rats. However, the induction of c-jun mRNA levels from dietary restricted old rats was similar to those in age-matched control rats. Therefore, the increase in IL-2 expression with dietary restriction correlates with the increase in binding activity of transcription factor NFAT and the increase in the expression of c-fos which constitutes the nuclear component of the NFAT protein complex.

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THE EFFECT OF DIET RESTRICTION ON THE CANINE IMMUNE SYSTEM. Greeley*, E.H., Kealy, R.D., Ballam, J.M, Lawler, D.F., and Segre, M. College of Veterinary Medicine, University of Illinois, Urbana, IL 61801 and Ralston Purina Company, St. Louis, MO 63164.

The effect of diet restriction on the canine immune system is being evaluated in a longitudinal study. A group of nine-year-old Labrador retrievers (30 females, 18 males), divided into age- and sex-matched pairs have been maintained on a diet restriction protocol since age 8 weeks. The restricted pairmate receives 75% of the total food consumed by the unrestricted control. Immune function has been monitored at regular intervals since age 4 yrs using a battery of tests including: lymphocyte blastogenic responses to mitogens; lymphocyte subset analysis, PMN phagocytosis, natural killer (NK) cell activity, and serum antibody responses. The immune data have been evaluated as a function of age, diet, gender, and body weight/percent body fat values. An age-related decline in both mitogen responses and natural killer (NK) cell activity has been observed, along with age-related increased in T-cell (4-7 yrs) and CD8 percentages. Diet-related differences in immune responsiveness have been sporadic; nevertheless, several notable trends have emerged. Mitogen responses reveal a treatment by gender interaction with restricted males exhibiting depressed responses at 4.5-8 years. Higher T-cell percentages are observed in the unrestricted group at 6-7 yrs, with females demonstrating higher T-cell (5-8 yrs.) and CD 8 (7-8 yrs.) percentages than males. Within females, both T-cell and CD 8 percentages correlate directly with body weight. In addition, the CD8 percentages of both the unrestricted dogs and the combined groups of dogs correlate directly with body fat percentages at 8 years. NK activity correlates inversely with both body weight and body fat percentages in the unrestricted and combined groups at 7-8 years. The phagocytic activity of PMN in restricted dogs exceeded that of unrestricted controls at 5 and 7 years. No age- or diet-related effects on antibody formation have been detected. The overall pattern of data suggests that the effects of diet restriction on the immune system may differ depending on gender. In addition, several parameters of canine immune aging (increased T-cell and CD8 percentages; decreased NK activity) tend to associate with increased body weight and/or fat.

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AGING AND THE GASTROINTESTINAL TRACT. Russell, R.M. Jean Mayer, USDA HNRCA at Tufts University, Boston, MA 02111.

Digestive and absorptive functions are, in general, quite well preserved during the aging process. Although there is diminished pancreatic secretion with aging, this change does not result in malabsorption of fat or protein to a significant degree. Breath hydrogen tests after a mixed carbohydrate meal are frequently positive (abnormal), but this could reflect small intestinal bacterial metabolism of carbohydrate rather than malabsorption. Although the gastrointestinal tract appears to have adequate reserve capacity to ensure normal absorption of macronutrients, aging does change the bioavailability of specific vitamins and minerals (vita infra).

One physiologic change which is common among the elderly and which affects the gastrointestinal metabolism of certain micronutrients is atrophic gastritis/gastric atrophy. Atrophic gastritis and hypochlorhydria are found in approximately 25% of those above the age of 60, and the prevalence approaches 40% of octogenarians. The physiologic consequences of atrophic gastritis include decreased intrinsic factor secretion, bacterial overgrowth of the small intestine, and increased proximal small intestinal pH. The mechanisms by which atrophic gastritis/gastric atrophy affects the bioavailability of micronutrients are: decreased solubility of nutrients; decreased dissociation of nutrients from fiber and food protein; decreased absorption of nutrients due to higher small intestinal pH; increased uptake of nutrients by small intestinal bacteria.

Bacterial overgrowth of the small intestine which occurs in atrophic gastritis, has both positive and negative affects in term of nutrient metabolism. Rarely does overgrowth result in fat malabsorption due to bile salt deconjugation. Moreover, bacteria which overgrow in the proximal small bowel in atrophic gastritis patients are able to synthesize folate and probably vitamin B6, vitamin K, and biotin, making more of these vitamins available to the host. However, the small intestinal bacteria have the ability to take up vitamin B12 for their own use, denying the host of this nutrient.

A second major physiologic change which takes place in the gut as a result of aging is an apparent change in the character of the unstirred water layer overlying the small intestinal epithelial membrane. It has been shown that the absorption of fat soluble substances from an isolated loop of small intestine increases with advancing age due to a change in the unstirred water layer which allows easier penetration of lipid soluble substances into the membrane. If hyperabsorption of lipid soluble substances were to take place as a result of aging, there could be a lower dietary need for certain fat soluble vitamins, particularly if this were coupled with decreased clearance of the vitamin by the liver or peripheral tissues (such has been seen with vitamin A).

The aging gut has not been shown to be more permeable than the gut of the young adult. Lactose mannitol urinary excretion ratios are not different in elderly subjects versus young people, and alpha-1 antitrypsin clearance has been shown to be the same over a broad adult age span.

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INTESTINAL CELL TURNOVER WITH AGING. Holt, P.R. St. Luke's-Roosevelt Hospital Center College of Physicians and Surgeons, Columbia University, New York, NY 10025

A phenomenon associated with aging is the development of epithelial cancers of the gastrointestinal tract. The development and growth of human cancers depend on aberrations of the control in tissue growth. Growth homeostasis in proliferative organs is determined by a balance between cell production and cell loss or cell death. Over the past decade we have studied changes in cell production (proliferation) and cell loss or death (apoptosis) in the small and large intestine of Fischer 344 rats ranging in age from 3 to 25-26 months. In contrast to expectations, epithelial cell production rates calculated from measurements of crypt cell production rate (CCPR), following administration of metaphase arrest agents in *ad-libitum* fed rats over the age of 21 months, was greater than in younger rats. Furthermore, the proliferative zone in the crypts of the aging animals was expanded compared to that seen in the young. Such an expansion of the zone of proliferation in gastrointestinal tissues is characteristic of the pre-neoplastic changes seen in the colons of animals and humans with an increased susceptibility to neoplasia. Prolonged food restriction to 60% of the *ad libitum* intake reduced the enhanced proliferation of the intestines of aging rats to levels seen in young animals until they reached the age of 33 months.

Measurement of epithelial cell numbers in small intestinal villi and in post-mitotic cells of the crypts of the large intestine indicated that total cell numbers in these tissues were similar in aging and younger rats. We then measured the migration and turnover of small intestinal epithelial cells from crypt base to villus tip in the small intestine and found no difference at the two extremes of age. We therefore were left with the conundrum that cell production was increased, cell mass unchanged without a change in cell loss as judged by the migration of cells containing *de novo* synthesized DNA. We thus hypothesized that aging was associated with premature destruction of epithelial cells within the cellular mass of the organ.

The most likely reason for such premature destruction of epithelial cells within the intestinal tissues was by apoptosis. We have measured apoptosis as judged by the technique of terminal uridine deoxynucleotide nick end-labeling (TUNNEL) staining as well as the concentration of pro and anti apoptotic gene products in the small and large intestine of young and aging rodents. Our preliminary data indicate a modest increase in apoptosis and apoptotic rates (recently confirmed by Higashide et. al. Gastroenterology 110:A805, 1996) without a significant change in the proapoptotic gene products BAK and BAX or the antiapoptotic gene products Bcl2 and $BclX_L$. These data suggest that enhanced apoptosis may be one factor leading to the increase of proliferation seen in aging animals. Such increased proliferation makes proliferative organs more susceptible to the development of neoplasia. Better understanding of the reasons for enhanced apoptosis or its possible control by antiapoptotic engineered products may reduce the susceptibility of such organs to neoplastic change.

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AGING: INCREASED SENSITIVITY OF THE GASTRIC MUCOSA TO EGF AND TGF-a. Majumdar*, A.P.N., Fligiel, S.E.G., and Tureaud, J. VA Medical Center, Allen Park, MI 48101; Departments of Medicine and Pathology, Wayne State University School of Medicine, Detroit, MI.

Results from this and other laboratories have demonstrated in the Fischer-344 rat model that aging is associated with increased gastric, small and large intestinal mucosal proliferative activity. Although the regulatory factor(s) remains unknown, at least in the gastric mucosa, this could not be attributed to either gastrin or bombesin, each of which stimulates mucosal proliferative activity in young rats. In fact, we have observed that aging is associated with a loss to the trophic action of both gastrin and bombesin. However, our recent data suggest a role for both EGF and TGF-a in gastric mucosal cell proliferation during aging. This inference is based on the following observations: (a) age-related increase in gastric mucosal proliferative activity is associated with a concomitant rise in tyrosine kinase (Tyr-k) activity and expression of EGF-R, the common receptor for both EGF and TGF-a (b) concentration of TGF-a needed to induce maximal stimulation of EGF-R Tyr-k activity in gastric mucosal membrane preparations (30,000 x g pellet) from aged (24-month) rats is at least 1/1000th of that required to attain the same in young animals (4-month) [1 x 10^{-8} M in young vs 1 x 10^{-11} M in aged], (c) in organ culture system, exposure of gastric mucosal explants to 1 x 10^{-8} M TGF-a for up to 45 min, which stimulates autophosphorylation of membrane proteins in young (4-month) rats causes a marked inhibition in aged (24-month) rats, (d) in organ culture system, EGF at a concentration of 5 x 10^{-11} M also caused a comparatively greater stimulation in tyrosine phosphorylation of gastric mucosal membrane proteins in aged than in young rats, and lastly (e) infusion (osmotic mini pump) of either EGF at a dose 250 ng/kg/h or TGF-a at a dose of 25 ng/kg/h, both of which markedly stimulated gastric mucosal proliferative activity in young rats, caused inhibition of the same in aged rats. We conclude that aging enhances gastric mucosal sensitivity to EGF and TGF-a. Endogenous EGF and TGF-a may induce a greater activation of EGF-R Tyr-k in the gastric mucosa of aged than in young rats leading to increased proliferative activity in the former age group.

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THE SIGNIFICANCE OF GUT BACTERIA IN THE AGING GI TRACT. Saltzman, J.R. Division of Digestive Disease and Nutrition, University of Massachusetts Medical Center, Worcester, MA 01655.

In healthy adults, the upper gastrointestinal tract is essentially sterile. There are two principle factors that prevent the growth of bacteria in the upper gastrointestinal tract: intestinal motility and gastric acid secretion. Conditions that lead to disordered intestinal motility include gastrointestinal surgery (Billroth II gastrectomy), jejunal diverticulosis and pseudo-obstruction syndromes (scleroderma and diabetes). Gastric acid normally inhibits the proliferation of ingested bacteria within the stomach. Atrophic gastritis is a common condition in the elderly which frequently results in bacterial overgrowth. Clinically significant bacterial overgrowth is most likely to occur when a lack of gastric acid is combined with a small intestinal motility disorder.

There is debate as to the clinical significance of small intestinal bacterial overgrowth in the elderly. Some studies have suggested that clinically significant malabsorption frequently occurs in the elderly due to intestinal bacterial overgrowth. Other studies suggest a more benign type of bacterial colonization in the elderly. We recently investigated the clinical significance of intestinal bacterial overgrowth in healthy, elderly subjects with hypochlorhydria due to atrophic gastritis or omeprazole-treatment and found that despite significant numbers of small intestinal bacteria, there was no evidence of fat or carbohydrate malabsorption.

The clinical syndrome of small intestinal bacterial overgrowth includes symptoms of abdominal pain, bloating, diarrhea and weight loss associated with steatorrhea, malabsorption, malnutrition and vitamin deficiencies. Steatorrhea may occur due to bacterial deconjugation of bile salts and vitamin deficiencies may result. Small intestinal bacteria compete for dietary vitamin B_{12}. In the elderly with atrophic gastritis, vitamin B_{12} malabsorption may also occur by malabsorption of protein-bound vitamin B_{12} and can be normalized after antibiotic treatment. Folic acid may be taken up and utilized by bacteria in the small intestine; however, these same bacteria also synthesize folic acid and thus serum folate levels may be paradoxically elevated.

The key to the diagnosis of the bacterial overgrowth syndrome is considering its possibility. The bacterial overgrowth syndrome should be considered in patients with any of the predisposing conditions who present with symptoms of unexplained abdominal pain, diarrhea, weight loss or malabsorption. Elderly patients with otherwise unexplained weight loss or clinical malabsorption should be suspected of possibly having intestinal bacterial overgrowth. The gold standard test for the detection of small intestinal bacterial overgrowth is quantitative cultures of small intestinal aspirates. Non-invasive breath tests may also detect small intestinal bacterial overgrowth (^{14}C-cholylglycine, lactulose and glucose hydrogen breath tests and the 1-g ^{14}C-xylose test), although these tests have varying sensitivities and specificities.

The goal of treatment in the bacterial overgrowth syndrome is the relief of clinical signs and symptoms. If the underlying condition that led to the development of bacterial overgrowth can be corrected, this should be done. Bacterial overgrowth can be reduced by antibiotic administration (typically tetracycline). Prokinetic medications may be used in patients with intestinal motility disorders. The nutritionally adverse consequences of bacterial overgrowth should be recognized so that appropriate nutritional support may be given.

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AGE-RELATED CHANGES IN INTESTINAL ABSORPTION OF DIETARY CALCIUM. Armbrecht* H.J., Boltz, M.A., Hodam, T.L., and Kumar, B.V.. Geriatric Research, Education, and Clinical Center, St. Louis Veterans Affairs Medical Center, St. Louis, MO 63125.

The capacity of the small intestine to absorb dietary calcium declines with age in both humans and rodents. In addition, the capacity of 1,25-dihydroxyvitamin D_3 (1,25$(OH)_2$D), the active metabolite of vitamin D_3, to stimulate intestinal absorption of dietary calcium declines with age. We

have used the F344 rat as an animal model in which to study the age-related decline in calcium absorption and the decreased responsiveness to $1,25(OH)_2D$. In the rat duodenum, the age-related decline in active calcium absorption is paralleled by a decrease in the expression of intestinal calbindin, the intestinal plasma membrane calcium pump (PMCA1), and serum $1,25(OH)_2D$ levels. Intestinal calbindin and the plasma membrane calcium pump are two vitamin D-dependent proteins which are thought to be involved in intestinal calcium transport. This would suggest that the decline in calcium absorption is secondary to the age-related decline in serum $1,25(OH)_2D$ levels and the expression of vitamin D-dependent proteins. However, administration of $1,25(OH)_2D$ does not stimulate duodenal calcium absorption in adult and old rats to the levels seen in young rats. Initial studies suggest that induction of key intestinal proteins by $1,25(OH)_2D$ may be diminished in older animals compared to young animals. In summary, these studies suggest that the age-related decrease in intestinal calcium absorption may be due to two factors - a decrease in serum $1,25(OH)_2D$ levels and a decreased responsiveness of the intestine to $1,25(OH)_2D$ with age. Understanding the biochemical basis of these age-related changes may suggest ways of improving calcium absorption by the small intestine and/or increasing intestinal responsiveness to $1,25(OH)_2D$.

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EFFECTS OF AGING ON LIVER STRUCTURE AND FUNCTION. Schmucker, D.L. Cell Biology and Aging Section, Department of Veterans Affairs Medical Center; The Department of Anatomy and The Liver Center, University of California, San Francisco, CA 94143 and the California Regional Primate Research Center, University of California, Davis, CA 95616.

The issue of whether or not liver function is compromised in the elderly remains unresolved. An extensive database documents a plethora of age-related changes in hepatic structure and function, but many of these observations are qualitative in nature, were made under less than optimal experimental conditions or are simply contradictory. On the basis of several comprehensive reviews of the literature, the late Hans Popper stated some years ago that "...aging exerts a limited effect on the constitutive functions of liver and more on its response to extrahepatic factors..." On one hand, alterations in a variety of hepatocellular structural parameters have been reported, e.g., increased hepatocyte size, appearance of mitochondria and the endoplasmic reticulum, more binucleate cells. On the other hand, quantitative morphological analyses have refuted many of these observations. Perhaps the only definitive change in hepatocyte structure is a marked increase in the number and volume density of dense bodies, i.e., secondary lysosomes and lipofuscin-containing residual bodies. There are few functional data that correlate with these structural changes. Clinical tests suggest that liver function is fairly well maintained in the elderly, including in the oldest old. Serum cholesterol appears to rise with age due, in part, to the reduced clearance of this sterol from circulating lipoproteins. However, the hepatobiliary secretion of cholesterol increases concomitant with reduced bile acid synthesis, creating an environment that predisposes the elderly to gall stones. The rate of liver regeneration declines in old animals and the elderly, although the regenerative capacity remains unchanged. The cause of this lag in liver regeneration may reflect a loss of hepatocyte sensitivity to growth factors or in the number of their receptors. This senescent change has important clinical implications with regard to surgical intervention for liver disease, e.g., resection or transplantation. However, most outcomes studies suggest that age alone should not be a determining factor in such clinical decisions. The geriatric population exhibits a decline in the hepatic clearance of certain drugs and a marked increase in the incidence of adverse drug reactions, especially since many in this age group are subject to polypharmacy regimens. Most of the evidence for an age-related deficit intrinsic to hepatic Phase I drug metabolism has been derived in studies using highly inbred male rats. Recent studies (a) failed to demonstrate significant declines in the *in vitro* metabolic efficiency of hepatic microsomal mono-oxygenases and (b) showed marked declines in liver volume and blood flow in the elderly. Changes in hapatic volume and perfusion most likely account for reduced drug clearance in this age group. In conclusion, the livers of elderly subjects are characterized by declines in (a) volume and perfusion, (b) adaptive responsiveness and (C) reserve capacity.

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ALCOHOL METABOLISM IN AGING. Seitz, H.K. Laboratory of Alcohol Research, Liver Disease and Nutrition, Department of Medicine, Salem Medical Center, D-69121 Heidelberg, Germany.

The rate of alcohol clearance from the blood depends predominantly on three major factors, namely (i) activity of hepatic alcohol metabolizing enzymes (ii) liver size, and (iii) blood flow through the liver. Blood ethanol concentrations also depend on the water distribution volume of the individual. Finally, ethanol undergoes a so-called First Pass Metabolism in the stomach and in the liver, and this depends on gastric alcohol dehydrogenase activity (ADH), gastric emptying time, and hepatic first pass metabolism. In advanced age, some of these parameters change, and as a result, ethanol metabolism and ethanol blood concentrations also change.

It has been shown that following the administration of oral or intravenous alcohol, ethanol blood levels are significantly increased in the elderly as compared to younger individuals. This is predominantly due to the decreased water distribution space in advanced age which leads to increased alcohol blood concentration. In the liver, alcohol is metabolized via ADH and cytochrome P-4502E1. The latter is inducible by chronic alcohol consumption and ethanol metabolism increases in the alcoholic. With advanced age, microsomal function decreases and, therefore, cytochrome P-4502E1 mediated ethanol metabolism also decreases which, however, does not lead to a decreased ethanol metabolism in non-alcoholics. Finally, at least in men, gastric sigma ADH activity decreases with advanced age, leading to a reduced First Pass Metabolism of alcohol which may result in an increased exposure of alcohol to the liver after oral intake. Since in the elderly, ethanol blood concentrations are higher and the liver is more susceptible to ethanol toxicity, chronic ethanol consumption may lead to liver injury earlier and at lower alcohol intake in this age group as compared to younger individuals. In addition, other organ systems such as the central nervous system and the gastrointestinal mucosa are also more sensitive to alcohol in advanced age. This is particularly relevant with respect to the interaction between ethanol and drugs. In the elderly, alterations of the central nervous function and organ injury such as mucosal lesions of the GI tract may occur even at relatively low ethanol intake as a reaction to drugs depressing central nervous function and/or those with potential toxicity to gastrointestinal mucosa (aspirine and non-steroidal antirheumatic agents).

In conclusion, ethanol metabolism in the elderly is changed, i.e., ethanol blood concentrations increase, and this may be associated with an enhanced possibility of ethanol associated organ injury.

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MUTANTS ALTERING LIFE SPAN IN *CAENORHABDITIS ELEGANS* AND OXIDATIVE STRESS. Adachi*, K., and Ishii, N., Bio. Science Research Center, LION Corp, Tajima 100, Odawara, Kanagawa 256, Japan and Dept. Molecular Biology, Tokai University School of Medicine, Isehara, Kanagawa 259-11, Japan.

The purpose of this study is to understand the role of oxidative stress in the aging process by determining oxidative molecular damage which is associated with the life expectancy and the ability of antioxidant defense. In the present study, protein carbonyl content both under normal and hyperoxia atmospheric conditions in some abnormal life-span mutants of a free-living nematode, *Caenorhabditis elegans (C. elegans)*, were measured. Stadtman has demonstrated that protein carbonyl modifications can be taken as a specific indicator of oxidative damage to cellular molecules. As a model for the analysis of the aging process, *C. elegans* offers several advantages as follows: (i) a 20-day mean life span, (ii) a three and a half day life cycle, (iii) an excellent system for genetic analysis, (iv) an excellent system for studying cumulative age-related cellular alterations because the somatic tissues in the nematode consist of long-lived postmitotic cells. A short-life span mutant, mev-1, whose activity of superoxide dismutase (SOD), a scavenging enzyme for oxygen radicals, was about half the wild type level. On the other hand, age-1 mutant has a long-life span and the activities of catalase and SOD were higher than wild type (P. Larsen). The carbonyl contents of young adults were similar between these mutants and wild-type animals and accumulated with increasing aging. The mev-1 mutant accumulated the damaged protein at a greater rate than wild-type and reversely less in age-1 mutant. Furthermore, the accumulation rates depended on concentration of oxygen. The carbonyl content of mev-1 mutant and wild-type were increased in response to hyperoxia; the highest increase occurred in mev-1 mutant. In

contrast, no increase in age-1 mutant occurred. The mean and maximum life spans of mev-1 mutant, wild-type and age-1 mutant were increased and decreased under low and high concentrations of oxygen, respectively. These results suggest that aging is due to oxidative damage, which is associated with the genes which regulate resistance to oxidative stress.

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[Abstract Withdrawn]

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EFFECT OF WATER EXERCISE ON DEVELOPMENT OF MUSCULAR STRENGTH AND ENDURANCE IN ELDERLY INNER CITY WOMEN. Binkley*, H., Doerr, E., Finestone, A., Pina, I. and Kendrick, Z.V. Biokinetics Research Lab, Temple Univ., Philadelphia, PA.

A 10-week water exercise program (WEP) (modified from the 12-week WEP of Ruoti et al., J Sports Phys. Therap, 1994) using the resistance of water to repetitive maneuvers as a mode of resistive training in elderly (mean age ± SEM, 70.5 ± 1.8 yrs) minority inner city women was used to determine the effects of water exercise on muscular strength and peak oxygen uptake. The WEP consisted of a 10-min warm-up, a 5-min cool-down, and a progressive increase in the duration of the water exercises from 15 to 45 min by 5 weeks of the 10-week WEP. WEP was performed at 50 to 70% of subjects' maximal functional capacity determined from a prescreening modified Naughton graded treadmill test and monitored by heart rate.

Muscular strength was evaluated using a 1-RM biceps curl, bench press, and leg press of the muscle groups involved with each of the maneuvers performed on Nautilus and Universal machines; muscular endurance was determined from timed (duration in seconds) repetitive shoulder vertical abduction//adduction and horizontal abduction/adduction maneuvers in the water prior to and following the WEP. Peak oxygen uptake was determined using a modified Naughton graded treadmill test. Paired t-Tests were used to determine significant ($p<0.05$) improvements.

Significant improvements in muscular strength were observed for the biceps curl (17.8 ± 1.9 to 23.5 ± 1.8 lbs) and leg press (117 ± 6.2 to 152 ± 6.3 lbs) with the bench press not changing significantly (56.7 ± 4.1 to 60.6 ± 2.9 lbs, $p<0.09$). Shoulder horizontal abduction/adduction maneuver improved significantly (185.0 ± 31.5 to 417.7 ± 70.9 sec). Nonsignificant improvements were observed for the shoulder vertical abduction/adduction maneuver (230.6 ± 47.4 to 310.3 ± 77.0 sec, $p<0.6$). The WEP had no effect on peak oxygen uptake (15.8 ± 1.5 to 16.8 ± 1.5 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$). We concluded that: 1) the WEP improved muscular strength and endurance in elderly inner city minority women; and, the WEP-induced increase in muscular strength and endurance had no effect on peak oxygen uptake.

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EFFECTS OF AGE AND DIETARY RESTRICTION ON HEPATIC ENDOGENOUS ANTIOXIDANT DEFENSES IN MALE LOBUND-WISTAR RATS. Chen*, L.H., Saxon-Kelley, D., and Snyder, D. University of Kentucky, Lexington, KY 40506 and Lobund Laboratory, University of Notre Dame, IN 46556.

Dietary restriction (DR) of 30% in caloric intake extends both median and maximum life span of male Lobund-Wistar rats for about 30%. DR retards the aging process, but the mechanism of action is not clearly understood. The effects of DR on major endogenous antioxidant defenses were studied in 80 male Lobund-Wistar rats at various ages throughout the life span. Two groups of rats were fed *ad libitum* (AL) or restricted diet (DR) from 6 weeks of age. Adult DR rats received 30% less diet with regard to calories per day when compared to adult AL rats. Eight rats in each diet group were killed at 6, 12, 18, 24 and 30 months of age. The livers were excised and prepared for the determinations of major endogenous antioxidant defense parameters including reduced glutathione (GSH), glutathione reductase (GR), Se-dependent glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase and quinone reductase (QR). Hepatic GSH levels were decreased with increasing age in the AL group, while DR eliminated this decrease. Activities of GR and Se-dependent GPx were not affected by age nor by DR. SOD and catalase activities decreased with increasing age in the AL group, while DR maintained the enzyme activities at about similar levels at all ages. QR activity increased with increasing age in the AL group, and DR further increased the enzyme activity. The results suggest that DR may contribute to the delaying of the aging process by improving hepatic endogenous antioxidant defense capability which decreases with aging in these male rats.

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EFFECTS OF CRUDE DRUG, GANODERMA LUCIDUM ON THE IMPROVEMENT OF LEARNING, MEMORY AND ANTIOXIDANT SYSTEM IN SAMR1 AND SAMP8. Choi*, S.J., Cho, K.H., Song, J.S., Kim, Y.B., Kang, D.R., Yu, J.K. and Han, S.S. Toxicology Research Center, CRICT Yusong PO Box 107, Taejon 305-606 Korea.

The present study was designed to investigate the effect of Ganoderma Lucidum (GL) with long-term treatment, especially in the alterations of behaviors and antioxidant system. The murine model of accelerated senescence, senescence-accelerated mouse (SAMR1 and SAMP8) were used for the study. Animals (N=12) were fed with food which was pre-mixed with 20 mg/kg (T1) and 200 mg/kg (T2) of powdered GL for 5 months starting from 7 months of age to 12 months of age. At the end of treatment, all animals were subjected to evaluation of the active and passive avoidance test for learning and memory capacity as a behavioral testing. The changes in the activities of antioxidant enzymes were biochemically analyzed to examine the anti-aging effect of GL. The results indicated SAMP8 with early onset and rapid advancement of senescence showed the improvement of short-term memory in GL treated group (T2: $p<0.01$). Its control group, SAMR1, also exhibited an increased percentage of avoidance response in active avoidance test in treated group (T2). According to biochemical assay, no significance was noted in antioxidant enzyme activities between treated and control groups of either strain. However, an increased survival rate was shown in GL treated group (T2) in SAMP8. The noticeable finding from the present study was the effect of GL on improving learning and memory was dose-dependent. Therefore, present investigation suggests that GL has a positive effect on improving the learning and memory capacity with increase in survival rate. This anti-aging effect in SAMP8 must be related to the other mechanism rather than the GL action on antioxidant enzyme systems.

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ANALYSIS OF THE BREAKPOINT REGIONS OF AGE-ASSOCIATED MITOCHONDRIAL DNA DELETIONS FROM SKELETAL MUSCLE AND BRAIN TISSUES OF C57bl/6 MICE. Chung*, S.S., Eimon, P.M., Weindruch, R., and Aiken, J.M. Department of Animal Health and Biomedical Sciences and Medicine and VA Geriatric Research, Education and Clinical Center, University of Wisconsin-Madison, Madison, WI 53706.

Mitochondrial genomes with multiple types of DNA deletions have been shown to accumulate with age in various tissues from humans, monkeys, rats, mice, and *C. elegans*. The deleted genomes have been classified based on the characteristics of the deletion breakpoints such as the presence (or absence) of direct repeat sequences. The prevalence of direct repeats precisely located at deletion breakpoints in human mitochondrial DNA deleted genomes has led several investigators to propose slip replication or recombination as mechanisms of deletion formation. Other sequence motifs such as topoisomerase II cleavage recognition sites and secondary or tertiary structures have also been implicated in aiding deletion formation. We have characterized, from mouse skeletal muscle and brain tissues, the breakpoint regions from 36 mitochondrial genomes with deletions. We have analyzed the breakpoint regions for topoisomerase II recognition cleavage sites, secondary structures and the presence of direct repeat sequences. We concluded that topoisomerase II recognition cleavage sites and secondary structures most likely do not play a significant role in deletion formation in mice. In contrast, we observed that the majority of the deletion breakpoints were precisely flanked by small direct repeats, indicating that they may be important in deletion formation. The prevalence of small (2-4 nucleotides) direct repeats suggests that another mechanism of deletion formation, other than slip replication or recombination, may predominate in mice.

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TWO POOLS OF SPHINGOMYELIN MAY DIFFERENTIALLY AFFECT VULNERABILITY TO OXIDATIVE STRESS. Denisova*, N.A., Arnio, E.B., Cantuti-Castelvetri, I., and Joseph, J.A. USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111.

Previous research has shown that membrane sphingomyelin (SPH) incorporation may dramatically increase deficits in Ca^{2+} regulation induced by oxidative stress (OS) in PC12 cells. Since membrane SPH

levels increase as a function of age, these findings suggest that interactions between OS and increased membrane SPH may be responsible for the age-related deficits seen in Ca^{2+} homeostasis. The present experiments were carried out to determine whether SPH metabolites, or alteration of the SPH level in the membrane may be involved with deficits in regulation of Ca^{2+} activity induced by OS. Therefore, PC12 cells were incubated under one of the following conditions: Growth medium (GM) alone; 500 mM SPH; 100 mM C2-ceramide (CER); 1 mM sphingosine-1-phosphate (S-1-P), 100 mU/ml *Staphylococcus aureus* sphingomyelinase (SPMase); 2mM L-Cycloserine (L-CS). Analysis of LDH activity (Sigma) and Live/Dead Eukolight kit (Molecular Probes) indicated that pretreatment of PC12 cells with these compounds did not affect plasma membrane integrity in the absence of H_2O_2. A subset of these cultures was then exposed to 300 mM of H_2O_2 in GM for 30 minutes. At the end of the incubation period, media with H_2O_2 was removed. Cells were washed three times with 1 ml fresh GM and subsequently analyzed for either lipid or Ca^{2+} image analyses (baseline - the initial CA^{2+} level before depolarizing the cell with 300 mM KCl; Increase - the increase in Ca^{2+} compared to the baseline, following KCl depolarization; Recovery - the ability of the cell to remove the Ca^{2+} to 20% of the baseline level). Our results show that these agents had differential effects on the ability of cells to recover after exposure to OS following depolarization. Only endogenously induced depletion of SPH by L-CS significantly increased the ability of the cells to recover.

The nature of this effect is not clear. It seems there are at least two different SPH pools (plasma membrane and new-synthesized in *cis-medial* Golgi stacks). Both pools affect Ca^{2+} homeostasis but only newly-synthesized SPH is able to significantly decrease cells' vulnerability to OS. It is possible that age-related increase in SPH synthesis, in part, may play an important role in regulation of cell viability.

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[Abstract Withdrawn]

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[Abstract Withdrawn]

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EFFECTS OF CALORIC RESTRICTION ON IMMUNE FUNCTION IN FISCHER 344 X BROWN NORWAY (F1) RATS. Gardner*, E.M., and Murasko, D.M. Department of Microbiology and Immunology, Allegheny University of the Health Sciences, Philadelphia, PA 19129.

The observation that immune function declines with advancing age has been well documented in both humans and rodents. Studies have also indicated that caloric restriction extends lifespan in rodents, presumably because of its positive effects on immune function. Based upon these observations, we have conducted a large, cross-sectional study in F1 rats to characterize the age-related decline in immune function and to determine whether this decline can be delayed by caloric restriction.

Rats were fed *ad-libitum* (AL) or fed a calorically-restricted (CR) diet (60% of AL diet supplemented with vitamins and minerals) that was initiated at weaning. AL and DR rats were divided into three age groups: young (<16 mo old), mid (16-34 mo old), and old (35+ mo old). Proliferative responses of splenocytes stimulated with either Concanavalin A (Con A) or calcium ionophore (Ca I) were measured after incubation with ^3H thymidine during the final 4 hr of culture. Supernatants were also collected from these cultures prior to the additon of ^3H thymidine and assayed for IFN-g production by a bioassay that measures protection from viral cytopathic effect.

Con A-induced proliferation of splenocytes from old AL rats was significantly lower ($p<0.04$) than that of young and mid AL rats. CR delayed the age-related decline in Con A-stimulated proliferation; the response of old CR rats was significantly higher ($p<0.02$) than that of old AL rats, but proliferation of old CR rats was not significantly different from that of young and mid CR rats. Con A-induced IFN-g production remained constant in AL rats at all three ages. However, Con A-induced IFN-g production increased with age in CR rats and was significantly higher ($p<0.04$) than that of AL rats at mid and old age. In contrast, Ca I-induced proliferation increased with age in both AL and CR rats, but proliferative responses of AL and CR rats in each age group were not markedly different. Likewise, an age-related increase in Ca I-induced IFN-g production was observed in both AL and CR rats.

These data indicate that CR delays the onset of the late age-related decline in proliferation and induces an age-associated increase in IFN-g production after stimulation with Con A. However, Ca I-induced proliferation and IFN-g production demonstrate an early age-associated increase that was not affected by CR. Collectively, these data suggest that CR may correct a defect in T cell receptor-dependent pathways evident at the later stages of aging, without altering the age-associated increase in T cell receptor-independent events.

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[Abstract Withdrawn]

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INFLUENCE OF CHEST PAIN PATTERNS AMONG AFRICAN AMERICAN, LATINO AND WHITE PATIENTS FROM TWO SOCIOECONOMIC STRATA. Haywood*, L.J., Ell, K., and deGuzman, M. LAC+USC Medical Center, Los Angeles, CA.

Differential behavior among racial/ethnic patients (pts) accounts for variable delay times in seeking acute heart care; delay times also vary by socioeconomic status (SES). We investigated symptom perception and chest pain presentation among 1,428 low and middle SES black, Latino and white patients seeking emergency care by questionnaire using the following categories: 1) increasing chest pain, 2) 'heart pain' by patient attribution, 3) angina by formal attribution (confirmed by physician), 4) Rose Questionnaire (RQ). Significant differences were found by SES within groups: low SES pts more often described increasing chest pain ($p<0.002$) and heart pain ($p<0.000$); middle SES patients more often had a history of angina ($p<0.001$) and a positive response to the RQ ($p<0.001$). Among acute MI pts, angina, prior CABG and PTCA were more common in the middle SES Group, while prior MI, chest pain or heart pain were more common in the low SES group by multivariate analysis ($p<0.001$). In MI pts, positive RQ were associated with prior MI and not predictive of MI confirmation. Low SES pts were younger and more likely to be current smokers ($p<0.001$).

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CHRONIC CHLOROQUINE ADMINISTRATION IN AGED MICE ATTENUATES EXCITOTOXIC BLOOD-BRAIN BARRIER DYSFUNCTION. Mielke, J.G., Murphy, M.P., Maritz, J., Bengualid, K.M. and Ivy*, G.O. University of Toronto, Scarborough College, Division of Life Sciences, 1265 Military Trail, West Hill, Ontario, Canada M1C 1A4.

A physiological correlate of both the aging process and Alzheimer's disease (AD) is blood brain barrier (BBB) dysfunction. Chloroquine (CHL), a common anti-malarial drug, causes manifestations of aging and AD in both cell culture and animals. In addition, CHL has shown proconvulsant properties in animals and humans that may result from BBB dysfunction in a fashion similar to that seen following administration of the excitotoxin kainic acid (KA). BCF1 and BDF1 male and female mice (aged 20-24 months) received i.p. injections with either CHL (45 mg/kg; n=18) or vehicle (0.9% physiological saline; n=24) once/day for six consecutive days. On the day following the final dose, half of the subjects from the two groups were injected i.p. with KA (36 mg/kg), while the remaining half received saline. Animals pretreated with CHL exhibited a tendency to develop seizure activity before the controls [$F(1,25)=3.81$, $p<0.06$]. Following 45 minutes of status epilepticus, the mice were sacrificed with an overdose of sodium pentobarbital and perfused transcardially with 4% paraformaldehyde. Coronal sections were taken (30 mm) from the septum to the hippocampus, and every eighth section was processed for immuno-cytochemical localization of serum proteins, such as IgG. KA injected subjects which received saline pretreatment exhibited intense IgG and serum protein incursions [$F(1,30)=8.56$, $p<0.0065$], while those that received CHL pretreatment displayed a dramatic attenuation of this effect [$F(1,17)=7.41$, $p<0.015$]. No strain or sex differences were noted. In addition to suggesting that CHL may exert a proconvulsant effect via BBB interaction, these results indicate that CHL administration may protect the BBB from damage under some circumstances.

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GLUTATHIONE LINKS OXIDATION TO PROTEIN DEGRADATION. Jahngen-Hodge*, J., Obin, M.S., Nowell, T., Gong, J., Abasi, H., Blumberg, J., and Taylor, A. Nutrition and Vision Research, Antioxidant Research, Jean Mayer USDA HNRCA, Tufts University, Boston, MA 02111.

Aging and age-related diseases appear to be causally related to oxidative damage. A manifestation of the damage is formation and accumulation of oxidatively modified, and potentially cytotoxic, proteins. The ubiquitin proteolytic pathway is the main proteolytic pathway respon-

sible for the selective removal of damaged proteins by conjugation of ubiquitin to substrate proteins. Oxidation-induced and age-related accumulation of endogenous ubiquitin-containing species, as well as the impaired ability to form ubiquitin protein conjugates *de novo*, have been noted; however, a link between oxidation and proteolysis has not been observed. The purpose of this study was to determine the effect of oxidative stress on two enzymes, E1 and E2, which are required for conjugation of ubiquitin to substrate proteins. We hypothesized that oxidized glutathione (GSSG) formed during H_2O_2 induced oxidation to the retina would inhibit ubiquitin conjugating enzymes (E1, E2) that contain active-site sulfhydryls. Using a retina organ culture model containing 0-1.4 m,mol H_2O_2/mg retinal tissue, reduced glutathione (GSH) levels fell from 8.4 nmol/mg protein to 3.3 nmol/mg protein in a dose-dependent manner. A corresponding 7-fold, H_2O_2 dose-dependent increase in GSSG (0.28-2.0 nmol/mg protein) was also measured. This resulted in a decrease in the GSH/GSSG ratio from 35 to 1.7. Endogenous ubiquitin conjugates also decreased in a H_2O_2-dose-dependent manner, with a corresponding increase in levels of free ubiquitin. *De novo* formation of ubiquitin conjugates, as well as E1 and E2 activities, were assessed in *in vitro* assays to which ^{125}I-labeled ubiquitin was added. Retinal E1~^{125}I-labeled ubiquitin thiol ester (120kDa) and E2~^{125}I-labeled ubiquitin thiol esters (31, 28, 26 and 23 kDa) were observed on nonreduced SDS polyacrylamide gels. Catalysis of the formation of E1 ~ and E2 ~^{125}I-labeled ubiquitin thiol esters was reduced up to 50% and 60%, respectively, in retinas treated with ≥0.75 mmol H_2O_2/mg tissue. This inhibition was also reflected in several-fold reductions in *de novo* retinal protein ^{125}I-labeled ubiquitin conjugates. Incubation of non-oxidized retinal supernatants with GSH/GSSG ratios equivalent to those produced during H_2O_2 oxidation caused a decrease in E1 ~ and E2 ~^{125}I-labeled ubiquitin thiol ester formation. This data indicated that mild oxidative damage to the retina inhibits the ubiquitin conjugating enzymes, presumably through modification of E1/E2 active-site sulfhydryls by GSSG. Confirmation of this conclusion was obtained with experiments in which <5 mM GSH and dithiothreitol were able to rescue H_2O_2-inhibited E1 and E2 activities.

This research indicates that the ubiquitin system, which is an important regulatory mechanism in virtually every cell in the body, responds quickly and dramatically to oxidative stress. In addition, GSSG is the moiety which effects the oxidation-induced modulation of ubiquitin conjugation. Since GSSG levels increase with age and oxidation in many cells, inactivation of critical ubiquitin conjugating enzymes by GSSG could result in the accumulation of damaged, and possibly cytotoxic, proteins.

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ANTISENSE REDUCTION OF GLUTATHIONE LEVELS RESULTS IN DOPAMINERGIC CELL DEATH VIA INCREASED CALCIUM INFLUX. Jurma, O.P., Hom, D.G., Andersen*, J.K. Division of Neurogerontology, Ethel Percy Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089-0191.

Glutathione (GSH) is a major regulator of the redox state of neuronal cells in the brain. Dopaminergic cells are particularly prone to oxidative stress, and lowered levels of GSH may be involved in dopaminergic cell loss associated with Parkinson's disease. We used PC12 cells as a model system to look at the effects of reducing GSH levels on dopaminergic neurons via antisense oligomers directed against the rate-limiting enzyme in GSH synthesis, glutamyl cyteine sythetase (GCS).

Disruption of dopaminergic cell redox by reduction of GHS levels resulted in increased hydroperoxide levels and free radical damage, as indicated by increases in both lipid peroxidation and protein oxidation. The increase in hydroperoxide levels was accompanied by a concomitant increase in intracellular calcium levels, along with a decrease in cell viability. The increased calcium influx could be prevented by pretreatment of cells with either GSH monoethylester or vitamin E. Cell death was calcium-mediated, as it could be prevented by depleting the media of calcium by either growing the cells in calcium-free media or by addition of the calcium-specific chelator, EGTA. Cell death could also be prevented by growing the cells in the presence of verapamil, an L-type calcium channel blocker but not by nifedipine, an N-type channel blocker.

Reduction of GSH levels resulted in increased cell death involving disruption of intracellular calcium homeostasis due to increased calcium influx through L-type calcium ion channels. The increased calcium entry is likely due to either direct free-radical mediated damage to the L-type voltage-dependent calcium channels themselves or damage to ion-transport ATPases which act to maintain membrane voltage, ultimately leading to depolarization and as a consequence calcium influx. Current work in lab is towards trying to delineate which mechanism is responsible for the increased calcium influx; preliminary data suggest the latter likely accounts for the calcium influx.

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NEW DIRECTIONS IN THE REVERSAL OF MEMORY LOSS. Khalsa, D.S. Alzheimer's Prevention Foundation, Tucson, AZ, 85749.

Alzheimer's Disease (AD) is an insidious brain disorder leading to global deterioration. Age associated memory impairment (AAMI), previously thought to be benign, is now known to progress to frank dementia in a sub-population of high risk individuals. With the aging of the population one of medicine's greatest challenges is to find a way to impact these diseases. Although researchers have been active, there has been little progress. Current thinking has focused on one drug therapy. The clinical study carried out by the APF was designed to investigate the effectiveness of a multi-modality medical program on cognitive decline. Fifteen patients with age-associated memory impairment (AAMI) and 6 patients with Alzheimer's disease (AD) are part of this uncontrolled pilot project.

The 7 step complementary medical program includes: 1) dietary modification 2) nutritional supplementation 3) newer nutrients and medicinal herbs 4) pharmaceuticals 5) hormone replacement therapy 6) cognitive exercise 7) mind/body exercise.

Every patient has been in treatment over 5 months, some as long as three years. The results are compelling. All have improved and none have progressed. The measures of clinical improvement utilized include the mini-mental state exam (MMSE), the global deterioration scale (GDS), follow-up interviews/examinations and self-reported measures.

The program attacks the problem of cognitive dysfunction at the level of the entire brain cell. Dietary modification, based on the work of Walford and others, has shown that a calorically limited nutrient-dense diet enhances mental function. This 20% fat diet lowers cholesterol, perhaps enhancing cerebral blood flow. Adequate scientific evidence suggests that supplementing the diet with anti-oxidants vitamin E, vitamin C and Coenzyme-Q-10 will support optimal brain health. B vitamins enhance neuronal function and improve neurotransmitter production and utilization. The use of newer nutrients, such as Acetyl-L-Carnitine and Phosphatidylserine (PS) are strongly supported in the medical literature. The former is currently in phase 3 clinical trials and the latter has been shown in double blind studies to improve attention, concentration, short term memory and activities of dail living in patients with mild to moderate dementia. The herb Ginkgo has had the benefit of close to 400 studies indicating an improvement in micro-vascular cerebral blood flow. L-Deprenyl, a MAO-B inhibitor, has recently been shown in a five-year double blind clinical trial to slow the progression of cognitive deterioration.

Hormone replacement therapy has been labeled "terribly exciting" by researchers. Pregnenolone and DHEA both have proven effective as part of our combined program. Cognitive exercise shows that mental stimulation helps improve the plasticity of the CNS by creating new dendritic growth. We use newspaper headline discussion, music, art, etc.

Aerobic conditioning improves some aspects of mental function from 20-30%. Mind/body exercises incorporating special breathing techniques and relaxation/meditation exercises, have been shown on PET scan to increase metabolic activity in the hippocampus. They have profound clinical effects.

Cognitive dysfunction is one of the 21st century's greatest medical challenges. The clinical work carried out by the Alzheimer's Prevention Foundation has demonstrated that a synergistic multidisciplinary medical program is effective in helping patients with AAMI and AD. We are in the process of organizing a controlled double blind multi-center clinical trial.

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EFFECT OF DECREASED MOBILITY ON BODY COMPOSITION IN PATIENTS WITH ALZHEIMER'S DISEASE. Khodeir*, M., Conte, E., Morris, J. and Volicer, L. Geriatrics Res. Educ. Clin. Center, E.N. Rogers Mem. Vet. Hospital, Bedford, MA 01730 and Boston University School of Medicine, Geriatric Medicine, Boston, MA 02118.

Elderly with dementia of the Alzheimer type (DAT) are at a significant risk of weight loss. Therefore, monitoring body weight of Alzheimer patients is an important indicator of clinical status. However, it is unclear

if weight standards developed for healthy elderly, assuming a normal level of activity, are applicable in the DAT population . With progression of DAT, patients become less able to ambulate and eventually are unable to ambulate even with assistance. We hypothesize that this decreased mobility may lead to muscle atrophy which would result in weight loss, even in the absence of malnutrition. The purpose of this study was to determine the interaction between mobility and nutritional status of DAT patients.

Body composition, using bioelectrical impedance plethysmography, dietary intake with caloric counts on two non consecutive days, anthropometric indices and laboratory indicators of malnutrition were measured in 50 institutionalized, DAT patients with varying levels of mobility.

We found a mean age of 73±7 years, duration of dementia, and duration of institutionalization were 8±3 and 1.8±1.7 years respectively. Ninety six percent of patients were men. Nutritional status indicators showed that none of the patients were malnourished according to biochemical indicators. Plasma albumin was >3 g/dl, hemoglobin >10.8gm/dl, TIBC >173 ugm/dl, transferrin >139mg/dl, vitamin B12 >179 pg/ml, and absolute lymphocyte count >472/cmm. The average total caloric intake was 2125 ± 398/day. However, some anthropometric indicators were below the 5th percentile of normal, consistent with loss of muscle mass.

Eighteen patients ambulated independently, 14 required assistance, and 18 were non-ambulatory. The mean lean and fat body mass for all patients were 62.5% and 37.5% of total body mass, respectively. We found a statistically significant positive correlation ($r=.59, p<.001$) between the degree of mobility and lean body weight and a negative correlation ($r = -.51, p<.001$) between body fat weight and degree of mobility.

These data support our hypothesis that patients with more advanced stages of dementia and compromised mobility tend to lose muscle mass and replace it with fat. We computed the lean body mass index, and found that it was dependent on the patient's age and mobility status. This relationship may be used to develop body weight recommendations for patients with Alzheimer's dementia at the different stages of disease. Patients who are ambulatory tend to have a body mass index (BMI) almost identical to the ideal standard BMI norms. However, in patients with impaired mobility status, a lower BMI than the recommended standard may be acceptable, without compromising the nutritional status of the patient.

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[Abstract Withdrawn]

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AGE-CHANGES OF ESR SIGNALS AND FREE-RADICAL PRODUCTION IN MITOCHONDRIA. Koltover, V.K. N. Semenov Institute of Chemical Physics, RAS Moscow Region, 142432 , Russia.

Up to date, low-temperature ESR spectroscopy has shown its great power as the most direct instrument in the definition, often discovery, of paramagnetic metal-ion components and free radicals in highly integrated whole-tissue preparations of all origins. The SKN enterosorbent (non-coated nitrogen-containing carbon), when regularly administered into animal diets, was found to increase the mean lifespan of male Wistar rats by 43% (Frolkis et. al., 1989). The mitochondrial ESR signals of liver, iron-sulphur proteins (SP), semiquinone radicals (SQ), and Mo-sulfite oxidase as well as the signals of endoplasmic cytochrome P-450, Mn-proteins, and iron-nitrosyl complexes are lower in their intensities for old rats (24-26 months) in comparison with the similar liver signals for adult animals (4-6 months). SKN remained the signal of sulfite oxidase of adult animals without change while the other signals diminished. Furthermore, the effects of a single administration of butylated hydroxytoluene (BHT), which is also known as the life-prolongating antioxidant, on the ESR signals of heart and blood were studied along with its effects on concentrations of hormones (ACTH, 11-OHCS, TSH, and T3) in blood plasma of the rats. The decrease of the signal of the heart ISP takes place during the first 6 hours after a single BHT injection while the SQ-signal tends to increase. Since the intensity of the ISP signal is directly proportional to the amount of these proteins in the reduced form, this is the evidence that BHT promotes oxygenation of the heart cells. BHT also decreased the ratio of the intensities of the ESR signals of transferrin and ceruloplasmin in the animals' blood. This decrease testifies the impact of BHT upon the redox-signaling system of the blood plasma. Moreover, the BHT-induced ESR signal of the NO-hemoglobin complex arose in the animals' blood. The intensity of this NO-signal was lower in the case of old rats. There were also significant BHT-induced changes in the plasma concentrations of ACTH, 11-OHCS, TSH and T3 hormones. It has also been shown that heart mitochondria become intensive generators of the superoxide radicals after transient anoxia/ischaemia conditions. The anoxia/ischaemia increases the reactivity of ubisemiquinones (UQ) of the mitochondrial respiratory chains to oxygen as it was documented by following intensity of the ESR-signals of the redox-cycling UQ (Nohl, Koltover, and Stolze, 1993). The loss of control of the electron flow correlated with the increase in the lipid membrane fluidity. As hypoxia/ischaemia is known to impair heart mitochondria and to trigger the release of toxic oxygen radicals, BHT may perform the indirect anti-oxidative protection by fostering optimal oxygenation of mitochondria in heart cells trough NO-cGMP-mediated mechanisms.

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[Abstract Withdrawn]

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DIETARY VITAMIN E SUPPLEMENTATION DECREASES LUNG VIRAL TITER AND H_2O_2 PRODUCTION IN OLD C57BL/6NIA MICE INFECTED WITH INFLUENZA. Hayek*, M.G., Han, S.N., Taylor, S.F., Bender, B.S., Meydani, M., Smith, D.E., Eghtesada, S. and Meydani, S.N., Jean Mayer, USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111; University of Florida, Gainesville, FL 32608.

Infections, particularly those affecting the respiratory system, rank among the leading causes of death in older adults. Influenza infection in mice causes a decrease in tissue levels of antioxidant nutrients including vitamin E (E). Oxidation may also affect protease cleavage of the HA protein on influenza virus, rendering it more virulent. The purpose of this study was to examine the effect of E supplementation on influenza infection in young (4 mo) and old (22 mo) C57BL/6NIA mice. Mice were fed diets supplemented with 30 or 500 ppm E for 8 weeks at which time they were intranasally infected with Influenza A/Port Chalmers/I/73 (H3N2). Mice were sacrificed on days 2, 5, and 7 postinfection and were examined for natural killer (NK) cell activity (day 2), cytotoxic T cell (CTL) activity (day 7), and pulmonary viral titers and E levels (days 2, 5, and 7). Old mice fed 30 ppm E had significantly delayed viral clearance compared to young mice fed 30 ppm E. Vitamin E reduced lung viral titers in young mice only on day 5, but E caused a greater reduction of viral titers in old compared to young mice. An age-associated decline in NK cell activity was reversed by feeding old mice 500 ppm E ($p<0.01$), but E had no effect on NK cell activity in young mice. Pulmonary CTL activity was not affected by age or E supplementation. Liver vitamin E concentrations decreased postinfection. However, old mice fed 500 ppm E maintained higher E levels on day 5 postinfection. To further determine the mechanism of E's effect on H_2O_2 production by zymosan stimulated lung cells from young and old mice before and after infection with influenza virus. Influenza infection increased pulmonary cell zymosan-induced H_2O_2 production, and old mice had significantly higher H_2O_2 production than young mice 1.81±0.03 in young vs 2.21±0.06 in old mice before infection and 2.4±0.28 in young versus 4.25±0.18 in old mice on day 7 postinfection. Vitamin E supplementation decreased H_2O_2 production (3.76±0.64 in mice fed 30 ppm E versus 1.97±0.57 in mice fed 500 ppm E). We conclude that vitamin E supplementation of old mice increases resistance to influenza infection by decreasing oxidative stress and preserving NK cell activity.

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MOLECULAR CLONING OF OXYGEN-INDUCIBLE GENES IN *CAENORHABDITIS ELEGANS*. Yanase, S., and Ishii*, N. Department of Biochemistry, Kanagawa Pref. College Nursing and Medical Technology, Yokohama, Kanagawa 241, Japan, Department of Molecular Life Science, Tokai University, School of Medicine, Isehara, Kanagawa 259-11, Japan.

Oxidative stress is a main candidate for the mechanism of aging. Aerobes have evolved a series of excellent defense systems against the oxygen stress. It is known that some defense components are shown to be inducible by oxidative stress in prokryotes. However, in spite of the importance of the oxygen stress response, less is known about the cellular response of gene expression of antioxidant enzymes in eukaryotic cells. In the present study, cloning of oxygen-inducible genes was performed to know the role of oxygen stress on the gene expression in nematode *Caenorhabditis elegans (C. elegans)*. *C. elegans* has proven a valuable organism for the study of basic biology, as both its molecular and Mendelian genetics have been defined and exploited.

Cloning was achieved using a modified method of PT-PCR differential display which has been shown to identify differentially expressed genes. Some genes including heat shock protein, calcium channel beta subunit and rDNA whose expression increased at high oxygen concentration were cloned. In these genes, a new gene, oxi-1, was homologous to yeast hypothetical 264.2kD protein in RAD-3-BMHI intragenic region. It is suggested that oxidative stress may be related with DNA repair or structural stability of chromatin.

C. elegans also offers several advantages for the study of aging as having a 20 day mean life span and an excellent system for studying cumulative age-related cellular alterations because the somatic tissues consist of long-lived postmitotic cells. There are some mutants altering life span in *C. elegans* like long-life span mutants, age-1 and daf-2 or a short-life span mutant, mev-1, which are also related to oxidative stress and the defense system. These mutants life spans may be related to these gene expressions.

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URINARY EXCRETION OF CALCIUM IN STUDENTS AND MATURE WOMEN TAKING SUPPLEMENTS. Cone, A., Danner, T., and Ordman*, A.B. Biochemistry Program, Beloit College, Beloit, WI 53511.

Calcium is an important nutrient for the prevention of osteoporosis. A Daily Value of 1,000-1,500 mg is widely reported to be beneficial, but use of supplements to achieve this intake remains controversial for many reasons. Supplemental calcium may be only partially absorbed from the intestine. It is unsafe for people with certain risk factors. It has unpleasant side effects for many other people. The purpose of this study was to determine a practical recommendation for calcium supplementation by identifying the minimum supplement dose necessary to elevate urinary calcium excretion.

Young students (ages 18-22) and mature women (ages 40-82) were given calcium carbonate supplements. Urine samples were collected and assayed by AA spectrophotometry to determine the level of calcium supplementation necessary to elevate urinary excretion of calcium on a continuous basis. Diet was recorded but not controlled, so that the effect of supplements for typical behavior could be determined.

Students taking supplements exhibited elevated urinary excretion of calcium at doses below 500 mg over a period of 12 hrs compared with controls. Elevated urinary excretion of calcium has previously been demonstrated to be an effective measure of intestinal absorption. Higher doses (comparable to most calcium supplements marketed commercially) did not elevate excretion further but often caused unpleasant side effects. A majority of the mature women who volunteered for the study were unable to participate because of risk factors associated with calcium supplements. Among those who did, approximately half did not have elevated calcium excretion even at high doses, while half did, at doses still well below most commercially available supplements.

Our results confirm that mature women may not absorb calcium from the intestine as well as young people. Anyone taking calcium supplements should limit intake to small enough doses to permit absorption and limit side effects.

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THE EFFECT OF IRON OVERLOAD ON MAMALONDIALDEHYDE (MDA) AND 0X08DE0XY-GUANOSINE LEVELS IN LIVER, KIDNEY, LUNG, HEART, BRAIN AND PLASMA IN YOUNG AND OLD RATS. Park*, J.Y.K., Youngman, L., and Ames, B.N. Division of Biochemistry and Molecular Biology, University of California, Berkeley, CA 94720-2302.

Iron in an oxygen-containing environment generates oxidants such as the hydroxyl radicals and the oxidants can have a variety of toxic effects including oxidative damage to important cellular macromolecules. Although iron accumulates in tissues with age, there is little information currently available on whether the tissue iron level is associated with the level of oxidative damage and whether the effect of iron dosing is the same in the elderly as in the young. The purpose of the present study is to determine the effects of iron dosing on oxidative damage to lipid (as measured by MDA) and DNA (as measured by oxo^{8}dG) in many different tissues including liver, kidney, heart, brain, and lungs as well as in plasma of both young and old rats. The results are: 1) Control old rats compared to young rats show an increased level of iron in all tissues analyzed, including liver (500%), kidney (400%), lungs (300%), heart (150%), and brain (150%). The iron accumulation in old rats is very significantly associated with their tissue MDA level per mg protein with highest level found in lungs and lower levels in kidney, heart, brain, and liver. 2) There is a positive association between the level of total tissue iron, MDA, and oxo^{8}dG in young rats when 20-fold excess iron was given via an intragastric route (IG) as ferrous sulfate among all the tissues analyzed. In old rats the association was negative, possibly due to their high oxidant level. 3) The 20-fold excess iron dosing in young rats via IG significantly increases oxidative damage as measured by MDA in liver, plasma, heart and brain, but not in kidneys and lungs. 4) When 1/3 of a lethal dose of iron was given intraperitoneally as ferric nitrilotriacetic acid to young rats, the iron damaged plasma, heart, lungs, and kidneys (18-fold increase in MDA), but not liver and brain.

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[Abstract Withdrawn]

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INVESTIGATION OF CHANGES IN GENE EXPRESSION DURING AGING OF THE RAT BRAIN BY DIFFERENTIAL DISPLAY. Salehi*, M.A.[1,2], Hodgkins, M.A.[2], Merry, B.J.[3], and Goyns, M.H.[1] [1]Molecular Gerontology Unit, School of Health Sciences, University of Sunderland, Fleming Building, Wharncliffe Street, Sunderland, SR1 3SD, UK, [2]Institute for Cancer Studies, Sheffield University Medical School, Beech Hill Road, Sheffield, S10 2RX, [3]Institute of Human Ageing / Environmental and Evolutionary Biology, Liverpool University, P.O. Box 147, Liverpool, L69 3BX, UK.

Purpose of study was to investigate changes in gene expression during aging of the rat brain. Methods: We have used the polymerase chain reaction (PCR) based technique of differential display to analyze changes in gene expression during aging of the rat brain (Liang & Pardee, 1992, Science, 257, 967-971). In this approach we have compared three young adult (6 months) with three old adult (20 months) animals. RNA preparations from the homogenized brains were subjected to reverse transcriptase (RT) - PCR using 40 different combinations of primer pairs. Any PCR product which was consistently found to be more prominent in the three young brains compared to the three old brains, and vice versa, was scored as potentially representing a gene which was differentially expressed during the aging of this tissue.

Results: out of a possible 2,000+ PCR products we identified 44 that might represent genes that exhibit differential expression during aging of the rat brain. An initial screen of these fragments, by Southern-blotting the PCR products and hybridizing them with cDNA probes derived from either young or old brain RNA preparations, indicated that 40% of the PCR products represented genes that were differentially expressed (Salehi et al., 1996, *Experientia*, In press). We are currently sequencing these PCR products and will use them to probe Northern blots and to screen cDNA libraries to isolate full-length clones.

Conclusions: We have found evidence for changes in gene expression in a number of genes, which we are currently characterizing. This approach is likely to prove invaluable for identifying cohorts of genes that show differential expression during the aging process.

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IMMUNOHISTOCHEMICAL SURVEY OF Ne-CARBOXYMETHYL-LYSINE-RELATED GLYCOXIDATION PRODUCTS IN RAT TISSUES: EFFECTS OF AGING AND DIETARY RESTRICTION. Shimokawa*, I, Higami, Y., Horiuchi, S., Iwasaki, M., Ikeda, T., and Tomita, M. Department of Pathology, Nagasaki University School of Medicine, Nagasaki 852, Japan; Department of Biochemistry, Kumamoto University Medical School, Kumamoto 860, Japan; and Panapharm Laboratories, Co., Ltd., Uto, Kumamoto, 869-04, Japan.

Modifications of cellular macromolecules by interplay between glycation/Maillard reactions and oxidation, referred to as glycoxidation, may be associated with many aspects of aging processes as well as diabetic complications. Restricting the dietary intake of rodents extends their life span by modulating many aging processes. By immunohistochemical survey of glycoxidation products in rat tissues with a monoclonal antibody to Ne-carboxymethyllysine (CML), we tested the following hypotheses: 1) that CML related glycoxidation products accumulate in a number of tissues of aged rats; 2) that dietary restriction suppresses

the accumulation. Frozen or paraffin sections of 4% paraformaldehyde-fixed tissues (cerebrum, cerebellum, lung, heart, kidney, pancreas, ileum, skin, subcutaneous tissue, and adrenal gland) of 6-month and 24-month old rats fed *ad libitum* (referred to as AL-Y and AL-O respectively) and 24-month old rats dietarily restricted from 1.5 months of age (DR-O) were subjected to immunohistochemistry. Immunoreactivity was scored by the amount of immunostained structures or the number of positive cells under light microscopy without knowing rat groups. In AL-Y rats, only a few glias and adrenal zona reticularis cells contained immunostained structures; no immunoreaction products were seen in the other tissues examined. In AL-O rats, immunoreactive structures were clearly observed in the cytoplasm of cerebellar Purkinje cells, some glias, adrenal zona reticularis cells, renal tubular cells, and hyaline casts in renal tubuli. IN DR-O, the immunoreactivities were similar to those in AL-O rats, except the kidney; the amount of immunostained structures in tubular cells and hyaline casts were apparently less as compared to those in AL-O rats. The present study demonstrated the accumulation of CML-related glycoxidation products with aging. The suppresisve effect of DR was, however, only observed in the kidney. Although further studies on the effect of dietary restriction will be needed, the present study provided a piece of evidence that glycoxidation processes are associated with some of the aging processes.

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DEFICITS IN PSYCHOMOTOR AND COGNITIVE PERFORMANCE PRODUCED BY DOPAMINE ADMINISTRATION UNDER REDUCED GLUTATHIONE CONDITIONS. Shukitt-Hale*, B., and Joseph, J.A. USDA, Human Nutrition Research Center on Aging at Tufts Univ., Boston, MA 02111.

Oxidative stress (OS) is thought to be a contributing factor to the behavioral decrements seen in aging; the 'free radical hypothesis of aging' maintains that these detrimental effects may be caused by generation and accumulation of reactive oxygen species, especially in the brain. Glutathione (GSH) is an endogenous antioxidant, present in most tissues, that acts to destroy reactive oxygen species and is capable of neutralizing free radicals generated by oxidative stress. Depletion of GSH in the brain may result in less OS protection and increased susceptibility to free-radical induced neuronal damage. It has been shown that administration of buthionine sulfoximine (BSO) selectively inhibits GSH biosynthesis, thereby inducing a GSH deficiency. Thus, the pro-oxidant effects of dopamine (DA), which rapidly auto-oxidizes to form reactive oxygen species, may increase. However, the behavioral consequences of reducing GSH brain levels with BSO in conjunction with DA administration, and their similarity to those seen in aging, have not been examined.

To test the motor and cognitive behavioral consequences of this reduced OS protection, BSO (3.2mg in 30ml Ringer's solution), followed by 6µl Ringer's solution, was administered to young male Fischer 344 rats via a cannula implanted in the right lateral ventricle every other day. In addition, DA (15ml of 500mM) was administered every day, either 1h after BSO (BSO+DA group) or 1h before BSO (DA+BSO group). Tests of psychomotor behavior (rod walking, wire suspension, and plank walking) and spatial learning (Morris Water Maze) were performed with different groups of rats at various intervals during the experiment.

BSO+DA administration impaired motor performance by decreasing latency to fall in the rod and plank walk tests compared to a vehicle only (Ringer's) group. BSO+DA rats also demonstrated cognitive impairment compared to a vehicle group in three important measures: 1) increased escape latencies to find the hidden platform, particularly on the first trial each day; 2) non-spatial strategies during the probe trials (60 sec swim with no platform) (e.g., fewer crossings and longer latencies to the previous platform location, as well as more time spent around the edge of the pool rather than in the zone that had contained the platform); and 3) longer escape latencies to learn a new platform location during reversal training. No differences were seen between the groups with respect to swim speed. In contrast, the performance of the DA+BSO group was not different from that of the vehicle group on any of the motor or cognitive measures.

Therefore, the behavioral consequences of reducing GSH brain levels with BSO in conjunction with DA administration depends on the order of administration. These findings are similar to those seen in aged rats, suggesting that the auto-oxidation of DA coupled with a reduced capacity to respond to oxidative stress may be responsible for the induction of age-related behavioral deficits.

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ARE TELOMERES USED TO GAUGE THE INTEGRITY OF GENOMIC DNA? Taylor* J., and Witkowski, J. University of Maryland and NASA/ GSFC Code 661, Greenbelt, MD 20771; University of Michigan, Geriatrics Center, MSRB III 6240, 1150 W. Medical Center Drive, Ann Arbor, MI 48109-0642.

Telomeres allegedly serve three purposes in eukaryotic cells: 1) to prevent loss of RNA-primed end genomic DNA at replication, 2) to protect end genomic DNA from damage (due to, e.g., end to end fusion), and 3) to serve as a "replication clock" in somatic cells with a Hayflick limit. Thus, once the length of telomeres falls below a critical permitted minimum, genes such as p53 presumably become active to initiate apoptosis. There are three main problems with this simple scenario: 1) it fails to explain how apoptosis is regulated in post-mitotic cells, of which comprise 80% of the cells in humans, 2) many post-mitotic cells are observed to have long telomeres - much longer than would be necessary for genomic capping, and 3) apoptosis can occur in cells with long telomeres, especially upon exposure to mutagens. We hypothesize that telomeres serve an additional purpose: to gauge the integrity, or incidence of mutations, of a cell's DNA. Since telomeres were originally formed from a well-defined repetitive template, we assume that any deviations from this template are due to mutations in the telomeric DNA. Provided the mutation rate of the telomeric DNA is approximately proportional to the mutation rate of the genomic DNA, the incidence rate of mutations in the telomeric DNA could enable certain factors to gauge the integrity of the genomic DNA. We propose that if the mutation rate rises above a critical threshold, such factors initiate apoptosis. This would insure that the genomic DNA integrity of surviving populations is preserved. If this hypothesis is correct, both the length and integrity of telomeres determine if a cell becomes apoptotic or malignant. Moreover, artificially inducing cellular immortality in a non-malignant cell would unfortunately be more complicated than simply adding telomerase unless telomerase were to correct errors in the telomeric DNA in addition to increasing its length. One test of the hypothesis would be to determine if the Hayflick limit is a simple, mono-tonically-decreasing function of mutagen con-centration, which would not occur if the Hayflick limit is a function of telomere size alone.

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LONG-TERM DIETARY SUPPLEMENTATION WITH ANTIOXIDANTS MODULATES CYTOKINE PRODUCTION BY PERITONEAL MACROPHAGES FROM OLD MICE. Wu*, D., Beharka, A.A., Meydani, A., Adolfsson, O, Smith, D.E., Han, S.N., Meydani, M., Meydani, S.N. Nutritional Immunology and Vascular Biology Laboratories, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA.

Aging is associated with dysregulation of immune and inflammatory responses. Macrophage (Mf) production of the multifunctional cytokine IL-6 becomes unregulated with advancing age. Unregulated production of IL-6 is involved in a variety of chronic inflammatory diseases, infectious processes, osteoporosis and certain types of autoimmune conditions. IL-10 is considered an anti-inflammatory cytokine. Reactive oxygen species have been shown to be capable of activating cytokine gene expression. We therefore hypothesized that antioxidant supplementation of old mice might modulate Mf production of cytokines involved in inflammation. To test this hypothesis, thirty 18-month old C57B1/6NIA mice were randomly assigned to one of the following dietary treatments: 1) control [30 ppm dl-a-tocopherol acetate (E)], 2)500 ppm E, 3) melatonin (0.001%), and 4) glutathione (0.5%). At 24 months of age, the mice were sacrificed and peritoneal exudate cells were removed by peritoneal lavage. Resident peritoneal Mf were enriched by adhesion. Mf were cultured for 48 hrs in RPMI 1640 supplemented with 10% FBS with and without 5 µg/ml LPS. Culture supernatants were analyzed for IL-6 and IL-10 by ELISA. Mf obtained from control mice cultured in medium alone produced significant amounts of IL-6. Spontaneous secretion of IL-6 by Mf from the mice fed 500 ppm vitamin E (1845+/-301 versus 729+/-311 pg/ml, respectively) or melatonin (1845+/-301 versus 674+/-260 pg/ml) was significantly lower ($p<0.05$) than that from the control mice. Glutathione had no effect on Mf IL-6 production. LPS stimulation induced Mf to produce significant amounts of IL-6; however, there was no difference in LPS stimulated IL-6 production between dietary groups. In contrast to IL-6 production, Mf cultured in medium produced little IL-10 spontaneously (average 200 pg/ml). LPS stimulation resulted in production of significant amounts of IL-10 by Mf from all the treatment groups (average 1372 pg/ml). Although Mf from

E- and melatonin-supplemented mice produced more IL-10 than those from the control mice, the increases were not significant. It is concluded that supplementation with vitamin E or melatonin decreases production of pro-inflammatory cytokine IL-6 while having no effect on anti-inflammatory cytokine IL-10.

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MUSCLE FUNCTION AND AGING: NUTRITIONAL INTERVENTIONS. Lukaski*, H.C. USDA, ARS, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58202.

As adult humans age, characteristic changes in body composition, physiological function and dietary intake of essential nutrients occur. Epidemiological studies suggest that decreases in skeletal muscle mass are associated with impaired strength, functional capacity and quality of life, and increased morbidity. Also, nutritional surveys indicate that many elderly consume less than recommended amounts of essential nutrients. The hypothesis that correction of inadequate nutrient intake ameliorates age-dependent deficits in physiological function has been examined for some nutrients. Women aged 66 to 79 years were fed diets containing either low (one-half the RDA) or adequate (RDA) dietary protein for 12 weeks. Low protein intake was associated with negative nitrogen balance, decreased fat-free mass and impaired muscle function. No alterations in nitrogen balance, body composition or muscle function were seen in the adequate protein group. Supplemental vitamin E (727 mg alpha tocopherol/d for 48 d) provided to young and older men significantly reduced exercise-induced skeletal muscle damage. Importantly, the beneficial effect of vitamin E was more pronounced in the older than the younger men. The effects of altered magnesium intake on muscle magnesium concenatrtion and energy expenditure during exercise were examined in postmenopausal women. As compared to a diet containing low magnesium (90mg/2000kcal/d) content, adequate magnesium (290mg/2000kcal/d) significantly increased skeletal muscle magnesium and increased the efficiency of energy production during ergocycle exercise. These examples emphasize the importance of adequate dietary nutritional intake to optimize physiological function in the elderly. Furthermore, because many older individuals fail to consume diets containing adequate amounts of essential nutrients, the opportunity exists for additional investigations to relate intake of additional nutrients to physiological function and quality of life in this population.

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MOLECULAR ASPECTS OF MUSCLE DAMAGE AND REPAIR. Cannon, J.G. Intercollege Physiology Program & Department of Kinesiology, Pennsylvania State University, University Park, PA 16802-6900.

Mature muscle fibers are not capable of cell division; therefore, regeneration of skeletal muscle tissue following damage depends upon the recruitment and activation of satellite cells. These normally-quiescent muscle precursor cells are usually located around the periphery of muscle fibers. Several peptide growth factors, including insulin-like growth factor, platelet-derived growth factor and fibroblast growth factor have been identified as important signals for satellite cell proliferation and differentiation. Some of these growth factors may be produced in an autocrine fashion by muscle cells themselves or may be delivered by inflammatory cells. Infiltrating macrophages release cytokines such as interleukin-1 and transforming growth factor beta as well as the growth factors mentioned above. These cytokines modulate muscle protein breakdown and synthesis, provide chemotactic gradients that guide the movement of satellite cells, and regulate the synthesis of the other growth factors and their receptors. Aging is associated with a decline in the number of satellite cells, presumably due to their gradual incorporation into new or existing fibers during normal growth and repair. Furthermore, the production of inflammatory cytokines is altered with age. These factors may contribute to the losses in skeletal muscle mass that accompany old age.

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DIETARY RESTRICTION ATTENUATES AGE-ASSOCIATED HISTOCHEMICAL ALTERATIONS IN RAT SKELETAL MUSCLE FIBERS. Lee*, C.M.[1], Weindruch, R.[2], Roecker, E.B.[3], Aspnes, L.E.[4], and Aiken, J.M.[1] Depts. of [1]Animal Health and Biomedical Sciences, [2]Medicine and VA Geriatric Research, Education, and Clinical Center, [3]Biostatistics, and [4]Nutritional Sciences, University of Wisconsin, Madison, WI 53706.

Biochemical analyses have detected age-associated decreases in several mitochondrial enzymatic activities in rodents. Histochemical localization of these enzymatic activities, however, has been lacking. In this study, cytochrome c oxidase (COX) and succinate dehydrogenase (SDH) activities were determined histologically in the vastus lateralis of two young (3-4 month), 15 control (30-32 month), and 15 age-matched dietary restricted (DR) Lobund-Wistar rats. In the control rats, specific fibers were found which had no detectable COX activity (COX-), were hyperreactive for SDH activity (SDH++), or were both COX- and SDH++. These animals contained an average of 1.5 COX-, 2.0 SDH++, and 1.1 both COX- and SDH++ fibers per vastus lateralis. The age-matched DR rats, however, contained significantly fewer abnormalities with an average of 0.3 COX- fibers, 0.3 SDH++ fibers, and 0.2 both COX- and SDH++ fibers per vastus lateralis. The effect of age and DR was also determined on fiber number and fiber type composition of the vastus lateralis. Age-associated decreases in fiber number and in the number of type I fibers were observed, both of which were attenuated in the DR animals. The results of this study indicate that late onset DR may be beneficial in delaying sarcopenia, or the age-associated muscle mass decline, which is so commonly observed in mammalian muscle.

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ULTRASTRUCTURAL FEATURES OF EXERCISE-INDUCED SKELETAL MUSCLE INJURY IN YOUNG AND OLDER ADULTS. Manfredi*, T.[1], Fielding, R.[2], Ding, W.[2], Fiatarone, M.[3], Evans, W.[4], Cosmas, A.[5], and Cannon, J.[4] [1]Exercise Science Laboratory, University of Rhode Island, Kingston, RI, 02881, [2]Sargent College of Allied Health Professions, Boston, University, Boston, MA 02115, [3]Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA 02111, [4]Noll Physiological Research Center, PA 16802, [5]School of Allied Health Professions, University of Connecticut, Storrs, CT 06259.

The purpose of this study was to examine the effect of exercise-induced injury on the skeletal muscle Z bands of young and older men and women, using the electron microscope and stereology procedures. We also examined the effect of injury on muscle fiber types and their respective size distributions using histochemistry and image analysis. Five and twelve days following an exercise protocol consisting of 45 minutes of downhill running or eccentric cycling at 78% of maximum heart rate, needle muscle biopsy specimens showed evidence of a shift in both type I and type II fibers toward a higher percentage of larger size fibers (>5,000 μm^2). There was also a significant increase in the percentage of damage Z bands [Pre: 16.9%, Post: 44.2%, 5 days: 37.3%, 12 days: 26.6%] following eccentric exercise. Age and gender had no effect on histochemical and ultrastructural expressions of muscle injury. This study presents ultrastructural and histochemical evidence which is consistent with earlier reports that prolonged protein degradation occurs following physiologic stress. The "fiber swelling", which was more evident in the fast twitch glycolytic fibers suggests an increased involvement and/or susceptibility of type II fibers to damage. Furthermore, the lack of agreement between these findings and that of an earlier study which reported greater sarcomere damage when quantified at the LM level, may be due to the greater severity of exercise imposed in the earlier study or the fact that this study quantified Z band damage using the electron microscope, which offers higher resolution. The moderate level of exercise used in this study suggests that older men and women are not differently affected by moderate exercise.

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MUSCLE DEGENERATION WITH AGING, RESPONSE TO TRAUMA, AND CAPACITY FOR RECOVERY. Reznick,A.Z. Musculoskeletal Research Laboratory, Division of Morphological Sciences, Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, 31096, Haifa, Israel.

Between the ages of 20 and 80 years, human beings lose about 30-40% of their skeletal muscle weight. This is observed primarily in the limb muscles, which lose a great deal of their metabolic and physiologic capacities in old age. Using aging Wistar rats 25-26 months old, we have developed a model for rapid muscle atrophy due to immobilization of the hindlimbs. Using the orthopedic technique of external fixation (EF), hindlimb muscles of old rats lost 35-45% of their weight in 4 weeks of immobilization. Muscles showed massive degeneration morphologically as well as changes in biochemical parameters such as an increase in acid phosphatase activity and a decrease in creatine phosphokinase level. In addition, oxidative damage to muscle proteins and lipids was observed. For example, there was a 215% increase in protein oxidation measured by the carbonyl assay and a 130% increase in lipid peroxidation in gastrocnemius muscles that underwent EF for 4 weeks. Administration

of rat growth hormone to the immobilized old animals could slow down some of the immobilization-assosicated muscle damage.

In another study, the capacity of young versus old animals to recover from immobilization was compared. After 4 weeks of immobilization, EF was removed and animals were allowed to recover for 4 weeks. Young animals could recover almost fully, judging by biochemical and morphological studies. However, the recovery of old animals was slower, especially in building up and renewing muscle mass and returning to preimmobilization levels of some biochemical and morphological parameters.

The findings of the above studies may have important implications for the wellness and quality of life of old people.

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NUTRITION, GENETIC MARKERS AND BONE LOSS IN THE ELDERLY. Wood, R.J. Mineral Bioavailability Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111.

Aging is universally associated with a reduction in bone mineral density. Reduction in bone mineral density beyond a critical threshold greatly increases the risk of osteoporotic fracture. Age-associated skeletal fracture is associated with significant morbidity and in some cases mortality. Bone mineral density measurements collected in a nationally representative survey in the United States (NHANES III) indicate that the prevalence of low bone mineral density among older white women is about 20%. Cross-sectional studies have shown that aging in black, white and Mexican-American women results in equivalent rates of bone loss. However, the higher peak bone mass achieved by blacks extends the time at which they reach a critically low BMD by about 20 years. This genetic difference in peak bone mass provides substantial protection for blacks from developing age-associated osteoporosis.

From the public health perspective, the long-term approach to the prevention of osteoporosis must consider factors that can influence the achievement of maximal bone mass, as well as factors that may attenuate age-associated bone loss. These environmental factors affecting BMD include level of physical activity, adequate sunlight exposure to assure proper vitamin D satus, medications that may affect bone metabolism, and diet. From the nutritional point of view, it is clear that large numbers of American girls and women do not consume diets supplying adequate amounts of calcium. Moreover, a recent National Institutes of Health Consensus Conference on Optimal Calcium Intakes recommended that optimal intakes of calcium are greater than the current RDA. Once peak bone mass has been achieved in early adulthood, high Ca intakes are needed to minimize bone loss. High Ca intakes can reduce the rate of bone loss in the elderly and reduce the incidence of bone fracture. Moreover, Ca and vitamin D supplementation has proven effective in reducing the incidence of hip fractures in persons in their 80s.

Osteoporosis is known to have an important genetic component. Recent studies have suggested that individual genetic markers may be useful in the prediction of BMD. For example, individuals with a particular polymorphism of the vitamin D receptor gene (BB homozygotes for Bsm-I restriction site) have been shown to have lower BMD, which could predispose them to earlier bone fracture, although the association with low BMD and osteoporosis is still controversial. The BB polymorphism of the VDR gene is found in blacks at a similar frequency as in whites and is associated with significantly lower BMD already in young women. The underlying differences in Ca and bone metabolism associated with the BB genotype are not completely understood, but may involve differences in Ca absorption.

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THE ROLE OF EXERCISE IN PREVENTING BONE LOSS AND BUILDING BONE MASS. Marcus, R. Aging Study Unit, Veterans Affairs Medical Center, Palo Alto, CA.

Because physical activity is widely believed to benefit the skeleton, exercise has been promoted as a means to preserve skeletal health and prevent age-related fractures. This view is supported by evidence of profound bone loss with immobilization, by comparisons of bone mass of athletes to that of sedentary people, and by exercise intervention trials. Over the past few years, intervention studies, encompassing young, middle-aged and older adults, generally, but not entirely, show that exercise either increases bone mass or maintains bone mass compared to losses in a control group. With few exceptions, increases in BMD have not exceeded 2%, a disappointing effect compared to that anticipated on the basis of cross-sectional comparisons. The reasons for these modest results are not clear. Early problems with exercise trials (non-randomization, inadequate bone density measurements, poorly quantified protocols) seem largely to have been corrected in recent trials, but other problems persist. Assessing the impact of experimental exercise on other habitual activities has not generally been done. Attrition rates remain high and protocol compliance by remaining participants is often poor. The skeletal loading actually achieved by exercise programs may not be much greater than baseline levels. Subjects in reported trials have usually been motivated volunteers who exercised under supervision, so exercise prescriptions for the general population may be even less effective. Thus, at present, the maximum potential for exercise to promote bone mass has not yet been accurately measured, nor do we have a sense of what can realistically be accomplished by endorsing an exercise program to the general public. Nonetheless, from the accumulated experience, it seems reasonable to conclude that regular exercise may promote skeletal integrity throughout life and attenuates age-related bone loss. In addition, exercise can modestly increase bone mass, but must be continued for gains to be sustained. Either resistance (e.g., weight training) or endurance activity (e.g., running) can improve lumbar spine BMD, but does not appear consistently to promote BMD at the hip.

Although exercise may give only modest increases, if any, in bone mass, its other benefits are substantial. Resistance exercise may improve flexibility, muscle strength, gait, balance, and stability, thereby lowering the risk for falls. Since >90% of hip fractures result from a fall, strategies to reduce falling should have a significant impact on reducing fracture incidence. Exercise is not a way of life for most adults. Strategies for the general population must be simple and flexible to accommodate busy people with average motivation and limited access to equipment. Although considerable recent progress has been made in understanding the relationship of physical activity to health, very little has yet been learned of how to motivate to motivate a sedentary population to incorporate exercise into daily life.

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EFFECT OF LONG-TERM CALORIC RESTRICTION AND EXERCISE ON PERIPHERAL MUSCLE BIO-ENERGETICS IN RATS. Horská*, A., Ingram, D. K., Roth, G.S. and Spencer, R.G.S. NIH/NIA-GRC, Baltimore, MD 21224.

Considerable interest is attached to long-term caloric restriction as a potential mechanism for life prolongation, and to exercise as a means of prevention of age-related decline of skeletal muscle mass. Although previous studies on short-term caloric restriction have revealed substantial changes in high-energy phosphate (HEP) metabolism, the effect of long-term caloric restriction on HEP's has not been investigated. ^{31}P NMR permits the non-invasive measurement of the intracellular phosphates ATP, phosphocreatine (PCr), and inorganic phosphate (P_i), as well as intracellular pH, during interventions such as muscle stimulation. Accordingly, we have studied the effect of 9-12 month caloric restriction on skeletal muscle HEP metabolism at rest and during electrical stimulation in sedentary and exercised rats.

Male Wistar rats were randomly assigned to four groups: *ad libitum*-fed sedentary (AS), *ad libitum*-fed exercised (AE), caloric restricted sedentary (RS) and caloric restricted exercised (RE). At 3 months of age, AE and RE rats were given free access to a running wheel. From 5 months of age, RS and RE received 30% less daily food intake than AS and AE rats. At the time of experimentation, rats were anesthetized with ketamine/acepromazine injected i.m. The gastrocnemius muscle (GM) was submaximally stimulated for two 8 min. periods separated by an 8 min. rest period. A 12 min. recovery period followed the second stimulation. ^{31}P NMR spectra were obtained with a 1.9 T, 31 cm Bruker Biospec system fitted with a homebuilt two-turn elliptical surface coil measuring 1.2 x 2.0 cm. Spectra were acquired successively over two minute periods throughout the entire protocol. Muscle fatigue was monitored by simultaneous measurement of the force developed by the lower leg muscles.

Both caloric restriction and exercise resulted in substantially lowered body weight (BW) in comparison to the control AS group (BW=605±41 g). The average BW in the AE group was 510±19 g, in the RS group 511±18 g and in the RE group 382±38 g. At rest, the relative concentrations of PCr and ATP were the same in all groups, indicating that neither of the two interventions affected the resting HEP metabolism. The PCr/P_i ratio decreased significantly during both periods of stimulation with no difference observed among the groups. The average relative concentration of ATP remained constant. However, pH was significantly higher in

the RE group during the first stimulation and during the first post-stimulation recovery period. In accord with previous observations, during the second stimulation the pH was in general higher in all groups. In addition, there was less fatigue of the GM in the RE group than in the other groups.

These results support the hypothesis that diet restriction can lead to improved substrate utilization for energy production. We conclude that dietary restriction and exercise act synergistically to improve muscle bioenergetics during physiological stress.

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EXERCISE AND LONGEVITY: FURTHER EVIDENCE FOR A THRESHOLD AGE. Cosmas*, A.C.[1], Edington, D.W.[2], McCafferty, W.B.[3], and Manfredi, T.G.[4] [1]School of Allied Health Professions, University of Connecticut, Storrs, CT 06269, [2]University of Michigan, Ann Arbor, MI 48109, [3]Department of Exercise Science, University of Massachusetts, Amherst, MA 01003 and [4]Department of Exercise Science, University of Rhode Island, Kingston, RI 02881.

One of the most critical questions that must be addressed in preventative medicine and aging research is: "Is there an age beyond which the effects of physical training may be minimal, or even detrimental to the organism?" In other words, is it conceivable that an exercise intensity which may be beneficial to the organism prior to a certain age may be of minimal benefit or even harmful once that organism has surpassed a certain age?

Our mortality data on rats suggest that a critical period is reached in their lifetime, beyond which the stress of physical training may be greater than their adaptive capabilities. We designate this critical period as the "Threshold Age." This concept is substantiated by myocardial mitochondrial size distribution analysis results which coincides with our mortality data. The size distribution analysis data indicate that during this critical time (approximately 450 days of age in the rat of the Charles River strain according to our mortality data), myocardial mitochondrial size distributions shift toward greater numbers of larger sized organelles in previously sedentary rats that have initiated a physical training program after 450 days of age. Utilizing the surface-to-volume ratio and applying it to cellular organelles, it would seem reasonable to predict that larger sized mitochodria would be less effective in the production of ATP. The distribution shift towards greater numbers of larger sized mitochondria in previously sedentary rats that were subjected to physical activity during the "critical age", was not evident in those rats that remained sedentary throughout their lifetime.

At "Threshold Age" certain structural and biochemical changes take place that apparently exceed the ability of the rat to adapt to physical activity. During this period, it appears that the rat may become locked into a metabolic pattern of cataclysmic decline that could conceivably culminate in death. This leads us to speculate the presence of a "survival factor" which appears to be age-related. We can assume that physical training produces an adaptation in younger rats which is significant as a survival factor. In contrast, in the older rats, either physical activity does not promote adaptation or does not alter the survival factor.

From the perspective of clinical medicine, should we re-examine our exercise prescriptions more closely and moderate them further once an individual reaches a certain age in order to minimize the onset or retard the progression of cardiovascular disease? The data from these studies imply that the physiological responses to exercise may be emphatically different once an organism has reached and surpassed the "Threshold Age".

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***IN VITRO* APPROACHES TO EXAMINING "ANTI-AGING" EFFECTS OF CALORIC RESTRICTION.** Roth*, G.S., Ingram, D.K., Lane, M.A., Kowatch, M.A., Eastman, H.B., and Holbrook, N.J. Laboratory of Cellular & Molecular Biology, Gerontology Research Center, National Institute on Aging, Baltimore, MD.

Caloric restriction (CR) is the most robust and reproducible intervention demonstrated to slow aging of both mammals and lower organisms. Age changes at levels ranging from the molecular to the organismic have been reported to be retarded by CR. Most studies at the cellular level have utilized short term/primary cultures of various cell types obtained from *ad libitum* (AL) fed and CR rodents. These have generally shown that cellular functions which become altered with age change at a slower rate in CR animals. However, longer term cultures of fibroblasts and various epithelial cells have yielded equivocal results.

We have utilized a system of primary rat hepatocyte cultures to examine the effects of *in vivo* CR on cellular functions which decline during aging, such as induction of stress proteins and stimulation of DNA synthesis. The latter has been shown to be a particularly sensitive index of altered hepatic responsiveness to various stimuli including growth factors, catecholamines, and various stresses, and age changes appear to occur distal to receptors in several signal transduction pathways. In addition, we have employed the same cell system (obtained from young animals) to examine the effects of reduced energy availability *in vitro* on time dependent changes in these function during culture.

Preliminary results suggest that while *in vivo* CR can improve or retard age/time related decrements in hepatocyte responsiveness, *in vitro* reductions in energy availability have no effect and may even be detrimental.

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RECENT ADVANCES IN MOLECULAR MECHANISM OF FOOD RESTRICTION IN RODENT MODELS. Richardson* A., McCarter, R. and Nelson, J.F. Department of Physiology, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78284.

Over the past three decades, the study of dietary restriction (DR) has passed through several phases. The major accomplishment of the first phase was to show that chronic restriction of food (energy) delays the onset of most age-related diseases, alters most physiological processes that change with age, and extends life span. These studies provided convincing evidence that DR increased survival by retarding aging. In the second phase of DR research, scientists began to study the mechanism(s) of DR, i.e., they sought to determine at the biochemical/molecular level mechanisms by which DR retards aging. These studies compared various processes in rodents fed *ad libitum* and caloric restricted diets in an attempt to identify those processes that are altered by DR. Using this approach, many laboratories have identified several potential mechanisms of DR. However, it is impossible to establish by a correlative approach which of these mechanisms are responsible for the anti-aging action of DR because DR affects such a large number of physiological and biochemical processes.

The next phase in DR will be to use experimental interventions to test specific mechanisms of DR. Such studies are particularly challenging because they require the long-term manipulation of specific processes in the intact organism. Transgenic mouse models give investigators an experimental system in which a specific process can be altered over a long term. We propose to use three transgenic models to study potential mechanisms of DR: (1) transgenic mice overexpressing the GLUT-4 glucose transporter protein to study the role that plasma glucose, glycation and oxidative damage plays in DR, (2) the CRH (corticosterone releasing hormone) knockout mouse model to test the hypothesis that elevated glucocorticoids play a role in the anti-aging action of DR, and (3) the DNA polymerase b knockout mouse model to determine if an accelerated accumulation of DNA damage/mutations accelerates aging and is retarded by DR.

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CALORIC RESTRICTION ATTENUATES AGE-ASSOCIATED MITOCHONDRIAL DNA DELETIONS IN SKELETAL MUSCLE GROUPS OF RATS AND MICE. Aspnes*, L.E.[1], Chung, S.S.[2], Havighurst, T.[3], Weindruch, R.[4], and Aiken, J.M.[2] Departments of [1]Nutritional Sciences, [2]Animal Health and Biomedical Sciences, [3]Biostatistics and [4]Medicine and VA Geriatric Research, Education and Clinical Center, University of Wisconsin-Madison, WI 53706.

Caloric restriction (CR) is the only known method of increasing both mean and maximum lifespans in a wide variety of mammalian and nonmammalian species. This has prompted several investigators to propose that CR affects fundamental processes of aging. Age-associated mitochondrial DNA (mtDNA) deletions have been characterized from a number of species. Whether these deletions are a causal factor in aging or a result of aging remains unknown. We have examined the accumulation of mtDNA deletions with age in control and CR fed rats and mice. We hypothesized that if mtDNA deletions play an important role in aging then they should be attenuated by CR. Four muscle groups from rats (epitrochlearis, adductor longus, soleus and extensor digitorum longus) and three muscle groups from mice (epitrochlearis, soleus and extensor digitorum longus) were examined from young, old CR and age-matched controls for the number of mtDNA deletions. Soleus and adductor longus from old CR rats and soleus and epitrochlearis from old CR mice contained significantly fewer mtDNA deletions than their age-matched controls. We conclude that CR can attenuate the number of age-associated mtDNA deletions in skeletal muscle.

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CALORIE RESTRICTION IN RHESUS MONKEYS: POTENTIAL FOR RETARDATION OF AGING IN LONG-LIVED SPECIES. Lane*, M.A., Ingram, D.K., and Roth, G.S. Gerontology Research Center, National Institute on Aging, NIH, Baltimore, MD 21224

Lifespan extension and reduction or delay of age-related disease by calorie restriction (CR) are among the most consistent findings in gerontological research and have been demonstrated many times in rodents and other short-lived species. However, until recently it was not known what, if any, effects CR had on physiological function in longer-lived species. To address the question if CR in longer-lived species had effects similar to the extensive findings in rodents, the NIA, in 1987, initiated a study of CR in nonhuman primates. Subsequently, similar CR studies focusing on adult-onset restriction were begun at the University of Wisconsin and the University of Maryland. Several studies from these ongoing investigations have shown that Rhesus monkeys on CR exhibit physiological responses to CR that parallel findings in rodents. In addition, several potential biomarkers of aging are being evaluated and preliminary findings suggest the possibility that CR in Rhesus monkeys could slow the rate of aging and reduce age-related disease, specifically diabetes and cardiovascular disease. The growing body of evidence from the monkey studies shows that R induces a wide variety of changes in physiological function that are consistent with data reported in rodent studies in which life span has been extended. Furthermore, it is interesting to note that many of these findings, such as reduced glucose and insulin, effects on 24-hr energy expenditure, and reduced body temperature, are consistent with physiological changes that could be related to possible mechanisms of CR in rodents. It will be several years before conclusive proof that CR slows aging and extends life span in primates is established; however, these studies suggest that possibility that the anti-aging effects of CR reported in rodents also occur in longer-lived species such as nonhuman primates and strengthen the possibility that this nutritional intervention will also prove beneficial in longer-lived species, including humans.

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CALORIC RESTRICTION IN BIOSPHERE II: EFFECTS OF ENERGY RESTRICTION ON LIPID AND LIPOPROTEIN LEVELS AND HDL SUBFRACTIONS. Verdery*, R., and Walford, R. Arizona Center on Aging, University of Arizona, Tucson, AZ, 85719 and Department of Pathology, UCLA School of Medicine, Los Angeles, CA 90024.

Data from Biosphere II were analyzed to assess the potential beneficial effects of calorie restriction on people.

Although calorie restriction prolongs the lifespan of rodents and other small animals, the potential benefits for people are not known. Eight subjects spent 2 years in Biosphere II, a closed environment in which participants lived and raised their own food isolated from outside resources. As previously reported, because of crop failures, the 8 Biosphereans were restricted to approximately 70-80% of their *ad lib* energy intake. Food eaten was calorie deficient but micronutrient dense and no malnutrition occurred. Subjects continued to work hard during this period of caloric restriction.

Calorie restriction in Biosphere II closely resembles calorie restriction which might be accomplished in free-living people. To study effects on lipid and lipoprotein levels, plasma was obtained before, during, and after closure of Biosphere II from all 8 participants. All samples were analyzed for cholesterol, triglyceride, and HDL cholesterol with standard clinical techniques. From 18 months after entry into Biosphere II until 18 months after exiting, blood from participants was analyzed for HDL subfractions. Other measurements included weight, height, skinfold thicknesses, food intake and blood pressure.

Results demonstrated significant decreases in triglyceride, cholesterol, and HDL cholesterol levels and increases in percent HDL_2 associated with calorie restriction. Triglyceride, cholesterol, and HDL cholesterol levels all decreased after the start of calorie restriction. Triglyceride and cholesterol levels reached nadirs after 12-16 months, but HDL decreased more quickly and began rising before exit. Analysis of follow-up data suggested that HDL_2 levels returned to baseline more slowly than triglyceride and cholesterol. These changes in lipid and lipoprotein levels paralleled changes in body-mass index and body fat as estimated from skinfold thickness measurements.

These results are similar to preliminary results previously reported from studies of calorie restriction in non-human primates. They show that some of the physiological responses to calorie restriction in humans and non-human primates are similar. Results support the notion that some of the beneficial effects of calorie restriction seen in small animal models of aging will be applicable to people. Calorie restriction will decrease risk of atherosclerosis whether or not it prolongs life.

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INTRODUCTION: ANTIOXIDANTS MODULATION OF AGING AND AGE-RELATED DISEASES. Meydani, M. Vascular Biology Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111.

Aging is a multifactorial process in which nutrition has been recognized to play an important role in the progressive age-related decline of several body functions It is becoming increasingly evident that free radicals are involved in the etiology of aging and age-related diseases including cancer, cardiovascular disease, stroke, arthritis, and cataract. Micronutrients and other non-nutritive dietary components with antioxidant properties appear to be important factors in the beneficial contribution of fruits and vegetables to the reduction of risk for the degenerative diseases associated with aging. Limited epidemiological studies and accumulating laboratory data suggest that the antioxidant vitamins E and C, carotenoids, and the B vitamins are important micronutrients, of which many elderly have an inadequate intake, involved in the protection of the body against oxidative stress and free radical damage as well as in the reduction of the risk for cardiovascular disease and cancer. In addition to these nutrients, a growing number of naturally occurring compounds, such as flavonoids in foods and beverages, have been shown to have antioxidant activity which may contribute to the body's multilevel defense against the deleterious effects of free radicals. Over 3,000 flavonoids have been identified, and the potential benefits to health of several of these compounds have come to be appreciated in many experimental situations. In addition to the protection of lipids, proteins, and DNA from oxidative stress, it has recently been recognized that antioxidants are involved in the signal transduction pathway and the gene expression of such mediators as eicosanoids and cytokines. These mediators are known to influence the aging process and the pathogenesis of age-related degenerative diseases, suggesting that antioxidants could play a significant role in prolonging life span and/or preventing or delaying the onset of such diseases as cancer, cardiovascular disease, or cataract.

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DIETARY ANTIOXIDANTS AND DEGENERATIVE DISEASES OF AGING: POPULATION STUDIES. Block, G. University of California, Berkeley, CA 94720.

Oxidative damage to DNA, cell membranes and plasma lipids appears to be an important factor in aging and the degenerative conditions that often accompany it, including cancer, heart disease, cataracts, and cognitive dysfunction. Numerous epidemiologic studies have found that persons with lower intake of antioxidant nutrients or the fruits and vegetables that provide them have a higher risk of almost every type of cancer. In many studies those with low intake had twice the risk of those with high intake. Recent clinical trials do not negate these findings. For cardiovascular disease, increasing evidence suggests an important role for antioxidants in prevention and even in treatment. In addition, several studies have found that low intake of antioxidant nutrients such as vitamin C and carotenoids had significantly increased risk of developing age-related eye diseases such as cataracts. A considerable proportion of older persons have very low intakes of antioxidants. Routine monitoring of intake and blood levels should be a standard component of care in older persons.

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ADULT LIFETIME DIETARY PATTERNS OF ANTIOXIDANT VITAMIN AND CAROTENOID CONSUMPTION IN A CASE CONTROL STUDY OF RISK FACTORS FOR ALZHEIMER'S DISEASE. Petot*, G.J., Cole, R., Conaway, C., Debanne, S.M., Esteban-Santillan, C., Koss, E., Lerner, A.J., Rowland, D.Y., Smyth, K.A., Whitehouse, P.J., and Friedland, R.P. Department of Nutrition, Department of Epidemiology and Biostatistics, School of Medicine and The Alzheimer Center, Case Western Reserve University, Cleveland, OH 44106.

The purpose of this study is to examine dietary patterns over adult lifetimes in a case control study of risk factors for Alzheimer's disease (AD). Oxidative stress has been proposed as a hypothesis for the pathogenesis of AD and other neurodegenerative disorders (Richardson, Ann NY Acad Sci 695:73 1993). Dietary components which function as antioxidants or as part of antioxidant enzymes include vitamins C and

E, carotenoids, zinc, copper and selenium. Long term dietary intakes of these nutrients have been shown to correlate positively with blood levels (Rauma et al. Am J Clin Nutr 62:1221 1995).

Dietary patterns throughout adult life are being investigated from a total of 270 cases and 406 friend and neighbor controls. A self-administered Life History Questionnaire© obtains medical, occupational, activity, education, smoking and diet histories from surrogates of cases and from controls for ages 20s and 30s (P1), 40s and 50s (P2), and 60+ years (P3). The Block Health Habits and History Questionnaire developed by the National Cancer Institute (Smucker et al, Am J Epidem 129:4455-49 1989) has been adapted to elicit food intake patterns for the three age periods. Responses are analyzed, using the Block software (DIETSYS.3.6) for daily nutrient consumption and patterns of foods and food groups that are significant contributors of these nutrients. We have completed four years of data collection and are reporting preliminary results for 117 cases and 239 controls.

Median values for percent of kilocalories from fat for each age period for cases and controls, respectively, are P1: 40.0, 41.8; P2: 39.6, 39.4: P3: 37.6, 33.4. This downward trend over time is consistent with US food consumption surveys over the same time periods, providing a measure of validity of the questionnaire. Mean adult lifetime (two or three time periods combined) consumption of selected vitamins, carotenoids and selected food groups is:

	CASES	CONTROLS	
Nutrients per 1000 kilocalories	n=104	n=223	p.Value
Vitamin A (RE)	855	983	.001
a Carotene (mcg)	294	389	<.001
b Carotene (mcg)	1921	2370	.003
Pro-A Carotene (mcg)	2231	2809	.001
Lutein (mcg)	972	1214	.015
Lycopene (mcg)	666	927	<.001
Vitamin C (mg)	74.6	86.7	.007
Vitamin E (a TE)	5.6	5.9	NS
Servings per day			
Yellow, green vegetables	2.0	2.3	.022
Vitamin C fruits, vegetables	2.4	2.6	NS

These preliminary results indicate significantly greater consumption by controls than cases of vitamins A and C and carotenoids, and more servings per day of foods that contain these nutrients. These data support the hypothesis that dietary antioxidants may be protective against free radical damage and thus delay or prevent the onset of a chronic disease. Data on nutrient supplements, body weight changes, education, smoking, and other life-style factors are also being collected and will be studied for relationships with long-term diet patterns.

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MELATONIN: A NOVEL ANTIOXIDANT AND ITS RELATION TO AGING. Reiter*, R.J. Department of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, TX 78284.

This presentation reviews the evidence that melatonin, a molecule produced in and secreted from the pineal, is an endogenous antioxidant which protects cells, by a variety of means, from oxidative damage inflicted by a number of chemical and physical toxicants. The paper will address melatonin's protective actions against free radical damage to DNA, proteins and lipids. The findings will be considered in the context of the free radical theory of aging.

The circadian production of melatonin in the pineal gland is characteristic of all mammals with high melatonin levels being produced during the night. Melatonin is highly lipid soluble and readily enters cells; furthermore, it easily crosses the blood-brain-barrier. During aging there is a gradual attenuation of the melatonin rhythm such that, in advanced age, endogenous melatonin levels are very low. At least one procedure that retards aging in animals, i.e., food restriction, also preserves the melatonin rhythm in later life. In humans, several neurodegenerative conditions which involve free radical damage correlate positively with low blood melatonin levels.

The reduction in melatonin throughout life may be relevant to the degenerative processes of aging because melatonin is a direct and indirect antioxidant. Melatonin is an effective hydroxyl (• OH) and peroxyl (LOO•) radical scavenger and a powerful inhibitor of oxidative processes. Thus, pharmacologically administered melatonin reduces damage to DNA following the treatment of animals with the carcinogen safrole and during the exposure of cells to ionizing radiation. Both safrole and ionizing radiation inflict their damage by generating toxic oxygen radicals. Melatonin resists lipid peroxidation in a variety of organs of rats treated with toxicants such as paraquat, carbon tetrachloride, bacterial lipo-polysaccharide, and Fenton reagents, as well as in models of ischemia-reperfusion and Parkinson's disease (MPTP administration). In all of these cases, free radical generation is known to be one of the processes by which these agents damage macromolecules.

Besides its direct free radical scavenging actions, melatonin also works indirectly as an antioxidant by stimulating the antioxidative enzyme glutathione peroxidase (GSH-Px) and seemingly superoxide dismutase (SOD), whose mRNA levels increase after melatonin administration. These enzymes reduce oxidative damage because they either metabolize toxic radicals or their reactive intermediates thereby reducing the associated damage. Most recently we have shown that melatonin influences the fluidity of cell membranes. Changes in membrane fluidity limit the movement of lipid peroxides and restrict the damage they inflict.

If accumulated free radical damage significantly contributes to the degenerative processes of aging, then a reduction in the total antioxidative capacity of the organism due to the loss of melatonin may be a contributing factor to aging. It has already been shown that giving melatonin throughout life increases longevity and maintains animals in better health throughout their life time.

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THE ANTIOXIDANT NETWORK AND AGING. Packer, L. Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720-3200

Free radicals, highly reactive and destructive chemical species, constantly form in almost every cell of the body at an astonishing rate. Antioxidant defense mechanisms have evolved to counteract free radical formation and reactions. Some antioxidants are vitamins or vitamin-forming compounds: vitamin E, vitamin C, and the carotenoids, which must be constantly replenished through the diet. Others, such as glutathione, lipoic acid, and ubiquinols, are manufactured by the body, but their levels can be bolstered through dietary supplementation. Vitamin E is the major chain-breaking antioxidant in membranes and blood lipoproteins, and vitamin C and the thiol antioxidants like glutathione are the major antioxidants in aqueous compartments.

The antioxidant network: Work in our laboratory and others indicates that there is a dynamic interplay among the antioxidant systems. For example, when vitamin E neutralizes a free radical in a membrane, it becomes itself a relatively harmless free radical, the tocopheroxyl radical, which no longer has antioxidant reactivity. However, vitamin C can regenerate vitamin E from the tocopheroxyl radical, in effect "recycling" vitamin E. Vitamin C becomes a radical (the ascorbyl radical) in the process, but it, too, can be recycled by interacting with other antioxidants such as glutathione or dihydrolipoic acid. It has been shown that these interactions occur *in vitro,* in artificial membrane systems such as liposomes and in biological membrane systems such as microsomes, low density lipoprotein, and erythrocyte membranes. Nutritional supplementation studies support this idea for the whole organism. Thus, there is emerging a picture of a complex interplay among the defense systems, with the various antioxidants cycles acting to prevent cell damage and disease. Our knowledge is far from complete but these findings already have implications in terms of recommendations for supplementation.

Aging: Individuals are exposed to oxidants from the moment of birth and even before. Evolutionary pressure optimizes antioxidant systems only toward survival long enough to reproduce, not necessarily toward long-term survival. This gap between antioxidant defenses and oxidant attack may explain the apparent oxidative basis of many of the diseases that appear in later life. I will discuss age-related diseases which are thought to have an oxidative component that does not appear until later in life. If oxidants cause pathology and antioxidants neutralize free radicals, then increasing levels of antioxidants should decrease pathology. Epidemiological and laboratory studies almost unanimously confirm this conclusion. Epidemiological studies consistently show that people who consume high dietary levels of vitamin C, vitamin E, and beta-carotene have lower risk of cancer and heart disease, and may even have a greater life expectancy, than those who consume low levels.

To optimize the body's antioxidant defenses we must understand physiologically functional foods and the major antioxidants. These include the major lipid-soluble antioxidants, vitamin E and ubiquinones, and the major water-soluble antioxidants, vitamin C and the thiol antioxidants such as glutathione and a-lipoic acid. Other key antioxi-

dants include the polyphenols, flavonoids, carotenoids, and other phytochemicals. Work from our laboratory and others indicates that antioxidants are interlinked in a complex series of interactive cycles, and underscores the importance of a comprehensive approach when analyzing aging, antioxidant defenses, and oxidative damage, as well as when approaching any measures aimed at preventing oxidative damage, such as dietary or other supplementation.

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IS THE EXTENSION OF LIFE SPAN OF RATS BY DEPRENYL CAUSALLY RELATED TO ITS EFFECT ON ANTIOXIDANT ENZYME ACTIVITIES? Kitani*, K.[1], Kanai, K.[2], Miyasaka, K.[2], Ivy, G.O.[3], and Carrillo, M.C.[2] [1]National Institute for Longevity Science, 36-3, Gengo, Morioka-cho, Obu, Aichi 474, Japan; [2]Department Clinical Physiology, Tokyo Metropolitan Institute Gerontolgy, Tokyo, 173, Japan; [3]University of Toronto at Scarborough, Toronto, Canada.

Recent reports including that of our own have shown that deprenyl increases the average life span (in some studies, the maximum) of rats, and that antioxidant enzyme activities [i.e., superoxide dismutase (SOD) and catalase (CAT) activities] in selective brain regions in the primarily dopaminergic system. The thesis originally proposed by Knoll that the latter is at least a partial cause for the former observation remains unresolved and difficult to prove experimentally. The present study aimed to elucidate this problem by examining the dose-efficacy relationships of these two effects.

Various doses of deprenyl (0.25mg/kg - 1.0 mg/kg, 3 times a week, s.c.) were administered in aging rats (18 to 26 months) for long term periods ranging from 3 months to 13 months. Antioxidant enzyme activities were determined after sacrifice. In other groups of rats, deprenyl was also administered in a similar manner beginning at 18 months of age, and the survival of animals was compared with saline injected controls.

While in our previous study, a 0.5 mg/kg (3x, a week) dose which was begun at the age of 18 months in Fischer-344 male (F-344) rats revealed a significant (34%) increase in the average life expectancy after 24 months of age, rats given a two times higher dose (1.0 mg/kg, 3x, a week) tended to die earlier than control animals showing after 13 months of treatment, 7 survivals among 12 animals in control rats in contrast with only 3 survivals among 12 at the age of 31 months. When the surviving animals were sacrificed at this age, SOD and CAT activities in all brain regions examined including the dopaminergic system were almost identical for control and treated groups. In contrast, F-344 female rats that were treated at a dose of 0.25 mg/kg (3x, a week), which was found to be an optimal dose for increasing enzyme activities in an experiment with a 6-month-treatment turned out to be significantly effective in increasing the average life expectancy after 24 months, when the treatment was started at 18 months.

These results, together with our past studies on mice showing much less effect on both life span and enzyme activities than was observed in rats, demonstrate that the effective doses of deprenyl for increasing enzyme activities and life span extension are in the same range in long term treatment experiments, showing that an excessive dose is ineffective for both effects and thus suggesting the possibility that the two effects are causally related to each other.

Furthermore, our mouse studies are in agreement with previously published works showing no significant effect in life span studies, suggesting that a greater difficulty in keeping enzyme activities higher than control animals in long term treatments, in mice, may be at least partial explanation for these past as well as our recent studies for life spans.

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AGE RELATED CHANGES IN LIPID PEROXID-ATION AND ANTIOXIDANT DEFENSE IN FISCHER 344 RATS. Bagchi*, M., Bagchi, D., Patterson, E.B., Tang, L., and Stohs, S.J. Creighton University Health Sciences Center and Department of Biomedical Sciences, Omaha, NE 68178.

Oxidative stress is believed to be associated with the aging process, and changes in antioxidant defenses may be involved in aging. In the present study, age-related alterations in antioxidant capacity including superoxide dismutase (SOD) and catalase (CAT) activities, glutathione (GSH) content, and lipid peroxidation (LP) were measured in the heart and liver of Fischer 344 rats at 4, 16, 22 and 29 months of age. LP was 2.4 to 3.5-fold higher in heart and liver tissues of 29 month-old rats as compared to 4 month old rats. CAT activity decreased by 42% to 63% in heart and liver subcellular fractions in 29 month-old rats. Small age-related changes were observed in SOD activity of liver but not in heart of aged rats. GSH content also decreased 25% to 77% in heart and liver of old rats. Protein kinase C (PKC) may be a target of ROS and altered cell proliferation, and therefore PKC was assessed. The total PKC activity in heart and liver of old Fischer 344 rats was approximately 1.5- to 2.0-fold higher than young rats. Aging significantly increased PKC activity in heart and liver. Heat shock protein formation increased with age as confirmed with the western blot by HSP-90 antibody in heart, liver, brain and retinal tissues. These results demonstrate that oxidative stress increases with age in heart and other tissues of Fischer 344 rats.

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UNDERSTANDING AND DELAYING AGING. Ames, B.N. University of California, Division of Biochemistry and Molecular Biology, Berkeley, CA 94720.

Aging appears to be due, in good part, to mutagenic oxidants that are by-products of normal metabolism (1). These oxidants, such as hydrogen peroxide, are the same mutagens that are produced by radiation, and cause damage to DNA, proteins, and lipids. The DNA in each cell of a normal rat receives on average about 100,000 oxidative lesions per day. DNA-repair enzymes continuously remove these lesions, but they do not keep up: an old rat has over one million oxidative lesions in the DNA of each cell. A human cell receives about ten times fewer lesions than a rat cell, which is consistent with the earlier age at which cancer appears, and shorter life span, in the rat (1). Mitochondrial decay, due to oxidation, appears to be a major contributor to cellular aging (2). Progress in reversing this oxidation will be discussed.

Diet has a major impact on the degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, and brain dysfunction, which have, in good part, an oxidative origin (1). Dietary antioxidants, such as Vitamins C and E and carotenoids, play a major role in minimizing oxidative damage; however, much of the world's population consumes inadequate amounts of them (1). Insufficiency of dietary antioxidants causes the same oxidative damage to DNA as radiation (1). g-tocopherol, lycopene, and plant phenolics will be discussed as electrophile traps and as complements to a-tocopherol and other antioxidants. The main source of dietary antioxidants, electrophile traps, and other essential vitamins, is fruits and vegetables. The quarter of the American population that eats the least fruits and vegetables has over twice the rate of most types of cancer as the quarter eating the most, as shown by about 200 epidemiological studies that are remarkably consistent (1,2,4). Thus a high percentage of the population, particularly the poor and their children, is eating insufficient fruits and vegetables (5 portions a day is advised). This lack also leads to a deficiency in other essential micronutrients including the vitamin folic acid. Folic acid deficiency causes cancer, heart disease, birth defects, and brain dysfunction (3). The chromosome breaks from folic acid deficiency, which could contribute to the other pathologies, are due to massive uracil incorporation (8M/cell) into human DNA resulting in nicks and breaks during repair.

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SURVIVAL OF RATS ON STANDARD LABORATORY DIET FED *AD LIBITUM* OR UNDER 30% RESTRICTION COMPARED TO A HIGH OAT FIBER DIET FED *AD LIBITUM*. Ingram*, D.K., and Roth, G.S. Gerontology Research Center, National Institute on Aging

Beginning about 4-5 months of age, male Wistar rats were fed NIH-07 rat chow at *ad libitum* levels or diet-restricted at levels 30% below ad libitum levels. A third group of rats was fed *ad libitum* on a special diet that contained 40% oat fiber. Body weight growth of rats on the normal diet fed *ad libitum* was much greater compared to either other diet groups. The diet-restricted group showed the least body weight gain with the oat fiber diet group at an intermediate level. Analysis of survival indicated the expected increase in mean and maximum survival of the diet restricted rats compared to the rats fed the normal diet *ad libitum*. However, rats fed the oat fiber diet showed significant increases in mean

and maximum survival compared to rats fed *ad libitum* on the normal diet despite the fact that food intake had been about 10% greater among those on the oat fiber diet compared to the normal NIH-07 diet.

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AGE-ASSOCIATED DECLINE OF ELECTRON TRANSPORT SYSTEM ACTIVITY AND RNA LEVELS IN *DROSOPHILA MELANOGASTER*. Schwarze*, S.R.[1], Feuers, R.J.[2], and Aiken, J.M.[1] [1]Department of Animal Health and Biomedical Sciences, University of Wisconsin, Madison, WI 53706 and the [2]National Center for Toxicological Research, Jefferson, AR 72079.

Insect aging is characterized by a reduction in fertility, decreased fitness, flight and locomotor activity. It has been proposed that mitochondrial dysfunction is responsible for the decreased physiological capacity in aging organisms. We, therefore, have analyzed various ages of adult male *Drosophila melanogaster* for electron transport system (ETS) function. The four multi-subunit complexes which comprise the ETS are products of both nuclear and mitochondrial origin, with the exception of complex II which is entirely nuclear encoded. Activity of complexes I, II, III and IV were measured spectrophotometrically and 45-day-old flies exhibited age-associated declines of 58%, 74%, 61%, and 84%, respectively, compared to one-day-old adults.

To determine if the decrease in ETS activity was due to declines in RNA levels for protein subunits comprising the complexes, Northern blot analysis was performed on total RNA extracted from flies 1, 15, 30, and 45 days of age. The abundance of steady-state levels of the mitochondrial-encoded transcripts NADH I, cytochrome *b*, cytochrome oxidase I and 16S mtRNA declined 5-10 fold during aging, similar to decreases observed in enzyme activity. Furthermore, steady-state levels of nuclear-encoded ETS transcripts of the succinate dehydrogenase Fe-S subunit and the b-ATPase component, decline 55% and 78%, respectively. Our data suggest that decreased abundances of both mitochondrial and nuclear ETS subunit RNAs are responsible for the age-related ETS decline and that mitochondrial gene expression may be a critical factor in determining *D. melanogaster* lifespan.

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EFFECTS OF OXIDATIVE STRESS ON EXPRESSION OF CYTOKINES BY PERIPHERAL BLOOD MONONUCLEAR CELLS IN RHESUS MONKEYS. Kim*,M.J.[1] Aiken, J.M.[2], Ershler, W.B.[3], and Weindruch, R.[3] Departments of [1]Nutritional Sciences, [2]Animal Health and Biomedical Sciences and [3]Medicine and VA-GRECC, University of Wisconsin-Madison, Madison, WI 53706.

Cytokines serve critical functions in immune responses. The plasma levels of several cytokines are altered with age and certain age-associated diseases. We have shown that dietary restriction (DR) attenuates interleukin-6 (IL-6) protein overproduction with advancing age in mice. Recently our data have shown that oxidative stress induces IL-6 production by peripheral blood mononuclear cells (PBMC) in rhesus monkeys. PBMC from old monkeys produce more IL-6 in response to this oxidative stress than those from young monkeys. DR attenuates IL-6 production in PBMC exposed to oxidative stress. These results suggest that free radicals may contribute to age-associated dysregulation of cytokines and that DR may attenuate this dysregulation.

To further elucidate the mechanism by which oxidative stress modulates cytokine production, we have investigated its influence on the gene expression of cytokines such as IL-6, interleukin-1 and interleukin-8 (IL-8). These cytokines play major roles in natural immunity such as inflammatory responses. A xanthine and xanthine oxidase (X/XOD) system was used to produce an oxidative burst in the cell culture. PBMC were incubated with or without X/XOD, and the mRNA levels of the cytokines were examined by RNase protection assays. We have shown that the mRNA levels of IL-6 and IL-8 increase with X/XOD treatment in PBMC from young monkeys. The effect of age was studied by examining normally fed young (5-7 yr) and old (>22 yr) rhesus monkeys. Since DR attenuates age-associated changes in IL-6 production and decreases free radical damage, we also investigated the DR effect by studying PBMC from middle aged monkeys (15-18 yr) that have either been fed normally or have been subjected to six years of DR (70% of *ad lib*).

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MORTALITY-RATE CROSSOVERS WHEN SUICIDE IS GENETICALLY PROGRAMMED. Hirsch[1]*, H.R.[1], Liu, X.[2], and Witten, M.[2] [1]Dept. of Physiology, University of Kentucky, Lexington, KY 40536-0084 & [2]Institute of Gerontology, University of Michigan, Ann Arbor MI 48109-2007.

Liu and Witten[1] examined the impact of a genetically predetermined maximum lifespan on patterns of human mortality. This topic is further explored in the present paper. Liu and Witten[1] considered two distinct population subgroups, one advantaged and the other disadvantaged, the survival function of the former being greater than that of the latter at all ages. They demonstrated mathematically that the existence of a maximum lifespan imposes the condition that mortality-rate functions describing the two groups must cross. Hirsch[2] obtained the same result with the use of different mathematical methods.

Liu and Witten[1] base their calculations on a survival function that is Gompertzian at early ages but departs progressively from the Gompertz form with increasing age, x, and approaches a vertical asymptote at the maximum attainable age, v. The mortality rate approaches infinity at v. In effect, aging is programmed to lead to zero survival.

Here we consider an alternative survival function which is perfectly Gompertzian until age v after which it drops instantaneously to zero. The mortality rate at v is described by an impulse. In this scenario, the condition that there is a genetically determined maximum lifespan is achieved by superimposing programmed suicide at age v on the mortality rate associated with normal Gompertzian aging. In the programmed aging model proposed by Liu and Witten[1], v is the maximum length of human life potential, while in the case of programmed suicide, it is the upper limit of life itself.

The conclusion that the mortality rates of the advantaged and disadvantaged groups must cross rests on the assumption that the survival functions of both are continuous over the closed interval $v \geq x \geq 0$. The suicide survival function does not satisfy this condition; it is discontinuous at age v. Examples of suicide survival functions describing the two groups show that the corresponding mortality-rate functions may cross at an earlier age but need not do so. In particular, the mortality rates of the groups do not cross if it is assumed that they are in constant ratio.

Thus, if the maximum lifespans of the groups are set by continuous survival functions, their mortality-rate functions must cross. However if genetically programmed suicide described by discontinuous survival functions occurs, mortality-rate crossovers are not implied by the existence a maximum lifespan.

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A RANDOMIZED OPEN TRIAL TO ASSESS THE INFLUENCE OF AN ALPHA-LACTABUMIN CONCENTRATE WHEY PROTEIN SUPPLEMENTATION ON THE ENDOGENEOUS MELATONIN SYNTHESIS Postaire*[1], E., Béjot, M.[1], Reiter, R.J.[2] [1]Oenobiol, 59 Bd Exelmans, 75016 Paris, France [2]Department of Cellular and Structural Biology, The University of Texas, Health Science Center, 7703 Floyd Curl Drive, San Antonio, Texas 78284-7762.

The amino acid pattern of human milk provides a high Trp/LNNA ratio, which induce high plasma Trp levels in relation to the bulk of other neutral amino acids, a high Trp transport to the blood-brain-barrier and thus significant serotonin synthesis. Serotonin is further metabolized in the pineal body by acrylaminoacetyltransferase and by acetylserotonin-methyltransferase to melatonin. Melatonin is of importance for the control of day- and night rhythms, serves as an intracellular scavenger of hydroxyl- and peroxyl radicals and influences apoptosis. It is also claimed to be active in the decay of age-dependent nerve degeneration and in preventing tissue damage in the course of tumor progression. Melatonin is specially produced by the pineal gland at night and can reset sleep onset through its synchronizing effect on the internal biological clock. Melatonin is rapidly metabolized by the liver and more than 85% is excreted in urine as 6-sulphatoxymelatonin (6-SMT). Urinary excretion of 6-SMT can therefore serve as a reliable measure of the serum melatonin profile. Design of the study: This study is an open randomized trial comparing the effects of the intake of two doses of a-lactalbumin with or without carbohydrate supplementation, with a Latin square design. This is a monocentre study which included 5 patients. The duration of study treatment was 10 days.

The biotransformation was determined by the increase of 6-SMT urinary excretion in various conditions of supplementation (dosage, carbohydrate intake) or without supplementation, each subject being their own control. Study treatment: Every subject took 0, 2 or 4 of

capsules containing 225 mg of a concentrate of α-lactalbumin (containing 3% of tryptophan) once a day with a glass of warm milk with or without sugar and within the Latin square design, one hour before desired bedtime. Results: Repeated measures ANOVA showed that melatonin secretion was not influenced by time of supplementation within each subject ($F = 0.64$, $df = 4$, $p = 0.81$).

Factorial ANOVA revealed significant rise in melatonin secretion for three subjects (S1, S3 and S5) after supplementation by 4 capsules daily, and one (S2) after supplementation by 2 capsules daily. Carbohydrate intake had no significant effect on 6-SMT excretion in urine. For one subject (S4) no significant difference has been revealed for each supplementation. A nutritional questionnaire indicated that this subject drank a glass of alcohol before bedtime. It is known that acute alcohol consumption (0.6 to 0.8 g/kg body wt dose) decreases circulating tryptophan concentration and availability to the sum of its competitors (Trp/LNNA ratio).

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THE QUANTITATIVE IMPACT OF ONE YEAR INCREMENTS OF AGE ON HOSPITAL RESOURCE UTILIZATION: BIAS OR SERVICE? McClaran*, J., Franco, E., and Berglas, R. McGill Centre for Studies in Aging, c/o Montreal General Hospital, 1650 Cedar Avenue, Room D13-157, Montreal, Quebec, H3G 1A4 Canada.

A total of 495 patients, 246 male and 249 females, mean age 74.6±6.8 years, admitted sequentially to an acute care university hospital constituted the study population. The mean length of stay was 45.00±35.73 days, range 11-269, median=33. Multivariate analysis revealed that each year of age contributed only .07 days to the hospitalization, so that even a 95-year old patient stayed only 2.1 days longer than a similar patient with the same diagnosis, and similar family supports, at the age of 65 (log to base e=3.44). In contrast, a surgical intervention not related to the reason for admission lengthened stay by 20.1 days (log to base e=3.94). A neurological diagnosis contributed 13.86 days (log to base e = 3.81). Having no adult children lengthened stay by 2.78 days (log to base e=3.53). Having no spouse lengthened stay by 1.65 days (log to base e=3.49). Social bias against senior populations has led us to believe that they consume more than their fair share of resources, especially in high technology settings. Our data suggest that utilization is not attributable to age.

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THE CORRELATION OF PHYSICAL ACTIVITY, FIBRINOGEN, AND GLYCATED HEMOGLOBIN IN POSTMENOPAUSAL WOMEN. Delmonico, M.[1], Manfredi*, T.[1], DiPietro, L.[2], Cosmas, A.[3], Dain, J.[1], Riebe, D.[1], and Lamont, L.[1] [1]University of Rhode Island, Kingston, RI, 02881, [2]J.B. Pierce Laboratory and Yale University, New Haven, CT 06519; [3]University of Connecticut, Storrs, CT. 062593.

Menopause is associated with an elevated risk for a host of age-related disorders, including cardiovascular disease (CVD) and diabetes. We reported earlier that age and fitness level had significant effects on glycated hemoglobin (GHbA1c) levels in men and not in women. We suggested that the lack of activity and age effects on GHbA1c levels in women may be due to menopausal status. Recently, epidemiological and clinical cohort studies reported associations between fibrinogen and CVD risk factors, suggesting that fibrinogen is a major independent risk factor. The purpose of this study was to determine the association between physical activity, GHbA1c, a clinical marker of glycation of proteins and glucose sensitivity, and fibrinogen in post menopausal women. We examined a host of physical and blood chemistry measurements in active and inactive postmenopausal women free of CVD and other metabolic-related diseases and not on estrogen therapy. The Yale Physical Activity Survey (YPAS) was used to estimate weekly energy expenditure. A score >175 kcal.kg-1. week. -1 was categorized as "active" and a score of <125 kcal.kg-1.week.-1 was categorized as "inactive" ($p<0.074$). Fibrinogen and GHbA1c did not correlate with weekly energy expenditure; however, fibrinogen correlated strongly with body mass index (BMI) ($r=0.65$) and with waist-to-hip ratio ($r=0.57$), two measurements associated with CVD and diabetes. BMI was considerably higher in the inactive women vs more active women (29.6 $kg.m^2$ vs 24.1 $kg.m^2$). Fibrinogen levels in the inactive vs active groups were 347 mg. dl-1 vs 288 mg. dl-1, respectively. HDL levels were 57 mg.dl-1 and 41.1 mg.dl-1 ($p<0.05$), respectively in the inactive vs active groups. Cholesterol, triglycerides, and LDL levels were considerably higher in the inactive women and all three showed strong inverse correlations with activity level. This study appears to support the contention of our earlier work that physical activity plays a significant role in altering clinical measures of CVD risk in post-menopausal women not on estrogen therapy. Perhaps estrogen provides a protective role regarding risk for metabolic disorders in inactive premenopausal women.

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EFFECT OF WATER EXERCISE ON KNEE FLEXOR AND EXTENSOR PEAK TORQUE, RESTING METABOLIC RATE, AND BODY COMPOSITION IN ELDERLY ADULTS. Kendrick*, Z.V., McGettigan, J.C., Paolone, A.M., Ruoti, R., Biokinetics Research Laboratory, Temple University, Philadelphia, PA 19122.

A 12-week water exercise program (WEP) (Ruoti et al, *J Sports Phys. Therap.*, 1994) using the resistance of water to repetitive maneuvers as a mode of resistive training in elderly adults (mean age± SEM, 65.5±5.2 yrs) was used to determine the effects of water exercise on peak torque of knee flexors and extensors, resting metabolic rate (RMR), and body composition. The age of control subjects was 54.8±6.9 yrs. The WEP consisted of a 10-min warm-up, a 5-min cool-down, and a progressive increase in the duration of the water exercises from 15 to 45 min by 5 weeks of the 12-week WEP. WEP was performed at 60% to 75% of subjects' maximal functional capacity determined from a prescreening graded stress test and monitored by heart rate.

Muscular strength of knee flexors and extensors was evaluated using a Cybex Dynamometer at 60, 120, and 210 degrees/sec. RMR was determined via indirect calorimetry using a SensorMedics metabolic cart. Body density was determined via underwater weighing with percent body fat calculated using the equation of Siri (1956). Data were analyzed using a 2 x 2 ANOVA with repeated measure for time (pre- and post-WEP).

A significant ($p<0.05$) main effect for exercise was observed for peak torque of both knee flexors and extensors. The greatest increases in peak torque following the WEP were observed for the knee extensors (between 14 and 22% greater peak torques for the three velocities over the values for the control subjects). The WEP had no effect on the RMR of subjects (pre-WEP, 1334±81 Kcal·day; post-WEP, 1375±81 Kcal·day). Body mass and percent body fat were not affected by the WEP (body mass: pre-WEP, 68.8±3.7 kg; post-WEP, 68.3±3.4 kg; percent body fat: pre-WEP, 40.7±2.1%; post-WEP, 39.0±1.7%). We concluded that: 1) the WEP of Ruoti et al provided sufficient exercise resistance to improve the peak torque of knee flexors and extensors in elderly adults; and, 2) the WEP had no effect on RMR and body composition.

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[Abstract Withdrawn]

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