32nd ANNUAL MEETING OF THE AMERICAN AGING ASSOCIATION

17th ANNUAL MEETING OF THE AMERICAN COLLEGE OF CLINICAL GERONOTOLOGY

June 6 – 9, 2003
Harbor Court Hotel
Baltimore, Maryland

Nutritional Modulation of Aging and Age-Related Diseases

Session 1:
The Relationship Among Diet, Aging and Age-Related Diseases
Chair: Jose Ordovas

1. Gladys Block: **Current Epidemiology of Diet and Aging-What are we eating now?**
2. Jose Ordovas: **Nutrigenomics and Healthy Aging**
3. Walter Willett: **Balancing Life-Style and Genomics Research for Disease Prevention in Later Life**
4. Carl W. Cotman: **Diet and Brain Aging in a Canine Model**
5. Andrew Greenberg: **The Triad of Diet, Obesity and Insulin Resistance: Molecular and Cellular Mechanisms**
6. Rob Friedland: **Dietary and Genetics Interactions in Alzheimer's Disease**
7. Susan Harris: **Vitamin D and Skeletal Health in Aging**

Session 2:
Oxidative Stress and Inflammation: The Gemini Twins of Aging
Chair: Mark Smith

8. Mark Smith: **Do Amyloid-b and Tau Serve Antioxidant Functions in the Aging and Alzheimer's Brain?**
9. Moussa Youdim: **Oxidative Stress and Inflammation in MPTP Parkinsonism and idiopathic Parkinson's Disease: Studies from cDNA Microarray Gene Expression**
10. Tammy Bray: **NFkB Activation and Diabetes**
11. John F. Keaney, Jr.: **Oxidative Stress. Inflammation and Cardiovascular Disease**
12. Regino Perez-Polo: **Stress Response in the Aged Rat Brain**

Session 3:
The Putative "Age-Friendly" Properties of Fruits and Vegetables
Chair: Mary Ann Lila

13. Mary Ann Lila: **Overview of the Antioxidant and Anti-Inflammatory Properties of Bioflavonoids**
14. Stephan Barnes: **Molecular Basis of the Anti-Inflammatory Properties of Isoflavones**

Session 4:
Can the Antioxidant and Anti-Inflammatory Properties of Fruits, Vegetables and Drinks be Expressed as Beneficial Effects in Aging and Age-Related Diseases?
Chair: Rui Hai Liu

Part 1: Fruits and Vegetables

15. Rui Hai Liu: **Health Benefits of Phytochemicals of Fruits and Vegetables**
16. James Joseph: **The Role of Fruit Polyphenolics in Brain Aging**
17. Kuresh Youdim: **Interaction Between Dietary Flavonoids and the Brain Barrier**
18. Helen Kim: **Proteomics Identification of Protein Targets of Polyphenols in Rodent Brain**
19. John Milner: **Mechanisms by Which Garlic and Allyl Sulfur Compounds Suppress Cancer**

Part 2: Nuts, Tea, Grape Juice, Wine and Cocoa

20. Joan Sabaté: **The Health Benefits of Nuts for an Aging Population**
21. Silvia Mandel: **Green Tea Polyphenol (-) Epigallocatechin-3Gallate; A Novel Neuroprotective and Neurorescue Agent for Treatment of Aging Brain**
22. Joseph Vita: **Tea, Cardiovascular Disease and Endothelial Cell Function** John Folts: **The Anti-Atherogenic Properties of Grape Products**

Paper Session
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23. Kenichi Kitani: **Green Tea Polyphenol (Sunphenon) Prolongs the Average Life Span of Male C57/BL Mice**
24. Susan McGuire: **Improving the Survival and Function of Grafted Dopamine Neurons: The Effect of Dietary Supplementation with Blueberry Extract**
25. Cathy Levenson: **Effect of Dietary Iron on Motor Behavior and Neuronal Death in an Experimental Model of Parkinsonism**
26. Wenzhen Duan: **Dietary Restriction Normalizes Glucose Metabolism and Brain Derived Neurotrophic Factor Levels, Slows Disease Progression and Increases Survival in Huntingtin Mutant Mice**
27. Rolf Martin: **The 2002 Blueberry Health Study: Daily Blueberries Improve Decision-Speed and Age-Related Health Indicators**
28. Isabel Newton: **Caloric Restriction Increases Brain-Derived Neurotrophic Factor Levels in Area Ca1 of the Rat Hippocampus**
29. Mary Ann Ottinger: **Avian Models for Evaluating the Effects of Calorie Restriction**

30. Neena Philips: **Skin Anti-Aging and Anti=Carcinogenic Effects of Lutein and Polypodium Leucotomos**
31. Xenia Tigno: **Age-Related Changes in Metabolic Parameters of Non-Human Primates**
32. Anna Aronis: **Regulation of Redox Parameter and Apoptosis in Calorically Restricted Rodents**
33. Gregory Tinker: **The Effects of Two Years Estrogen Loss and Replacement on Cholinergic Neurons and Cortical Cholinergic Fibers in Monkeys**
34. Aubrey D. N. J. de Grey: **Mutant Mammals with Normal Lifespan: an Underappreciated Resource**

Session 5:
Fats: The Good, the Bad and the Ugly
Chair: Ernst Schaefer

35. Lloyd Horrocks: **Putative "Age Beneficial" Effects of Omega Oils**
36. Penny Kris-Etherton: **Health Benefit of Monounsaturated Fats: Food Based Research on MUFA's**
37. Ernst Schaefer: **Fats: the Good, the Bad and the Ugly**

Session 6:
Supplements in Aging in Age-Related Diseases
Chair: Balz Frei

38. Mohsen Meydani: **Polyphenol Antioxidants in Green Tea and Oats: Potential Roles in Angiogenesis and Atherosclerosis**
39. Paul Bernstein: **Carotenoid-Based Nutritional Interventions Against Age-Related Macular Degeneration: Beyond AREDS**
40. Steve Bondy: **Age-Related Changes in Murine CNS and mRNA Gene Expression are Modulated by Dietary Melatonin**
41. Aron Troen: **The Potential for Neuroprotection through Folate Supplementation and Homocysteine-Lowering in Alzheimer's Disease**
42. Balz Frei: **Antioxidant Vitamins and Chronic Disease: Who Needs Supplements?**
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Annual Luncheon and Awards

2003- Nicolai Awardee – Gregory Tinker
"The Effects of Two Years Estrogen Loss and Replacement on Cholinergic Neurons and Cortical Cholinergic Fibers in Monkeys" from the Department of Anesthesiology and Department of Neurobiology and Anatomy, Wake Forest University School of Medicine, Winston-Salem, NC

2003- Nicolai Awardee – Christa Studzinski
"Why its Harder for an Old Dog to Learn New Tricks" from the Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada

2003-Nicolai Runner Up – Joseph Araujo
"Canine Working Memory Performance is Impaired by Scopolamine in an Age and Task Dependent Manner" from the Department of Pharmacology, University of Toronto, Toronto, ON, Canada

2003 Glenn Awardee – Tracy Ann Perry
"The Glucagon-like Peptides: A New Genre in Therapeutic Targets for Intervention in Age-Related Central & Peripheral Neurodegenerative Disorders" from the Section of Drug Design & Development, Laboratory of Neurosciences, Gerontology Research Center, National Institute on Aging, Nation Institutes of Health, Baltimore, MD

2003 Glenn Runner Up – Rafael de Cabo
"Age and Caloric Restriction Alter the Response to LPS of Peritoneal Macrophages from C57BL/6 Mice" from the Laboratory of Experimental Gerontology NIA, NIH, Baltimore MD and the. Division of Pediatric Surgery, Johns Hopkins University School of Medicine, Baltimore MD

Denham Harman Award Lecture

SIMIN NIKBIN MEYDANI, DVM, PhD
"Age Related Immune Dysregulation:
Molecular Mechanisms and Reversal
by Antioxidant Nutrient Intervention"

Distinguished Achievement Award

WILLIAM DONALD SCHAEFER
Comptroller of Maryland
Former Governor of Maryland

1.

CURRENT EPIDEMIOLOGY OF DIET AND AGING – WHAT ARE WE EATING NOW?

Gladys Block, PhD (P), School of Public Health, University of California, 426 Warren Hall, Berkeley, CA 94720

The 1999-2000 National Health and Nutrition Examination Survey (NHANES 99-00) provides the most recent dietary data from a large nationally representative sample. Trends in energy and nutrient intake across the life span are described. Energy intake decreases considerably with age, resulting in substantial declines in nutrient intake, especially in certain demographic groups. Food group patterns and vitamin supplement usage also vary with age. Certain serum nutrient levels are described, and compared with data from NHANES, 1988-94.

2.

NUTRIGENOMICS, LIPID METABOLISM AND CARDIOVASCULAR DISEASE

Jose M Ordovas, PhD (P) Nutrition and Genomics Laboratory. JM-USDA-HNRCA at Tufts Univ., Boston, MA

Nutrigenomics is an emerging and promising multidisciplinary field that focuses on studying the interactions between nutritional, genetic factors, and health outcomes, using the new technical and conceptual developments derived, in part, from the human genome project. The ultimate goal of nutrigenomics is to elaborate more efficient individual dietary intervention strategies aimed to preventing disease and improving health status. To date, gene-diet interactions have been carried out using the "candidate gene" approach. Our studies using the Framingham Heart study as well as other population and intervention studies have found already significant evidence for interactions between dietary factors, genetic variants and biochemical markers of cardiovascular disease. The traditional approach of recommending low fat, low cholesterol diets for the entire population has been the subject of heated discussion, based on the fact that some populations with relatively high intakes of non-saturated fats have very low rates of cardiovascular diseases and other chronic disorders. Now, we can begin to characterize individuals that may respond better to one type of recommendation or another. Therefore, a low fat, low cholesterol strategy may be especially beneficial in terms of lowering plasma cholesterol levels to those subjects carrying the apoE4 allele at the APOE gene. The levels of HDL are modulated also by dietary, behavioral and genetic factors. We have recently reported that the effect of dietary PUFA intake on HDL-cholesterol concentrations is modulated by a common genetic polymorphism in the promoter region of the APOA1 gene. Thus, subjects carrying the A allele at the –75 G/A polymorphism show an increase on HDL-C concentrations with increased intakes of PUFA; whereas those homozygotes for the more common G allele have the expected lowering on HDL-C levels as the intake of PUFA goes up. We have also found significant interactions between intake of fat and variability at the hepatic lipase locus that could also shed some light to the different ability of certain ethnic groups to adapt to new nutritional environments. At this regard we are carrying gene-diet interactions studies in Singapore, a country inhabited by three ethnic groups (Chinese, Indians and Malays). Our data is being contrasted with those obtained in Framingham in order to gain more understanding about potential gene-diet-ethnicity interactions. This knowledge could pave the way for most successful dietary recommendations based on genetic factors that may help to reduce cardiovascular risk more efficiently than the current universal recommendations

3.

BALANCING LIFESTYLE AND GENOMICS RESEARCH FOR DISEASE PREVENTION IN LATER LIFE.

Walter C. Willett, M.D (P), Harvard School of Public Health

Both genetic and environmental factors, including diet and lifestyle, contribute to cardiovascular diseases, cancers, and other major causes of mortality, but various lines of evidence indicate that environmental factors are most important. This evidence includes observations that rates of these diseases differ dramatically among countries, that migrants from low to high risk countries almost always achieve the disease rates of their new environment, and that studies of twins within countries indicate a greater contribution to risk than genetic factors. Much of the genetic contribution seen in twin studies may be spread across hundreds of polymorphisms, each with a minor contribution that has little practical importance. Overly enthusiastic expectations regarding the benefits of genetic research for disease prevention have the potential to distort research priorities and spending for health. However, integration of new genetic information into epidemiologic studies can help to clarify causal relationships between both lifestyle and genetic risk factors and risks of disease. Thus, a balanced approach should provide the best data to make informed choices about the most effective means to prevent disease.

4.

DIET AND BRAIN AGNG IN A CANINE MODEL.

Cotman, C.W.[1] (P), Head, E[1]., Muggenburg, B.A[2]., and Milgram, N.W[3].

[1]University of California, Irvine, Irvine, CA 92697; [2]Lovelace Respiratory Research Laboratory, Albuquerque, NM 87108-5127; [3]University of Toronto, Scarborough Ontario, Canada M1C 1A4

Advanced age is accompanied by cognitive decline indicative of central nervous system dysfunction. One possibly critical causal factor is oxidative stress. Accordingly, we studied the effects of dietary antioxidants

Abbreviations:
(P) Denotes Presenter
(G) Denotes Post-doctoral Candidate for Glenn Award
(N) Denotes Pre-doctoral Candidate for Nicolai Award

and age in a canine model of aging that parallels the key features of cognitive decline and neuropathology in humans. Old and young animals were placed on either a standard control food, or a food enriched with a broad spectrum of antioxidants and mitochondrial enzymatic cofactors. The dietary components included vitamin E, vitamin C, fruits, vegetables, alpha-lipoic acid and l-carnitine. At multiple time points during the 2.5 year intervention study, changes in different types of learning abilities (landmark discrimination, oddity discrimination, size discrimination and reversal learning, intensity discrimination learning and reversal) and spatial memory were examined. Overall, old animals performed more poorly than young animals. However, this age-associated decline was reduced in the animals fed the enriched food, particularly on the more difficult learning tasks. These results indicate that maintenance on foods fortified with complex mixtures of antioxidants can partially counteract the deleterious effects of aging on cognition. A series of neurobiological studies are currently underway. The first experiments indicate that the diet rich in antioxidants slowed, but did not reverse, senile plaque formation in the aged canines. The largest reduction in Aβ was achieved in posterior cortical regions including the parietal and occipital cortices, which accumulate plaque pathology at later ages in the canine. These regions showed reduced Aβ deposition in the antioxidant-treated aged animals. Thus, a diet rich in antioxidants may slow cognitive decline and reduce senile plaque formation in a higher mammalian species and may be useful for promoting successful brain aging in humans.

5.

THE TRIAD OF DIET, OBESITY AND INSULIN RESISTANCE: MOLECULAR AND CELLULAR MECHANISMS. Andrew S. Greenberg (P), JM-USDA HNRCA at Tufts University, Boston, MA and Susan K Fried, University of Maryland, Baltimore, MD.

One of the major metabolic problems facing aging individuals is the regulation of glucose homeostasis. Both insulin sensitivity and adequacy of insulin secretion can be affected during the aging process. Insulin sensitivity may be regulated and/or decreased by alterations in body composition. Increases in fat mass and/or fat redistribution may promote insulin resistance. Insulin resistance appears to be a central component of the metabolic syndrome, which appears to affect approximately 40% of individuals over the age of 60 years. The metabolic syndrome is increasing at an alarming rate and is a major risk factor for heart disease. As a result of alterations in body composition, adipocyte metabolism may become dysregulated. Enlarged fat cells produce less perilipin and adiponectin but increased quantities of interleukin-6 (IL-6) and tumor necrosis factor (TNF). Perilipin, which coats the surface of intracellular triacylglycerol in adipocytes, blocks the lipolytic actions of lipases. Enlarged adipocytes have reduced perilipin protein levels, which may facilitate increases in fatty acid release (FA). Increased circulating FA promotes insulin resistance in skeletal muscle and liver. Both adiponectin and IL-6 act as hormones released by adipocytes. Adiponectin increases oxidation of fatty acids in peripheral tissues. IL-6 increases adipocyte lipolysis and block insulin actions in peripheral tissues. Thus a reduction in adiponectin and increased production of IL-6 both promote insulin resistance. TNF appears to act locally in adipose tissue and increases adipocyte lipolysis. Regulation of fatty acid metabolism appears to be a critical regulator of insulin sensitivity. The mechanisms that underlie the reduction in insulin secretion with aging remain unclear. Thus actions to maintain fatty acid homeostasis are critical in preventing insulin resistance and countering any reduced insulin secretory capacity that may be associated with aging.

6.

DIETARY AND GENETIC INTERACTIONS IN ALZHEIMER'S DISEASE.

R.P. Friedland (P), F. Traore, S.M. Debanne, T. Fritsch, A.J. Lerner, K.A. Smyth, G.J. Petot. Laboratory of Neurogeriatrics, Dept. of Neurology, Case Western Reserve University, University Hospitals of Cleveland.

Genetic factors, especially the apolipoprotein E (Apo E) e4 allele, are important determinants of Alzheimer's disease (AD). However, many people with the e4 allele do not get the disease and many AD patients do not have the e4 allele. The role of Apo E as a lipid transport protein suggests that lipid homeostasis is an important factor in the disease. In our CWRU case-control study of 121 AD cases and 227 controls we investigated genetic and environmental risk and protective factors and their interactions. Diet was studied in mid-adult life using a life history questionnaire adapted from the Block Health Habits and History instrument. We have found that: 1) controls with Apo E e4 consumed less fat than controls without e4 ($p < .01$); 2) higher fat intake was protective in those without e4 [OR = 0.42 (95%CI 0.2-0.89)]; 3) a diet pattern identified from factor analysis characterized by low fat/high antioxidant intake was protective [OR = 0.45 (.21-.96) for highest tertile); 4) midlife BMI and fish consumption were not related to disease, and 5) plasma total homocysteine values were also not related to disease. Other important issues to be considered include physical and mental exercise, smoking, education and occupational demands. Lipid levels may influence the crucial process of clearance of the amyloid Beta protein from brain to blood through competition for binding sites on Apo E. Genetic factors and diet and their interactions are important determinants in the causal web of AD.

7.

VITAMIN D AND SKELETAL HEALTH IN AGING

Susan Harris, DSc (P), New England Research Institute

Abstract not available

8.

DO AMYLOID-ß AND TAU SERVE ANTIOXIDANT FUNCTIONS IN THE AGING AND ALZHEIMER BRAIN?

M.A. Smith[1] (P), G. Casadesus[2], J.A. Joseph[2], G. Perry[1]. [1]Institute of Pathology, Case Western Reserve University, [2]USDA-Human Nutrition Research Center on Aging at Tufts University.

Amyloid-ß and tau, the major components of senile plaques and neurofibrillary tangles, respectively, have historically been considered central mediators of the pathogenesis of Alzheimer disease. However, efforts to understand disease mechanisms through understanding either the processes involved in amyloid-ß deposition as senile plaques or on the phosphorylation and aggregation of tau as neurofibrillary tangles may be inappropriate. In fact, rather than initiators of disease pathogenesis, both lesions occur consequent to oxidative stress as relatively later events in neuronal pathogenesis. Moreover, there is increasing evidence that the lesions function as a primary line of antioxidant defense in both the aged and diseased brain. Given this, it is perhaps not surprising that the increased sensitivity to oxidative stress in the aged brain, even in control individuals, is invariably marked by the appearance of both amyloid-ß and tau. Additionally, in Alzheimer disease, where chronic oxidative stress persists and is superimposed upon an age-related vulnerable environment, one would predict, and there is, an increased lesion load. The notion that amyloid-ß and tau function as protective components brings into serious question the rationale of current therapeutic efforts targeted toward lesion removal.

9.

OXIDATIVE STRESS AND INFLAMMATION IN MPTP PARKINSONISM AND IDIOPATHIC PARKINSON'S DISEASE: STUDIES FROM cDNA MICROARRAY GENE EXPRESSION.

*[1]Moussa B.H. Youdim (P), [2]Gila Maor and Silvia Mandel [1]Eve Topf and US National Parkinson Foundation Centers of Excellence for Neurodegenerative Diseases Research and Department of Pharmacology and [2]Department of Cell Biology, Technion-Faculty of Medicine, Haifa, Israel.

Cell death whether it occurs in systemic organs or CNS involves a set of complex processes, many of which have not been identified biochemically. In Parkinson's disease environmental factors and genetic vulnerability of nigro-striatal dopamine-containing neurons have been implicated. It is possible that both events are involved. At the present biochemical techniques cannot adequately establish this. However, the advent of cDNA microarray or microchips (genomics) and proteomics, by which the expression of thousands of genes and their proteins can be measured at once to give a global assessment of the disease pathology progress, is simplifying this. We have employed these techniques to study the mechanism of neurotoxicity induced by MPTP and 6-hydroxydopamine in neuronally-derived cells in culture and in the animal models of Parkinson's disease, and the neuroprotection initiated by the monoamine oxidase-B inhibitor/anti-Parkinson drug rasagiline, iron chelators (R-apomorphine, green tea polyphenol EGCG and VK-28) and other neuroprotective drugs. Our studies have clearly indicated that MPTP-induced early (first 24 hr) gene expression, prior to nigro-striatal dopamine neuron death, are a prerequisite for the >50 late gene changes implicated at the time of neuronal death. The latter include genes involved in iron metabolism, oxidative stress, inflammatory processes, glutamatergic excitotoxicity, nitric oxide, growth factors, cytokines, transcription factors, cell cycle, apoptosis, intermediatory metabolism and others (huntingtin, prostaglandines and neurotrophic factors), previously not identified. The expression changes of many of the latter genes, also identified by *in-situ* hybridization, is prevented when the animals are pretreated with the aforementioned neuroprotective drugs, such as R-apomorphine, EGCG and rasagiline. These studies clearly show that neurodegeneration is a complex cascade of "domino effect" in which single neuroprotective drug treatment may not be adequate in clinical therapy. But rather, similar to the treatment of cancer, AIDS and cardiovascular diseases, a cocktail of neuroprotective drugs may be of greater effectiveness for the treatment of neurodegenerative diseases. Our goal is to understand the interplay of early gene changes with the process of neurodegeneration and neuroprotective pharmacological activities.

10.

NFκB ACTIVATION AND DIABETES.

Tammy M. Bray (P), College of Health and Human Sciences and LPI, Oregon State University, Corvallis, OR 97331

Although many risk factors can trigger the development of type I diabetes, it is likely that reactive oxygen species (ROS) are involved in ß cell death and disease progression. Development of type 2 diabetes is predominantly genetic predisposed and dietary activated, the pathogenesis of the long term disease outcome is also related to ROS. This presentation will focus on the role of antioxidant defense systems in the susceptibility to type I diabetes and on ROS as cellular messengers that regulate the expression of genes leading to β cell death. Accumulating evidence indicates that increased antioxidant defense systems reduce the susceptibility to diabetes in animal models or in human study. It is suggested that pancreas-specific ROS productions play a critical role in signaling the cellular autoimmune/inflammatory response by activating the transcription factor, NFκB. Various diabetogenic factors may lead to an increase in ROS production, which activates the redox-sensitive NFκB. This may be the initial event for the expression of cytokines and chemotactic agents involved in the inflammatory response. It is believed that this cascade results in a cyclic amplification of ROS and eventually leads to apoptosis and/or necrosis of ß cells. The specificity of antioxidants to inhibit NFκB activation and the hyperglycemic response emphasizes the importance of selectivity in antioxidant therapy. Our understanding of the cellular and mechanistic role of ROS in the etiology of diabetes will help us lead to the development of better prevention strategies.

11.
OXIDATIVE STRESS, INFLAMMATION IN CARDIOVASCULAR DISEASE.

J.F. Keaney, Jr. (P), Boston University School of Medicine

Information regarding cardiovascular risk factors associated with systemic oxidative stress and inflammation has largely been derived from highly selected samples with advanced stages of vascular disease. Thus, it has been difficult to evaluate the relative contribution of each cardiovascular risk factor to both systemic oxidative stress and inflammation to determine if such risk factors act independently and are applicable to the general population. We examined subjects from the Framingham Heart Study and measured systemic markers of both oxidative stress and inflammation. Age- and sex-adjusted multivariable regression models were used to assess clinical correlates of oxidative stress and inflammation. In age- and sex-adjusted models, increased markers of oxidative stress and inflammation were positively associated with a number of established risk factors for cardiovascular disease as well as body mass index. In contrast, some well-known risk factors for cardiovascular disease were not associated with oxidative stress such as age, total cholesterol, and hypertension. These data suggest the relation between cardiovascular disease, oxidative stress, and inflammation is complex and warrants evaluation in large-scale studies. Moreover, these data also implicate obesity as a state of heightened oxidative stress and inflammation.

12.
STRESS RESPONSES IN THE AGED RAT BRAIN.

J. Regino Perez-Polo, (P) University of Texas Medical Branch, Galveston, Texas

The aged nervous system displays both impaired cognitive functions and recovery from challenges to its ability to maintain homeostasis. Our hypothesis is that aging-associated chronic oxidative stress serves to alter the dynamic ability for the nervous system to respond to acute insults and return to energy homeostatic setpoints. Here we examine the response of aged rat hippocampi to normobaric hyperoxia treatments and demonstrate an attenuation in the DNA binding activity of the NF-kB transcription factors, which are important components of stress response signal transduction pathways and can determine shifts in cellular commitments to necrosis, apoptosis, or functional recovery in the central nervous system. Hyperoxia is an oxidative stressor that triggers signaling cascades via changes in promoter activation by transcription factors. The transcription factor NF-kB has been shown to regulate transcription of many genes that play a role in inflammation and recovery from acute or chronic trauma. The aged-associated changes in NF-kB action may account for some changes in cognitive function.

13.
OVERVIEW OF THE ANTIOXIDANT AND ANTI-INFLAMMATORY PROPERTIES OF BIOFLAVONOIDS.

Mary Ann Lila (P), University of Illinois, Urbana, IL 61801

The flavonoids are a category of polyphenolic phytochemicals that are naturally-occurring and widely available in a wide range of edible plant foods including fruits, vegetables, nuts, honey, and teas. A typical daily intake in a healthy diet is 1-2 g. Plants synthesize flavonoids as a defensive reaction to stress (UV light, extreme temperatures, etc.), or to help attract pollinators, but for the humans that consume flavonoid-rich plants, these extranutritional constituents (a.k.a. bioflavonoids) exert significant preventative, health-promoting, and therapeutic properties for health maintenance. The best known and most widely publicized property of flavonoids is the antioxidant capacity, yet flavonoids can also act as prooxidants, enzyme inhibitors, antiadhesins, enzyme activators, anti-inflammatory agents, antiproliferative agents, vasoprotective agents, and more. These versatile compounds have unfortunately been extremely difficult to study due to their large, complex structures, ephemeral nature, and the potentiation effects created when different compounds interact to exert biological activity. Creative labeling of key flavonoids prior to feeding may provide a means to do the metabolic tracking and quantification necessary to making valid recommendations for consumption.

14.
MOLECULAR BASIS OF THE ANTI-INFLAMMATORY PROPERTIES OF ISOFLAVONES.

S. Barnes (P), T. D'Alessandro, J. Prasain, R. Moore II, M. Kirk, N.P. Botting, R.P. Patel, V.M. Darley-Usmar, Departments of Pharmacology & Toxicology and Pathology, Purdue-UAB Botanicals Center for Age-Related Research, and Comprehensive Cancer Center Mass Spectrometry Shared Facility, University of Alabama at Birmingham, Birmingham, AL 35294, and Department of Chemistry, St. Andrews University, Fife, Scotland.

Isoflavones are members of the polyphenol family and are present in a limited number of foods, principally soy-derived products Their consumption is associated with a lowered incidence of chronic diseases such as atherosclerosis, cancer, cognitive decline and osteoporosis. In many of these diseases, inflammation is an important associated event. Polyphenols have long been assigned antioxidant and antiinflammatory properties. However, there is a discordance between their in vitro properties and their effects in vivo - the concentrations needed in vitro far exceed the amounts present in the blood. Since inflammation is a localized event around inflammatory cells, we have examined the hypothesis that circulating isoflavones alter the chemistry of that local environment and in doing so are converted to new pharmacophores. Isoflavones contain two aromatic rings both of which are analogous to the phenolic tyrosine residues on proteins. They are converted to relatively stable free radicals and can in turn react with other antioxidants such as vitamin C, thereby increasing their antioxidant potency. In mod-

els of inflammation, protein tyrosines in the vicinity of the inflammatory cells are chlorinated (by HOCl generated by neutrophils) and nitrated (by peroxynitrite generated by several inflammatory cell types). The addition of HOCl and peroxynitrite to isoflavones leads to rapid chlorination and nitration. Activation of neutrophils by phorbol esters results in rapid chlorination of isoflavones. In a rat lipopolysaccharide model of cholestasis, extensive nitration of genistein is observed in the lung. Chlorinated daidzein and genistein are more potent antioxidants in models of LDL oxidation. However, they have 1-2 orders of magnitude lower effects on estrogen receptor-dependent gene expression than their parent compounds. In summary, a rationale for the larger biological effects of isoflavones than can be detected in vitro was developed - this may arise from (1) synergistic interaction with other antioxidants, (2) conversion at local sites of inflammation to stronger antioxidants, and (3) formation of weaker estrogenic metabolites. These events may each have important roles in the prevention of aging

15.

HEALTH BENEFITS OF PHYTOCHEMICALS OF FRUITS AND VEGETABLES.

Rui Hai Liu, M.D., Ph.D. (P) Department of Food Science, 108 Stocking Hall, Cornell University, Ithaca, NY 14853

Epidemiological studies have consistently shown that regular consumption of fruits and vegetables is associated with reduced risk of chronic diseases, such as cancers, cardiovascular disease, stroke, Alzheimer's disease, cataracts and some of the functional declines associated with aging. In 1989, a report from the National Academy of Sciences on diet and health recommended consuming five or more servings of fruits and vegetables daily for reducing the risk of both cancer and heart disease. The Five-a-Day program was developed as a tool to increase public awareness of the health benefits of fruit and vegetable consumption and promote adequate intakes of known vitamins. Plant-based foods, such as fruits, vegetables and whole grains, which contain significant amounts of bioactive phytochemicals, may provide desirable health benefits beyond basic nutrition to reduce the risk of chronic diseases. It is now widely believed that the actions of the antioxidant nutrients alone do not explain the observed health benefits of diets rich in fruits and vegetables because taken alone, the individual antioxidants studied in clinical trials do not appear to have consistent preventive effects. Work performed by our group and others have shown that fruit and vegetable phytochemical extracts exhibit strong antioxidant and antiproliferative activities; we proposed that the additive and synergistic effects of phytochemicals in fruits and vegetables are responsible for these potent antioxidant and anticancer activities, and that the benefit of a diet rich in fruits and vegetables is attributed to the complex mixture of phytochemicals present in whole foods. Our hypothesis may explain why no single antioxidant can replace the combination of natural phytochemicals in fruits and vegetables to achieve the health benefits. We believe that the evidence suggests that antioxidants are best acquired through whole food consumption.

16.

THE ROLE OF FRUIT POLYPHENOLICS IN BRAIN AGING.

James A. Joseph[1](P), Gemma Casadesus[1], Rachel L. Galli[2] and Barbara Shukitt-Hale[2]

[1]United States Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111; [2]Department of Psychology, Simmons College, Boston, MA 02115

Dietary supplementation (S) with fruit or vegetable extracts high in antioxidants (e.g., blueberry, BB, spinach) can decrease the enhanced vulnerability to oxidative stress (OS) that occurs in aging and these reductions are expressed as improvements in neuronal signaling and behavioral. In addition, recent examinations using striatal or hippocampal tissue isolated from BB supplemented aged animals, have shown that striatal slices show reductions in H_2O_2-induced decrements in muscarinic receptor sensitivity decrements, while the hippocampal slices show decreases in baseline levels of HSP-70 and increases in HSP-70 responsiveness to lipopolysaccharide (LPS) exposure. Moreover, there are also indications that BB supplementation can also reduce the sensitivity to neurotoxic agents (kainic acid) that induce oxidative stress and inflammation. Additional experiments suggest that BB effects also may include enhancement of neuronal signaling, and that these increases can offset the putative deleterious consequences of amyloid beta deposition in APP/PS-1 mice. Therefore, it appears that polyphenolic compounds such as those found in BB may exert their beneficial effects by enhancing the endogenous anti-inflammatory, antioxidant and neuronal signaling capabilities of the organism. In addition, recent work from our laboratory has indicated that one of the most striking effects of BB supplementation may involve increases in neurogenesis. It is known that factors such as head injury, depression and stress that lead to decreases in neurogenesis are all associated with greater rates of cognitive decline. Conversely, exercise and environmental enrichment can improve both neurogenesis and cognitive function in aging. The results of our recent work has indicated that aged BB-supplemented rats, tested in the radial arm water maze (RAWM) and given injections of BrdU showed that the number of proliferating cells in the dentate gyrus were significantly higher ($p < 0.05$) in the BB-fed rats. Moreover, these findings were correlated with improvements in the RAWM performance such that as the number of proliferated cells increased, the number of memory errors decreased (reference memory errors: $r = -0.654$, $p < .05$, working memory errors: $r = -0.646$, $p < .05$, total memory errors: $r = -0.587$, $p = .08$). Subsequent research has indicated anthocyanins from BB supplementation can localize in various brain regions and that the number of the anthocyanins that localize in regions such as the cortex are negatively correlated with the amount of errors made in the Morris water maze on the probe trials. Taken together, these findings suggest that antioxidant-rich fruits such as BBs may improve cognitive performance by increasing proliferation of neural precursor cells in the hippocampus (i.e., RAM Kempermann, 2002) and en-

hancing signaling in areas such as cortex, hippocampus, and striatum which may offset deficits in in aging or via genetic modifications.

17.

INTERACTION BETWEEN DIETARY FLAVONOIDS AND THE BRAIN BARRIER.

Kuresh Youdim, PhD (P), M. Zeeshan Qaiser, Catherine A. Rice-Evans & N. Joan Abbott

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Recent studies highlight an exciting role with respect to the neuroprotective actions of dietary flavonoids. The mechanisms associated with flavonoid neuroprotection is complicated by the lack of information about their ability to enter the CNS. However, we have shown recently, using an *in vitro* blood-brain barrier (BBB) model (ECV304 co-cultured with C6 glioma cells) that flavonoids including their physiologically relevant metabolites exhibit high apparent permeability (P_{app}) across the brain endothelium. Using [^{3}H] naringenin and [^{14}C] quercetin as model substrates we have also shown *in vivo*, accumulation into 7 brain regions (cerebellum, cortex, hippocampus, hypothalamus, striatum, superior colliculus, and medulla). Total uptake (K_{in} µL min^{-1} g^{-1}) of naringenin was high in all regions studied, suggesting significant passive permeability. In contrast quercetin uptake was comparable to the P-glycoprotein (P-gp) efflux transporter substrate, colchicine. The potential influence of efflux transporters in mediating quercetin permeability *in vivo,* was studied by pre-administering animals with P-gp inhibitors PSC833 and GF120918 (10mg/kg). Only GF120918 significantly effected uptake. A closer examination of the role played by efflux transporters on flavonoid flux across the BBB was performed using MDCK-MDR1 and rat brain endothelial cells (RBE4) (both expressing functional P-gp). Our in vitro findings support observations in vivo, where flavonmoid accumulation was greatest in cells exposed to GF120918. In conclusion, these studies demonstrate that flavonoids are able to permeate the BBB but that some are possible substrates for efflux transporters, which limit their CNS bioavailability.

18.

PROTEOMICS IDENTIFICATION OF PROTEIN TARGETS OF POLYPHENOLS IN RODENT BRAIN.

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Many botanically-derived dietary supplements are thought to have health benefits due to the anti-oxidant activities of constituent polyphenolic compounds; examples of such polyphenols are the catechin in teas, the procyanidins in grape seeds, and the isoflavones in soy. Previous findings by others indicated that oxidative stress is a risk factor for age-related cognitive decline, as well as for Alzheimer's disease.

We hypothesized that dietary supplementation with polyphenols would have neuroprotective actions in rodent brain that would be manifested as either enhancement of expression or modifications of proteins important for neuronal viability, and/or reduction of expression or modifications of, proteins linked with neuropathology. Normal female adult rats were maintained on 5% grape seed extract-supplemented diets for six weeks, after which they were sacrificed, and homogenates of their whole brains analyzed by proteomics technology (2-dimensional electrphoresis followed by mass spectrometry) to assess protein differences between the two sets of brain homogenates. Software-assisted image analysis of the 2D gel images identified several gel "spots" that quantitatively differed in intensity or in horizontal position between the two sets of gels representing the dietary groups. Matrix-assisted laser-desorption ionization time of flight mass spectrometry (MALDI-TOF MS) followed by quadrupole time of flight mass spectrometry (Qtof MS) identified the proteins contained in the gel spots of interest. With the exception of one novel protein, and a cytoskeletal protein, all the proteins identified in this study were previously implicated in either Alzheimer's disease, or mouse models of neurodegeneration. Moreover, the direction of the differences determined in this study were in the opposite direction to those detected for these proteins in the disease tissues. These results strongly suggest that ingestion of components in grape seed extract was neuroprotective.

19.

MECHANISMS BY WHICH GARLIC AND ALLYL SULFUR COMPOUNDS SUPPRESS CANCER.

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Garlic [Allium sativum] is among the oldest of all cultivated plants. Throughout history it has been revered for its medicinal properties with benefits proposed to encompass antimicrobial, antithrombotic, hypolipidemic, antiarthritic, hypoglycemic and antitumor effects. In some parts of the world the sales of garlic preparations ranks with those of leading prescription drugs. A variety of epidemiological and preclinical studies support the ability of allium foods, particularly garlic, to retard the cancer process. While epidemiological evidence suggests allium foods may retard the risk of cancer at several sites the strongest evidence exists for the gastrointestinal tract. It is unclear if the variability in epidemiological observations relates to the quantity, duration or to the type of garlic preparation provided or maybe to a modified response resulting from interactions with a host of dietary or environmental factors or with the consumer's genetic (nutrigenetic) profile. Nevertheless, a recent report suggests that reduced risk of prostate cancer that was associated with enhanced

allium vegetables consumption was independent of body size, intake of other foods, and total calorie intake. Fresh garlic extracts, deodorized garlic preparations, garlic oil and a number of individual organosulfur compounds derived from garlic have been found to modify one or more aspects of the cancer process in model systems. While the mechanism(s) by which garlic and its organosulfur constituents brings about these effects remains elusive several mechanisms are plausible. Among the possible mechanisms are alterations in drug metabolizing enzymes, reduced oxidative damage, suppressed tumor proliferation, enhanced apoptosis and suppressed angiogenesis. The shifts in carcinogen metabolism are frequently accompanied by a reduction in the activity of some cytochrome P450s enzymes, an increase in the activity of glutathione S-transferases carcinogens and an accompanying reduction in DNA adducts in several target tissues. Carcinogen induction studies reveal that the benefits of garlic are not limited to a specific species, to a particular tissue, or to a specific type of carcinogen. The antiproliferative activity associated with garlic and related organosulfur compounds has been observed in several tumor cell lines and may relate to changes in cell cycle regulation resulting from a reduction in p34 (cdc2) kinase activity as a result of posttranslational changes in phosphorylation. The nutrigenomics effects of selected allyl sulfur compounds are evident by the widespread effects on the expressions of genes involved with various cancer processes. Biochemical analysis of tumors revealed several characteristic features of apoptosis, including the formation of DNA ladders, compaction of nuclear DNA, and the activation of caspase-3. Recently diallyl sulfide (DAS), an organosulfur compound present in garlic was reported to inhibitangiogenesis in tumor bearing mice. The exact molecular target for any of the alteration in the cancer process remains to be determined. Transgenic and knockout models may offer special insights into the sites of action of garlic and its allyl sulfur components. Overall, while compelling preclinical evidence supports garlic and its organic allyl sulfur components are effective inhibitors of the cancer process, considerably more controlled intervention studies in humans are needed to truly define who will benefit from garlic and its components and under what circumstances.

20.

THE HEALTH BENEFITS OF NUTS FOR AN AGING POPULATION.

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Coronary heart disease and diabetes are leading causes of morbidity and mortality in the aging population in the U.S. Four large prospective epidemiological studies have reported consistent and substantial protective effects of nut consumption on risk of CHD. Persons eating nuts 5 or more times per week have a 30 to 50% reduction in risk for myocardial infarction or CHD death, compared to those eating nuts infrequently. The protective effect of nuts on CHD has been found in men and women, adults and the elderly. Also, recent epidemiological findings have linked nut consumption to the prevention of type 2 diabetes.

Presently, there is a growing interest in the scientific community about the mechanisms that may explain the cardioprotective effects of nuts. Nuts are complex plant foods and a rich source of many nutrients. The matrix of nutrients and bioactive substances present in nuts suggests that multiple mechanisms may exert their beneficial effects on cardiovascular risk.

A number of well controlled clinical trials and other less controlled human nutrition studies indicate that the inclusion of nuts in the diet lowers total and LDL cholesterol blood levels and improves the lipoprotein profile. The cholesterol-lowering effect of nut-rich diets seems to be greater than predicted for their fatty acid composition. Thus, other lesser-known constituents in nuts may have also beneficial effects. Nut-rich diets do not seem to affect body weight. Fewer studies have been conducted on other factors that affect cardiovascular disease risk such as the oxidizability of LDL, lipoprotein (a). Available data, however small, also indicates favorable effects of nuts on them.

Additional research is needed to further understand the cardioprotective effect of nuts and mechanisms by which nuts may lower the risk of type 2 diabetes.

21.

GREEN TEA POLYPHENOL (-)-EPIGALLOCATECHIN-3-GALLATE; A NOVEL NEUROPROTECTIVE AND NEURORESCUE AGENT FOR TREATMENT OF AGEING BRAIN.

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We have recently reported that both green tea extract, as well and its main polyphenol constituent, (-)-epigallocatechin-3-gallate (EGCG) possess potent neuroprotective activity in cell culture and in mice MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) model of Parkinson's disease, part of which may be explained by their radical scavenging-iron chelating actions. However, the cell signaling mechanism implicated in this action is unknown. We have extended our in vivo studies to neuronal cell culture employing the neurotoxins, 6-hydroxydopamine (6-OHDA,50μM), 1-methyl-4-phenylpyridinium (MPP^+, 400μM) and amyloid-beta peptides ($A\beta_{1\text{-}25}$, $_{1\text{-}40}$, $_{1\text{-}42}$, 10μM) to induce cell damage. Pretreatment with EGCG (0.1-10μM) markedly attenuated human neuroblastoma (NB) SH-SY5Y and rat PC12 cell death, induced by 24 h exposure to the toxins or by serum withdrawal, as assessed by nucleosome formation and mitochondrial function analysis. EGCG was able not only to prevent, but also to rescue the neurons when applied after Aβ–induced cell damage or 4 days after serum deprivation. Potential cell signaling candidates involve in this neuroprotective effect were further examined. EGCG restored the reduced protein kinase C (PKC) and extracellular signal-

regulated kinases (ERK1/2) activities, caused by 6-OHDA toxicity. Furthermore, EGCG (0.1-10μM) considerably increased (~8-fold) the secretion of the non-amyloidogenic soluble form of the amyloid precursor protein (sAPPα) into the conditioned media in both cell lines. Thus, EGCG may affect APP metabolism by shifting the balance of α-secretase-mediated APP processing towards a presumably non-pathogenic pathway. The positive effect of EGCG on cell survival and sAPPα secretion, was abolished by pretreatment with PKC inhibitor GF109203X (1μM), indicating the involvement of PKC in the stimulated effect. In addition, customized cDNA microarray expression analysis and confirmatory real-time PCR and protein determination revealed that EGCG decreased the expression of pro-apoptotic Bcl-2-family members Bax, caspase-1, cyclin dependent kinase (cdk) inhibitor p21 (Waf1), mdm2, growth arrest and DNA-damage-inducible protein GADD45β (gadd45β) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) mRNAs. We also demonstrated a concentration-dependent correlation between EGCG structurally-related antioxidant compounds and modulation of cell survival/ cell death-related gene pathways. Given the potent antioxidant, neuroprotective , neurorescue and enhancing sAPPα release properties of EGCG, we suggest that this polyphenol may significantly and beneficially delay the progression of neurodegenerative disorders such as Alzheimer's, Parkinson's and Lewy body diseases.

22.

TEA, CARDIOVASCULAR DISEASE AND ENDOTHELIAL CELL FUNCTION

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Epidemiological studies suggest that consumption of tea and other flavonoid-containing foods is associated with reduced risk of cardiovascular disease events including myocardial infarction and stroke. There also are data to suggest that tea consumption reduces extent of atherosclerosis in the aorta and the risk of recurrent complications following myocardial infarction. The mechanisms responsible for these beneficial effects remain uncertain. Studies that attempted to demonstrate that tea has an antioxidant effect in vivo in human subjects have had mixed and largely negative. These findings have prompted investigators to consider other possible explanations.

One area of particular interest has been the vascular endothelium. Recent studies have emphasized that the endothelium plays a central role in the regulation of vascular homeostasis. Loss of normal endothelial function may promote the development of atherosclerotic lesions and the conversion of quiescent plaques to active plaques that are responsible for acute ischemic syndromes, including myocardial infarction and stroke. There recently has been considerable interest in the effects of certain water-soluble antioxidants on endothelial function. For example, studies indicate that short and long term exposure to ascorbic acid enhance endothelium-dependent vasodilation in patients with coronary artery disease. These studies prompted us to examine the effects of tea consumption on endothelial function. In a randomized crossover clinical trial, we observed that tea consumption improves endothelial function in patients with coronary artery disease. These findings were not attributable to the effects of caffeine and appear to be specific for the endothelium, rather than vascular smooth muscle. Similar findings have been reported for patients with mild hypercholesterolemia, and for other flavonoid-containing beverages. Ongoing studies will exam the effects of specific components in tea in an attempt to determine how tea is exerting this effect. Tea consumption has beneficial effects on other clinically relevant aspects of the cardiovascular system. For example, tea consumption has been shown to reduce systemic markers of platelet activity and collagen-induced platelet aggregation.

Thus, there is growing evidence that tea consumption has effects on endothelial function and possibly platelet function that would tend to reduce the risk of cardiovascular disease. While there are insufficient data to specifically recommend tea consumption for treatment or prevention of coronary artery disease, the evidence is growing. As tea is a vegetable-derived beverage, however, a recommendation to drink tea would be consistent the dietary guidelines of the American Heart Association to increase consumption of fruits and vegetables as a strategy to reduce the risk of heart disease.

23.

THE ANTI-ATHEROGENIC PROPERTIES OF GRAPE PRODUCTS.

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Many factors are thought to stimulate the vascular biology of atherogenesis in multiple interacting ways. Platelets and other cells release mitogenic growth factors, which stimulate Vascular Smooth Cells (VSMC) to multiply and migrate toward the lumen. Healthy endothelial cells (EC's) inhibit the stimulation of VSMC and inhibit the entry of LDL into the wall of the artery. Healthy EC's also secrete Nitric Oxide, which relaxes VSMC and allows the artery to dilate thus improving blood flow. However when the endothelial layer becomes dysfunctional, i.e. Dysfunctional EC'S (DEC's) this antiatherogenic protection is lost. Free radicals, elevated LDL and Homocystein produce DEC's. DEC's do not secrete normal amounts of Nitric Oxide but can secrete Endothelin 1, which promotes atherogenesis and causes vasoconstriction. In addition, DEC's release inflammatory mediators that draw Monocytes into the arterial wall where they become Macrophages and take up LDL, which is oxidized by free radicals. These Macrophages become foam cells and lead to fatty streak formation. We and others have shown that the Flavonoids in Grape products can inhibit platelet aggregation, improve endothelial function, protect LDL from oxidation, inhibit VSMC proliferation and inhibit the activation of Monocytes, which become the Macrophages. Thus Grape Flavonoids from Red Wine, Purple grape juice, or grape seeds and skins may inhibit the initiation and progression of Atherosclerosis by multiple mechanisms. The anti-platelet properties of Flavonoids may also inhibit the platelet-mediated thrombosis that occurs in Acute Coronary Syndromes. The

initiation of experimental atheromas in Hypercholesterolemic animals has also been diminished by feeding them Red Wine or Purple grape juice. Thus the "French Paradox" may be a real phenomenon.

24.

GREEN TEA POLYPHENOL (SUNPHENON) PROLONGS THE AVERAGE LIFE SPAN OF MALE C57/BL MICE.

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Although the Free Radical Theory of Aging initially proposed by Harman half a century ago has been increasingly supported in recent years, a direct proof for the thesis is still lacking. If the administration of a nutritional antioxidant is shown to increase the life span of animals, it may serve as a significant progress for the theory. At the same time, it may also provide a means for prolonging the life span (at least health span) of humans. Despite this expectation, past efforts in this direction have been mostly unsuccessful and the general consensus of experimental gerontology is that the only reproducible means to prolong the life span of animals is caloric restriction.

In our previous work, we reported that an oral administration of tetrahydrocurcumin (a more active antioxidant metabolite of curcumin contained in turmeric) can significantly prolong average life span as well as the 10% longest survival of male C57/BL mice. In the present study, we examined the effect of green tea polyphenols (PP). Male C57/BL mice were imported from Harlan, USA and had been maintained in the clean conventional animal facility until use. Animals were divided into two groups, one, control animals given normal drinking water, the other given water containing green tea PP (Sunphenon1), Taiyokagaku, Yokkaichi, Japan), both pasteurized by g-ray irradiation. Animals started to receive treatments at the age of 13 months. Sunphenon is made from green tea water extract which contained different PPs (>70%).

The average life span (days) of green tea PP fed mice was significantly longer days than that of control animals (801.1±121.5, control vs. 852.7±88.2 PP, each n=50, $P<0.01$, t test). At three continuous months (24~26 months), numbers of surviving animals were significantly greater than corresponding values of control animals ($P<0.01$, chi-square test). On the other hand, the 10% longest survival of animals was not significantly different between the two groups ($P>0.05$). Average body weights were almost identical for the first 10 months of treatment and then tended to be slightly higher in the following several months in PP fed animals; however, a statistically significant difference was observed only at the months of 24 ($P<0.05$). At 32 months, the average body weight was lower in PP fed mice ($P<0.05$). Except for these minor differences, body weights were almost identical for both groups at corresponding months of age.

Green tea PPs have been shown to be potent antioxidants and to be effective in preventing a number of experimentally induced pathologies including atherosclerosis, cancer, etc. Further, a recent epidemiological study also suggested a beneficial effect of drinking green tea in lowering the incidence of malignant neoplasms. The results of the present study have shown for the first time that green tea PPs can prolong the average life span of animals. Since aging mice do not develop many of the age-associated pathologies observed in humans and yet PPs are effective in increasing the life span of these animals, a similar (or even a greater) effect of prolonging the health span of humans is expected to be present for green tea PP. Ref. 1) Sakanaka S et al., Agric Biol Chem 53:2307-2311

25.

IMPROVING THE SURVIVAL AND FUNCTION OF GRAFTED DOPAMINE NEURONS: THE EFFECT OF DIETARY SUPPLEMENTATION WITH BLUEBERRY EXTRACTS.

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Transplantation of embryonic dopamine (DA) neurons into the striatum is a viable treatment for Parkinson's disease (PD). However, transplanted cells survive poorly, with ~90% of transplanted cells dying within the first four days after transplant. Cell death is exacerbated by ~75% in aged animals, resulting in transplants that provide little to no therapeutic benefit. Although the exact mechanism underlying cell death is not known, oxidative stress and inflammation are hypothesized as major contributing factors. Multiple studies have attempted to improve cell survival by pre-treating the cell transplant material with various anti-apoptotic or antioxidant compounds. This study provides evidence that dietary supplementation with blueberry extract (BBE), a fruit extract with antioxidant and anti-inflammatory properties, provides an efficacious, easily administered and well tolerated therapy that can be used to treat the transplant recipient, thus improving survival of the transplanted cells. Young adult (4 months, n=10) and aged rats (24 months, n=3) were unilaterally lesioned with 6-OHDA to deplete striatal DA and allowed to recover from surgery for 2 months. Animals with stable, amphetamine-induced rotational values, indicating unilateral striatal DA depletion, were assigned to one of two dietary treatments that consisted of custom formulated rat chow with or without 2% BBE. After six weeks of dietary treatment, sub-optimal numbers of primary embryonic (gestational day 14) ventral mesencephalic cells, including the developing midbrain DA neurons, were transplanted into the denervated striatum. Rats were assessed for amphetamine-induced rotational behavior at two week intervals for 8 weeks post-transplantation. Young, BBE-fed rats exhibited fewer rotations per minute than did control-fed rats ($P<0.05$), indicating the presence of a functional graft. However, no behavioral benefit was noted in either group of aged rats. Morphological analysis revealed a greater than two-fold in-

crease in DA neuron survival within the grafts in both young and aged BBE-fed rats ($P<0.05$) as assessed by tyrosine hydroxylase immunoreactivity (THir). BBE-fed animals also tended to have increased transplant areas ($P=0.1$) with individual graft-derived neurons exhibiting increased THir ($P=0.1$). These data provide evidence that dietary supplementation of the host with BBE can provide an easily tolerated, non-invasive treatment for the graft recipient that has beneficial effects on neural graft survival and function. Supported by NS 42125 (TJC)

26.

EFFECT OF DIETARY IRON ON MOTOR BEHAVIOR AND NEURONAL DEATH IN AN EXPERIMENTAL MODEL OF PARKINSONISM.

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Because of the role of iron in oxidative processes, it has been speculated that high dietary iron may make neurons more vulnerable to damage and exacerbate neurodegenerative disorders associated with aging. This hypothesis is supported by the fact that high iron levels have been implicated in the development of Parkinson's disease (PD). Thus, this work was designed to test the impact of three levels of dietary iron on motor behavior and the development of an experimental model of PD. Two-month-old male C57BL/6 mice were fed diets containing low (4 ppm), normal (48 ppm) or high (400 ppm) iron for 6 weeks prior to the administration of MPTP, a mitochondrial toxin that causes the death of nigro-striatal dopaminergic neurons and induces PD-like symptoms. As expected, in mice fed the normal iron diet, MPTP impaired motor behavior as determined by the rotarod test ($p<0.05$). The low iron diet appeared to provide protection against the effects of MPTP on motor behavior, as the number of falls was not different from controls. Six weeks of iron supplementation significantly impaired motor behavior ($p<0.001$), and the combination of high dietary iron and MPTP treatment proved to be lethal. To understand the mechanisms that may be responsible for iron-induced neuronal death, primary cultures of rat hippocampal neurons were treated with increasing levels of iron. Iron treatment resulted in a dose-dependent decrease in neuronal survival that had the morphological characteristics of apoptosis, and was accompanied by increases in the generation of mitochondrial reactive oxygen species (ROS), and expression of the chaperone protein Hsp 70 and the pro-apoptotic tumor suppressor protein p53. Furthermore, cholesterol loading of cells prior to iron treatment significantly increased ROS production and decreased cell survival suggesting that iron, cholesterol, and its oxidative products may act synergistically to impair neuronal survival. In conclusion, these data suggest that dietary iron may play a role in the vulnerability of neurons to insults associated with PD and other neurodegenerative disorders.

27.

DIETARY RESTRICTION NORMALIZES GLUCOSE METABOLISM AND BRAIN DERIVED NEUROTROPHIC FACTOR LEVELS, SLOWS DISEASE PROGRESSION AND INCREASES SURVIVAL IN HUNTINGTIN MUTANT MICE.

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Huntington's disease (HD) is a progressive neurodegenerative disorder characterized by uncontrolled body movements, psychiatric disturbances, and dementia resulting from degeneration of neurons in the striatum and cerebral cortex - it is caused by polyglutamine repeat expansions in the huntingtin protein. In addition to neurological deficits, HD patients exhibit abnormalities in glucose metabolism suggestive of a hypermetabolic state. We now report that the progression of neuropathological (formation of huntingtin inclusions and apoptotic protease activation), behavioral and metabolic (glucose intolerance) abnormalities in huntingtin mutant mice, an animal model of HD, are retarded when the mice are fasted every other day resulting in an extension of lifespan. Dietary restriction increases levels of brain-derived neurotrophic factor (BDNF) and the protein chaperone HSP-70, which are depleted in HD mice fed a normal diet. These findings establish links between food intake, brain BDNF levels and glucose metabolism in a mouse model of HD, and suggest that mutant huntingtin promotes neuronal degeneration by impairing cellular stress resistance.

28.

THE 2002 BLUEBERRY HEALTH STUDY: DAILY BLUEBERRIES IMPROVE DECISION-SPEED AND AGE-RELATED HEALTH INDICATORS

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Joseph and collaborators reported in 1999 that blueberry diet supplementation significantly reduced cognitive decline in older laboratory rats. Our research group observed decision-speed improvement during a pilot study with thirteen multiple sclerosis patients (2001). The 2002 Blueberry Health Study was then conducted to determine if blueberries provide health benefits to a larger number of adults, aged 60 and over.

The 2002 study was a sixteen-week randomized, multicenter, crossover trial involving 100 participants and positive and placebo controls. The protocol in-

cluded four 4-week steps: baseline measurement, treatment period 1, treatment period 2, and post-treatment follow-up.

To begin the study, Connecticut residents were invited to public meetings at the New Fairfield and Mansfield Senior Centers, and to the study web site at BlueberryStudy.com. Recruitment ended approximately 30 days after newspaper, radio and television announcements were made.

Participants were randomly assigned to receive individually quick frozen wild Maine blueberries (1 cup/day or 10 lbs/month), rice powder (500 mg/day), coenzyme Q10 (30-mg/day) or an antioxidant mixture containing mixed tocopherols (500 IU total/day) plus lipoic acid (25 mg/day). To equalize blueberry and control group expectations, participants were sent email messages citing evidence of health benefits from vitamin E, lipoic acid and our other control supplements. Participants conducted weekly measurements of single-choice decision speed, provided estimates of their aches and pains, energy-level, mood, sharpness, peacefulness, sleep quality, and overall health, and made three separate decision-speed predictions each week to allow expectations and motivation to be measured. Errors and decision speed were recorded before and also after predictions during each measurement session, to provide additional measures of participant expectations and motivation.

Ninety seven participants completed the protocol. Among those receiving wild blueberries, significant improvement occurred in decision speed (t-test $p = 0.025$) and self-reported aches and pains ($p = 0.017$), energy level ($p = 0.002$), sharpness ($p = 0.001$), sleep quality ($p = 0.017$), mood ($p = 0.010$), peacefulness ($p = 0.005$) and overall health ($p = 0.001$). Blueberry group response times improved by 4.2%, decreasing from 39.96 to 38.27 centiseconds, more than twice the improvement in the control group. Actual decision speeds were not displayed during the study so speed improvement could not influence self-reported health. Adjustments to balance decision speed predictions, error rates and within-measurement decision-speed improvement (our measures of expectation and motivation) in blueberry and control groups did not significantly change these results. Two blueberry recipients reported hearing improvement that was confirmed by an independent observer, and three reported their prostate serum antigen level declined. Significant changes were not observed in any control group.

Results of this study indicate that blueberries consumed regularly for four weeks can improve a number of health indicators related to aging, including decision speed, aches and pains, and energy level.

We greatly appreciate assistance provided by Bill Holme, Phil Fichandler, Kathy Hull, Marilyn Gerling and many others at the New Fairfield and Mansfield Senior Centers.

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29. (N)

CALORIC RESTRICTION INCREASES BRAIN-DERIVED NEUROTROPHIC FACTOR LEVELS IN AREA CA1 OF THE RAT HIPPOCAMPUS.

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Caloric restriction (CR) extends lifespan and retards age-related diseases and degenerative processes in the body and the brain. Moreover, CR prevents the decline in behavioral measures of hippocampus-dependent learning and memory after middle age in Fischer 344 x Brown-Norway (F344xBN) rats (Markowska et al, 2002). CR may protect learning and memory by modulating neurotrophic activity. Brain-derived neurotrophic factor (BDNF) is a candidate to mediate age- and CR-dependent effects on learning and memory, since it influences potential substrates of hippocampal function, including synapse density, synaptic efficacy and long-term potentiation. The literature is inconclusive regarding the effects of age or CR on BDNF levels in the hippocampus, in part because previous measurements have lacked the resolution to detect changes within hippocampal subfields, which exhibit very different levels of BDNF immunoreactivity. One might speculate that the age-related decline in learning and memory reflects a decrease in BDNF levels and that CR preserves hippocampal function by increasing BDNF levels as compared to age-matched AL controls. We measured BDNF in the hippocampal subregions dentate gyrus (DG), area CA3 and area CA1 of male F344xBN rats at ages 10, 18 and 29 months using an electrochemiluminescence immunoassay (ECLIA) (N=5 rats/condition). Surprisingly, BDNF levels were found to increase with age in DG of both AL and CR rats. No age-related change in BDNF levels was detected in CA3 or CA1. In area CA1 of old rats, BDNF levels were greater in CR versus AL rats. CR rats exhibited a trend of higher BDNF levels in all regions and at all ages as compared to AL rats. In conclusion, the observed increase in DG BDNF levels across ages, independent of diet, may constitute a compensatory response to age-related degenerative processes in this area. Furthermore, CR may preserve hippocampal function after middle age in the F344xBN rat by increasing CA1 BDNF levels in old age. NIA AG11370& AG019886

30.

AVIAN MODELS FOR EVALUATING THE EFFECTS OF CALORIE RESTRICTION.

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Calorie restriction has been used routinely in domestic species as a means of enhancing lifetime productivity and health. The use of calorie restriction has become increasingly critical in domestic breeds of poultry as the birds have been selected for rapid growth. If unchecked, the rapidly growing strains of poultry develop a number of clinical problems, including poor reproduction, joint inflammation, and other obesity related conditions. In

birds destined to become breeders, calorie restriction is routinely initiated in the first few weeks of life, often in an alternate day or skip-a-day feeding regimen. When the birds are photostimulated to induce reproduction, then they are fed on a daily calorie restricted paradigm. We conducted studies on heavy poultry breeders as well as on Japanese quail to ascertain level of calorie restriction that benefits long term reproduction and minimizes health problem and stress. Results showed that moderate levels (15-25%) of calorie restriction delayed sexual maturation, whereas more stringent restriction (35+%) resulted in a lack of sexual maturation, especially in males. Although delayed in the initiation of reproduction, moderately calorie restricted birds showed long term benefits in reproduction, with production of more chicks and longer reproductive function. These differences between groups was reflected in plasma steroid hormone levels. Studies in Japanese quail showed similar responses, with the more severe calorie restriction being associated with elevated corticosterone levels, suggesting activation of a stress response. These studies provide evidence for similar benefits of moderate calorie restriction across phyla.

31.

SKIN ANTI-AGING AND ANTI-CARCINOGENIC EFFECTS OF LUTEIN, AND POLYPODIUM LEUCOTOMOS.

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Lutein, a carotenoid, and Polypodium leucotomos, a fern extract, are potent antioxidants and anticarcinogenic agents. The extracellular matrix (ECM) maintains the structural integrity of the skin. Skin aging is associated with the degradation of the ECM by the matrixmetalloproteinases (MMPs), and reduced cellular response to signals. MMPs are also involved in the metastasis of cancer cells. Identification of agents that inhibit MMPs can be beneficial in the inhibition of skin aging and cancer.

The purpose of this research is to investigate the dose dependent effects of lutein and Polypodium leucotomos on the proliferation and expression of MMP-1 (degrades structural collagen) and MMP-2 (degrades the basement membrane) in normal dermal fibroblasts (early and late passage) and melanoma cells. In-vitro cell passage is thought to mimic in-vivo aging.

Fibroblasts and melanoma cells were exposed to 0, 0.1uM, 0.3uM, 1uM, 3uM, and 10uM lutein for 24 hours, and examined for cell proliferation, MMP-1 and MMP-2 protein levels, and MMP-1 promoter activity. Lutein did not significantly alter the proliferation of normal skin fibroblasts or melanoma cells. It inhibited MMP-1, and MMP-2 protein levels, dose responsively, from 0.3uM to 10uM in fibroblast and at 0.1uM to 1uM in melanoma cells. Lutein (0.1uM to 10uM) inhibited MMP-1 promoter activity in normal fibroblasts.

Early and late passage fibroblasts, and melanoma cells were treated with 0, 0.01%, 0.03%, 0.1%, 0.3% and 1% Polypodium leucotomos for 24 hours, and analyzed for cell proliferation and protein levels of MMP-1 and MMP-2. In early passage fibroblasts, Polypodium leucotomos from the lowest to the highest concentrations (0.01% to 1%) inhibited the proliferation and expression of MMP-1 and MMP-2, dose responsively. In late passage fibroblasts, higher concentrations of Polypodium leucotomos (0.1 to 1%) were required to mediate the inhibition of proliferation and MMPs, in comparison with the early passage cells. Polypodium leucotomos was also effective in inhibiting the proliferation and MMP expression in melanoma cells.

Lutein, and Polypodium leucotomos are effective skin anti-aging agents. Lutein inhibits the MMPs. Polypodium leucotomos inhibits the proliferation and expression of MMPs in early and late passage fibroblasts. However, the late passage fibroblasts are less responsive and require higher concentrations (0.1%-1%) of Polypodium leucotomos for effects, in comparison with the early passage fibroblasts (0.01%-1%). Lutein, and Polypodium leucotomos are effective anticarcinogenic agents, for the prevention/treatment of melanoma. Lutein at lower doses (0.1uM-1uM) inhibits MMPs, and thereby metastastic potential. Polypodium leucotomos inhibits proliferation and the expression of MMPs in melanoma cells.

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32.

AGE-RELATED CHANGES IN METABOLIC PARAMETERS OF NON-HUMAN PRIMATES.

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Type 2 diabetes is closely associated with aging, making it difficult to distinguish which of the symptoms attributed to type 2 diabetes are actually manifestations of the aging process. A colony of rhesus monkeys with ages ranging from 2 to 32 years, some of whom spontaneously develop obesity and diabetes, was followed up for a period of 10 or more years. Routine clinical chemistry and hematology, intravenous and oral glucose tolerance testing, and the euglycemic, hyperinsulinemic clamp procedure, were performed periodically to assess metabolic status. Comparing values obtained by averaging over two-year intervals, no age- related changes in Fasting Plasma Glucose (FPG) and 2-hours post-OGTT glucose could be demonstrated among non-diabetic (N) monkeys. On the other hand, mean values of FPG as well as 2-hours post OGTT –glucose were already significantly elevated over the normal group by age 14 for monkeys who eventually developed diabetes (DM). An age - related decline in Fasting Plasma Insulin levels, the acute insulin response (AIR), the two-hour post-OGTT- insulin levels, and the glucose disappearance rate post- IVGTT (Kg) was evident in both the N and DM groups. Insulin sensitivity (M –rate), however, did not appear to change. Circulating triglyceride levels did not

change significantly with age in the non-diabetic group, but was significantly higher among the diabetics from age 14. Similarly, no age- related elevation in total cholesterol, LDL- cholesterol, VLDL-cholesterol, and VLDL- Triglyceride levels could be detected among the non-diabetics; however, HDL-cholesterol levels appeared to be higher among the younger subjects (age < 10 years) than among the elderly. Longitudinal assessment of the clinical status of this colony of non-human primates makes it possible to differentiate which changes in the physiologic variables may be attributable to diseases of aging, including the metabolic syndrome, and which are normal correlates of the aging process.

33.

REGULATION OF REDOX PARAMETERS AND APOPTOSIS IN CALORICALLY RESTRICTED RODENTS.

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Caloric restriction (CR) can extend the life span of multiple species and is the only intervention known to attenuate aging in mammals. Mechanisms mediating the CR influence are as yet unknown. There is strong evidence that some of these mechanisms are associated with mitochondrial functions. The purpose of our study was to search parameters of apoptosis and redox in caloric restriction. We used two models: 1) alpha-MUPA transgenic mice previously reported to spontaneously eat less and live longer compared to their wild type (WT) control. We used this model to search apoptotic aspects in young mice (5-7 months old). To prove the similarity of this model to caloric restriction we measured some parameters in calorically restricted WT mice. 2) Old calorically restricted (CR) Sprague-Dawley rats compared to ad libitum old and young rats. The CR rats were restricted at the age of 10 months for 60% of their average food consumption (40% caloric restriction); the restriction continued for 1 year. Here we report that compared to their ad-libitum fed WT, young alpha-MUPA mice and short-term (8 weeks) caloric restricted WT mice showed increased susceptibility to calcium-induced high amplitude swelling of isolated liver mitochondria and increased cytochrome c release, as well as enhanced caspase-3 activity of fresh liver homogenates and increased DNA fragmentation in hepatocytes. In addition, alpha-MUPA mice showed significantly decreased rate of spontaneously occurring tumors at the old age, and significantly reduced level of plasma IGF-1. Old calorically restricted rats showed a lower level of lipid peroxidation and a higher rate of mitochondrial reactive oxygen species (ROS) production compared to their ad libitum control. Long-term caloric restriction in old rats approximated these parameters to young rats. These results provide an indication that CR can moderately enhance mitochondria-dependent apoptotic capacity and improve redox parameters. Collectively, the results are consistent with the possibility that a combination of long lasting, moderately extended apoptosis, decreased oxidative damage (lipid peroxidation) and normalized ROS production that is important for cell signaling could play a role in the CR-induced anti-aging influence.

34. (N)

THE EFFECTS OF TWO YEARS ESTROGEN LOSS AND REPLACEMENT ON CHOLINERGIC NEURONS AND CORTICAL CHOLINERGIC FIBERS IN MONKEYS.

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Women can expect to spend one-third of their lives postmenopause, yet the neurobiological consequences of chronic estrogen loss and replacement are poorly understood. Additionally, only a handful of studies investigating this issue have been conducted in humans or non-human primates. The basal forebrain cholinergic system (BFCS) contains estrogen receptors and is sensitive to changes in estrogen state in rodents. We have found that normal performance on a visuospatial attention task is dependent upon the BFCS or estrogen. The current study investigated the effects of two years estrogen loss and replacement on neurons in the nucleus basalis and their cortical cholinergic projections in surgically menopausal adult monkeys. Using stereological methods we analyzed the number and volume of the cholinergic neurons of the nucleus basalis, and also analyzed cholinergic fiber length and density in cortical regions that are part of a frontoparietal visuospatial attention network, in intact monkeys (n=6) and ovariectomized monkeys treated with placebo (n=6) or estrogen (n=6) for two years. We did not find changes in the number or size of nucleus basalis cholinergic neurons, or of cholinergic fiber length or density in parietal cortex (area 7a) or ventrolateral principal sulcus (area 46v), following 2 years of estrogen loss or replacement. In contrast, we did find a layer-specific decrease in cholinergic fiber length and density in the dorsal bank of the principal sulcus (area 46d) following two years of estrogen loss. Daily oral treatment with estrogen prevented this decrease. The results suggest that long-term estrogen loss may have subtle but significant neurobiological consequences in the primate cholinergic system that may be prevented with long-term estrogen replacement.

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35.

MUTANT MAMMALS WITH NORMAL LIFESPAN: AN UNDERAPPRECIATED RESOURSE.

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Biogerontology proceeds largely by designing interventions to alter the life expectancy, or sometimes the maximum lifespan, of an experimental population. Interventions designed to extend an organism's lifespan are often claimed to be far more informative than ones

designed to shorten it, because shortening lifespan can be done in innumerable ways that do not necessarily have anything to do with what limited the controls' lifespan, whereas to extend lifespan one must modulate all the organism's life-limiting processes. I suggest that this overlooks a critical point. Our goal is to test hypotheses concerning which mechanisms do and do not greatly influence lifespan. An intervention that extends lifespan does not generally do this, because putative markers of lifespan-limiting processes (PMLPs) that are postponed may be causal in determining lifespan or may be mere bystanders, while ones that are not postponed may be unimportant for lifespan or may be important but better tolerated because of the intervention. Moreover, interventions designed to extend lifespan but which fail to do so also tell us nothing, because PMLPs that they postpone are not shown to be irrelevant (only to be not the only ones that are relevant), while ones that are not postponed are again neither supported nor challenged (they could either matter or not for lifespan). When the intervention is designed to shorten lifespan, on the other hand, information can be gained that truly falsifies hypotheses. True, when lifespan is indeed shortened we learn little, since the organism may have been killed by something irrelevant to controls: thus, no PMLPs are falsified. But if lifespan is unaffected by the intervention, the situation is totally different: any PMLP that is accelerated is unambiguously eliminated as being relevant to the determination of lifespan in that organism. I will discuss the many mammalian examples of this "failed shortening of lifespan" that are now available and a number of tests of prominent hypotheses that they make possible.

36.

PUTATIVE "AGE BENEFICIAL" EFFECTS OF OMEGA OILS.

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Most polyunsaturated fatty acids (PUFA) are either n-6 (omega-6) or n-3 (omega-3). The n-6 PUFA include arachidonic acid, 20:4 n-6, the precursor of the most common prostaglandins and leukotrienes, the eicosanoids. These have essential functions, but are also involved in inflammation and promote cell division. The dietary precursor of 20:4 n-6 is linoleic acid, 18:2 n-6. The n-3 PUFA include the dietary precursor, alpha-linolenic acid, 18:3 n-3, EPA, 20:5 n-3, and DHA, 22:6 n-3. About one-twentieth of the dietary 18:3 n-3 is converted to DHA. This conversion requires peroxisomal enzymes. EPA and DHA are also obtained from fish and eggs. EPA is anti-inflammatory because it competes with 20:4 n-6 as a substrate for the formation of a prostaglandin. The prostaglandin from EPA is not inflammatory.

DHA promotes cell differentiation and stimulates the synthesis of the peroxisomal enzymes necessary for plasmalogen synthesis. Plasmalogens are involved in signaling mechanisms in the brain. DHA turns over rapidly in the brain, but most is then recycled back into phospholipids. DHA is very concentrated in the retina where it is involved in the transduction of light to electrical signals. DHA is also enriched in the nerve-endings where it is mostly in the ethanolamine plasmalogens. Adrenoleukodystrophy (ALD) is a genetic disease of peroxisomes in which DHA, and thus plasmalogens, are not formed. The infants develop mental retardation because myelin, which requires plasmalogen, is not formed. ALD can be treated with DHA.

The first lipid changes in Alzheimer disease (AD) are decreased levels of DHA and ethanolamine plasmalogens. The breakdown of ethanolamine plasmalogens may enable the abnormal cleavage by gamma-secretase to produce Abeta. The lack of plasmalogens also leads to the loss of nerve-endings. Some success has been had with AD treatment with DHA supplements.

37.

HEALTH BENEFIT OF MONOUNSATURATED FATS: FOOD-BASED RESEARCH ON MUFAs.

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Significant progress has been made to reduce the incidence of cardiovascular disease (CVD) in the U.S. However, CVD remains the leading cause of death, accounting for more deaths than the other leading causes of death collectively. Thus, there is still a pressing need to develop effective intervention strategies to further reduce CVD morbidity and mortality markedly. Diet remains a cornerstone in prevention and treatment efforts. It is without question that lowering dietary saturated fatty acids (SFA), trans fatty acids and cholesterol are essential to lower LDL cholesterol levels, a major CVD risk factor. There is a growing literature that indicates that monounsaturated fatty acids (MUFA) have beneficial effects (when substituted for SFA) on other CVD risk factors beyond lowering LDL cholesterol levels. Compared with a low fat diet, a moderate fat diet low in SFA and high in MUFA decreases triglyceride (TG) levels and attenuates the HDL cholesterol, and in particular, the HDL (2) decrease. A blood cholesterol-lowering diet high in MUFA favorably affects hemostatic factors, including fibrinogen and coagulation factors VIIc, XIIc, XIIa, and Xc. There is evidence that MUFA diets also significantly improve insulin sensitivity compared with a high SFA diet, providing that total fat does not exceed 37-38% of calories. A limited body of evidence indicates that MUFA reduces blood pressure. There also is emerging evidence that following a MUFA diet lowers chylomicron remnant particle response to a high fat meal. Finally, there is exciting evidence that a calorie restricted, moderate fat diet high in MUFA favorably affects weight loss and maintenance of reduced body weight compared with a low fat diet. Despite the numerous beneficial effects of MUFA on multiple risk factors for CVD, there is some evidence of potentially adverse effects. In monkeys fed a high MUFA atherogenic diet for five years, coronary artery atherosclerosis was comparable to that of monkeys fed an athero-

genic diet high SFA; atherosclerosis was minimal in animals fed a similar diet high in polyunsaturated fatty acids. In addition, in human studies a high MUFA fat load decreases flow mediated dilation (FMD) in the brachial artery. However, this response is markedly attenuated when antioxidants are included in the fat load. In summary, a diet that emphasizes MUFA has many attributes that could reduce CVD mortality especially within the context of a diet that has other cardioprotective nutrients/components. Because of the worrisome monkey data, however, an intervention study assessing atherosclerosis in humans is needed to conclusively resolve the role of MUFA in a cardioprotective diet. Nonetheless, even without these data, MUFA can be included in moderation in a heart healthy diet that is rich in many cardioprotective nutrients. This position is consistent with our current thinking that there are many diverse diets that can be designed to prevent CVD.

38.

FATS: THE GOOD, THE BAD AND THE UGLY.

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Fats are an integral part of the diet and comprise about 20-40% of caloric intake in the U.S (mean about 34%). Fats are eaten as triglyceride (TG, 3 fatty acids attached to a glycerol backbone), absorbed as fatty acids (FA) after lipolysis, reconstituted as TG, transported as chylomicrons, which undergo lipolysis in the capillary bed, where FA goes to muscle and fat cells, and the chylomicrons become remnant particles which pick up cholesterol ester (CE), and then deliver the CE along with fat soluble vitamins to the liver. The liver makes TG rich particles known as very low density lipoproteins (VLDL) which undergo lipolysis to form low density lipoproteins (LDL). VLDL is the major TG carrying lipoprotein in fasting plasma, while LDL is the major cholesterol carrying lipoprotein, and delivers cholesterol to tissues. A protein known as apo(a) can attach itself to VLDL and LDL to form lipoprotein (a) or Lp(a). High density lipoproteins (HDL) serve as acceptors of cholesterol from tissues, and can deliver CE to the liver or it can be transferred to other lipoproteins via cholesterol ester transfer protein. Risk factors for coronary heart disease (CHD) include increased age, hypertension, smoking, diabetes, elevated LDL C (> 160 mg/dl), and decreased HDL C (< 40 mg/dl). Emerging risk factors for CHD include elevated Lp(a) (>30 mg/dl), remnant cholesterol (>10 mg/dl), C reactive protein (> 3.0 mg/L) and homocysteine (> 12 micromoles/L) levels. LDL is the bad cholesterol particles; HDL is the good cholesterol particle, TG, remnants, and Lp(a) are the ugly particles that also confer increased risk. FA are also found on phospholipids and CE, and are critical for determining membrane fluidity. Saturated fat (mainly palmitic 16:0), has no double bonds, raises LDL, and decreases membrane fluidity, monounsaturated fat (mainly oleic 18:1n9) has one double bond, and has little effect on LDL or membrane fluidity, while the polyunsaturated n6 fatty acids (mainly linoleic, 18:2n6) have two or more double bonds, lower LDL C, and increase membrane fluidity. The polyunsaturated n3 fatty acids (eaten mainly as alpha linolenic acid 18:3n3 in vegetable oils or as eicosapentaenoic acid or EPA 20:5n3 and docosahexaenoic acid or DHA 22:6n3 in cold water fish or fish oil) lower TG, decrease CHD risk, and increase membrane fluidity. Replacing saturated fat with polyunsaturated fat has been shown to reduce CHD risk. Fish oil supplementation has been shown to decrease CHD death. Low plasma DHA levels, associated with low fish intake, in our studies in Framingham are associated with an increased risk of Alzheimer's disease and all-cause dementia. When polyunsaturated fats are hydrogenated through long term heating, the double bonds can be converted from the cis (hydogens on the same side) to the trans (hydrogen on opposite sides of the carbon chain) position, and then they behave in a deleterious manner by raising LDL C. Therefore the good fats are the essential fatty acids and their derivatives (linoleic, alpha linolenec, EPA, and DHA) in the right balance, the bad fats are the saturated fatty acids (especially palmitic, myristic, and lauric acids), and the ugly fatty acids are the trans fats (trans versions of linoleic and oleic). The top of the USDA food pyramid should be changed to read: "Use animal fats, trans fats, and sugars sparingly."

39.

POLYPHENOL ANTIOXIDANTS IN GREEN TEA AND OATS: POTENTIAL ROLES IN ANGIOGENESIS AND ATHEROSCLEROSIS.

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Cancer and cardiovascular diseases (CVD) are the major causes of morbidity and mortality among middle aged and older people in Western societies. Epidemiological and experimental evidence indicate that dietary habits play important roles in the risk of these diseases. Polyphenols with antioxidant capacity have recently been recognized for their potential health effects. Green tea contains catechins and oats are unique for their avenanthramides content; both are polyphenolics with powerful antioxidant capacity. Consumption of green tea, which is associated with a reduced risk of certain forms of cancer, can inhibit carcinogenesis and tumor growth and metastasis by suppressing angiogenesis. We have recently elucidated the molecular mechanism by which epigallocatechin gallate (EGCG), one of the green tea catechins, inhibits angiogenesis. EGCG inhibited tyrosine phosphorylation of vascular endothelial (VE)-cadherin in vascular endothelial cell growth factor (VEGF)-induced tube formation. In addition, EGCG inhibited the signaling pathway involving VEGFR, PI3-kinase, β-catenin, and VE-cadherin complex. We have recently discovered that oats, in addition to their health benefits for CVD through their soluble fiber content, have potential anti-inflammatory and antiatherogenic properties due to their avenanthramides content. We have found that avenanthramides reduced expression of adhesion molecules such as ICAM-1, VCAM-1, and E-selectin, inhibited adhesion of mono-

cytes to endothelial cells, and reduced expression of proinflammatory cytokines such as IL-6, IL-8, and MCP-1. Thus, inclusion of green tea and oats in the daily diet during a lifespan may reduce the risk of cancer and CVD, the two major age-related diseases. Supported by NCI grant# 1 R03CA94290-01 and USDA contract# 58-1950-9-001.

40.

CAROTENOID-BASED NUTRITIONAL INTERVENTIONS AGAINST AGE-RELATED MACULAR DEGENERATION: BEYOND AREDS.

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Current treatments for advanced age-related macular degeneration (AMD) have limited effectiveness, and the majority of treated patients still end up legally blind in the affected eye. Thus there is considerable interest in preventative strategies to slow or halt the progression of AMD before significant visual loss has occurred. The recent AREDS study demonstrated that antioxidant supplementation with high doses of zinc, vitamin E, vitamin C, and beta-carotene can decrease the likelihood of AMD progression and visual loss in patients with the intermediate stages of AMD. While AREDS has had significant impact on the management of AMD, it is just the first step since formulations need to be optimized and other nutrients need to be examined. The most promising of these other nutrients include lutein and zeaxanthin, xanthophyll carotenoids derived from green, orange, and yellow fruits and vegetables that are specifically concentrated in the macula of the human eye that may protect against AMD by acting as antioxidants and photoprotectants. Clinical and basic science studies on the protective roles and biochemistry of lutein and zeaxanthin in the macula will be presented with special emphasis on a novel noninvasive method to measure macular pigment levels developed at the University of Utah using resonance Raman spectroscopy.

41.

AGE-RELATED CHANGES IN MURINE CNS AND mRNA GENE EXPRESSION ARE MODULATED BY DIETARY MELATONIN.

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The profile of gene expression is not constant throughout the life cycle. Gene array analysis has been used to quantitatively estimate the extent to which genes are expressed in the cerebral cortex of both young (4-month) and old (27 month) B6C3F1 male mice. A stringent degree of significance was obtained using an Affymetrix program and six different comparisons. Out of 12423 mRNA levels, only 25 changed significantly with age. Nine of these genes coded for inflammatory proteins, all of which were elevated in aged, relative to younger mice. Melatonin (200 ppm) was included in the diet of aged animals for 8 weeks. Such supplementation with melatonin is able to elevate levels of melatonin in both serum and cortical tissue. This treatment reversed 13 of the 25 genes altered with age. Levels of 8 of the 9 inflammatory genes elevated with age, were reduced by melatonin. In no case did melatonin potentiate age-related changes in gene expression.

Concentrations of mRNAs for inflammatory cytokines interleukin-6 (IL-6) and TNFa, were not present in the microarray in sufficient amounts for quantitation. These were therefore assayed by Northern blotting. Both mRNA species were greatly elevated with age but lost their ability to respond to an exogenous inflammogen, lipopolysaccharide (LPS). Dietary melatonin reversed this age-related increase in basal levels of IL-6 and TNFa mRNAs. Melatonin also restored the ability of these mRNAs to be expressed more in the presence of LPS.

The restoration of a more youthful gene profile to brains of aged animals by melatonin treatment, to a large extent, involves reversal of age-induced elevation of basal inflammatory parameters. Supported by NIH Grants AG16794 and ES 7992.

42.

THE POTENTIAL FOR NEUROPROTECTION THROUGH FOLATE SUPPLEMENTATION AND HOMOCYSTEINE-LOWERING IN ALZHEIMER'S DISEASE.

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Alzheimer Disease (AD) is a devastating and debilitating neurodegenerative condition, and the most common cause of dementia among the elderly. Despite considerable advances in the cellular and molecular pathology of AD, little progress has been made in understanding the primary causes of the disease, and it remains incurable. Strong epidemiological evidence for an association of AD, stroke and other neurological disorders with low blood levels of folate and elevated plasma total homocysteine (a risk factor for vascular disease and stroke) is of considerable interest in this regard. These associations implicate disrupted homocysteine metabolism as a factor in age-related cognitive decline. Several theoretically plausible mechanisms have been proposed to explain how low folate or high blood homocysteine might promote neurodegeneration. However, these mechanisms have yet to be adequately demonstrated *in vivo* and it remains uncertain whether they are causally related to cognitive decline. Nevertheless, plasma homocysteine can be safely lowered by nutritional intervention using vitamin supplements, thus raising the possibility of lowering the risk of dementia by specific vitamin therapy. Several intervention trials are currently underway to determine the efficacy of dietary folate supplements in combination with other B-vitamins in slowing cognitive decline in Alzheimer's disease and stroke.

43.

ANTIOXIDANT VITAMINS AND CHRONIC DISEASE: WHO NEEDS SUPPLEMENTS?

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The antioxidant vitamins E and C, and certain carotenoids, flavonoids and other phytochemicals may exert beneficial effects in age-related diseases such as cancer, cardiovascular diseases (CVD), eye diseases and other chronic diseases that are caused, in part, by oxidative or nitrative stress. This abstract will focus on vitamin C as an example of an antioxidant vitamin. Vitamin C (ascorbate) has several well-defined biological functions, e.g., it is required for collagen, catecholamine and carnitine biosynthesis. Ascorbate also is a potent antioxidant capable of scavenging numerous reactive oxygen and nitrogen species, and regenerating other small molecule antioxidants. For example, ascorbate is the most effective water-soluble antioxidant in human plasma exposed to different types of oxidative stress; it effectively protects low-density lipoproteins from oxidative modification, a process implicated in atherosclerosis and CVD; and vitamin C supplementation in humans dose-dependently increases the resistance of plasma to ex vivo lipid peroxidation and, in some studies, lowered in vivo levels of F2-isoprostanes, a biomarker of lipid peroxidation. Vitamin C may also lower CVD risk by preventing endothelial dysfunction and increasing synthesis of endothelium-derived nitric oxide. Over thirty double-blind, placebo-controlled clinical studies have shown that vitamin C treatment restores normal endothelial function and vasodilation in patients with CVD or coronary risk factors. Related to these findings, vitamin C treatment also lowers blood pressure in moderately hypertensive patients.

In light of these findings and the proven safety of vitamin C supplements – other than occasional gastrointestinal upset or mild diarrhea – plasma and tissue saturation of vitamin C appears desirable, as it would maximize the antioxidant benefits derived from the vitamin. Pharmacokinetic data indicate that in young, healthy, non-overweight males and females, 200 mg/d of vitamin C is sufficient to saturate cells and tissues, and 400 mg/d to saturate plasma. These intake levels can be achieved by dietary means, especially by eating five to nine servings of fruits and vegetables. However, detailed pharmacokinetic data for vitamin C are not available for elderly persons, patients suffering from infections or chronic diseases such as CVD, diabetes or cancer, obese people or smokers. It is known that smokers have a higher requirement for vitamin C, as reflected by their RDA, which is 35 mg/d higher than for non-smokers. In addition, in the above clinical studies of vitamin C and endothelial function or hypertension, daily doses of 500 mg or intravenous infusion of large amounts of vitamin C were used, but no detailed dose-response studies were performed. Given these uncertainties, and the facts that vitamin C supplements are safe and most people do not consume the recommended five to nine daily servings of fruits and vegetables, vitamin C supplementation, and supplementation with a multivitamin/ multimineral in general, is recommended.

44.

THE SULFORAPHANES AND PHASE 2 ENZYME INDUCTION.

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Multiple lines of evidence link oxidative damage with rates of aging and age-related chronic diseases such as cancer, but the ability to modulate these processes by administration of conventional and naturally occurring *direct* antioxidants is not persuasive. The transcriptional activation of Phase 2 genes (e.g., coding for glutathione transferases, NAD(P)H:quinone reductase, glucuronosyltransferases, heme oxygenase 1, etc.) and those involved in glutathione synthesis are emerging as major strategies for protection of cells against the damaging effects of both oxidants and electrophiles, including neoplastic transformations. The activation of Phase 2 genes results in versatile, long-lasting antioxidant protection. This *indirect* antioxidant protection is catalytic, unlike direct antioxidants which are consumed in radical scavenging processes. Fortunately many edible plants, some of which are eaten in substantial quantities, contain potent phytochemical inducers of the Phase 2 response, and can inhibit carcinogenesis in animal models. It is tempting to attribute the rather special protective qualities of cruciferous vegetables to their high content of such inducers. Sulforaphane, an isothiocyanate isolated from broccoli and other crucifers (existing in the intact plant as its glucosinolate precursor) is a very potent Phase 2 inducer. The molecular mechanism of induction involves covalent interaction of inducers with certain specific cysteine thiols of Keap1 which is a protein anchored in the cytoplasm to the actin cytoskeleton. Keap1 is a multidomain protein that normally binds powerfully to the transcription factor Nrf2, thereby retaining Nrf2 in the cytoplasm. Upon modification by inducers, Keap1 undergoes conformational changes, releases Nrf2 which migrates to the nucleus where it binds to the AREs (Antioxidant Response Elements) of Phase 2 genes and (in dimeric combination with other factors) activates the transcription of these genes. The resultant elevation of the Phase 2 response protects mammalian cells against the toxicities of electrophiles and oxidants. Evaluations in multiple animal models and high risk populations are in progress (Supported by NIH Grants CA 94076, CA 93780, American Institute for Cancer Research, and the Lewis B. and Dorothy Cullman Foundation).

45.

"12 WEEKS TO MENTAL AND PHYSICAL STRENGTH" – IS IT TIME FOR A CONTROLLED EVALUATION OF THE 'BODY FOR LIFE' FITNESS PROGRAM?

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In 1997, the owner of a fitness magazine sponsored a twelve week physique transformation contest. 54,000 entrants submitted descriptions of the programs they followed, and the entries were compared. A list of

strategies used by the most successful entrants was compiled and used to create a fitness program. Anecdotal evidence suggests that this program leads to large decreases in fat mass with concomitant increases in lean mass in only three months, and a book describing the program has been on the New York Times bestseller list for four years. This presentation discusses several reasons why these claims, which at first seem too dramatic to be given credence, should be taken seriously. It goes on to suggest several approaches that could be taken to evaluate the claims in controlled studies. The overall effectiveness of the program relative to a more typical fitness program, such as one based on the current American College of Sports Medicine guidelines, should be evaluated initially in a crossover study. If results are positive, the possibility that the components of the program interact synergistically should be examined. This possibility influences the approach that must be taken in study design. Rather than adding one variable at a time to a sedentary baseline, it will be necessary to begin with the combination claimed to provide the highest rate of success and remove one variable at a time. This presentation concludes with a discussion of the practicality and utility of such a program in the prevention of sarcopenia and frailty.

46. (N)

CANINE WORKING MEMORY PERFORMANCE IS IMPAIRED BY SCOPOLAMINE IN AN AGE AND TASK DEPENDENT MANNER

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We have previously found that cholinergic blockade using the non-specific muscarinic antagonist scopolamine (15 mcg/kg; SC) causes deficits in visuospatial working memory in aged dogs, but spares spatial perception and reference memory. The present study further investigated the role of the cholinergic system in working memory performance by comparing the effects of scopolamine on the performance of young and aged dogs on working memory tasks of increasing difficulty. Seven aged dogs (> 10 years of age) and seven young dogs (< 5 years of age) were over-trained on a novel three- component delayed-non-matching-to-position (DNMP) task, which tests visuospatial working memory. Subjects were tested on a variable-delay procedure using delays of 5, 55, and 105 s. Scopolamine (15 mcg/kg; SC) impaired performance in aged dogs in a delay-independent manner. By contrast, scopolamine did not affect the performance of young dogs on this task, although the baseline performance levels of the young animals did not differ from that of the aged animals. The effect of scopolamine on the performance of young dogs was also investigated on a delayed-non-matching-to-sample (DNMS) task, using a fixed delay of 30 s. This task provides a measure of object recognition memory. Young dogs were impaired after scopolamine (15 mcg/kg; SC) compared to control conditions. Lastly, four young and six aged animals were tested on a serial list learning (SLL) task, which, tests visuospatial working memory. Scopolamine (15 mcg/kg; SC) significantly impaired performance on the task independently of age. An error analysis indicated that scopolamine caused an increase in errors towards the end of the test session, which suggests an increase in proactive interference. The results on the DNMP task indicate that visuospatial working memory in aged dogs is more sensitive to cholinergic blockade than in young dogs. Furthermore, the finding that young animals are impaired by scopolamine on the DNMS and SLL task indicates that sensitivity to cholinergic blockade may increase with working memory demand and extends our previous findings that working memory performance is particularly sensitive to cholinergic blockade. This pattern of task-based sensitivity parallels previous findings from our lab that indicate the DNMS and SLL tasks are more difficult to acquire than the DNMP. Collectively, these findings suggest that cholinergic function is diminished in an age-dependent manner in dogs and that scopolamine-induced impairments may serve as a model for cholinergic dysfunction. The absence of delay-dependent scopolamine impairment and the possible increase in proactive interference on the SLL under the scopolamine condition support the hypothesis that impaired cholinergic transmission may be linked to deficits in attention and not memory per se.

47.

THE PUTATIVE EFFECTS OF BLUEBERRY OR STRAWBERRY SUPPLEMENTATION AGAINST INCREASES IN OXIDATIVE STRESS AND INFLAMMATION INDUCED BY IRRADIATION

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Research has indicated that exposing young rats to irradiation enhances indices of oxidative stress and inflammation. Previous research has suggested that diets high in antioxidants, particularly those supplemented with 2% blueberry or strawberry extracts, have the ability to retard and even reverse age-related deficits in behavior and signal transduction in rats. Therefore it became of interest in the present experiment to examine the protection offered by these diets under conditions of oxidative stress and inflammation produced by insults possibly similar to aging. This study evaluated the efficacy of antioxidant diets on the performance of young (2 mo) Sprague Dawley rats on spatial learning and memory using the Morris water maze (MWM) and on measures of signal transduction including oxotremorine-enhanced K(+)-evoked release of dopamine (DA) from striatal slices, and oxidative stress and inflammation markers such as the transcription factor NF-kappa B, Human Interleukin-1 (beta) [IL-1 (beta)] and stress-inducible Heat Shock Protein (HSP70) one month following irradiation with 1.5 Gy of 1 GeV/n 56Fe particles.

Just as we have observed previously in aging, irradiation impaired performance in the MWM and measures of DA release; these deficits were protected by the antioxidant diets. The cognitive measures affected by radiation

included longer escape latencies to learn a new platform location during reversal training on Day 4 when the platform was moved to the opposite quadrant, and spatial strategies during the probe trials (60s swim with no platform), specifically fewer crossings and longer latencies to the previous platform location and decreased percent time in the platform location and platform quadrant. The strawberry diet offered protection against deficits in using spatial strategies in that the strawberry-fed animals were better able to retain place information (a hippocampally mediated behavior) compared to controls. The blueberry diet, on the other hand, seemed to improve reversal learning, a behavior more dependent on intact striatal function.

These data suggest that 56FE particle irradiation causes deficits in behavior and DA release in rats, which were ameliorated by an antioxidant diet. We are also examining if radiation increased markers of inflammation and oxidative stress in the brain, and if the blueberry and strawberry antioxidant diets were able to protect against these adverse effects.

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48.

DELAY CLASSICAL EYEBLINK CONDITIONING IS FACILITATED IN OLD F344 RATS WITH LOCALIZED CEREBELLAR INFUSION OF SP-CAMP, BUT YOUNG RATS ARE IMPAIRED BY INFUSION OF RP-CAMP.

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F344 rats show age-related changes in noradrenergic modulation of cerebellar Gaba inhibition of Purkinje cell firing. We have shown previously (Gould and Bickford, 1997) that addition of Sp-cAMP, an activator of cAMP dependent PKA, restores the ability of isoproteronol (a selective beta-noradrenergic receptor agonist) to modulate Gaba inhibiton of cerebellar Purkinje neurons in vivo. Here we have used the cerebellar-dependent form of eyelid conditioning (EC)to test the hypothesis that a deficit in cAMP activation of PKA may account for some learning deficits in older animals. Conversely, we have tested the hypothesis that blocking activation of PKA with Rp-cAMP will cause a deficit in learning in young animals.

Rats were 19 months or 3 months old at the time of training. All animals were operated to fix two eyelid EMG monitoring wires to a headstage on the cranium. Training included a 3kHz 85dB tone CS and 10psi airpuff US, with a 400msec isi. Guide cannulae were placed to deliver 1uL infusions into HVI and interpositus nucleus each day emediately before training. Young animals given Rp-cAMP showed acquisition of conditioned responses on day one only, and made no further improvement. The control group showed normal acquisition across days of training. Old animals receiving Sp-cAMP showed an increasingly higher percent of conditioned responses compared to control animals beginning on day four of training. We conclude that activation of PKA during learning may contribute to processes that establish long term memory storage for EC.

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49.

SPHINGOLIPIDS AND STEROLS IN THE REGULATION OF DEVELOPMENT AND AGING IN *C. ELEGANS*

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Using *C. elegans* as a model, we set out to test the hypothesis that aging could be in part caused by the accumulation of certain lipid growth-factors. We searched for lipids that are initially required for an organism to reach sexual reproduction, but then continues to accumulate and push the developmental program of the organism past optimum performance into dysfunction and death. There is a strong correlation between the maximum life span of a species and its developmental rate, which suggests that these processes may share a common mechanism. Many transgenic and dietary restriction/antioxidant experiments indicate that a reduction in certain growth-factors can slow developmental events while increasing health and extending maximum lifespan. In this study we are focusing on the lipid growth-factor classes of sphingolipids and sterols. Sphingolipid and sterol metabolism is largely regulated by insulin like growth-factors and oxidative stress. Using electro-spray ionization tandem mass spectrometry we have observed an accumulation of long-chain ceramides and sphingomyelins with development and aging in the worms. Using confocal microscopy we quantified the accumulation of fluorescent lipofuscin pigments with development and aging. We then used these markers to determine the development rate of worms grown at various temperatures, exposure to sterol and sphingolipid substrates, and small interfering RNAs directed against genes encoding enzymes involved in sphingolipid synthesis. Our results suggest that the development rate as determined by the rate at which the worms develop through each life-cycle stage and reach sexual maturity is regulated by the metabolic rate and the amount of sterols and sphingolipids that are being metabolized/synthesized. We have found that limiting the amount of sterols in the diet and/or inhibiting de novo sphingolipid synthesis results in a dose dependant delay in early development and a significant extension of mean and maximum lifespan in the *C. elegans*.

50. (G)

AGE AND CALORIC RESTRICTION ALTER THE RESPONSE TO LPS OF PERITONEAL MACROPHAGES FROM C57BL/6 MICE.

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Aging involves an interaction of complex processes resulting in decreased homeostasis, a gradual enhanced vulnerability to damage over time, and an increased probability of death. A diet based on a significant reduction in caloric uptake without essential nutrient deprivation, coined caloric restriction (CR) diet, has been shown to increase life span and reduce the incidence and delays the onset of age-related pathologies in numerous studies of various mammalian models. The attenuation of biological aging processes in animals on CR is due to better maintenance of cellular functions. The aim of this study was to evaluate the role of aging and CR in the inflammatory response triggered by the endotoxin, lipopolysaccharide (LPS). Male C57BL/6 mice (4 weeks old) were randomly assigned to one of the following diet groups: (1) fed *ad libitum* (AL); or (2) CR diet (40% of *ad libitum* food consumption). At the age of 72 or 96 weeks, mice were sacrificed and peritoneal macrophages (PMϕ)) were isolated. As control, younger mice (8 weeks old), fed *ad libitum* were used. PMϕ were incubated with LPS (100ng/ml) for 1 to 5 h. For all AL groups, PMϕ derived from 72 or 96 weeks old mice showed reduced LPS-induced TNF-α, IL-1β and IL-10 levels in comparison with 8-weeks old animals. In PMϕ derived from CR mice, LPS-induced IL-1β and IL-10 levels were elevated, whereas TNF-α was reduced compared to responses of PMϕ from AL mice of the same age. Detection of caveolin-1 and surface CD14 by immunostaining of PMϕ demonstrated a reduction in the levels of these proteins in older mice compared to 8 week-old mice. The reduced expression of these proteins was partially reversed in PMϕ isolated from CR mice. The reduced expression of caveolin-1 and CD14 was not related to alterations of lipid rafts by age or diet. These results demonstrated clear age-related alterations in the inflammatory response of macrophages in response to LPS. However, CR could attenuate this reduced response. We were also able to replicate the response to LPS of these ex-vivo macrophages using a macrophage-like cell line, J774 and serum derived from AL or CR fed animals, our previously described in vitro model of CR.

51. (G)

CCR5 CHEMOKINE RECEPTOR EXPRESSION INCREASES IN THE MURINE THYMUS WITH AGING

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Age-related thymic involution is characterized by decreased thymopoiesis, fat accumulation in the interlobular spaces and alterations in thymus architecture. Although the aged thymus still maintains some capacity to generate immunocompetent T cells, alterations in the ability of thymocytes and stromal cells to express various hormones and growth factors have been described during this process. Chemokine receptors are expressed by a variety of thymic cells and are involved in both intrathymic and extrathymic T cell trafficking. Particularly, CCR5 is known to play a role in primary T cell and Th1 cell migration and serves as a co-receptor for HIV infection. In the current study, we examined the expression of CCR5 within the murine thymus over the aging process. Based on our results using cDNA microarray and real time RT-PCR analysis, we found that CCR5 mRNA expression increased in the thymus of mice ranging from 2 to 18 months of age. This age-associated change in CCR5 levels does not appear to be due to alterations in thymocyte CCR5 expression as total thymocytes and thymocyte subsets appear to express similar receptor levels (~ 1-5%) on their cell surface regardless of the age examined. However, upon immunohistological analyses, we visualized $CCR5^+$ cells in the cortex and medullary areas of the thymus in both young and old mice. Additionally, a substantial increase in $CCR5^+$ cells (predominantly exhibiting a preadipocyte phenotype) was observed in the septa and thymic parenchymal regions with age. These $CCR5^+$ cells also positively stained for PPAR-γ and ERTR7, a fibroblastic cell marker, suggesting that some form of adipocyte differentiation may be occurring within the aging thymus. In addition, the number of preadipocytes appeared to be increased within the thymus with progressive aging. Finally, analysis of the aging thymi for CCR5 ligand mRNA expression using real time RT-PCR demonstrated an increase in the levels of RANTES, MIP-1α and MIP-1β message with age. This increase was further supported by the increased level of MIP1-β protein observed within the parenchyma of aged thymi. Together, our results demonstrate that an age-associated increase in CCR5 expression occurs within the murine thymus possibly playing an active role in the process of adipocyte deposition and thymocyte loss during thymic involution.

52.

ATTENUATION OF A KAINIC ACID INDUCED LEARNING IMPAIRMENT IN RATS ON DIET ENRICHED WITH BLUEBERRIES

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Blueberries are among a group of fruits and vegetables that contain phytochemicals with high levels of antioxidants believed to be important in delaying the onset of age-related diseases and retarding aging processes. In the present study, 5 mo old male Fischer 344 rats (n=28) were fed a diet consisting of either a 2% blueberry (BB)extract or a control diet consisting of a 2% corn (CN) extract. All rats were maintained on these diets for 13 weeks before being assigned to one of the following four groups that recieved a bilateral intracranial injection to dorsal hippocampus of either phosphate buffered saline (PBS) or the neurotoxin, kainic acid (KA): a blueberry PBS group (n=8), a corn PBS group (n=8), a KA blueberry group (n=6), and KA corn group (n=6). One week later these rats were trained in a straight runway to run to avoid footshock (0.8 mA). On the following day, each rat received 20 massed practice trials in a 14-unit T-

maze using footshock motivation (0.8 mA). A 1-way ANOVA of mean errors per trial across the 20 trials revealed a significant effect of group, $F(3,24)=4.26$, $p < 0.05$. Dunnett's post-hoc analyses revealed that only the KA corn group differed from the control group (PBS corn). The other performance measures of run time, shock frequency and druation revealed a similar profile. These behavioral results suggest that the blueberry group received a significant level of neuroprotection from this neurotoxic insult.

53.

ATTENUATION OF OXIDATIVE STRESS IN SLED DOGS SUPPLEMENTED WITH BLUEBERRIES

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Oxidative damage from free radicals play an important role in several diseases such as Alzheimer's disease and heart disease. Exercise may also contribute to oxidative stress. Fruits, such as blueberries, are good antioxidants because they contain phenolics and polyphenolics that preferentially react with free radicals. Maintaining proper antioxidant levels by supplementing the diet with blueberries may prevent oxidative stress and muscle damage during exercise. The goal of our study was to compare antioxidant levels in blueberries and to observe any short-term effects supplementing the diet with blueberries had on the health of sled dogs.

Total antioxidant power (TAP), haptoglobin, isoprostane and other blood parameters were measured in plasma samples taken from racing sled dogs before exercise, post-exercise, 24 hours post-exercise, and 48 hours post-exercise. The antioxidant concentrations of samples were determined by extrapolation from a standard curve developed from known concentrations of uric acid. Isoprostanes, prostaglandin-like compounds that are produced by free radical mediated peroxidation of lipoproteins, were determined by an ELISA competitive enzyme linked immunoassay. Haptoglobin, creatine kinase, and other blood parameters were also monitored.

Results show that dogs fed blueberries had a significant ($p=0.05$) increase in plasma TAP 24 hours post-exercise, with levels returning to baseline by 48 hours. This indicates that dogs fed blueberries while exercising, compared to dogs fed a control diet, are better protected against oxidative damage. Plasma haptoglobin levels 24 hours after exercise in dogs fed blueberries increased significantly ($p=0.05$) from pre-exercise, and returned to baseline by 48 hours.

(Funded in part by Nestle Purina, the UAF graduate school, and the UAF Department of Chemistry and Biochemistry)

54.

INDIVIDUAL DIFFERENCES AND POPULATION TRENDS IN LONGITUDINAL PATTERNS OF BASAL CORTISOL IN CALORIE RESTRICTED AND *AD LIBITUM* FED RHESUS MACAQUES .

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Stress and its endocrine consequences are implicated as causes of aging and age-related disorders ranging from diabetes to dementia, but evidence is inconsistent regarding the relationship between age and cortisol concentrations in humans and nonhuman primates. The glucocorticoid cascade hypothesis suggests that chronic elevation of cortisol results in the death or dysfunction of neurons containing Hypothalamic-Pituitary-Adrenal (HPA) axis regulatory receptors in the hypothalamus and hippocampus. This progressive damage is thought to lead to obesity, hypertension, dislipidemia, and other disorders through impaired regulation of cortisol, insulin, glucose, and the other endocrine elements of the dysmetabolic syndrome that is increasingly common in western cultures. Spontaneously occurring adult-onset diabetes can be prevented in nonhuman primates through dietary control, but weight stabilization through calorie restriction is often implemented too late in non-experimental settings to prevent progression to diabetes. Here we report analyses of the relationship between basal morning cortisol concentrations and age in rhesus macaques maintained for many years to study obesity, diabetes, and aging. The database included cortisol values for 141 macaques ranging in age from 4 to 40 years. The relationship between age and cortisol values for the entire dataset, including all values for all individuals was statistically significant, but modest ($r=.20$); however, regression analyses of cortisol by age for 30 male rhesus macaques for whom at least 10 years of longitudinal data were available (at ages ranging between 5 and 33 years) were more illuminating. Six of these animals were calorie restricted for weight stabilization and had not become diabetic. The other 24 monkeys (12 diabetic and 12 non-diabetic) had been fed *ad libitum*. Cortisol increased significantly with advancing age in 5 of the 6 calorie restricted animals (83%), 9 of 12 *ad lib* fed non-diabetic individuals (75%%), and only 4 of 12 diabetic individuals (33%). Cortisol declined in only three of the 30 animals (10%)—all of whom became diabetic. Fasting glucose increased significantly with age in all diabetic animals and in no non-diabetic animals. Most rhesus males exhibited significant direct relationships between cortisol and age, but some exhibited the opposite effect, which would be predicted by the glucocorticoid cascade hypothesis never to occur. These results warn against reliance on pooled datasets for characterization of the biological correlates of aging and suggest caution regarding acceptance of the glucocorticoid cascade hypothesis. Our data support attention to individual patterns of longitudinal variation, as practiced in the assessment of "allostatic load."

55.

LEARNING MORE FROM EACH PRIMATE: AN INTEGRATIVE BIOINFORMATICS OF AGING INITIATIVE. J. M. Erwin[1,3,4,5] (P), J. Robertson[1,5], D. P. Perl[2,5], P. R. Hof[2,5], J. Ely[4,5], and B. C. Hansen[3,5], [1]Virginia-Maryland Regional College of Veterinary Medicine at Virginia Tech, Blacksburg, VA; [2]Mount Sinai School of Medicine, New York, NY; [3]Obesity and Diabetes Research Center, University of Maryland School of Medicine, Baltimore, MD; [4]BIOQUAL, Inc., Rockville, MD; and [5]Foundation for Comparative and Conservation Biology, Hagerstown, MD.

The promises of aging research involving nonhuman primates will not be fully realized without parallel advances in phenotypic characterization and an integration of biological and behavioral information from multiple systems and levels of organization. Databases, such as the Primate Aging Database, must be developed, refined, and effectively mined. Aging primate tissue banks must be established and managed to optimize access. Primate genomics programs must be initiated and advanced. Emerging imaging technologies must be applied to primates of many species and all ages. Coordination and analysis of data from all these sources provides an extraordinary, but essential, bioinformatics challenge. To begin to meet this challenge, we have entered into a collaborative effort to integrate several projects and programs that promote database building and sharing and the creation of tissue banks that will enhance detailed postmortem studies. Efforts to date include, (1) development of a CNS tissue bank as "A Comparative Neurobiology of Aging Resource" at BIOQUAL, Inc., and Mount Sinai School of Medicine (including brain imaging and detailed stereologic cell measures), in cooperation with the nonprofit Foundation for Comparative and Conservation Biology; (2) initiation of "An Aging Primate Tissue Bank" at the Virginia-Maryland Regional College of Veterinary Medicine (Virginia Tech) and plans for development of integrative bioinformatics coordination; (3) further development of the "Obesity, Diabetes, and Aging Animal Resource" at the University of Maryland School of Medicine, including a colony of aging rhesus monkeys, an archive of tissue specimens, a database of clinical chemistries and other physiological measures, and a well-developed corps of collaborating scientists. This coalition is intended to grow by adding more participating institutions and to refine research by improving efficiency and increasing the amount of information yielded by each individual primate. This approach will amplify the value of each life by enhancing considerate care, promoting understanding of biology and behavior, and improving the quality of life for all primates.

56.

NEUROINFLAMMATION IN AMYOTROPHIC LATERAL SCLEROSIS: NEW THERAPEUTIC OPPORTUNTIES FOR SELECT NATURAL PRODUCTS

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Multiprobe ribonuclease protection assays (RPAs) were used to investigate expression of 36 different cytokines and apoptosis-related genes in spinal cords of mice that ubiquitously express human SOD1 bearing a glycine to alanine substitution at residue 93 (G93A-SOD1). Mice were studied at late presymptomatic stage (80 D), and at 120 D when the animals experience severe hindlimb paralysis. Spinal cord tissue from G93A-SOD1 mice expressed a subset of macrophage-typical cytokines (monokines) including IL1a, IL1b and IL1RA at 80 D increasing by 120 D. Contrastingly, T-cell derived cytokines (lymphokines) including IL2, IL3 and IL4 were detected at low levels in nontransgenic mice but these were not elevated in G93A-SOD1 mice even at 120 D. Apoptosis-related genes were generally unaffected at 80 D but caspases and death receptor components were upregulated at 120 D; a notable exception being the TNF-RI which was upregulated at 80D and increased further at 120 D. These data indicate that in the G93A-SOD1 mouse (1) cytokine expression changes precede bulk protein oxidation and apoptosis gene expression; (2) lymphocyte contributions to cytokine expression in FALS are likely minor; and (3) TNFa and its receptors may link inflammation to apoptosis in ALS. Based on these findings, a microglial cell culture model was developed to identify anti-TNFa compounds that might prove efficacious in ALS. One natural product to emerge from the cell culture screen, nordihydroguairetic acid, increased survival and improved prognosis of G93A-SOD1 mice when administered orally or intraperitoneally. Supported by the Amyotrophic Lateral Sclerosis Association (ALSA), the National Institute on Aging (RO3-AG20783), and the Oklahoma Center for Neuroscience (OCNS).

57.

2003 FLOW CHART OF BIOCHEMICAL AND PHYSIOLOGICAL INTERACTIONS IN HUMAN AGING.

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The many observable signs and symptoms of human senescence have been hypothesized by various researchers to result from several primary causes. Close inspection of the biochemical pathways associated with each of the hypothesized causes reveals several parallel cascades of events with multiple interactions and feedback loops among them.

As an aid to keeping track of the many processes and interactions, a flow chart is presented. Promising intervention points for the development of new therapeutics are also highlighted on the flow chart.

The mechanisms which are incorporated into this flow chart include:

— Nonenzymatic glycation of long-lived proteins and nuclear DNA.

— Mutations accumulate in the mitochondrial genomes of postmitotic cells.

— Increasing acetylation of histones opens heterochromatin, permitting inappropriate expression of nuclear genes.
— Lipofuscin accumulates in lysosomes of postmitotic cells, and leaks into cytoplasm.
— Increased redox poise alters signaling and enzyme activities.
— Redox damage and crosslinking of long-lived macromolecules in postmitotic cells and extracellular matrix.
— Stiffer blood vessels promote stroke and heart disease.
— Nuclear DNA damage and telomere shortening induce altered phenotype and arrest cell division in some cells.
— Apoptosis, necrosis, and cell loss lead to tissue wasting, neurodegeneration, and organ malfunction.
— Alterations in neuroendocrine and immune systems.
— Rate of repair & turnover of macromolecules & organelles slows.
— Arrested cells export toxic reactive species and inflammatory cytokines.
— Abnormal aggregations of proteins damage brain cells.
— Consequences include atherscIerosis, cancer, stroke, macular degeneration, and other age-associated diseases.

Theoretically powerful points for the development of new interventions include:
— Slowing or reversing the accumulation of lipofuscin in lysosomes of postmitotic cells.
— Slowing or reversing the accumulation of AGE crosslinks in extracellular collagen, elastin, and blood proteins.
— Preventing takeover of postmitotic cells by mutant mitochondrial DNA.
— Enhancing turnover of damaged macromolecules and organelles.

This flow chart is continuously maintained on the Web as a reference to researchers, and is updated as new information comes to light.
[w w w . L e g e n d a r y P h a r m a . c o m / senescence.html#Mechanisms]

58.
CORRELATING BRAIN/BEHAVIOR RELATIONSHIPS IN AGING: THE EFFECTS OF DIETS HIGH IN BERRYFRUIT POLYPHENOLICS

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Dietary supplementation with fruit or vegetable extracts can ameliorate age-related declines in measures of learning, memory, motor performance and neuronal signal transduction in our rat model. In this study, groups of 19 month old male Fischer rats were fed a well balanced control diet, or the diet supplemented with 2% extract from either blueberry, cranberry, black currant, or boysenberry fruit. During weeks 10 & 11 on the diets, animals were tested in the Morris water maze (MWM) and on psychomotor tasks. Brain regions were excised and examined for striatal dopamine release (a measure of signal transduction sensitive to age and oxidative stress) and a hippocampal marker of inflammation (IL-1beta). Production of Heat Shock Protein 70 (HSP70) was induced by an in vitro inflammatory challenge (LPS) and levels of the neuroprotective repair protein assessed in hippocampal tissue.

Correlations within and across diet groups explored the connection between the neuronal and the behavioral effects of nutritional supplementation targeting age-related changes. The black currant, blueberry, and cranberry diets enhanced neuronal signal transduction, and the blueberry and cranberry diets were effective at ameliorating changes in hippocampal HSP70 neuroprotection and IL-1beta levels. All of the diets affected MWM measures of spatial learning and memory, and the blueberry and cranberry diets improved motor performance.

The four berryfruits tested are rich in color and correspondingly high in anthocyanins and other polyphenolic compounds. These phytochemicals protect the growing fruit from solar radiation and in laboratory assays demonstrate antioxidant capabilities. Several anthocyanins have been detected in the brains of rats fed a 2% blueberry extract supplemented diet. Ongoing studies seek to characterize which polyphenolic compounds, or relative combination and concentration of compounds, may contribute to the significant anti-aging effects seen with blueberries and some other fruits and vegetables.

59.
GENE EXPRESSION PROFILING OF AGING AND CALORIE RESTRICTION ON THE PHERIPHERAL BLOOD T LYMPHOCYTES IN RHESUS MONKEYS USING OLIGONUCLEOTIDE ARRAY.

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Immune function declines with increasing age and immune senescence causes a variety of age-related disorders. T lymphocytes from aged human and animal subjects exhibit impaired response to various mitogens and expression of delayed hypersensitivity. The mechanisms of age-related immune dysfunction in T lymphocytes are unknown. The aim of this study is to investigate the effects of aging and calorie restriction (CR) on the gene expression profile in peripheral blood T lymphocytes of rhesus monkeys through the use of oligonucleotide array representing 12,558 human genes and expressed sequence tag (ESTs). This study involved in 20 male rhesus monkeys. The aging was studied in two groups (n=5) of monkeys given free access to Purina Monkey Chow. These monkeys were young (9-12 years old) and old (29-33 years old). For the CR study, middle aged feeding control (21 years old) and middle aged CR (21 years old) from our long term CR study initiated in 1989 at the Wisconsin National Primate Research Center. The CR monkeys had 30% lower calorie intakes than the controls based on the measurements of individual

chow diet caloric intakes. Peripheral venous blood was obtained from each monkey. CD4+ (Helper) and CD8+ (Cytotoxic) T lymphocytes were sorted by using magnetic beads. The amount of 5 microgram total RNA from CD4+ and CD8+ T lymphocytes was conversed into cDNA.

Biotin–labeled cRNA were synthesized to hybridize to the Affymetrix Human U95A Array which was derived from selected genes and ESTs from Unigene, GeneBank and TIGR databases. After washing, staining, and scanning, analysis was performed by an Affymetrix algorithm with Affymetrix GeneChip analysis software. To determine the effect of age, each young monkey (n=5) was compared to each old monkey (n=5) by twenty-five pairwise comparisons.

Likewise, the effects of CR were determined by comparing each middle aged CR monkey (n=5) to each middle aged feeding control monkey (n=5), generating twenty-five pairwise comparisons. We applied to criteria for selecting significant genes based on nonparametric bootstrap testing with a *P* value (<0.05) and average fold change (e"1.5 fold). Aging in CD4+ T lymphocytes resulted in a downregulation of genes involved in adhesion molecules and development regulatory protein in CD4+ T lymphocytes. On the contrary, aging in CD8+ T lymphocytes resulted in a upregulation of genes involved in adhesion molecules and development regulatory protein. CR decreased genes involved in adhesion molecules and development regulatory protein in CD8+ T lymphocytes. These data suggest that aging is associated with specific transcriptional pattern in CD4+ and CD8+ T lymphocytes and CR can attenuate the age-related changes.

60.

AGE-RELATED CAPILLARY CHANGES IN WHITE MATTER OF MOUSE BRAINS.

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In this cross-sectional study we used modern morphometric methods to assess the effects of aging on angiogenesis in mouse brain. Novel stereological approaches using isotropic sphere probes combined with unbiased sampling have been recently introduced to quantify the total length of thin linear features (nerve fibers, capillaries) in defined brain regions. These techniques overcome the disadvantages of previous methods including the requirements for inconvenient section orientations, complex counting rules at multiple focal planes, and differential shrinkage of age versus young tissue (Mouton et al., *J. Microscopy*, 206:54-64, 2002). Brains from male C57BL/6J mice (n = five 9-mos old and five 26-mos old) were perfusion fixed and sectioned in a systematic-random manner, and total number of capillary segments and total length of the capillary vessels were quantified in the corpus callosum. Preliminary findings suggest a significant age-related increase in the number of capillary segments in the white matter of aged mouse brain ($p<0.01$). These results provide support for the idea of increased angiogenesis as a function of age, possibly in response to increased demand for perfusion.

61.

SENIOR CENTERS CAN ADMINISTER AND INCREASE THE STATISTICAL POWER OF LONGEVITY STUDIES.

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Today's modern senior centers have networked computers, computer classes and volunteer teachers who can train and assist study participants. They regular meetings that greatly speed recruitment. And they have a corps of reliable study participants and professional volunteers who can work with scientists to implement study protocols.

Our experience conducting the 2002 Blueberry Health Study at the New Fairfield and Mansfield Senior Centers suggests that large-scale studies of the kind described over 30 years ago by Alex Comfort (1969) can be conducted, at virtually no cost, if scientists work with senior center leaders. Their skill, dedication and willingness to perform tasks required during human studies — reminding participants to provide weekly reports, assisting with computer tasks, typing answers for those who are unable, communicating regularly with scientists — tie everyone's efforts together and lead to a level of reliability that is more than sufficient for high-quality studies.

Recruitment at both centers required approximately 1 month, rather than six to twelve months typical for this kind of study. The baseline period dropout rate was 25% when PF worked with Mansfield participants, and 60% for all other participants. Overall test-retest reliability for decision speed was 0.92, far higher than the average reported reliability of 0.67 for other cognitive studies. The final confidence limit for decision-speed improvement during the blueberry study was 0.025. Without data from senior center participants, the confidence limit would have been 0.077.

The formula we found successful included (1) public meetings at each center, at the start, middle and end of the study, (2) weekly email messages to participants and center leaders, who posted and announced them for those without email, (3) formation of a review committee so that the leaders and participants could review and amend each weekly draft message before it was released, (4) weekly phone conversations between

scientists and center leaders, to respond rapidly to problems and questions, and (5) involvement of both the center director and one or two leaders, including the individual in charge of computer instruction.
Dr. Comfort published his research plans, repeatedly, but could not implement them (Kirkwood, 2001). Today's senior centers appear to have the hardware and human talent and dedication needed to carry out studies of the kind he so eloquently described. Scientists and Senior Center members who wish to join our research group are invited to contact us at BlueberryStudy.com.
Citations: Comfort, A. (1969) Lancet: Dec 27;2(7635):1411-4. Kirkwood, TB Exp Gerontol 1998 Jan-Mar; 33(1-2):135-40.
Acknowledgments: We thank Phil Fichandler, Howard Raphaelson, William Holme, Marilyn Gerling, Kathy Hull and Catherine Pappas for inventing their roles as leaders/scientists and enabling the 2002 Blueberry Health Study to be completed. We thank also the MMT Corp. for contributing the study software, Capsugel Inc., for donating all capsules for the study, the Wild Blueberry Association of North America for providing all blueberries, and the Danbury A&P Superfoodmart, Sherman IGA Supermarket, Mansfield Big Y Supermarket, Happy Rainbows Herbs and the New Fairfield and Mansfield Senior Centers for serving as our regular blueberry distribution centers.

62.

DESIGN OF A STUDY TO DETERMINE THE EFFECT OF VITAMIN C AND E INTAKE ON PROTEIN CARBONYLS, TBARS AND HEINZ BODIES

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A clinical trial was conducted to compare the antioxidant effectiveness of three vitamin C dosages in the presence of 400 mg vitamin E (alpha-tocopherol). Dosages of vitamin C were 200 mg daily in food, 500 mg twice a day as supplements, and 1,000 mg twice a day as supplements, the Upper Limit established by the Food and Nutrition Board. Ten volunteers participated. Each spent 3 weeks at each dosage before blood samples were taken on two consecutive days, 4 -12 hours after supplementation. Participants were initially given instruction on portion size and vitamin C content of food and drinks available. Compliance was measured by food diaries and supplement time logs maintained during the days before blood draws.
Discussion of appropriate endpoints involved investigators at four institutions. Endpoints selected were protein carbonyls, TBARs, and Heinz body formation in RBCs.
Protein carbonyls did not change significantly between the three vitamin C dosages. Even with daily vitamin E supplementation, Heinz body formation was significantly lower at 500 mg of vitamin C twice a day. Heinz body formation increased at either higher (200 mg) or lower (1,000 mg twice a day) intakes of vitamin C. Results indicate that even when vitamin E intake is 400 mg per day, it is possible to distinguish the effect of different levels of vitamin C on hemoglobin oxidation.

63.

IMPACT OF AGING AND OF CALORIC RESTRICTION ON T-CELL IMMUNITY IN PRIMATES

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A prominent theory of immune senescence holds that repeated antigenic stimulation and decreased production of naïve cells combine to progressively exhaust the reserve of lymphocytes available to fight new pathogens, culminating in an accumulation of lymphocytes that achieved replicative senescence. It is not known whether (and to what extent) caloric restriction can reverse the manifestations of immune senescence.
Here, we investigated phenotypic and functional T-cell aging in the rhesus macaques (RM), and the impact of caloric restriction on those processes. Our results show that sharp differences exist between the CD8 and CD4 T-cell subsets in: (i) cell cycle programs (as assessed by both in vitro proliferation and in vivo turnover measurement); (ii) CD28 regulation upon cell cycle entry; (iii) accumulation of immediate effector cells amongst the CD28- cells, believed to be close to or at replicative senescence. Moreover, our experiments confirm that CD28- cells continue to turn over in vivo, even in deep senescence, stressing the poor reliability of CD28 as a marker for senescence. Much of the aging phenomenology in RM, therefore, can be ascribed to accentuation over time of the inherent differences in activation programs in CD8 and CD4 T-cells. While caloric restriction did not impact upon all of these changes, some were clearly ameliiorated by it.

64.

ANTIOXIDANT ACTIVITY OF UYAKU (LINDERA STRYCHNIFOLIA), A NATURAL EXTRACT USED IN TRADITIONAL MEDICINE.

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Uyaku (Lindera strychnifolia: Sieb. et Zucc.) is known as a traditional Asian medicine, used in the treatment of stomach and renal disease, neuralgia, rheumatism and aging. We hypothesized that Uyaku may have antioxidant activities.
In this study, we have examined the effects of Uyaku extracts on reactive oxygen species, lipid peroxidation, and protein carbonyl formation. The dry roots and leaves of Uyaku were extracted by hot-water (100 °C, 10 min) or ethanol (40 or 80%)-H_2O extract (23 °C, 24h). The extract was lyophilized. The effects of Uyaku on hydroxyl ($HO\cdot$) and superoxide ($O_2^{\cdot-}$) radicals were examined using the electron spin resonance (ESR) spectrometer with spin trap, 5,5'-dimethyl-1-pyrroline-N-oxide (DMPO). The nitric oxide ($NO\cdot$) scavenging activity was estimated spectrophoto-metrically (545 nm) by flow injection analysis using Griess reagent. The peroxynitrite ($ONOO^-$) scavenging activity was assessed using fluorescence spectroscopy by measuring the oxidation product of dihydrorhodamine 123 (Ex: 500 nm; Em: 536 nm). The inhibitory effect on

Fe^{2+}-induced lipid peroxidation was estimated by measuring the thiobarbituric acid reactive substances (532 nm). The inhibitory effect on (Fe^{2+} + H_2O_2)-induced protein carbonyl formation was examined by measuring the reaction product of 2,4-dinitrophenylhydrazine and carbonyl groups in oxidatively modified protein (370 nm). Linolenic acid and bovine serum albumin were used as standard materials, respectively. The inhibitory effect on 3-morpholinosydnonimine (SIN-1)-induced lipid peroxidation in neuronal cells (ATCC#LLC107: rat, glial tumor) was examined by the measurements of malonaldehyde and 4-hydroxyalkenals (MDA+4-HNE; 580 nm).
The extracts directly scavenged HO· generated from Fenton reagent (roots: ID50=1.2, 0.5 and 0.4 mg/ml for hot-water extract, 40 and 80%-ethanol extract) (leaves: ID50=0.9, 0.9 and 0.4 mg/ml for hot-water extract, 40 and 80%-ethanol extract). In comparison, the ID50 of trolox (water-soluble vitamin E) and ascorbic acid were each 0.3 mg/ml (1.2 mM) and 0.002 mg/ml (0.011 mM). The direct scavenging activity was confirmed by analyzing ESR spectra using different concentrations of spin trap DMPO. The activity of the extract (roots or leaves, hot-water extract) corresponds to approximately 100 SOD-equivalent units/mg of extract.
The hot-water extract of both roots and leaves scavenged NO· in a dose-dependent manner (ID50=3~4 mg/ml). The extract (roots) showed direct ONOO⁻ scavenging activity in a dose-dependent manner (ID50=0.035 mg/ml). Both extracts inhibited lipid peroxidation (ID50=0.08 mg/ml). In comparison, the ID50 of alpha-tocopherol was 0.03 mg/ml (0.06 mM). The extract (leaves) inhibited lipid peroxidation in neuronal cells (ID50=2 mg/ml and 1 mg/ml for 4 and 36 h after the addition of SIN-1 respectively). Both extracts inhibited protein carbonyl formation (ID30=0.029 and 0.12 mg/ml each for roots and leaves).
In conclusion, the Uyaku extracts showed potent scavenging activity against $O_2^{-\cdot}$ and $ONOO^-$, and effectively inhibited lipid peroxidation and protein carbonyl formation. These results may hold some importance in age-associated diseases induced by excess reactive oxygen species.

65. (G)
THE GLUCAGON-LIKE PEPTIDES: A NEW GENRE IN THERAPEUTIC TARGETS FOR INTERVENTION IN AGE-RELATED CENTRAL & PERIPHERAL NEURODEGENERATIVE DISORDERS.
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Glucagon - like peptide - 1 (7-36) amide (GLP-1) may represent a novel approach for reversing or halting the neurodegenerative processes commonly associated with age-related diseases of the central and peripheral nervous systems. GLP-1 is an endogenous insulinotropic peptide that is secreted from the gastrointestinal tract in response to the ingestion of food. It enhances pancreatic islet beta-cell proliferation, glucose-dependent insulin secretion, and lowers blood glucose and food intake in patients with type 2 diabetes mellitus. GLP-1 receptors, are coupled to the cyclic AMP second messenger pathway, and are expressed throughout the brain of rodents and humans. GLP-1 and exendin-4; a naturally occurring, long-acting analog of GLP-1 that binds at the GLP-1 receptor, possess neurotrophic and protective properties in cultured neuronal cells, and attenuate the cholinergic neuron atrophy in the basal forebrain in a well-established rodent model of neurodegeneration.
In line with their insulinotropic and neurotrophic properties, we now demonstrate that pharmacological doses of GLP-1 and exendin-4 offer significant protection against the sensory neuropathy induced by pyridoxine (vitamin B6) toxicity. This rodent model of peripheral nerve degeneration reflects one aspect of human peripheral neuropathies, such as that commonly associated with type 2 diabetes mellitus and the numerous neuropathies of unknown etiology, for which there is currently no effective treatment.
In addition, we now report that GLP-1 and exendin-4 exhibit anti-oxidant properties; both peptides dose-dependently protect cultured hippocampal neurons against death induced by amyloid beta-peptide (A-beta) and iron. Moreover, GLP-1 dose-dependently reduces endogenous levels of A-beta in mouse brain, and lowers secreted and cellular beta-amyloid precursor protein (beta-APP) levels in cultured neuronal cells. These data indicate that GLP-1 can modify APP processing and protect against oxidative injury, two actions which suggest a novel therapeutic target for intervention in Alzheimers disease (AD). Together with the neurotrophic effects of these peptides, which we have previously shown to be facilitated through the GLP-1 receptor that, unlike TrkA, is not documented to be lost in the AD brain, we suggest a novel alternative and potentially valuable approach for the treatment of central, as well as peripheral nervous system disorders.

66. (N)
THE GENERATION OF MITOCHONDRIAL HSP70 MICE
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Emerging research indicates that mitochondria play a central role in the aging process. The epicenter of oxidative damage is the mitochondrion, where radical oxygen species (ROS) are created as byproducts of energy metabolism. ROS can damage proteins, lipids and DNA. Severe damage can lead to mitochondrial dysfunction, such as alterations in mitochondria structure, membrane potential, release of cytochrome *c* from the inner membrane matrix and induction of the apoptotic pathway. Cytosolic inducible HSP70 (heat shock protein of 70 kDa) has shown a protective role against protein damage, oxidative stress, ischemia, apoptosis and mitochondrial dysfunction in cell lines, drosophila, and/or mice. The HSP70 family is large and highly conserved throughout evolution. First discovered as a

response to heat shock, HSP70 is now known to have multiple functions. Of the human HSP70 family, mitochondrial HSP70 (MTHSP75) is most homologous to bacterial Hsp70 (DnaK). MTHSP75 is constitutively transcribed in the nucleus but localized to the inner membrane of the mitochondria, where it serves as an ATPase transporter of proteins into the mitochondria. Once proteins arrive inside the mitochondria, MTHSP75 is able to fold them into their proper conformation. It also serves as a chaperone of proteins synthesized on mitochondrial ribosomes and can help degrade misfolded proteins within the mitochondrion. Under stress, MTHSP75 refolds denatured proteins properly and prevents aggregation. MTHSP75 is also known as glucose regulated protein of 75 kD (GRP75). Another grp family member, GRP78, is a resident endoplasmic reticulum HSP70 family member involved in Ca++ homeostasis. GRP75 (MTHSP75) may contain a calcium binding domain and also be involved in mitochondrial Ca++ homeostasis. By creating a transgenic mouse overexpressing human MTHSP75 we are studying the role of this protein in protection against protein oxidative damage/aging and maintaining the integrity of the mitochondrion. The human MTHSP75 cDNA was cloned into the pCAGGS vector. Expression is driven at high levels in the heart and muscle, and at lower levels in the brain, by the chicken Beta-actin promoter and CMV enhancer. Five transgenic mice were generated, two of which died after birth prior to weaning. A high copy number of MTHSP75 leads to smaller animals and in some cases an early death. A low copy number founder of MTHSP75 has thus far shown no phenotype varying from wildtype C57Bl/6 mice. Northern analysis shows expression in muscle, heart and brain. Western analysis and immunohistochemistry confirm these results. Electron microscopy has shown that the protein is localizing to the mitochondria. Currently functionality assays to demonstrate a possible protection by the MTHSP75 transgene in cardiomyocyte cell lines to hydrogen peroxide damage are being performed. Further assays will be run to test protection of the MTHSP75 transgene against varying stressors such as gamma-irradiation and paraquat both in cell lines and in vivo. Future experiments will include I/R injury stressing experiments and aging timepoint microarray analysis of the MTHSP75 transgenic mice to further analyze for resistance to protein oxidative damage, retarding of aging and/or resistance to mitochondrial dysfunction under stressful conditions.

67.

INFLUENCE OF AGE AND DIET ON THE DISRUPTION OF FIXED-RATIO RESPONDING IN RATS EXPOSED TO 56FE PARTICLES.

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Exposing rats to ^{56}Fe particles produces "accelerated aging", both in terms of dopaminergic function and in terms of behavior. Maintaining rats on antioxidant diets ameliorates the neurochemical and behavioral effects of both aging and irradiation. The present experiment was designed to evaluate the interaction between age and diet on the disruption of operant responding by exposure to ^{56}Fe particles.

Rats were maintained on either a control diet or diets containing 2% blueberry or strawberry extract for two months prior and 1 week following exposure to 1.5 Gy of ^{56}Fe particles. When tested on an ascending fixed-ratio schedule 7 months following exposure (approx. 11 months old), all radiated rats fed both control or antioxidant diets showed response patterns similar to that ofthe unirradiated animals. When tested 12 months following irradiation (approx. 16 months old), the radiated rats fed the control diet showed a deficit in responding at the higher ratios. In contrast, the rats fed the strawberry diet, but not the blueberry diet, showed no significant differences in the pattern of responding compared to the non-irradiated rats. These results suggest that maintaining rats on a diet containing strawberry extract can prevent changes in fixed-ratio responding that occur in older rats following exposure to ^{56}Fe particles.

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68.

ROSIGLITAZONE IMPROVES CONTEXTUAL FEAR CONDITIONING IN AGED F344 RATS.

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There is a strong evidence from the literature describing a significant inflammatory component in aging-related disorders. Multiple approaches aimed at decreasing the inflammatory response have been proposed. Experimental, clinical and epidemiological studies have suggested a beneficial role of non-steroidal anti-inflammatory drug (NSAID's) in Alzheimer's disease. Growing evidence suggests that NSAID's might act via their interaction with peroxisome proliferator-activated receptors (PPARs) PPARg activation has been shown to inhibit the production of proinflammatory and neurotoxic products elaborated by b-amyloid-stimulated microglial cells and monocytes, as well as the activation of nuclear factor kB. However, although PPARs have been shown to affects many biological functions little it is know about their effects on learning and memory deficits associated with aging. In this study we explore the effect of a synthetic ligand that acts as PPARg agonist, rosiglitazone, on learning and memory processes in aged F344 rats. Rosiglitazone was administered via the rat chow at a dose of 10 mg/kg/day for 2 months prior to behavioral testing. Rats were tested in a contextual fear conditioning paradigm that involves exposing the rat to a mild footshock on the first day of testing. Forty eight hours later rats are then placed back into the same chamber and tested for freezing. Young rats show a high degree of freezing in this environment as they have a memory for the place in which the footshock was administered. Aged rats show diminished levels of freezing on this task, however aged rats that were on the rosiglitazone

diet showed significantly higher freezing demonstrating an improvement in memory.

69. (N)
WHY IT'S HARDER FOR AN OLD DOG TO LEARN NEW TRICKS

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Dogs show a pattern of age-dependent cognitive decline, which is similar in many respects to the pattern seen in humans. As dogs age, they are slower to learn complex tasks and exhibit deficits in memory storage. The present study used measures of within-session learning and between-session retention to characterize the effect of age on size discrimination and reversal learning in young and old dogs. To quantify within-session learning, we calculated mean difference scores between the number of correct responses made in the first half of a session and the number of correct responses made in the second half of the same session. Positive scores represent learning occurring within a session. To quantify between-session retention, a measure of memory, we calculated the mean difference between the number of correct responses made in the second half of session 'n' and the number of correct responses made in the first half of session 'n + 1'. For this measure, positive scores represent remembering of information and negative scores represent forgetting.

Age differences occurred on both the size discrimination task and the size reversal task; the old dogs showed forgetting, whereas the young dogs showed retention. Old and young dogs did not differ in the within-session learning score but there was a trend towards increased within-session learning in the old animals on the size discrimination task. Altogether, aged dogs show increased within-session learning and decreased between-session retention scores when compared to young dogs. These findings suggest that age-dependent discrimination learning deficits reflect impaired retention of previous information. The retention measure used in the present study is unable to distinguish between consolidation and retrieval; we cannot conclude, therefore, the extent to which deficits in these processes are responsible for the age-dependent retention impairment noted. In contrast, the decreased within-session learning scores in young animals reflect the animals' increased ability to remember the previously learned information. Therefore, the present study suggests that impaired retention is responsible for age-dependent deficits in discrimination and reversal learning in old dogs.

70.
CALORIC RESTRICTION, ANTIOXIDANT THERAPY, OXIDATIVE STRESS AND BRAIN AGING

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Previous studies in our laboratory on the effects of in old mice have demonstrated that short-term caloric restriction can reverse oxidative damage accumulation and improve psychomotor performance of old mice. Based on these observations, we decided (i) to further investigate the effects of short-term caloric restriction on the levels of oxidative damage in mitochondria, (ii) to determine whether short-term antioxidant therapy would exhibit the same effects as caloric restriction. Vitamin E, a-lipoic acid and coenzyme Q10 (CoQ10) were studied alone or in combination in C57BL/6 mice and in Sprague-Dawley rats. The results indicated a significant augmentation of the endogenous levels of the antioxidants in various tissue homogenates and mitochondria in both species. The different treatments with antioxidants exhibited no effect on oxidative stress measurements. On the other hand, short-term caloric restriction demonstrated a significant decrease of protein oxidative damage in brain mitochondria. Behavioral assessments revealed a significant improvement in the discriminative avoidance task for the supplementation combining a-tocopherol and CoQ10, whereas other treatments did not have effects on discriminative avoidance or on other cognitive and psychomotor tasks. Thus, individually, the various antioxidant treatments led to significant increases of their endogenous concentrations in old mice, but failed to improve the cognitive and psychomotor functions of these animals and did not reverse the accumulation of oxidative damage. However, the improvement in cognition found in the á-tocopherol + CoQ10 study suggested the possibility of a synergistic action of the antioxidants to improve brain function during aging.

71.
INTERMITTENT FASTING AND DIETARY SUPLLEMENTATION WITH 2-DEOXY-D-GLUCOSE IMPROVE CARDIOVASCULAR AND NEUROENDOCRINE RESPONSES TO STRESS

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The present study examined the effects of reduced levels of energy intake and bioavailability on cardiovascular and neuroendocrine responses to stress. Energy restriction was induced by either intermittent fasting (IF, every other day feeding regimen) or dietary supplementation with the non-metabolic glucose analogue, 2-deoxy-D-glucose (2-DG), for 6 months. Adult male Sprague-Dawley rats were implanted with radiotelemetry probes that allow monitoring of arterial blood pressure (BP), heart rate (HR), core body temperature (BT), and general activity (GA). Blood samples from animals were analyzed to determine the effects of the diets on stress-responsive neuroendocrine systems and glucose metabolism. IF rats exhibited significant decreases in basal HR and BP, which were evident on both fasting and non-fasting days. Basal BT was decreased on fasting days, but not on non-fasting days. 2-DG rats showed significant decrease in basal

BP with minimal influences on other physiological parameters. In response to a single 1 h immobilization stress, the magnitudes of stress-induced increases in HR, BP, and GA in IF rats were significantly reduced compared to those in ad libitum (AL) control rats. In response to repeated daily immobilization stress sessions, IF rats exhibited not only a significant reduction of magnitude in stress-induced physiological responses but also a fast adaptation during and post-stress periods. In response to a cold water swim stress, IF rats exhibited a full behavioral response and a reduced magnitude of cardiovascular responses. The effects of 2-DG on physiological responses to various stresses were intermediate to those of groups IF and AL. The effects of IF and 2-DG on hormonal alterations were similar: the basal levels of glucose and insulin were decreased, whereas ACTH and corticosterone were increased compared to AL rats. Immobilization and cold water swim stresses generally increased levels of ACTH, corticosterone and epinephrine, except that the levels of those hormones were decreased after exposure to repeated immobilization in diet-restricted rats, particularly in the IF rats. These findings suggest that energy restriction induces a mild stress response which may optimize a number of functions over a long period. We conclude that improvements in cardiovascular risk factors and cardiovascular and neuroendocrine stress adaptation occur in response to energy-resticted diets.

72.

EFFECTS OF AGING AND CALORIE RESTRICTION ON OBJECT DISCRIMINATION AND REVERSAL LEARNING IN RHESUS AND SQUIRREL MONKEYS

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Beginning in 1987 the NIA has conducted a longitudinal study of aging and calorie restriction (CR) in two nonhuman primate species (*Macaca mulatta*) and squirrel monkeys (*Samiri sciureus and Samiri boliviensis*). CR is the only intervention consistently demonstrated to increase lifespan, reduce the incidence and onset of age-related chronic disease, increase protection against stress and toxicity, and attenuate age-related decline in many physiological functions in laboratory animals. Evidence emerging from the NIA study and a similar study at the University of Wisconsin has suggested that CR can also retard many aspects of aging in species closely related to humans. Control monkeys in the NIA study have been provided a highly nutritious diet while monkeys on CR have had their intake of this diet reduced 30 percent from the level of comparable aged controls. Monkeys were introduced to the study at different stages in the lifespan, including juvenile, adult, and aged groups of monkeys. A major objective in the NIA study is to assess the effects of long-term CR on brain and behavioral function. To this end, we present results outlining age and CR effects on performance in two simple learning tasks, two-object discrimination (2-OD) and two-object reversal (2-OR). The objects are presented on a tray attached to the home cage of the monkeys. To make a response, the monkey must reach out of the cage to displace an object on the tray and retrieve a food reward located under the object designated as the correct object. If the monkey displaced the wrong object, then it was not permitted to retrieve the reward. In 2-OR testing, the reward was placed under the object that had not been rewarded in 2-OD testing. Performance was measured as the number of errors made prior to achieving a criterion. In two rounds of 2-OD and 2-OR testing using different objects for each round, a significant age-related decline (10-31 years) in performance was observed in both male and female rhesus monkeys in the control group except in first round of 2-OD testing in females. During one round of testing, the performance of control male squirrel monkeys in the 2-OD showed a nonsignificant age-related increase across a narrow age range (17-24 years) but showed nonsignificant decrease with age in the 2-OR test. When monkeys of comparable ages in the control and CR groups were compared in performance, we found no significant effects of diet in either species. The learning tasks described here are initial steps in a training program progressing toward a more complex task, the delayed matching-to-sample task (DMS) for assessment of memory. The lack of diet effects on performance in these simple learning tasks will permit assessment of memory that should prove unconfounded by any diet effects on noncognitive performance variables, including motivation for food.

73.

CELL CYCLE ACTIVATION MEDIATES NEURONAL DEATH INDUCED BY DNA DAMAGE.

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Increasing evidence indicates that neurodegeneration involves the activation of cell cycle machinery in postmitotic neurons. Recently, evidence of DNA replication in neurons in the brains of patients with Alzheimer's disease and in cultured cortical neurons exposed to amyloid b-peptide has been reported. However, the role of cell cycle-associated events in neuronal apoptosis is still unclear. We therefore tested the hypothesis that cell cycle activation and DNA replication are critical components of the mechanism involved in DNA damage-induced neuronal cell death. Different DNA damaging compounds (etoposide, methotrexate and homocysteine) induced DNA replication in cortical neurons which preceded cell death. In contrast, staurosporine and colchicine induced neither DNA damage nor cell cycle activation in the neurons, although both agents induced apoptosis. Caffeine, a blocker of ataxia telangiectasia mutated (ATM), a proximal component of DNA damage induced cell cycle check point pathways, blocked both apoptosis and cell cycle activation in neurons exposed to DNA damaging

agents. Caffeine did not prevent apoptosis induced by staurosporine or colchicine. Our data suggest that the cell cycle activation is an obligatory step in the apoptotic pathway activated by DNA damage in postmitotic neurons.