TWENTY-SEVENTH ANNUAL MEETING — American Aging Association
TWELFTH ANNUAL MEETING — American College of Clinical Gerontology
Friday through Monday
October 17-20, 1997
Warwick Hotel
Philadelphia, Pennsylvania

PROGRAM

SYMPOSIUM I:
Free Radical Response During Exercise and Physical Activity
Chair: Fielding, R.:

1 Leeuwenburgh, C.: **Exercise Induced Oxidative Damage of Mitochondrial Proteins**

2 Kanter, M.: **Exercise and Anti-Oidant Supplementation**

3 Hall, D.: **Radical Generation with Elevation of Core Body Temperature**

4 Ji:, L.: **Anti-Oxidant Regulation During Exercise**

Allegheny Health, Education and Research Foundation Institute on Aging
INVITED PRESENTATIONS

5 Kelly, S.: **The Effects on Senescent Osteoblasts of IGF-I for use in Giodegradable Polymers**

6 Lee, R.: **Influence of Blood Flow on Muscle metabolism in Elderly Patients with Peripheral Vascular Disease**

7 Sell, C.: **Activation of the Insulin-Like Growth Factor Type I Receptor by Deletion of Amino Acids 870-905**

SYMPOSIUM II:
Neural Control of Movement and Motor Learning in Aging
Chair: Bickford, P.

8 Woodruff-Pak, D.: **Cerebellar Involvement in Aging, Learning and Timing**

9 Bickford, P.: **Noradrenergic Involvement in Motor Learning**

10 Hoffer, B.: **Effects of GDNF in Aging and Animal Models of Parkinson's Disease**

11 Moxon, K.: **Neural Population Activity in Sensorimotor Cortex Can Control an External "Arm" Movement System**

INVITED PRESENTATIONS

12 Floyd, R.: **HPLC-Electrochemical Array Detection of Oxidative Biomarkers in the Central Nervous System: Application to the Understanding and Assessment of Neurodegenerative Diseases.**

13 Kitani, K.: **The Lateral Diffusion Coefficient of Con-A Receptor Protein in the Skeletal Muscle Membranes also Declines with Age as in Hepatocyte membranes in C57BL Mice**

14 Reiter, R.:**Melatonin Reduces Oxidative Stress Mediated Tissue Damage**

15 Hansen, B.: **Chronic IGF-I Treatment of Aging Type 2 Diabetic Monkeys: Effects on Muscle and Adipose Tissue**

SYMPOSIUM III:
Structure/Function Relationships in Exercise and Aging
Co-Chairs: Cosmas, A. and Manfredi, T.

16 Cosmas, A.: **Biomarkers of Aging and An Innovative Theory Based on the Threshold Age Concept**

17 Farrar, R.: **Muscle Plasticity and Aging: At What Point Does Stimulus Become Stress**

18 Manfredi, T.: **Morphological Features of Aging Skeletal Muscle**

SYMPOSIUM IV:
Effects of Exercise in Women with Aging
Chair: Schwendner, K.

Schwendner, K.: **Introduction — Women, Exercise and Aging**

19 Drinkwater, B.: **Bone Basics: The Role of Exercise, Nutrition and Hormones in Preventing Osteoporosis**

20 Tate, C.: **Are There Gender Differences in Older Adults' Response to Exercise?**

21 Brown, M.: **Exercise Efficacy for Older Women**

Allegheny Health, Education and Research Foundation Institute on Aging

Chair: Cristofalo, V.; Keynote Speaker, Goldberg, A.: **Health Benefits of Regular Exercise Across a Spectrum of Fitness Levels in the Elderly**

SYMPOSIUM V:
New Methods of Evaluating Muscle Metabolism in the Elderly
Chair: McCully, K.

22 Chance, B.: **Quantitation of Exercise Improvement in the Geriatric Population**

23 Conley, K.: **Why Exercise Training Improves Elderly Muscle Performance: Noninvasive Insights from Magnetic Resonance**

24 Price, T.: **Carbon 13 NMR to Measure Carbohydrate Metabolism and Its Potential for Aging Studies**

25 McCully, K.: **Age-Related Changes in Muscle Metabolism and Blood Flow: Noninvasive Measurements**

Submitted Papers — Poster Session

Competition for the Nicolai Prize and Glenn Award

SYMPOSIUM VI:
Effects of Exercise on Body Composition and Functional Capacity of the Elderly
Chair: Evans, W.

26 Evans, W.: **High Intensity Exercise in the Elderly**
Khort, W. : ***Body Composition in the Frail Elderly***

27 Di Pietro, L.: **Epidemiological Studies of Exercise in the Elderly**

28 Nelson, M.: **Exercise Interventions and Body Composition in Older Women**

Hayflick Lecture
Leonard Hayflick — Not titled

Annual Luncheon and Awards:

Walter Nicolai Prize
Sung Nim Han, Ph.D. candidate:
"Effect of Long-Term Dietary Antioxidant Supplementation on Influenza Infection"
Kristen D. Kewitt, Ph.D. candidate:
"Genetic Manipulation of Skeletal Muscle Glut4 Glucose Transporters and Physical Activity"
Maria Tresini, Ph.D. candidate:
"Defect in the Ras/MEK/MAP Kinase Signaling Pathway During Cellular Senescence"

Glenn Award
Dr. S.H. Kim
"Modulation of Oxidative Status and Exercise Glut-4 is Over-Expressed in Transgenic (TG) Mice."
Dr. Alison Beharka
"Long-Term Supplementation with Vitamin E Modulates Macrophage Production of Pro-Inflammatory Molecules"

Harman Research Award Lecture
Dr. V.J. Cristofalo
"The Blind Men and the Elephant, or What Have We Learned About Aging from the Study 'Serially Propagated Human Fibroblasts?' "

SYMPOSIUM VII — ROUNDTABLE DISCUSSION:
Exercise Strategies for Healthy Aging: Where Are We Now?
Moderator: McCarter, R.
Panelists: Evans, W., Khort, W. and Di Pietro, L.

POSTER PRESENTATIONS

29 Kharlamov, A.,* Kharlamov, E., Khera, D., Armstrong, D.M.: **Age-Dependent Response of the Rat Brain to Ischemic Insult Produced by Photochemically Induced Thrombi**

30 Ikonomovic, M.,* Mizukami, K., Davies, P. Hamilton, R., Sheffield, R., and Armstrong, D.: **Conformational Change in Tau Precedes β-Amyloid Deposits in the Hippocampal Formation of Alzheimer's Disease Cases**

31 Heraux, T.M.,* Mossey, J.M., Knott, K.A., Higgins, M., and Talerico, K.: **An Evaluation of the Effectiveness of Short-Term Interpersonal Counseling for Subdysthymic Depression in Medically Ill Elderly**

32 Wong, A.,* Cristofalo, V., and Tresini, M.: **Comparison of CDC25A mRNA in Young and Old WI-38 Cells**

33 Furth, J.J.,* Allen, R.G., Tresini, M., and Cristofalo, V.J.: **Phenotypic Variation in Dermal Fibroblasts Derived from Fetal and Postnatal Skin.**

34 Zwick, O.,* O'Connell, B., Smith, T.W., and Lippa, C.E.: **Perforant Pathway Synaptophysin Immunoreactivity is Reduced in Alzheimer's Disease Relative to Lewy Body Disease**

35 Jarrett, J.,* O'Connell, B., Hulette, C., and Lippa, C.E.: **The Medial Temporal Lobe in Pick's Disease: Is Measurement of Cross-Sectional Area Useful in Diagnosis?**

36 Peric-Stepcic, G.,* O'Connell, B., Smith, T.W., and Lippa, C.E.: **In Regional Variation in Nigral Pathology Alzheimer's Disease Patients with Early Parkinsonism**

37 Breen, A.,* Matthews, M.K., Weiss, M.K., Ryan, B., and Lippa, C.F.: **Donepezil in the Treatment of Alzheimer's Patients: The Auhs Dementia Program Experience**

38 Allen, R.G.,* Tresini, M., Volker, C., and Cristofalo, V.: **Development and Age-Associated Differences in Electron Transport Potential and Consequences for Oxidant Generation**

39 Tresini, M.,* Allen, R.G., and Cristofalo, V.J.: **Defect in the RAS/MEK/MAP Kinase Signaling Pathway During Cellular Senescence**

40 Sanders, T.,* McCully, K., and Leiper, C.: **The Effects of Peripheral Vascular Disease on Gait**

41 Cai, G.,* Wang, H., and Friedman, E.: **Coupling of Adenosine A1 Receptors ot G Proteins is Reduced in the Aged Rat Heart**

42 Zhen, X.,* Uryu, K., Cai, G., and Friedman, E.: **MAPK Pathway is Impaired in Aged Brains of Fischer 344 Rats**

43 Tresini, M., Mawal-Dewan, M., Cristofalo, V.J., and Sell, C.: **A Phosphatidylinositol 3-Kinase Inhibitor Induces a Senescent-Like Growth Arrest in Human Diploid Fibroblasts**

44 Walter, R. and Sierra, F.: **Increased Levels of Nuclearly Located, Inactive Stat3 and ERK Proteins in the Liver of Aged Rats**

45 Li, M.* and Sierra, F.: **Defects in MAPK Signal Transduction in Splenocytes with Aging**

46 Li, M.,* Torres, C., Walter, R., Murasko, D.M., and Sierra, F.: ***T-Kininogen is a Biomarker of Senescence in Rats***

47 Torres, C.* and Sierra, F.: **T-Kininogen Inhibits the RAS/MAP Kinase Pathway by Increasing the Stability of MKP-1**

48 Snyder, D.,* Gao, E., Horwitz, J., and Roberts, J.: **Gender Differences in the Age-Related Decline in Beta-Adrenergic Receptor Function in the Rat Heart**

49 Landsberg, L.A.,* Wolfe, K., Rohrbach, J., Knupp, A., Youth, H., and Posner, J.D.: **Predictors of Average Weekly Weight Loss in Members of a Weight Management Program**

50 Francis, M.K.,* Volker, C., and Cristofalo, V.J.: **EPC-1 Expression and Aging: Effect on Proliferation**

51 Volker, C.,* Fertala, A., Sieron, A., and Cristofalo, V.: **Purification and Characterization of EPC-1 Protein**

52 Johnsen, P.T.*: **What Influence Does Consanguineous Mating have on Human Longevity?**

53 Balin, S.,* Severino, J., Balin, A., and Cristofalo, V.J.: **Expression of β-Galactosidase *In Vitro* and *In Vivo***

54 Severino, J.,* Francis, M.K., and Cristofalo, V.J.:**Expression of the EPC-1 Gene and Its Role in the Etiology of Ovarian Carcinoma**

55 Laury-Kleintop, L.D. and Tulenko, T.N.*: **Expression of Prolyl-4-Hydroxylase α-Subunit and hnRNP-K in Atherosclerotic Arterial Smooth Muscle Cells: A Link to Vascular Disease in Aging**

56 Ara, J., Horwitz, J. and Ischiropoulos, H.: **Peroxynitrite-Mediated Modifications of Tyrosine Hydroxylase in PC12 Cells**

57 Murasko, D.M. and Gardner, E.*: **Use of Immune Parameters as Biomarkers of Aging**

58 Plett, P.A.* and Murasko, D.M.: **Strain and Tissue Difference in Basal and IFNa/β Induced NK Activity in Young and Old Mice**

59 Bernstein, E.,* Murasko, D.M., and Abrutyn, E.:**Decreased Efficiency of Influenza Vaccine on the Elderly: Reproducibility of Responses Across Several Years**

60 Abstract not received.

61 Komninou, D.,* Leutzinger, Y., Reddy, B.S., and Richie, Jr., J.P.: **Enhanced Colon Carcinogenesis in Aging Rats**

62 Beharka, A.,* Wu, D., Martin, K.R., Han, S.N., Adolfsson, O., Smith, D.E., Cao, G., Prior, R.L., Meydani, M., and Meydani, S.N.: **Long-Term Dietary Supplementation with Vitamin E Modulates Macrophage Production of Pro-Inflammatory Molecules**

63 Han, S.N.,* Meydani, M.D., Wu, K.R., Martin, B.S., Bender, D.E., Smith, G., Cao, R., Prior, and Meydani, S.N.:**Effect of Long-Term Dietary Antioxidant Supplementation on Influenza Infection**

64 Utley, S., Manfredi, T., Cosmas, A., Lamont, L., Bronson, R., and Lipman, R.:**Age and Caloric Restriction Effects on Mouse Skeletal Muscle Mitochondrial Volume Density**

65 Abstract not received.

66 Abstract not received.

67 Chang, K.S., Kewitt, K.D.,* Tsao, T., Charron, M., and McCarter, R.:**Genetic Manipulation of Skeletal Muscle Glut4 Glucose Transporters and Physical Activity**

68 Snyder, D.L.,* Gao, E., Horwitz, J., and Roberts, J.: **Gender Differences in the Age-Related Decline in Beta-Adrenergic Receptor Function in the Rat Heart**

69 Kim, S.H., Kim, J.D., Chang, K.S., Charron, M.J., McCarter, R.J.M., Yu, B.P., and Fernandes, G.: **Modulation of Oxidative Status in Exercised Glut-4 Over-Expressed Transgenic (TG) Mice**

70 Avula, C.P.R., Yoshida, M., Azar, R., and Fernandes, G.*: **Source of Dietary Fat and Treadmill Exercise Modulates Antioxidant Enzymes and Apoptosis in ICR Male Mice**

71 Yoshida, M., Ernani, F., Azar, R., and Fernandes, G.: **Effect of Corn Oil and Fish Oil Enriched Diet on the Immune Function in Sedentary and Treadmill Exercised Female and Male Mice**

72 Pahlavani, M.A. and Harris, M.D.: **Oxidative Stress Induced by Xanthine-Xanthine Oxidase and Hyperthermia Suppresses the Induction of IL-2 Expression and Nuclear Signal Transduction in T Cells from Young and Old Rats**

73 Lee, C.M., Lopez, M.E., Weindruch, R., and Aiken, J.M.: **Electron Transport System Abnormalities Are Associated with Deleted Mitochondrial Genomes in Rhesus Monkey Skeletal Muscle**

74 Lee, C.M., Chung, S.S.,* Lopez, M.E., Aspnes, L.E., Weindruch, R., and Aiken, J.M.: **Characterization of Aged Muscle Atrophy and Electron Transport System Abnormal Fibers in Rhesus Monkeys and Rats**

75 Walton, K.G., Fields, J.Z., Harris, D.A., Pugh, N.D.C., and Alexander, C.N.: **Reduced Signs of Aging in Subjects Using the Maharishi Vedic Approach to Health (MVAH): Improvements in the Hypothalamic-Pituitary Adreno-Cortical (HPA) Axis as a Likely Mechanism**

76 Matz, M.L., Schwantes, L.G., Thorp, S.C., and Ordman, A.B.: **Excretion of Vitamin C by Cigarette Smokers**

77 Gonzales, E.W.: **The Relationships of Appraisal of Disruptive Behaviors, Learned Resourcefulness and Anxiety Among Family Caregivers of Relatives with Alzheimer's Disease**

1

EXERCISE-INDUCED OXIDATIVE DAMAGE OF MITOCHONDRIAL PROTEINS. Leeuwenburgh, C., Hansen, P., Holloszy, J. O., Heinecke, J. Department of Internal Medicine, Washington University School of Medicine,St. Louis, Missouri 63110.

The overall goal of this project was to the utility of exercise as a physiological source of oxidative stress, with the long-term aim of using the model to explore the effectiveness of antioxidant interventions. Rats were subjected to a single bout of sustained exercise. Control and exercised animals were sacrificed before and after exercise, mitochondria were isolated from cardiac muscle of the animals, and the levels of oxidation products in mitochondrial proteins was determined using stable isotope dilution gas chromatography-mass spectrometry. In vitro studies demonstrated that the oxidized amino acids o,o_-dityrosine is a stable marker for protein oxidation by tyrosyl radical and o-tyrosine is a stable marker of protein oxidation by hydroxyl radical. In addition, the level of amino acid oxidation products in the urine of control and exercised animals was determined. Exercise lead to a 2-fold increase in oxidation as monitored by the levels of o-tyrosine and o,o'-dityrosine in mitochondrial proteins. Moreover, there was a similar increase in the level of o,o'-dityrosine in the urine of the exercised animals. These results indicate that exercise is a physiologically relevant oxidative stress, and raise the exciting possibility that this model will be useful for studying the effects of antioxidant interventions in vivo.

2

EXERCISE AND ANTIOXIDANT SUPPLEMENTATION. Kanter, M.,Principal Scientist Clinical Nutrition and Scientific Communications, The Quaker Oats Co., Barrington, IL.

During the past 25 years, numerous epidemiological and experimental studieshave strongly implicated free radicals in the development of diverse disease conditions such as cancer, cardiovascular disease, and autoimmunediseases, as well as the aging process. Studies conducted since the early1980's have also established a relationship between free radicals and physical exercise. However, it is difficult to draw definitive conclusions regarding the link between exercise and free radicals, largely because of differences in experimental protocols and the equivocacy of markers used to measure free radicals in living systems.

Nevertheless, the suggestion that free radicals may, in essence, represent the "dark side" of physical activity has led to the extensive study of nutritional antioxidants as a means of diminishing the potential harmful effects of reactive oxygen species. Research conducted with "mainstream" nutrients such as the vitamins C and B have produced contradictory findings, although studies conducted with elderly subjects have been promising. Research with "non-traditional" antioxidant nutrients such as genestein, lycopene, and various other phenolic compounds is in it's infancy, but it is possible that future research will uncover additional antioxidant compounds that can benefit the physically active individual.

3

RADICAL GENERATION WITH ELEVATION OF CORE BODY TEMPERATURE. Hall, D., Glennan Center for Geriatric Medicine and Gerontology, Eastern Virginia Medical School, Norfolk, Va.

We have established that whole body hyperthermia stimulates free radical production within splanchnic tissues *in vivo*. The nature and evolution of these radicals suggests that hyperthermia produces hypoxia in splanchnic tissues, increases nitric oxide synthesis, and elevates cellular concentrations of redox active transition metals. The resultant oxidative stress evolves through complex interactions between nitric oxide, superoxide anion, hydrogen peroxide, and active metal species.

4

ANTIOXIDANT REGULATION DURING EXERCISE. Ji, L. L., Dept. of Kinesiology, University of Wisconsin, Madison, WI 53706.

Strenuous exercise is characterized by an increased oxygen consumption and disturbance of intracellular prooxidant-antioxidant homeostasis. At lease three biochemical pathways, i.e., mitochondrial electron transport chain, xanthine oxidase, and polymorphoneutrophil, have been identified as potential sources of intracellular free radical generation during exercise. These deleterious reactive oxygen species pose a serious threat to the cellular antioxidant defense system, such as diminished reserve of antioxidant vitamins and glutathione, and has been shown to cause oxidative damage in exercising and/or exercised muscle and other tissues. However, enzymatic and non-enzymatic antioxidants have demonstrated great versatility and adaptability in response to acute and chronic exercise. The delicate balance between prooxidants and antioxidants during exercise may be altered with aging. Study of the complicated interaction between aging and exercise under the influence of reactive oxygen species would provide more definitive information as to how much aged individuals should be involved in physical activity and whether supplementation of nutritional antioxidants would be desirable.

5

THE EFFECTS ON SENESCENT OSTEOBLASTS OF IGF-I FOR USE IN BIODEGRADABLE POLYMERS. Kelly, S. J., * Attawia, M., Laurencin C. T. Department of Orthopaedics, Allegheny University of the Health Sciences, Philadelphia, PA 19129.

As patients age, osteoblast proliferation decreases, and patients are more prone to severe fractures. Our laboratory has developed several degradable polymer matrices that hold great promise for the treatment of bone defects. The incorporation of osteogenic growth factors into these polymer matrices will help to promote growth and healing of the surrounding bone. This study serves as a pilot for the development of degradable polymer matrices which incorporate growth factors for use in the repair of bone defects and fractures.

The growth, proliferation, and phenotypic expression of osteoblasts appear to be regulated by the complex interrelationship of a series of cytokines and growth factors. A number of recent studies have demonstrated the importance of Insulin Like Growth Factor I (IGF-I) and Bone Morphogenic Proteins (BMPs) as important mediators of osteoblast growth and proliferation. IGF-I appears to increase the rate of osteoblast growth and differentiation in both an autocrine and endocrine manner. Stimulation of osteoblasts with IGF-I could be used clinically to promote bone healing or arrest the development of osteoporosis.

Recently, studies of osteoblasts isolated from fetal rat calvaria indicate that IGF-I does increase the rate of growth and proliferation of osteoblasts. However, the effect of IGF-I on senescent cells in vitro has not been extensively characterized due to the difficulty of culturing such cells. One study has shown that IGF-I will up-regulate the proliferation of senescent stromal cells in vitro; however, IGF-I's effect on differentiated senescent osteoblasts' growth and phenotypic expression was not characterized.

This study attempts to determine the effect of the growth factor IGF-I on the growth, differentiation, and phenotypic expression of senescent osteoblasts. Osteoblasts were harvested from the tibiae of 3-, 6-, and 12-month-old Fischer 344 rats using an explant culture technique. The harvested osteoblasts from each age group will be grown in media containing a concentration of either 5 ng/ml or 50 ng/ml of IGF-I. The growth, proliferation, and phenotypic expression of cells grown in media containing the IGF-I will be compared to osteoblasts grown in media without IGF-I.

Growth, proliferation and phenotypic expression will be measured using a number of techniques. Cell counts using a trypan blue exclusion technique will be performed at 24 hours, 4 days, and 7 days. At days 7, 14 and 21, the expression of alkaline phosphatase, osteocalcin, and mineralization will be measured. The osteoblasts will be observed using SEM and light microscopy to characterize their attachment and growth.

The isolation of osteoblasts from the tibiae of the animals proved to be highly successful. Once additional optimization of the maintenance of the cultures is performed, the effects of IGF-I on senescent osteoblasts will be demonstrated.

6

INFLUENCE OF BLOOD FLOW ON MUSCLE METABOLISM IN ELDERLY PATIENTS WITH PERIPHERAL VASCULAR DISEASE. Lee, R. K., * McCully, K., Gahtan, V., Posner, J., Department of Medicine, MCP◆Hahnemann School of Medicine and AHERF Institute on Aging, Philadelphia, PA 19129.

This study was designed to develop a methodology to produce small quantifiable changes in blood flow while measuring muscle metabolism. The methodology was then used to gather preliminary data concerning the relative importance in muscle metabolism of blood flow versus mitochondrial function on subjects with either normal circulation or with peripheral vascular disease (PVD). Previous studies have shown that it has been difficult to quantify the relative contributions of oxygen delivery and mitochondrial capacity for oxidative metabolism in muscle tissue. The study population was comprised of five subjects with normal circu-

lation and two subjects with PVD. Subjects performed plantar flexion exercise using their left medial gastrocnemius muscle. Phosphocreatine (PCr) resynthesis after the exercise was used as an index of muscle metabolism and was measured using magnetic resonance spectroscopy. Post-exercise blood flow of the popliteal artery to the medial gastrocnemius was controlled via a blood pressure cuff inflated to 50, 60, 70, 80 and 90mmHg. All subjects showed a hyperemic response after the exercises compared to resting blood flow measurements, though there was a stronger response in the normal subjects. Blood flow was not affected dramatically until the cuff was inflated to approximately 70mmHg, resulting in a 22% reduction in normal subjects and 57% for PVD subjects. Large blood flow restrictions (>70mmHg cuff inflation) resulted in PCr recovery time constants (Tc) that were increased substantially. However, small reductions in blood flow to muscle (corresponding to cuff pressures between 50-70mmHg) had comparatively little effect on the PCr Tc in normal subjects. These preliminary data suggest that in healthy non-PVD people, blood flow is in slight excess and the limiting factor in muscle performance is mitochondrial capacity.

7

ACTIVATION OF THE INSULIN-LIKE GROWTH FACTOR TYPE 1 RECEPTOR BY DELETION OF AMINO ACIDS 870-905. Li, S. Hoff, H.*, Sell, C., Center for Gerontological Research, Department of Pathology and Laboratory Medicine, MCP◆Hahnemann School of Medicine, Allegheny University of the Health Sciences, 2900 Queen Lane, Philadelphia, PA 19129.

We have created a deletion mutant of the insulin-like growth factor type 1 receptor (IGF-1R) which lacks the 36 amino acids immediately C-terminal to the transmembrane domain (aa870-905). This region has been found to exert a negative effect on the function of an avian sarcoma virus gag-IGF-1R fusion. We sought to determine whether this region plays a similar role in the intact IGF-1 R. Analysis of the tyrosine kinase activity of the IGF-1RD870-905 indicates that the mutant receptor is constitutively autophophosphorylated and processing of the receptor is decreased, resulting in accumulation of a high molecular weight precursor (containing both a and b subunits). The major substrate of the IGF-1R, IRS-1, is also constitutively phosphorylated by the IGF-1RD870-905, and phosphoinositide 3-kinase activity is increased in the absence of IGF-1. In contrast, ERK-1 activity was not elevated in the absence of IGF-1. The mutant receptor promoted mitogenesis only in the presence of IGF-1. We conclude that this deletion increases the basal activity of the IGF-1 receptor tyrosine kinase and stimulates the PI 3-kinase but is unable to stimulate MAP kinase activation in the absence of ligand. This mutation verifies observations made in the gag-IGF-1R fusion implicating the extracellular domain of the IGF-1R in negative regulation of the receptor.

8

CEREBELLAR INVOLVEMENT IN AGING, LEARNING, AND TIMING. Woodruff-Pak, D. S, Department of Psychology, Temple University, Philadelphia, PA.

The structure essential for learning in eyeblink classical conditioning is the cerebellum ipsilateral to the conditioned eye. Extensive research using a variety of neuroscientific techniques in rabbits has demonstrated that specific regions of the cerebellum are essential for acquisition and retention of conditioned responses, and studies using human neurological patients and normal adults with imaging have identified similar circuitry in humans. In normal aging in humans and rabbits, eyeblink conditioning becomes impaired. In rabbits this age-related impairment in conditioning is highly correlated with Purkinje cell loss in cerebellar cortex. Age-related changed in cerebellar cortex appear to affect the timing of responses as well as learning.

9

NORADRENERGIC INVOLVEMENT IN MOTOR LEARNING. Bickford, P.C., Department of Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262.

Norepinephrine is known to act as a neuromodulator in the cerebelar cortex because it can increase the effect of neurotransmitters such as GABA. This neuromodulatory effect of NE is a possible substrate for an effect of NE on cerebellar plasticity. We have examined cerebellar plasticity using a motor learning behavior which consists of rats running on aluminum rods in a fixed pattern. Rats that have received 6-hydroxydopamine lesions to deplete central stores of NE do not learn this task as quickly as normal controls. In addition, aged rats that have impaired noradrenergic neurotransmission also show impairments in performance on this motor task. This supports the role of NE in cerebellar plasticity.

10

EFFECTS OF GDNF IN AGING AND ANIMAL MODELS OF PARKINSON'S DISEASE. Hoffer, B. J. NIH/NIDA, Division of Intramural Research, Addiction Research Center, PO Box 5180, Baltimore, MD 21224.

Aging is accompanied with declines motoric function which may be the result of deficits in central nervous system dopaminergic function. Glial Cell-Line Derived Neurotrophic Factor (GDNF) has been shown to have neuroprotective and restorative effects on dopaminergic neurons of the nigrostriatal pathway in young rats. In this study, 10, 40, 60 mg GDNF or vehicle was injected inrastriatally in 16-17 month old F344 rats. Coordination and muscle strength as determined by performance on an inclined balance beam and a wire grip strength test were monitored for up to 5 weeks post-injection. GDNF elicited dose-dependent improvements in motor coordination without concurrent increases in strength. The highest dose tested produced >79% improvement in motor coordination, resulting in performance scores approaching those achieved by 3 month old rats tested concurrently. These findings indicate GDNF produces profound improvement in the motoric function of mature rats, which may be related to dopaminergic circuits.

11

NEURAL POPULATION ACTIVITY IN SENSORIMOTOR CORTEX CAN CONTROL AN EXTERNAL "ARM" MOVEMENT SYSTEM. Moxon, K.A., and Chapin, J.K., Department of Neurobiology, Allegheny University, Philadelphia PA.

Neural activity recorded from populations of cortical cells was used to control a mechanical arm that provided a water reward to a rat. Neuronal ensembles were simultaneously recorded through chronically implanted arrays of electrodes in the forelimb areas of the primary motor (MI) cortex and the ventrolateral (VL) thalamus of rats trained to obtain a water reward by pressing a bar which was configured to proportionally move a mechanical arm (MA) from a water source to the rat's mouth. The activity of 32 single units were simultaneously recorded in the MI and VL during performance of this task. The resulting data were subjected to a principal components analysis (PCA) to extract an eigenvector weighting matrix which weighted these neurons according to their task related activity. The weighted responses were processed through a 32 channel spike integration circuit whose output, a neural population vector (NPV), was then used to control the MA. When control of the MA was switched from the bar press to the NPV, the rat was able to obtain its water reward by direct real-time translation of ongoing MI and VL neural ensemble activity. When under NPV control, the MA movement accurately followed the phasic activity of these neurons around the onset of arm movement, but were not maintained during the position holding phase. Nonetheless, the rats NPV was able to move the MA and obtain the water reward with a nearly 100% reliability. Over 90% of the recorded neurons were found to contribute to the smooth control of the MA, its precision increasing a function of the number of neurons used. The precision increased still further when clusters of 2-4 units were used rather than single units. These results suggest that large neural populations in the MI and VL can precisely encode the onset phase of forelimb movement.

12

HPLC-ELECTRO-CHEMICAL ARRAY DETECTION OF OXIDATIVE BIOMARKERS IN THE CENTRAL NERVOUS SYSTEM: APPLICATION TO THE UNDERSTANDING AND ASSESSMENT OF NEURODEGENERATIVE DISEASES. Hensley, K. Oklahoma Medical Research Foundation, Oklahoma City, OK 73142, Maidt, M.L., Oklahoma Medical Research Foundation, Oklahoma City, OK 73142, Markesbery, W. Sanders Brown Center on Aging, University of Kentucky, Lexington, KY 40526, and Floyd, R.A. Oklahoma Medical Research Center on Aging, University of Kentucky, Lexington, KY 40526.

An emerging paradigm holds that Alzheimer's disease and perhaps other neurodegenerative disorders involve some component of oxidative stress that may be causally linked to an inflammatory etiology. Such an hypotheses is supported by a growing body of empirical data, however, it remains to be proven to a high degree of confidence. Validation of the

"oxidative stress and inflammation" hypotheses of neurodegeneration requires the development of bioanalytical techniques for the precise and routine quantitation of oxidative stress biomarkers within the central nervous system (CNS). Toward this goal, methods have been developed for the measurement of tyrosine oxidation products in human cerebrospinal fluid and other tissue. Free and protein-bound tyrosine, 3,3-dityrosine, 3-nitrotyrosine, 3-chlorotyrosine, 0- and m-tyrosine and 3,4-dihydroxyphenylalanine were measured by high performance liquid chromatography with coulometric electrochemical array detection (HPLC-EC) in the ventricular fluid (VF) and brain tissue (hippocampus, superior and middle temporal gyri, inferior parietal lobule and cerebellum) of subjects afflicted with clinical and postmortem histological manifestations of Alzheimer's disease (AD, N-11), as well as age-matched normal subjects (N-5). Results of this work indicate a significant ($p < 0.02$) 2.5 -fold elevation of dityrosine / tyrosine ratio in the AD VF relative to control VF. Since dityrosine is biosynthesized by activated macrophages as well as produced nonenzymatically by Fenton chemistry, the alterations in dityrosine / tyrosine ratio may indicate oxidative stress resulting from macrophage/microglial activation. VF and brain levels of methionine homocysteine (a possible biomarker of vascular compromise), uric acid (a putative biomarker of antioxidant capacity), nitrite and nitrate were also measured. Preliminary results indicate a decrease in VF levels of uric acid and nitrite in the AD subjects, with an increase in the nitrate/nitrite ratio. Analyte concentration in the VF are currently being correlated with brain concentrations in all subjects. These data support the "oxidative stress and inflammation" hypotheses and suggest that HPLC-EC may be a useful tool for the clinical evaluation of neurodegenerative diseases.

13

THE LATERAL DIFFUSION COEFFICIENT OF CON-A-RECEPTOR PROTEIN IN THE SKELETAL MUSCLE MEMBRANES ALSO DECLINES WITH AGE AS IN HEPATOCYTE MEMBRANES IN C57BL MICE. National Institute for Longevity Science, 36-3, Gengo, Morioka-cho, Obu-shi, Aichi 474, Japan; and Tanaka, S. National Institute for Longevity Science, 36-3, Gengo, Morioka-cho, Obu-shi, Aichi 474, Japan; Nagy, J. Zs., National Institute for Longevity Science, 36-3, Gengo, Morioka-cho, Obu-shi, Aichi 474, Japan and Department of Gerontology (VILEG Hungarian Section), University Medical School, Debrecen, H-4012, Hungary.

The authors have previously shown that the lateral diffusion coefficient of proteins (and more recently of lipids) of hepatocyte surface membranes declines in a linear fashion in various rodent models such as rats, mice, Peromiscus, and mus Musculus. In order to examine whether this change also occurs in other cell types, the present study examined the protein lateral diffusion coefficient in the skeletal muscle membranes in mice of both sexes and of different ages. The lateral diffusion coefficient (D_p) of the Con-A-receptor protein was measured in the sarcolemma of the quadriceps femoris (QF) muscle of male and female C57BL/6J mice in 4 age groups between 2 and 26 months of age. Freshly prepared, ex vivo taken muscle strips were stained with Con-A-FL conjugate for 10 minutes, and fluorescence recovery after photobleaching (FRAP) measurements were carried out on 20-30 cells per animal, at 37°C. Using this technique, D_p and the fractional recovery (mobile fraction = FR%) of these proteins can be measured. In the youngest male and female age-groups, D_p = 5.72E-10, and D_p -5.43E-10cm²/sec, while FR% = 43.3 and 36.3% were found, respectively. D_p displayed a characteristic, significant, negative, linear age correlation in both sexes with the D_p values being 3.11E-10 and 3.07E-10 in oldest (26 months) mice and female mice respectively. The slope of the linear regression line calculated per month of age was 1.06-11, and 0.96E-11 cm²/sec for males and females respectively; both of them differed from zero highly significantly. FR% values which remained unchanged in hepatocyte surface membrane proteins tended to increase slightly with age, yet the estimated average D_p extrapolated to the total Con-A-receptor pool D(FR), maintained its significant, negative, linear age-correlation. We conclude that the linear decline with age of protein diffusion coefficient, previously found by us for hepatocyte surface membranes occurs with other cell types as well.

Reference:

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MELATONIN REDUCES OXIDATIVE STRESS MEDIATED TISSUE DAMAGE. Reiter, R. J., Department of Cellular & Structural Biology, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78284.

Melatonin, a phylogenetically ancient indoleamine found throughout the animal kingdom as well as in plants, exhibits significant free radical scavenging and antioxidant activities. As an electron donor, melatonin reported scavenges the hydroxyl radical (•OH) with a rate constant of $2{,}7x10^{10}$ M/sec. Secondarily, it also neutralized the superoxide anion radical (O_2-•). While melatonin provides significant antioxidative protection against lipid peroxidation, its efficacy as a direct peroxyl radical (LOO•) scavenger is debated. In addition to these actions, melatonin scavenges singlet oxygen ($^1O_2$) and the peroxymitrite anion (ONOO-) and stimulates two antioxidative enzymes, glutathione peroxidase and glutathione reductase, while it inhibits nitric oxide synthase, a prooxidative enzyme. This combination of actions coupled with melatonin's synthase, a proxidative enzyme. This combination of actions coupled with melatonin's ability to be readily absorbed, cross all morphosphysiological barriers and possibly distribute throughout the cell, permits melatonin to protect cells from oxidative stress induced by a variety of agents. While these pharmacological actions of melatonin are well documented, whether endogenously produced melatonin provides significant protection against oxidative stress remains unknown. To date, exogenously administered melatonin has been shown to reduce hepatic, gastrointestinal and neural damage due to ischemia/reperfusion, to limit excitotoxicity in the central nervous system, to protect against α-amyloid protein of Alzheimer's disease, to reduce MPTP-toxicity in the model of Parkinson's disease, to curtail oxidative damage due to hyperbaric hyperoxia, to limit lipid peroxidation products and 2-deoxyguanosine levels that result from toxin exposure, and to lower oxidative stress during acute exercise. Naturally occurring melatonin analogues, for the most part, are less efficient antioxidants than is melatonin. Melatonin has no known significant acute of chronic toxicity. Endogenously, its production diminishes with age.

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CHRONIC IGF-I TREATMENT OF AGING TYPE 2 DIABETIC MONKEYS: EFFECTS ON MUSCLE AND ADIPOSE TISSUE. Hansen, B.C. Bodkin, N.L., and Levin, N. University of Maryland, Baltimore, MD 21201 and Genentech, San Francisco. CA 94080,USA.

Aging-associated declines in growth hormone, insulin-like growth factor I (IGF-1) and DHEAS are well recognized and are likely to affect muscle and adipose tissue metabolism. Clinical trials of GH replacement do not, to date, suggest that growth hormone therapy is likely to be attractive, and such GH treatment is limited by adverse effects, including increased insulin resistance, gynecomastia, and carpal tunnel syndrome. The possibility that IGF-1 administration could have positive effect on skeletal muscle and adipose tissue in the elderly diabetic subjects was examined in the present study. The effects of IGF-1 are mediated principally by its own receptor which is structurally similar to the insulin receptor. With aging, insulin sensitivity declines, and it declines further with the development of Type 2 diabetes mellitus. Circulating IGF-1 levels also decline with age (Bodkin et al, Metabolism 40:1131-1137, 1991) and may be of importance in cellular aging (Rosen and Kessenich, Endocrinology 6:102-108, 1996 and Parrizas, Saltiel, and LeRoith, J. Biol. Chem. 272:154-161, 1997).

We have carried out a long term (>6 months) study of IGF-1 administration to middle aged and older diabetic monkeys to examine several aspects of the effects of chronic IGF-1 treatment. IGF-1 was administered to fourteen non-insulin dependent diabetic monkeys using a dose of 40 micrograms per kilograms bid. For many monkeys the dose of insulin could be reduced during IGF-1 treatment (average dose of insulin reduced by thirty percent) while total IGF-1 plasma levels were increased by 2-3 fold (Hansen, Levin, Taylor and Bodkin, Diabetologia 40:A347, 1997).

In muscle and bone IGF-1 is known to play a role in tissue differentiation and we have examined its effects on the expression of several genes considered important to adipcyte differentiation, as well as IGF-1 effects on skeletal muscle GLUT-4 which could be involved in the insulin resistance of aging and diabetes.

After 25-30 weeks of IGF-1 treatment (and co-therapy with insulin) skeletal muscle GLUT-4 mRNA was not difference from the control (insulin only) conditions. The adipose tissue expression of peroxisome proliferator activated receptor (PPAR) and of lipoprotein litpase (LPL)

were significantly reduced during IGF-1 treatment (p's ,0.05). Further several other genes related to adipocyte differentiation and metabolism tended to be reduced (although not significantly) including GLUT-4, FAS, CEBP.a and adipsin.

We conclude that IGF-1 is likely to be associated with fewer incidents of hypoglycemia compared to insulin and may have positive effects to increase protein synthesis and reduce adipocyte differentiation. Consideration must be given to increased risk of neoplasms with IGF-1 treatment, a possibility not yet addressed fully to date in this or other prior studies. The potential role of exogenous IGF-1 treatment in aging and in aging-associated diabetes mellitus deserves further study.

16

BIOMARKERS OF AGING AND AN INNOVATIVE THEORY BASED ON THE THRESHOLD AGE CONCEPT. Cosmas, A. C., School of Allied Health Professions, The University of Connecticut, Storrs, CT 06269. Manfredi, T. G., University of Rhode Island, Exercise Science Program, Kingston, RI 02881.

Aging may be regarded as a process of gradual disorganization among a number of intrinsic complex biochemical cellular systems that are normally responsible for maintaining cellular homeostasis. Even though detailed patterns of aging within the mammalian systems are incomplete, there appears to be a consistent pattern of age-related changes that occurs throughout a normalized life span beginning during middle age.

This discussion will serve as an introduction for this morning's session in the context that it will review a number of the specific biomarkers of cellular aging that occurs at the transcriptional and translational levels within the cell. Each speaker in the symposium will present his current research on aging biomarkers.

It is reasonable to assume that somatic cells that undergo senescent changes must differ in their expression of certain genes. Moreover, that this selective gene expression increases their vulnerability to the aging process is understandable. Is it possible to alter the products of selective gene expression, and even more fundamental, what is the potential of altering gene expression; thereby affecting the cellular aging process? As an example, the general consensus gives us the confidence to make the general statement that the OXPHOS system declines in activity as age progresses. We are confident that intense physical activity results in some degree of hypoxia. One of the consequences of hypoxia-reperfusion injury may be the accelerated production of reactive oxygen species (ROS) which could result in mitochondrial DNA mutations and eventually accelerated aging. Is it conceivable that physical activity may actually act as a prime catalyst in this process? If so, does this process occur consistently throughout the organism's life or is there a critical period, a "Threshold Age," prior to which the organism can successfully adapt to the stress, but beyond which it becomes too stressful and may accelerate aging? In other words, at what period during the organism's life and at what intensity does physical activity cease to be a positive stimulus and become a stress factor. At a certain age, hypoxia caused by physical activity may affect mitochondrial-nuclear dynamics to the point where mitochondrial sizes are altered and the organelle's ability to provide for OXYPHOS becomes compromised resulting in increased numbers of ROS generated and accelerated aging.

Another rather interesting area relates to the effect of diet manipulation on the aging process. It appears that diet restriction extends the median and maximum life spans, at least in part, by altering genetic expression at the transcriptional level causing the expression of genes for the synthesis of specific antioxidant enzymes. Caloric restriction (CR) is an experimental manipulation that increases longevity and reduces lesions in mice as compared to animals fed ad libitum (AL). Morphologically, the appearance of tubular aggregates, centrally displaced nuclei, nuclear chains along the sarcolemmal borders, interfascicular wedging, longitudinally split fibers and cellular infiltrates are biomarkers of cellular aging and are dependent, to some degree, at least to age and diet.

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MUSCLE PLASTICITY AND AGING: AT WHAT POINT DOES STIMULUS BECOME A STRESS? Farrar, R. P. Department of Kinesiology and Health, University of Texas, Austin, TX 78712.

As longevity increases it becomes more important to maintain quality of life for the older individual. Exercise has grown in popularity as a modality to help maintain physiological function and attenuate the declines normally associated with aging. Over the last 25 years research reports have documented that the skeletal musculature maintains a significant level of plasticity. Endurance training induces equivalent increases in aerobic capacity as evidenced by elevated enzyme activities of muscle homogenates and mitochondrial content if the increased conractile activity is the same for young and old. This is true in both human and animal models, utilizing either endurance training or chronic tonic stimulation. Muscle mass is lost during the aging process in much of the skeletal musculature, although there are some muscles in rodent models that do not demonstrate loss of muscle mass. The loss of muscle mass in humans occurs through the loss of both fibers and cross sectional area of the fibers, with a greater percentage of loss occurring in the fast fibers. In rodent models, the muscles that demonstrate atrophy do so through loss in cross-sectional area of the muscle. Both human and rodent models demonstrate the ability to respond to strength training with significant increases in muscle mass and force output. The muscles in aging organisms are more susceptible to injury, however, and have a poorer regenerative capacity. Eccentric exercises place a greater force per cross-bridge and thus a greater stress on the weakest portion of the muscle or in sarcomeres where inhomogeneities exist. The slowed rate of relaxation may make the aging muscles stiffer and thus more susceptible to damage. The reduced antioxidant capacity of the muscles exacerbates the damage that occurs. The reduced regenerative capacity of the aging muscle makes this damage more significant.

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MORPHOLOGICAL FEATURES OF AGING SKELETAL MUSCLE. Manfredi, T.*, Cosmas, A., Lamont, L., and Riebe, D., Univ. of Rhode Island, Exercise Science, Kingston, RI 02881; Univ., Connecticut, Allied Health Professions, Storrs, CT. 06269.

The lack of mobility in older adults compromises a quality lifestyle and increases risk for metabolic and immune disorders (ex. cardiovascular disease, diabetes, osteoporosis). Resistance training in frail elderly adults enhances strength and mobility. Diet restriction has also been reported to lower the incidence of tumors and increase life span. However, little information is available regarding the relationship of tissue morphology to biochemical and immune responses to dietary intervention. Furthermore, research relating ultrastructural adaptations of older adults to resistance training will broaden our understanding regarding the plasticity of senile muscle. Our collaborative experiments explore the effects of various diet and exercise interventions on myocardium and skeletal muscle ultrastructure of young and older animal and human tissue and attempts to relate these findings to cell function.

Aging human and animal skeletal muscle is characterized by alterations in substrate content and mitochondrial mrophometry. These changes can affect responses to physical stress and diet. For example, we reported that older adults with lower oxidative capacity and muscle mass show evidence of greater muscle damage than their younger counterparts when subjected to an exercise protocol designed to impose myofilament stress. This implies compromised tissue plasticity in older adults, explained in part by a lowered muscle mass. We also examined skeletal muscle from mice fed ad libidum (AL) and a 40% restricted diet (DR) and reported that DR appears to lessen the age related effect on muscle fiber atrophy. However, age matched DR mice showed evidence of lowered capillary density, implying a lowering of oxidative capacity. To this end we explored the effect of DR on mitochondrial density, a morphological feature of tissue oxidative energy production and found that although DR did not appear to have any effect on the density of mitochondria, older DR mice appear to have fewer larger size mitochondria, suggesting a more efficient metabolic tissue in DR mice. This tempering of the age associated increase in mitochondrial size supports findings from other studies exploring the effect of chronic exercise on myocardial mitochondria.

We recently reported the effects of dietary manipulation and exercise on rat myocardial capillary receptor morphometric profiles and found that receptor numbers are altered with cholesterol feeding, exercise and age. Exercise apparently increases vessel receptor numbers; however in older animals this is not true, suggesting that physiologic stress may not be as effective in enhancing receptor numbers. Collectively, these studies imply that exercise and diet restriction are powerful regulators of skeletal muscle and myocardium ultrastructure. Furthermore, careful examination of young and older tissues suggests that diet and exercise interventions can be effective interventions in older adults if properly prescribed.

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BONE BASICS: THE ROLE OF EXERCISE. NUTRITION, AND HORMONES IN PREVENTING OSTEOPOROSIS. Drinkwater, B. L., Dept. of Medicine, Pacific Medical Center, Seattle, WA.

Osteoporosis is a "silent" disease. The gradual loss of bone that places women and men at risk for this disease is imperceptible but with time can result in serious fractures leading to years of dependency, discomfort, and depression. Rather than emphasizing treatment for a disease which is essentially nonreversible, emphasis must be focused on prevention. The three most important factors that contribute to healthy bones are hormonal, nutritional, and mechanical. If there is a deficiency in any one of these three areas, bone loss is likely to occur.

Exercise: Exercise has many beneficial effects for women and men of all ages and has the potential to help them reach their biological potential for bone mass. Although the absence of gravitational force or mechanical loading on the skeleton results in a rapid and marked loss of bone, there is no evidence that exercise beyond that required for activities of daily living can increase bone mass or attenuate bone loss enough to protect women from osteoporotic fractures. While physically active women usually have above average bone mass, any increase in BMD of sedentary women enrolled in a training program is minimal. The low vertebral bone density observed in young amenorrheic women suggests an important interaction between estrogen and exercise. Normal estrogen levels, physical activity, and adequate calcium may work together to maximize peak bone mass in the early adult years and slow bone loss as women age. Even when an exercise program is started during the postmenopausal years, there may be a significant increase in bone mass if the women are on estrogen therapy. How effective this small increase in density will be in preventing or delaying osteoporotic fractures is unknown. However, exercise may also decrease the likelihood of fractures indirectly by improving neuromuscular coordination, balance, and muscular strength, thereby decreasing the chances of falling and/or minimizing the damage from a fall.

Calcium: Current evidence suggests that adequate calcium intake is important to ensure that each woman attains her biological potential for peak bone mass during childhood and adolescence, maintains that level during her adult years, and minimizes bone loss in her later years. Beneficial effects have been reported for women age 60 and older. Several studies report that the combination of calcium and exercise was more effective in increasing bone mass than either factor individually.

The NIH Consensus Statement on Optimal Calcium Intake lists the recommended daily calcium intake for children, men, and women and can be obtained by calling 1-800-644-6627.

Estrogen: Maintenance of a normal reproductive cycle prior to menopause is important in ensuring an adequate level of endogenous estrogen. Women who diet excessively or who incur an energy deficit by exercising strenuously without matching energy intake with energy output frequently become amenorrheic. Bone loss can be rapid and extensive. While the structure and function of the cardiovoascular system change with mammalian aging. With a focus on the heart, we will review the literature and our own work putting into context potential gender differences. Specifically, we will center the discussion on the diastolic dysfunction (impared relaxation) commonly observed in the elderly and the impact of a regular program of exercise. Finally, we will outline potential avenues for additional study. Exercise alone does not prevent bone loss in the postmenopausal years, the combination of estrogen replacement and exercise has a more beneficial effect on bone than either alone. The complete picture of how these three important variables – estrogen, calcium, and estrogen – interact to preserve bone mass remains to be determined.

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ARE THERE GENDER DIFFERENCES IN OLDER ADULTS' RESPONSE TO EXERCISE? Tate, C., Vice Povost, Academic Programs and Faculty Affairs, University of Houston, Houston, TX.

The structure and function of the cardiovascular system change with mammalian aging. With a focus on the heart, we will review the leterature and our own work putting into context potential gender difference. Specifically, we will center the discussion on the diastolic dysfunction (impaired relaxation) commonly observed in the elderly and the impact of a regular program of exercise. Finally, we will outline potential avenues for additional study.

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EXERCISE EFFICACY FOR OLDER WOMEN. Brown, M., Department of Physical Therapy, Washington University, St. Louis, MO

For a variety of reasons (e.g., less strength, cardiovascular endurance), older women are more at risk than men for functional decline with advancing age. Functional compromise frequently includes difficulty with activities of daily living and slowing of gait. The decline in functional capacity with aging has been associated with deficits in strength, balance, range of motion, and declines in speed of movement and reaction time, all of which are modifiable with exercise. The intent of this presentation is to highlight changes that occur with low-intensity exercise in women who are young-old (60-70 years) and in women who are old (78-92 years). The low-intensity program emphasized proximal stability, balance, modest strengthening, flexibility and speed of movement and gains in each of these domains were seen following exercise. For the older group changes in strength, range of motion, balance and speed of movement were associated with improvements in functional tasks. For a sub-group of the younger women, the 3 month low-intensity exercise program was followed by 9 mo of endurance training. Half of the subgroup was on estrogen replacement therapy during the one year exercise period which did not appear to promote improvements in strength over and above those seen with exercise along. Findings suggest that factors contributing to functional decline (e.g., strength, range of motion) are modifiable and that loss of physical independence is preventable.

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QUANTITATION OF EXERCISE IMPROVEMENT IN THE GERIATRIC POPULATION. Chance, B.[1], Nioka, S.[1], Lech, G.[1], Long, H.[1], Bank, W.[2], McCully K.[3] [1]Department of Biochemistry & Biophysics, University of Pennsylvania, School of Medicine, Philadelphia, PA 19104,[2]Department of Neurology, Hospital of the University of Pennsylvania, Philadelphia, PA 19104, [3]Allegheny University of the Health Sciences, Department of Medicine, Philadelphia, PA,

The general concept that exercise will improve the quality of life in the geriatric population can be validated in a simple, safe and affordable noninvasive study with near infrared spectroscopy (NIR) of muscle tissue. Magnetic resonance spectroscopy affords a safe and accurate approach to the quantification of the velocity of ATP synthesis in the human limb, but has three drawbacks: (1) the horizonal position in which the exercise is to be performed is awkward; (2) Accommodation within the cylindrical hole of the magnet is unacceptable to a portion of the population, (3) The expense is significant. Ultrasound precisely measures structures, but not functions. The novel NIR technique of spectroscopy and imaging is not only safe and effective, but significantly affordable and in tune with the times of HMO support. The present NIR devices, which will be described and detailed by those contributing to this symposium will examine the quantification of exercise-induced activation of muscle function in this important population. The methods are generally similar, activation of muscle function for example, by treadmill exercise for the mobile subject or plantar flexion exercise for the immobile subject causes increased oxygen delivery and oxygen utilization in the muscle under optical monitoring. In cases of oxygen utilization in excess of oxygen availability, hemoglobin deoxygenates in the exercised muscle to an extent depending upon the exercise ability and capability of the subject. In peripheral vascular disease, excessive deoxygenation occurs and the consequences of claudication are readily discernable. The most useful and quantitative response occurs at the cessation of an exercise interval adequate to induce significant deoxygenation. At this time the rate of restoration of oxygen in the tissues or phosphocreatine to the muscle fibers gives a robust measure of the oxidative capacity of the limb and is furthermore a sensitive indicator of changes therein induced by exercise-training regimens. In special cases where the energy metabolism is disabled by disease or by genetic defects, then the pattern of response is quite different, namely oxygen delivery exceeds oxygen demand and oxygenation of hemoglobin occurs. In this case, on cessation of exercise, the recovery (which is in fact a deoxygenation) only poorly represents the capability of the energy metabolism. Thus exercise with cuff for occluding circulation is much preferable. Just as in the case of nuclear magnetic resonance, spectroscopy was first embodiment, but then imaging took over and became the clinical manifestation of the technology, so as near infrared studies, spectroscopy is now being replaced by imaging. The initial studies of the imaging of muscle function may have special uses in the study of adaptation to exercise. Particular portions of the muscle are clearly shown be activated to a greater extent

than others. it may be that these 'islands" off activity will he where the improvements of muscle function due to exercise will be most noted. In summary, the field of noninvasive methods for studying muscle metabolism and its enhancement in the elderly population is moving ahead rapidly with safe, economical and affordable methods appropriate to today's geriatric medicine.

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WHY EXERCISE TRAINING IMPROVES ELDERLY MUSCLE PERFORMANCE: NON-INVASIVE INSIGHTS FROM MAGNETIC RESONANCE. Conley, K. E. Departments of Radiology, Physiology & Biophysics and Bioengineering, Box 357115, University of Washington Medical Center, Seattle, WA, 98195-7115

Exercise training has proven to be an effective way to reverse many of the structural and functional deficits that accompany the aging of muscle. The purpose of this talk is to show how magnetic resonance provides views into the changes in muscle properties that explain the adaptation of elderly muscle to training. We trained elderly subjects for six months using either strength or endurance exercise. Two critical muscle properties were found to change with exercise training: muscle size and energetics. The well resolved images of the cross-section of limbs possible with magnetic resonance imaging reveal surprisingly small changes in the area and volume of muscle (10%) with a strength training program. This small structural change indicates that the large increase in leg press force production (1 RM, 63%) that we found with strength training is due to neuronal control rather than hypertrophy of the muscle. Spectroscopy of the phosphorylated "high-energy" compounds in the muscle reveals that the major sources and sinks for the fuel for contraction, ATP, also adapt to exercise training. Three ATP fluxes that are critical to muscle function can be quantified with new analytical methods applied to magnetic resonance information: 1) ATP cost of contraction, 2) glycolytic ATP synthesis and 3) the ATP recovery time (oxidative phosphorylation capacity). The ATP cost of contraction increases following strength training and indicates a faster force generation capacity. In contrast, endurance training results in reduced contractile costs, lower glycolytic flux and faster recovery times from exercise. This reduction in ATP demand and increase in ATP supply are the basis of the higher aerobic capacity and higher sustained performance seen in these subjects after endurance training. The non-invasive picture of the structural and metabolic changes provided by magnetic resonance reveals how intracellular properties are reflected in muscle performance. The larger size and higher contractile costs reflect the faster and greater force production capacity of elderly muscle following high resistance training. The reduced contractile costs, lower glycolytic flux and faster recovery times are consistent with greater endurance and higher sustained force production in elderly muscle following aerobic training. Thus both muscle size and energetics are adaptable to chronic exercise training in the elderly and this adaptability provides a means to reverse the loss of muscle function.

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13C NMR TO STUDY CARBOHYDRATE METABOLISM AND ITS POTENTIAL FOR AGING STUDIES. Price, T.B. Yale University, New Haven, CT 06510

Natural-abundance 13C nuclear magnetic resonance (NMR) spectroscopy is a noninvasive technique that enables *in vivo* assessments of muscle and/or liver glycogen concentrations. When directly compared with the traditional biopsy technique; NMR was found to be more precise. Over the last several years, we have developed and used 13C-NMR to obtain information about human glycogen metabolism with diet and exercise. because NMR is noninvasive, we have been able to obtain more data points over a specified timecourse, thereby dramatically improving the time resolution. This improved time resolution has enabled us to document subtleties of the resynthesis of muscle glycogen following exercise that had not been observed previously. We have tracked muscle and liver glycogen concentrations in several different human populations under a variety of conditions that include: 1) Muscle glycogen recovery from heavy localized exercise with normal insulin and with insulin repressed, 2) Muscle glycogen recovery in an insulin resistant population, 3) Muscle glycogen depletion during prolonged low-intensity exercise, 4) Effect of a mixed meal upon post-prandial muscle glycogen synthesis, 5) Effect of training upon insulin stimulated muscle glycogen synthesis in healthy, insulin resistant and obese populations, 6) Liver glycogen depletion/synthesis patterns over a 72-hour fasting period and 7) Patterns of muscle glycogen synthesis and glucose transport associated with a standard carbohydrate loading protocol. This presentation will focus upon basic 13C NMR, give results from selected studies and point out potential applications study of an aged population. The noninvasive nature of 13C spectroscopy allows, for the first time, highly resolved timecourses of both muscle and liver glycogen synthesis and depletion. The normal metabolic changes that occur in the elderly can be tracked in a noninvasive manner to yield information that was previously unavailable.

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AGE-RELATED CHANGES IN MUSCLE METABOLISM AND BLOOD FLOW: NONINVASIVE MEASUREMENTS. McCully, K. Department of Medicine, Allegheny University of the Health Sciences, Philadelphia PA 19131.

A key determinate of skeletal muscle metabolism is oxygen delivery. Because of this, it is important when evaluating muscle metabolism to also evaluate potential changes in oxygen delivery and muscle blood flow. The use of noninvasive measurement techniques are especially important when designing studies of elderly humans. This presentation will: discuss the influence of reduced skeletal muscle blood flow on muscle metabolism, give an overview of current noninvasive methodologies to measure skeletal muscle blood flow, and to review previous studies on age-related changes in skeletal muscle blood flow. Blood flow capacity is often not thought to be a factor limiting muscle metabolism under normal conditions in young individuals. However, it is not clear what happens with healthy aging. In addition, reduced blood flow does occur in several age-related disease states. The consequence of this in order of severity of blood flow limitation are: reduced oxidative capacity, limited muscle performance, muscle atrophy, and clinical problems. Several new methodologies have been developed to evaluate muscle blood flow, including magnetic resonance angiography (MRA), Doppler duplex imaging, and near-infrared spectroscopy (NIRS). MRA is an expensive and technically challenging method that promises a wealth of information, including: vessel location and patency, flow measurements in large arteries, and muscle perfusion measurements. Doppler offers arterial flow measurements at slightly less cost and inconvenience. NIRS is an inexpensive and simple technology that can be applied to studies of frailer and more difficult to study subjects. Current studies of blood flow and oxygen delivery have provided hints of age-related changes in healthy individuals while large changes are seen with cardiovascular disease. One of the difficulties in studying healthy aging is identifying when cardiovascular disease first begins to impact on muscle function. Age related changes in blood flow and its effect on muscle metabolism may well prove important in understanding some of the reduced exercise tolerance that is seen in the elderly.

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EFFECTS OF EXERCISE ON BODY COMPOSITION AND FUNCTIONAL CAPACITY OF THE ELDERLY. Evans, W. J., Nutrition, Metabolism and Exercise Laboratory; Reynolds, D. W., Department of Geriatrics, University of Arkansas for Medical Sciences.

Frailty in the elderly is characterized, in part, by greatly reduced muscle mass and strength, as well as chronic conditions which predispose to impaired mobility and function. For men and women between the ages of 65 and 69, almost 30% and 46% of their remaining years will be spent dependent on others. Muscle strength is highly related to function in the very old. Data from the Framingham study indicate that 40% of women between 55-64, 45% between 65-74 and 65% between 75-84 could not lift 4.5 kg. In this study similar percentages of the population reported an inability to perform heavy household work. This striking decrease in strength among the oldest members of our society place them at great risk of dependence and institutionalization. In population studies a significant negative correlation between strength and chosen normal walking speed for both sexes has been seen. In very frail, institutionalized men and women, muscle strength and leg power are closely related to walking speed, chair stand time , and stair climbing ability. Nursing home residents who have been classified as fallers have been shown to be significantly weaker in all of the muscle groups of the knees and ankles. Thirty five percent of a population of 1,042 home-dwelling men and women older than age sixty reported on one or more falls in the preceding year. Among a host of factors that distinguished fallers from nonfallers, such as polypharmacy and arthristis, handgrip strength of the dominant hand emerged as the most important factor. It

appears that the age-related loss of muscle mass may be an important determinant in the reduced maximal aerobic capacity seen in elderly men and women.

Since the sedentary lifestyle of a long-term care facility may exacerbate losses of muscle function exercise training should help the oldest old increase muscle strength and endurance. Research on very old and frail nursing home residents has shown that after 8 weeks, subjects increased muscle strength by 174% and muscle size by 9%. In this population, increased muscle strength also increases gait speed and balance. The fact that significant muscle hypertrophy was seen in this population indicates that age does not decrease the capacity to adapt to a progressive resistance training program. Thus exercise may minimize or reverse the syndrome of physical frailty which is so prevalent among the oldest old. More recently in a population of 100 nursing home residents, a randomly assigned high intensity strength training program resulted in significant gains in strength and functional status. In addition, spontaneous activity, measured by activity monitors increase significantly in those participating in the exercise program while there was no change in the sedentary control group.

This symposium will also focus on newer exercise interventions to increase aerobic capacity in the very old as well as interventions to improve bone health.

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EPIDEMIOLOGICAL STUDIES OF EXERCISE IN THE ELDERLY. DiPietro, L. The John B. Pierce Laboratory and Yale University, New Haven, CT 06519.

A primary goal of studies of successful aging is to identify the modifiable factors related to plasticity of higher physical function; however, little is known about the relationship between habitual physical activity (especially that of lower- to moderate-intensity) on the maintenance of daily function in older people. The question of how patterns of activity and exercise translate into altered physical function among healthy older populations is of considerable interest. Physical activity is defined as a behavior, whereas physical function is defined as performance, comprised of a series of increasingly integrated steps. Recent data from several epidemiologic studies suggest that physical activity is associated with the maintenance of more basic components of physical function, as well as with higher-order task- or goal-oriented functions in healthy older people. Moreover, higher levels of physical activity appear to be associated with better functioning even in those with already-established chronic disease. Thus, regular participation in activities such as walking, gardening, or house/yardwork should be encouraged among the general older community.

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EXERCISE INTERVENTIONS AND BODY COMPOSITION IN OLDER WOMEN. Nelson, M. E. The Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111.

Women are at a distinct disadvantage with men when it comes to body composition. Women have less muscle and bone and naturally have more body fat yet they live longer than men. Because of this difference in body composition, women disproportionately suffer more from disability, frailty and osteoporosis. Aerobic exercise has been shown to improve cardiovascular health in older women but does not seem to influence muscle mass or muscle strength; whereas, progressive resistance training has been shown to increase these factors. In addition progressive resistance training seems to have a much more potent positive influence on bone density and dynamic balance than does aerobic exercise. There is now evidence that both aerobic and strengthening exercises have the ability to improve depressive symptoms and sleep problems in older individuals. Optimizing mood and quality of sleep are important for overall quality of life. The best exercise prescription for improving overall health in older women is a program that incorporates both aerobic exercise and strength training. An exercise program including aerobic and strength training will improve body composition, decrease risk for heart disease, diabetes, osteoporosis, frailty, weight gain with age, mood disorders and will improve symptoms of arthritis. It is important to realize that the burden of chronic disease is not a contraindication for exercise in older women.

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AGE-DEPENDENT RESPONSE OF THE RAT BRAIN TO ISCHEMIC INSULT PRODUCED BY PHOTOCHEMICALLY INDUCED THROMBI. Kharlamov, A.,* Kharlamov, E., Khera, D., Armstrong, D. M. Center for Neurosciences Research, Allegheny University of the Health Sciences, Allegheny Campus, Pittsburgh, PA 15212.

We hypothesize that there is an age-dependent increase in the vulnerability of neurons following brain injury. Underlying this vulnerability in the aged brain is the failure of plasticity in the aged brain, which is exacerbated during an extreme condition such as ischemic stroke which requires the mobilization of all neuroplasticity reserves. The photochemical model of inducing cerebral infarctions is ideally suited for aged rats because it is relatively non-invasive and results in a very low mortality. In addition, the model results in a highly reproducible infarct which, when positioned within sensory-motor area of neocortex, results in specific behavioral deficits. In the present study we produced photochemically induced focal ischemic damage in the brain of rats 4, 20 and 28 months of age in order to investigate the age-dependent increase in neuronal vulnerability. In this study the size of the infarcted area provided an index of neuronal vulnerability while glial response (vinementin immunostaining) and behavior performance on rota-rod provided indices of compensatory mechanism/recovery of function. 24 hours post-lesion the size of the infarcted area was similar between the three groups although there was a trend for a larger lesion in the 20- and 28-month-old rats. 7 days post-lesion, the size of the infarction in the 4-month-old group was the same as that observed 24 hours post-lesion. In contrast, in the rats 20 and 28 months of age the lesion was significantly larger (i.e., 23%) 7 days post-lesion as compared to 24 hours. These data support an age-related increase in infarct volume over time. Correlating with these latter data is marked absence of vimentin-positive profiles in the area penumbra of old animals compared to young. In addition, performance on rota-rod supports a sustained deficit in the 28-month-old rats compared to 4-month rats. Thus, these data demonstrated that, as a result of lack of neuronal plasticity, in old rats, the pathological consequences of photothrombotic brain ischemia were more severe than in young animals. Contributing to this increased vulnerability is a delayed and/or inadequate response of reactive glia.

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CONFORMATIONAL CHANGE IN TAU PRECEDES β-AMYLOID DEPOSITS IN THE HIPPOCAMPAL FORMATION OF ALZHEIMER'S DISEASE CASES. Ikonomovic, M.,* Mizukami, K., Davies, P., Hamilton, R., Sheffield, R. and Armstrong, D. Center for Neurosciences Research, MCP◆Hahnemann School of Medicine, Allegheny University of the Health Sciences, Pittsburgh, PA.

The goal of the study was to determine whether conformational changes in tau, detected with mab MC1, precede the deposition of amyloid b protein (AbP), detected with R1280. MC1 was selected for the study because it has been shown to be a very early marker of neurofibrillary pathology. All cases received a Braak score (i.e., I-VI) indicating pathologic severity. Consistent with this rating scale, MC1 immunostaining clearly delineated the progressive nature of the neuropathology in the hippocampal formation (HF) such that immunolabeled cells were first observed in the entorhinal cortex (EC) followed by the subiculum (SUB)¾CA1¾CA2¾CA3/4¾dentate gyrus. Notably, these early appearing MC1-labeled cells (i.e., those in SUB/CA1 of a stage I, CA2 of a stage II, etc.) were characterized by normal neuronal morphology and in no instance were associated with AbP immunolabeling. In contrast, in regions displaying more advanced pathology (i.e., EC of a stage I, EC, SUB/CA1 of a stage II, etc.), MC1 antibodies mainly labeled neurons with a tangle-like morphology as well as numerous dystrophic neurites. In these latter regions, as well as throughout the HF of stage IV-VI cases, numerous AbP-labeled deposits were also observed. In every instance, these deposits were co-distributed with clusters of MC1-labeled dystrophic neurites. Of importance, a number of clustered dystrophic neurites were observed free of AbP. These data led us to conclude that within the HF, early neurofibrillary changes precede SP formation. The exception to this rule lies in the presubiculum, where we observe numerous AbP deposits with little or no neurofibrillary pathology.

Brain tissue was provided by UPMC ADRC.

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AN EVALUATION OF THE EFFECTIVENESS OF SHORT-TERM INTERPERSONAL COUNSELING FOR SUBDYSTHYMIC DEPRESSION IN MEDICALLY ILL ELDERLY. Heraux, T. M. *, Mossey, J. M., Knott, K. A., Higgins, M. and Talerico, K. MCP◆Hahnemann School of Medicine, Philadelphia, PA.

Subdysthymic depression occurs in up to half of all hospitalized elderly and is associated with physical and social disability, delayed recovery, and excessive usage of health services. This is a much higher rate than major depression, yet little is known regarding the nature of such depressive symptomatology, or its responsivity to treatment. Studies have been conducted that demonstrate the effectiveness of psychosocial intervention through interpersonal counseling. This report is an evaluation of that case-finding strategy targeted to hospitalized, medically ill, older individuals. Those having elevated depressive symptoms noT meeting DSM-III-R criteria for major depression or dysthymia were enrolled into a randomized clinical trial comparing a brief psychosocial intervention, Interpersonal Counseling (IPC), with Usual Care (UC).

It was observed that adaptability to the physical limitations as well as a clear understanding and acceptance of the changes associated with the process of aging resulted in a quicker recovery from subdysthymic depression. Short-term psychotherapy, which ranged from six to ten counseling sessions proved to be a highly beneficial treatment. Treatment included education of their primary illness, validation of their mood, introduction to means of support which are available throughout the community, stress management, conflict resolution, and development of personal practical skills to live actively in retirement. The result of the IPC showed a statistically significant difference in the rate of improvement in Geriatric Depression Scale (GDS) scores compared to UC members. Therefore, implementation of IPC interventions for hospitalized elderly is recommended.

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COMPARISON OF CDC25A mRNA IN YOUNG AND OLD WI-38 CELLS. Wong*, A. Cristofalo, V., Tresini, M. Center for Gerontological Research, MCP◆Hahnemann School of Medicine, Allegheny University of the Health Sciences, Philadelphia, PA.

Cdc25A is a recently characterized member of a gene family that act as phosphatases to activate cyclin/cdk complexes with dual threonine and tyrosine specificity. Cdc25A is postulated to act at the G1-S transition and is essential for S-phase entry in cells. Recent studies have indicated a role for cdc25A in c-myc-regulated apoptotic pathways. A preliminary study of relative levels of cdc25A mRNA through the WI-38 cell cycle is described here. Additionally, cdc25A levels in three different WI-38 cell lines were compared. Young confluent (CPDL=28.8), young subconfluent (CPDL=28) and old (CPDL=53.5) WI-38 cell lines were "stepped down" and then stimulated after three days. RNA was harvested at 0, 2, 4, 8, 16, 20, and 26 hours post-stimulation and a Northern blot analysis is being carried out.

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PHENOTYPIC VARIATION IN DERMAL FIBROBLASTS DERIVED FROM FETAL AND POSTNATAL SKIN. Furth, J. J.*, Allen, R.G, Tresini, M. Cristofalo, V. J. Center for Gerontological Research, MCP◆Hahnemann School of Medicine, Allegheny University of the Health Sciences, Philadelphia, PA 19129.

Twenty-nine skin fibroblast lines established from fetal, young, and old donors were studied throughout their proliferative life. Donor ages ranged from 12 gestational weeks to nonagenarian. Considerable variation in the morphology of the various cell lines, independent of changes ascribable to proliferative senescence, was observed. The fetal lines could usually be distinguished from the postnatal lines; postnatal lines from the skin of young and old donors could not be readily distinguished.

The steady-state mRNA abundance of a1(I) procollagen, a1(III) procollagen, actin and p21Sdi1 was determined in these lines. The abundance of a1(I) procollagen mRNA was decreased in cell lines from both young and old donors compared with fetal lines; the abundance of a1(III) procollagen mRNA was lower in postnatal lines from old donors compared with fetal lines. The abundance of actin mRNA was also lower in lines established from postnatal donors compared with fetal lines. In contrast, the abundance of p21Sdi1 mRNA was higher in lines established from postnatal donors. Variation was observed in the abundance of these mRNAs in the individual cell lines. Variability was greater in cell lines derived from postnatal skin than in the fetally derived lines.

These biochemical and morphological differences could reflect phenotypic variation in fibroblasts obtained from different individuals. The marked differences between cell lines derived from fetal and postnatal donors could reflect biochemical changes associated with development that had not occurred at the time the fetal cells were used to establish the cultures.

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PERFORANT PATHWAY SYNAPTOPHYS IN IMMUNO REACTIVITY IS REDUCED IN ALZHEIMER'S DISEASE RELATIVE TO LEWY BODY DISEASE. Zwick, O.[1]*, O'Connell, B.[1], Smith, T.W.[2], C.F. Lippa[1]. Department of Neurology, Allegheny University Hospitals, MCP, Philadelphia, PA 19129; University of Massachusetts Medical Center, Worcester, MA.

The hippocampal perforant pathway originates in the entorhinal cortex and terminates in the outer molecular layer of the dentate gyrus (DG). It is the major source of afferent tracts to the hippocampus. We previously demonstrated severe neuronal losses at the origin of the perforant pathway in Alzheimer's disease (AD), with mild perikaryal losses in Lewy body disease (LBD).

We determined immunoreactivity for the synaptic-terminal specific protein, synaptophysin (Boehringer Mannheim, monoclonal; 1:10) in the outer molecular layers of the DG. Immunohistochemistry was performed on 8 pure LBD cases and results were compared to data derived from 9 AD cases. Synaptophysin immunoreactivity in the outer DG was compared to that in the adjacent inner molecular layer, which remains normal in AD. In all cases post-mortem delays were less than 12 hours. Results were quantitated using an Image-Pro automated image analysis system interfaced with a light microscope. In AD, synaptophysin immunoreactivity was visibly reduced in the outer 2/3 of the DG in all cases. In LBD, differences in immunoreactivity between the inner and outer molecular layers were not conspicuous; in most cases immunoreactivity appeared similar to that seen in control cases. Densitometric analysis confirmed a greater reduction in synaptophysin immunoreactivity in the outer DG in AD when compared to LBD (10.5 units compared to 5.1 units; $t=-2.327$, $p=0.03$).

We conclude that in LBD there is nearly normal synaptophysin immunoreactivity at the site of termination of the perforant pathway, probably reflecting nearly normal densities of synapses. In AD the accelerated neuronal losses within the perforant pathway may overwhelm the ability of compensatory mechanisms to operate. In LBD the more subtle perforant pathway neuronal loss appears to be compensated.

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THE MEDIAL TEMPORAL LOBE IN PICK'S DISEASE: IS MEASUREMENT OF CROSS-SECTIONAL AREA USEFUL IN DIAGNOSIS? Jarrett, J.*, O'Connell, B.[1], Hulette, C.[2], Lippa, C.F.[1] [1]Department of Neurology, Allegheny University Hospitals, MCP, Philadelphia, PA 19129; [2]Duke University Medical Center, Durham, NC 27704.

Pick's disease (PiD), a form of frontotemporal dementia, can be characterized by gross frontal and temporal lobe atrophy, and microscopically by the presence of Pick bodies and swollen (ballooned) neurons (Pick cells). Previous studies have indicated that the medial temporal lobe (MTL) undergoes severe atrophy in Alzheimer's disease (AD). We investigated MTL size in PiD to determine whether MTL cross-sectional area measurement can be useful in differentiating these two conditions from each other or from normal aging.

Using an ImagePro image analysis system and an observer who was blinded to diagnosis, we measured the cross-sectional area of the MTL from 16 patients with PiD (mean age 68.2 years at death, range 40-85) and compared results to data from 20 AD cases (mean age 69.9 years, range 50-95) and 18 control cases (mean age 72 years, range 52-91). We used coronal H&E stained sections at two rostrocaudal levels. Both AD and PiD cases had roughly similar disease durations and severities.

The MTL in both PiD and AD was smaller than control MTL at both rostral (mean MTL areas 0.88, 1.00 and 1.89 cm2, respectively; $F=34.028$, $p<0.001$) and caudal (0.83, 0.94 and 1.59 cm2, respectively; $F=16.559$, $p<0.001$) levels. MTL areas in PiD and AD did not differ. We also note variability in cross-sectional area with overlap between the three groups.

We conclude that MTL atrophy is marked in most (but not all) cases of PiD. MTL cross-sectional area determination may be a useful adjunct in distinguishing PiD or AD from normal aging, but is less useful in distinguishing PiD from AD.

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IN REGIONAL VARIATION IN NIGRAL PATHOLOGY ALZHEIMER'S DISEASE PATIENTS WITH EARLY PARKINSONISM. Peric-Stepcic, G.*, O'Connell, B.[1], Smith, T.W.[2], Lippa, C. F.[1]. [1]Department of Neurology, Allegheny University Hospitals-MCP, Philadelphia, PA 19129; [2]University of Massachusetts Medical Center, Worcester, MA.

Neurons within the substantia nigra (SN) show topographical organization with the medial SN projecting principally to the caudate, and the lateral SN to the putamen. In Parkinson's disease (PD), dementia is related to neuronal loss in the medial SN, possibly due to disruption of cortico-subcortical projections between the caudate and frontal lobes. The anatomic substrate for parkinsonism in the Alzheimer's disease (AD) patient is unknown.

We evaluated neuropathological changes of the SN - pars compacta in a group of four patients with AD with early parkinsonism (ADP), four AD patients lacking parkinsonism (ADLP), and six control patients. Using formalin-fixed, paraffin-embedded tissue, six micrometer sections were obtained from the SN at the level of the red nuclei posteriorly and the third nerve anteriorly. The pars compacta was divided to ventromedial, ventrolateral and dorsal subregions. We examined the tissue at 20x using antibodies directed against tau (Sigma, monoclonal, 1:750) for neurofibrillary tangles and neuropil threads, and amyloid (Dako, monoclonal, 1:50) for Ab plaques.

All pathology was more severe in patients with ADP compared with ADLP in all nigral subsectors. This trend was most marked with neurofibrillary tangles in the ventromedial region (19.6, 7.07 and 0.0 /field in ADP, ADLP and controls, respectively) and Ab plaques in the dorsal subregion (18.4, 3.8 and 0.1 /field in ADP, ADLP and controls, respectively). Our next step will be to evaluate neuronal loss and levels of synaptic proteins in the SN to determine their influence on AD patients with and without parkinsonism.

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DONEPEZIL IN THE TREATMENT OF ALZHEIMER'S PATIENTS: THE AUHS DEMENTIA PROGRAM EXPERIENCE. Breen, A.*, Matthews, M.K., Weiss, B., Ryan, B., Lippa, C.F. Allegheny University of the Health Sciences, Philadelphia, PA 19129.

Donepezil is the most recently approved drug for use in the cognitive impairment of Alzheimer's disease (AD). It is a reversible acetylcholinesterase inhibitor, reported to work in over 80% of patients (using the ADAS-cog and CIBIC plus). We retrospectively studied the effectiveness of donepezil in the first 50 AUHS Dementia Program patients started on this medication. Determination of a "best dose" was done using three criteria: 1) the patient's change in score on the Mini Mental Status Examination (MMSE); 2) the patient's perception of efficacy; 3) the family's perception of efficacy. We also evaluated predictors of outcome including depression, hallucinations, delusions, degree of fluctuation of symptoms, extrapyramidal symptoms, insomnia, wandering and prior loss of consciousness, which were recorded for each patient in the AUHS Dementia Database. Each patient was given the MMSE exam immediately before initiating donepezil at 5 mg daily. The first follow-up was at 5-8 weeks, and the second (at 10 mg daily) was after an additional 5-8 weeks. Of the 50 patients, 14 reached a best dose of 10 mg and 7 at 5 mg; 10 have not yet established a best dose. Medication was discontinued due to side effects in 9 patients and due to lack of efficacy in 6 patients. Four patients were lost to follow-up. Side effects requiring discontinuation included belligerence (n=3), GI upset (n=2), hallucinations (n=1), and insomnia (n=1). Side effects which limited dosage were all related to GI upset (n=3). 57% of patients' MMSE scores had improved while on 5 mg. 30% improved further on 10 mg. 32% patients' MMSE scores got worse while on 5 mg, and 45% of patients' MMSE scores decreased while on 10 mg. Unless there were significant side effects, 76% of patients and/or their families perceived that the patient had improved on donepezil, whether or not the patient had actually improved on the MMSE exam. We were not able to show that any pre-existing symptoms could serve as positive or negative predictors of outcome.

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DEVELOPMENT AND AGE-ASSOCIATED DIFFERENCES IN ELECTRON TRANSPORT POTENTIAL AND CONSEQUENCES FOR OXIDANT GENERATION. Allen, R.G.*, Tresini, M., Volker, C. and Cristofalo, C. Center for Gerontological Research, MCP◆Hahnemann School of Medicine, Allegheny University of the Health Sciences, Philadelphia, PA 19129.

We determined the activities of NADH dehydrogenase, succinate dehydrogenase and cytochrome c oxidase in 29 skin fibroblast lines established from donors ranging in age from 12 gestational weeks to 94 years. These enzymes regulate the rate of electron flow through the electron transport chain (ETC), which is believed to be a primary determinant of the rate of cellular oxidant generation. The results of this study demonstrate that all three of the enzyme activities examined are greater in adult-derived fibroblasts than in the fetal cell lines. The ratio of enzyme activities that control electron entry into and exit from the ETC varied directly with lucigenin-detected chemiluminescence (an indicator of .O2- generation) and inversely with H2O2 generation. Chemiluminescence was greatest in fetal cell lines, while H2O2 generation was greater in cell lines established from adults. The results indicate a clear difference between the capacity of fetal and adult fibroblasts to generate oxidants; they also reveal a difference in the predominant oxidant species generated during fetal and adult stages of life. To evaluate regulatory mechanisms that might account for differences observed in fetal and postnatal cells, we examined the mRNA abundances of different components of the ETC complexes. Both NADH dehydrogenase and cytochrome c oxidase are mosaic enzyme complexes that are encoded by both the nuclear and mitochondrial genomes; succinate dehydrogenase is encoded by the nuclear genome only. For the mosaic enzymes, we observed higher abundances of mitochondrial-encoded mRNAs (COX 1 and ND 4) in cell lines established from adults than in fetal cells. No differences in the mRNA abundances of the nuclear-encoded sequences (COX 4 and ND 51) were observed in fetal and postnatal-derived lines. Succinate dehydrogenase mRNA abundance was greater in cell lines established from postnatal donors than in fetal cell lines. No significant differences between cell lines established from young and old adults were detected in any of the parameters examined.

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DEFECT IN THE RAS/MEK/MAP KINASE SIGNALING PATHWAY DURING CELLULAR SENESCENCE. Tresini, M.*, Allen, R.G., and Cristofalo, V.J., Center for Gerontological Research, MCP◆Hahnemann School of Medicine, Allegheny University of the Health Sciences, Philadelphia, PA 19129.

Human diploid cells have a limited replicative lifespan in culture. This loss of replicative capacity, known as in vitro senescence, is characterized by dramatic phenotypic alterations, with the most profound being the inability of senescent cells to proliferate in response to mitogens. Although there is a significant amount of evidence suggesting that senescent cells are blocked in a state with characteristics of the G1/S boundary, it is also established that certain events of the early G1 phase of the cell cycle fail to occur in senescent cells. A typical example is the inability of serum-stimulated senescent fibroblasts to upregulate the early response gene c-fos to levels similar to those seen in young cells. It should be emphasized, however, that certain stimuli, such as UV light and the antioxidant N-acetyl cysteine, can induce c-fos expression to similar levels in both young and senescent fibroblasts. The above observations lead to the hypothesis that senescent cells have impairments in some, but not all, of the signaling pathways normally utilized by the cells to transmit signals from the environment to their nuclei. We have used various stimuli (such as serum, growth factors and the antioxidants N-acetyl cysteine and Nordihydroguaiaretic acid) in young and senescent WI-38 human fibroblasts, to induce signal transduction pathways known to lead to transcriptional induction of c-fos. Our results indicate that the activation, but not the protein abundance, of the Mitogen Activated Protein Kinases p44mapk (ERK1) and p42mapk (ERK2) decreases as a function of in vitro age. This decrease indicates that senescent cells are impaired in responding to signaling events that utilize the ras/MEK/MAP kinase signaling pathway.

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THE EFFECTS OF PERIPHERAL VASCULAR DISEASE ON GAIT. Sanders, T[1]*, McCully, K.[1], Leiper, C.[2] [1]Department of Medicine, Allegheny University Hospitals, MCP, and AHERF Institute on Aging, Philadelphia, PA and [2]Department of Physical Therapy, Beaver College, Philadelphia, PA.

This study was designed to determine whether patients with peripheral vascular disease (PVD) have gait abnormalities. A previous study on humans with PVD found no abnormalities while significant gait changes were seen with a rat model of PVD. The study population was comprised of 7 controls and 5 PVD subjects (all males). All PVD subjects

experienced more intense pain in their right leg. Subjects ranged in age from 55-82 years of age, with a mean age of 70. The GaitMat II system was used to measure both spatial and temporal parameters of gait. Plantar flexion strength was also measured. Subjects walked across the mat, four to six times, at their comfortable walking speed. PVD subjects then walked on a treadmill until they experienced moderate claudication pain and felt they had to stop (pain levels between 6-8 with maximal pain = 10). Control subjects walked for ten minutes without pain. All subjects repeated the gait tests on the GaitMat II system immediately after treadmill walking. All PVD subjects reported claudication pain during the second gait test. PVD subjects had decreased velocity compared to controls (1.09 versus 1.24 m/s, $p = 0.008$) and reduced plantar-flexor strength ($p=0.004$ left and $p= 0.001$ right). In addition, PVD subjects showed asymmetrical relative step length ($p=0.013$) and base of support ($p=0.015$). The abnormalities in PVD subjects were not different in the presence or absence of claudication pain. While preliminary, this study suggests that PVD is associated with measurable changes in gait.

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COUPLING OF ADENOSINE A1 RECEPTORS TO G PROTEINS IS REDUCED IN THE AGED RAT HEART. Cai, G.*, Wang, H., and Friedman, E. Department of Pharmacology, MCP◆Hahnemann School of Medicine, Philadelphia, PA 19129.

Adenosine A1 receptor (A1-AdoR) function has previously been shown to decrease with age in rat ventricles. In order to explore the underlying mechanism, an immuno-coprecipitation technique was employed to directly assess the coupling of A1-AdoR with G proteins in the ventricular myocardium of 6-, 12- and 24-month-old male Fischer 344 rats. Ventricular membranes were incubated with 1 mM of the specific A1-AdoR agonist, N6-p-sulfophenyladenosine (SPA) or with buffer prior to solubilization and immuno-precipitated with antisera raised against specific Ga proteins. The A1-AdoR was assessed in the immunoprecipitates by binding with the specific A1-AdoR antagonist ligand, [3H]8-cyclopentyl-1,3-dipropylxanthine ([3H]DPCPX). Specific [3H]DPCPX bindings were detected in the immunoprecipitates of anti-$G\alpha 3$ and $G\alpha o$ antisera but not with antibodies for the other $G\alpha$ proteins. The basal immuno-coprecipitation of A1-AdoR with $G\alpha 3$ and $G\alpha o$ proteins was found to be decreased by 14% and 12% in ventricular membranes of 12-month-old rats and by 22% and 21% in 24-month-old rats when compared to 6-month-old animals. Pre-incubation of ventricular membranes with SPA increased the coprecipitation of A1-AdoR with $G\alpha 3$ and $G\alpha o$ proteins by 287% and 245%, respectively, in 6-month-old rats, but only by 208% and 205% ($p<0.05$), respectively, in 12-month-old animals and by 129% and 140%, respectively, in 24-month-old animals ($p<0.01$). No age differences in the levels of ventricular $G\alpha$ proteins, as measured by Western blots, were found. Thus, reduced immuno-coprecipitation of A1-AdoRs with their associated $G\alpha$ proteins in the absence of changes in $G\alpha$ proteins or A1-AdoR levels suggests that A1-AdoRs are uncoupled from Gi3 and Go proteins and this may contribute to the age-related decrease in ventricular A1-AdoR function.

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MAPK PATHWAY IS IMPAIRED IN AGED BRAINS OF FISCHER 344 RATS. Zhen, X.*, Uryu, K. Cai, G. and Friedman, E. Department of Pharmacology, MCP◆Hahnemann School of Medicine, Philadelphia, PA 19129.

The extracellular signal-regulated kinases (ERK) are a subfamily of mitogen-activated protein kinases (MAPK) which are expressed highly in CNS neurons. Thus, an important functional role of these kinases in postmitotic neuronal cells has recently been suggested. During aging the activity of a number of brain protein kinases such as PKC and PKA have been shown to change. A decline in MAPK activity was also reported in rat hepatocytes. In the present study, we investigated the effect of aging on tyrosine-phosphorylated ERK in brain of the Fischer 344 rat. The results show that basal tyrosine-phosphorylated ERK2 (active ERK) in cortex was significantly reduced ($p < 0.05$) in 24-month-old rats, compared to 6-month-old rats. This decline is not related to a change in protein level of this kinase, since immunoreactivity of ERK was not altered by aging. Moreover, no age-related changes in brain tyrosine phosphorylated PLCgn1 was found, implicating a selective impairment of the ERK pathway during aging of the brain. Immunohistochemical observations indicate no significant age-related changes in ERK immunoreactivity level and pattern of localization in brain. Moreover, life-long caloric restriction prevented the age-related decrease in basal phosphorylated ERK (active ERK). The activation of ERK in response to stimuli (EGF, PMA, NaF) was also reduced in cortical brain slices of aged rats. These results demonstrate that there is an age-associated selective impairment in the ERK signal pathway, and that long-term caloric restriction can attenuate this defect.

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A PHOSPHATIDYLINOSITOL 3-KINASE INHIBITOR INDUCES A SENESCENT-LIKE GROWTH ARREST IN HUMAN DIPLOID FIBROBLASTS. Tresini, M., Mawal-Dewan, M., Cristofalo, V. J. Sell, C.*, Center for Gerontological Research, Department of Pathology and Laboratory Medicine, MCP◆Hahnemann School of Medicine, Allegheny University of the Health Sciences, 2900 Queen Lane, Philadelphia, PA 19129.

The signal transduction cascade initiated by the activation of phosphoinositide 3-kinase (PI-3 kinase) is implicated in mitogenic and anti-apoptotic signaling generated by growth factors in a variety of cell types. We have examined the consequences of an inhibition of this pathway in human diploid fibroblasts. We find that a specific PI-3 kinase inhibitor (LY294002) causes growth arrest in these cells accompanied by changes in gene expression that are similar to those seen during cellular senescence. A second inhibitor, PD58029, which is specific for the mitogen-activated protein kinase kinase 1 (MEK-1), also induces a growth arrest, but does not induce the same spectrum of gene expression. The pattern of gene expression in the presence the MEK-1 inhibitor is similar to that seen during growth arrest induced by serum starvation.

The specific phenotypic changes seen following inhibition of PI-3 kinase are: an increase in beta galactosidase activity, a decrease in EPC-1 gene expression and a dramatic increase in collagenase gene expression. Thus, growth arrest with a PI-3 kinase inhibitor induces a senescent-like phenotype which is not seen when cells are growth arrested by either serum starvation or a MEK-1 inhibitor.

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INCREASED LEVELS OF NUCLEARLY LOCATED, INACTIVE STAT3 AND ERK PROTEINS IN THE LIVER OF AGED RATS. Walter, R.*, and Sierra, F. Center for Gerontological Research, Allegheny University of the Health Sciences, 2900 Queen Lane, Philadelphia, PA, 19129.

We are interested in the effect of age on activation of different signal transduction pathways in rat liver. Western blot analysis of total cellular proteins prepared from the liver of young (4 months) and old (31 months) Brown Norway rats indicates that the steady state levels of MAP kinases (ERK-1, ERK-2, JNK, and p38) are not significantly affected by aging. However, subcellular fractionation indicates that these proteins accumulate to higher levels within the nuclei of old animals as compared to their young counterparts. Therefore, aging results in nuclear accumulation of several signal transduction proteins whose activation normally results in nuclear translocation. We then measured the basal level of activities of MAP kinases in our nuclear preparations. Surprisingly, we find that these proteins are functionally inactive, probably due to a concomitant increase in the levels of nuclear MKP-1. Furthermore, activation of the corresponding signal transduction pathways by LPS leads to a gradual decline in the nuclear levels of these proteins in old animals, while young animals show the expected increase in nuclear localization. By 4 hrs after induction, the steady state levels of these signal transducers are indistinguishable among rats of different ages. The accumulation of these proteins in the nuclei of cells from old animals is specific, as evidenced by normal levels of several transcription factors analyzed, with the notable exception of Stat3 and the p65 subunit of NF-kB. These factors, which are normally present in a latent form in the cytoplasm and are translocated into the nucleus upon activation, also accumulate in the nucleus in old animals. In contrast, Stat5, which is normally present in high levels in the nuclei of uninduced young rats, is present at much reduced levels in older animals. Our results suggest an impairment of nuclear/cytoplasmic trafficking in the liver of old rats.

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DEFECTS IN MAPK SIGNAL TRANSDUCTION IN SPLENOCYTES WITH AGING. Li, M.,* and Sierra, F. Center for Gerontological Research, Allegheny University of the Health Sciences, 2900 Queen Lane, Philadelphia, PA, 19129.

T lymphocyte activation and proliferation depends on activation of the IL-2 gene and the autocrine effects of IL-2. It has been established that aging results in a decrease in both IL-2 gene expression and T cell

proliferation, and that the age-related decrease in IL-2 expression is controlled at the transcriptional level. The IL-2 promoter is regulated by several transcription factors which are activated through a series of signal transduction pathways, such as MAPK (Mitogen Activated Protein Kinases) and Ca2+/ Calcineurin. Limited studies suggest an age-related disturbance in the T cell receptor (TCR)-proximal events leading to activation of ERK/ MAPK. This pathway is believed to be crucial to the control of mitogenesis and cell division. We decided to further investigate the defects in MAPK signal transduction with age. By using phorbol esters (TPA) plus Ca Ionophore (Cal) as a signal, we bypass the TCR, thus allowing us to assess if there are any age-related defects in signaling downstream of the TCR-proximal defects already described in the literature. Furthermore, there are no reports in the literature concerning the effects of aging on activation of JNK or p38, the other known MAPK family members. Using spleen lymphocytes from young and old C57BL/ 6 mice as a model, we have measured both the basal levels of MAPK family members and their activities upon stimulation with TPA plus Cal. For this, we have used western blot analysis and 'in gel' kinase assays, respectively. Our results suggest that 1. Splenocytes from old mice have a disturbance in MAPK signal transduction pathways downstream of TCR-proximal defects, since bypassing the TCR still results in diminished activity of the pathways. 2. Specifically, we found a reproducible and statistically significant 50% decrease in both ERK2 and JNK2 activities upon stimulation, while ERK1 and JNK1 activities are not significantly affected by age. 3. The abundance of these signal transduction proteins is not affected by age. 4. In accordance with the literature, we find a corresponding 50% reduction in the mitogen-induced proliferation capacity in splenocytes from old mice.

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T-KININOGEN IS A BIOMARKER OF SENESCENCE IN RATS. Li, M.[1]*, Torres, C.[1], Walter, R.[1], Murasko, D. M.[2] and Sierra, F.[1] [1]Center for Gerontological Research and [2]Department of Microbiology and Immunology, Allegheny University of the Health Sciences, 2900 Queen Lane, Philadelphia, PA 19129.

We have previously reported the identification of T-kininogen (T-KG) as a gene whose expression is increased during senescence in male Sprague-Dawley (S-D) rats. Serum T-KG levels increase 2.5 to 4 months before the time of death for any given animal, irrespective of the actual age of the animal at the time of this event, and dietary restriction (DR) delays, but does not prevent, the increase in serum T-KG levels. In the present study, we have assessed whether or not the age-related increase in T-KG is a common feature of senescence in other strains of rat. In a cross-sectional study, we have analyzed hepatic T-KG mRNA levels in male Fischer 344 (F344) rats, as well as in both male and female (Fischer 344 x Brown Norway) F1 rats (called F1). Furthermore, we have conducted a longitudinal study in F344 rats of both genders. The cross-sectional study indicates that there is a dramatic increase in hepatic T-KG mRNA levels when male F1 rats approach senescence. In the case of females, young F1 rats fed ad libitum (AL) show a statistically significant ($p=0.0008$) 2.6-fold higher level of T-KG mRNA, as compared to their male counterparts. Thus, while we still observe an age-related increase in this parameter in both AL and DR female F1 rats, the difference is statistically significant ($p=0.0001$) only in DR animals.

In F344 rats, the cross-sectional study indicates that the increase in T-KG mRNA levels occurs earlier in life in this strain, and is not strongly affected by DR. The longitudinal study, however, indicates that the increase in serum T-KG levels occurs in both genders of F344 rats, and that this age-related increase is indeed delayed by DR, as observed in the other strains analyzed. We conclude therefore that in these commonly used rat strains, the increase in T-KG gene expression is a common feature of senescence and it represents a reliable biomarker of aging. Since the increase in T-KG gene expression does not appear to correlate with inflammatory processes, and since different strains of animals succumb to different pathologies, these results further suggest that the increase in T-KG expression might be related to the process of aging per se, rather than to any given age-related pathology.

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T-KININOGEN INHIBITS THE RAS/MAP KINASE PATHWAY BY INCREASING THE STABILITY OF MKP-1. Torres, C.*, and Sierra, F. Center for Gerontological Research, Allegheny University of the Health Sciences, 2900 Queen Lane, Philadelphia, PA 19129.

Cell growth in vitro is modulated primarily by the presence of growth factors in the culture medium. Their presence is sensed by receptors, and this information is transmitted to the nucleus by signal transduction processes. The main mechanisms utilized for this purpose are the Ras/ MAP kinase and Stat pathways. The role of proteolysis on these pathways is poorly understood, but we have previously shown that T-kininogen (T-KG), a strong and specific inhibitor of cysteine proteinases, inhibits cell proliferation. In an attempt to determine the possible mechanism(s) by which this might occur, we have used western blot analysis to measure the steady state levels of several proteins involved in these signal transduction pathways. Our results indicate that T-KG leads to a significant increase in the steady state levels of MEK and ERK isoforms. In contrast, T-KG has little or no effect on the steady state levels of Ras, Raf-1, PKCa, JNK-1 or Stat3. Surprisingly, and in spite of the dramatic increase in the level of both ERK1 and ERK2, we have observed a significant decrease in MAP kinase activity in T-KG-expressing cell lines. In an attempt to reconcile these findings, we have established that T-KG-expressing cell lines contain an elevated level of MKP-1, the phosphatase responsible for ERK inactivation. Furthermore, we have established that the rates of degradation of ERK-1, ERK-2 and MKP-1 are diminished in the presence of T-KG. Our results are consistent with the hypothesis that inhibition of cysteine proteinases by T-KG leads to a disturbance in both the steady state levels and activities of ERK and MKP, resulting in an overall inhibition of the activity of this pathway. Since we have previously established that, at least in rats, T-KG gene expression increases during the process of aging, our results suggest that this could lead to a disruption in the activity of the Ras/MAP kinase pathway, leading to the decreased cell responsiveness to the environment characteristic of old age.

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GENDER DIFFERENCES IN THE AGE-RELATED DECLINE IN BETA-ADRENERGIC RECEPTOR FUNCTION IN THE RAT HEART. Snyder, D.,* Gao, E. Horwitz, J., and Roberts, J. Department of Pharmacology, MCP•Hahnemann School of Medicine, Allegheny University of the Health Sciences, Philadelphia, PA 19129.

Beta-adrenergic receptor-mediated stimulation of adenylyl cyclase activity as well as beta-adrenergic receptor-independent activation of adenylyl cyclase activity decline with age. The aim of the present study was to determine if these age-related changes differ in male and female rat hearts. Ventricular membranes were prepared from the hearts of male and female F344 rats of 6, 12, and 24 months of age. 100 uM isoproterenol (ISO), a beta-adrenergic receptor agonist, and 10 um forskolin (FOR), which stimulates adenylyl cyclase independent of the beta-adrenergic receptor, were used to stimulate cAMP production. These assays are based on the conversion of 32P-ATP to 32P-cAMP. Data were expressed as pmol/mg protein/minute obtained in a 30-minute incubation from 6 - 8 animals in each age and gender group. All data were analyzed by ANOVA with significance set at $p < .05$. The response to ISO was significantly greater in female rats compared to male rats at all ages. Significant reductions in the response to ISO occur between 6 and 12 months in male rats and 12 to 24 months in female rats. The response to FOR was comparable in male and female rats at 6 months; however, in male rats there was a greater age-related decline, so that by 24 months female rats had a significantly greater response to FOR than the male. In male rats, ISO and FOR stimulation of cAMP production declined by 42% and 34% by 12 months, respectively, and by 58 and 57% by 24 months, respectively. In female rats, the decline was only 11% and 18% by 12 months, respectively. In female rats, the decline was only 11% and 18% by 12 months, respectively, and 44% and 34% by 24 months, respectively. These data suggest that the age-related decrease in postsynaptic beta-adrenergic receptor-mediated responses and in adenylyl cyclase activity occurs to a lesser degree in the female than in the male rat heart.

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PREDICTORS OF AVERAGE WEEKLY WEIGHT LOSS IN MEMBERS OF A WEIGHT MANAGEMENT PROGRAM. Landsberg, L.A.*, Wolfe, K., Rohrbach, J. Knupp, A., Youth, H., Posner, J.D. Department of Medicine, Division of Geriatric and Rehabilitation Medicine, Allegheny University of the Health Sciences, Philadelphia, PA 19131.

We studied predictors of average weekly weight loss (WWC) in 93 members of a medically supervised weight management program of the Division of Geriatric and Rehabilitation Medicine. Members of the pro-

gram were given bimonthly nutrition counseling, thrice-weekly supervised exercise, and prudent use of anorectic medications if appropriate. Members had follow-up visits with a program physician twice a month.. Eighty percent of the members were female (n=74). The average age at entry was 47.6 + 7.9 years for males, and 45.9 + 9.4 years for females. Average weight at entry was 265 + 33 lbs for males (range: 207 to 328 lbs) and 223 + 48 lbs for females (range: 146 to 359 lbs). Baseline Body Mass Index (BMI) calculated as wt(kg)/ht(meter2) was 39.9 + 5.0 for males and 39.0 + 7.9 for females. Members' average weekly rate of weight change was calculated after their progress was followed for a minimum of 2 weeks to a maximum of 98 weeks. WWC was negatively correlated with length of time in program (r=-.30, p<.01), with greater loss occurring earlier in the program. Men lost an average of 19.8 lbs (+ 12.0 lbs) and women lost an average of 26.3 lbs (+ 21.2 lbs) while in the program. This weight loss translated to an average weekly weight change of .82 lbs (+.8 lbs) for men and .96 lbs (+ .9 lbs) for women. Half the members were compliant with the prescribed exercise regimen, exercising aerobically 3 x week or more. Members who exercised at least 3 x week had WWC twice that of members exercising 2 or fewer times per week but...in a linear multiple regression analysis, WWC was best predicted by baseline BMI, baseline resting VO2, and exercise at least 3 x week during the program (p's<.05). After all variables were included in the analysis, WWC was not predicted by age, gender, or daily fat intake < 20 grams. WWC was also not predicted by psychological scales measuring depression, attitudes towards exercise, self-motivation, or the Yale Readiness to Lose Weight index. This study suggests that exercising at least 3 times a weeks significantly increases average weekly weight loss.

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EPC-1 EXPRESSION AND AGING: EFFECT ON PROLIFERATION.
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Normal human diploid fibroblasts (HDF) have been well characterized as having a limited proliferative lifespan and are frequently used as an in vitro model to study cellular senescence. This cell culture model has been used to identify genes that are differentially expressed during the aging process in vitro. The expression of one of these genes, EPC-1 [Pignolo, et al., J Biol Chem, 268: 8949], is detected as a 1.4 kb transcript in young (early passage), serum-deprived WI-38 cells at levels greater than 100-fold higher than similarly treated senescent ("old") WI-38 cells. EPC-1 is a 50 kD secreted protein with approximately 30% sequence identity to serpins, a family of secreted mammalian serine protease inhibitors.

In order to understand the implications of the loss of EPC-1 expression with age, we are initially focusing on its function in young cells. Our underlying premise is that the secreted EPC-1 protein is an age-associated inhibitor of cellular proliferation. This idea is supported by data generated in our laboratory and shows that: 1. In 80% of ovarian carcinomas examined, EPC-1 expression is lost at both the protein and RNA level and appears to be the result of a chromosomal deletion (our work and others); 2. The number of cells entering S phase in confluent cells can be increased by the addition of antibodies to EPC-1 (R. Pignolo, Ph.D. thesis); 3. The number of cells entering S phase in growing cells can be reduced upon the addition of recombinant EPC-1 protein by approximately 50%; and 4. Overexpression of EPC-1 in mouse fibroblasts leads to a significant reduction in proliferation.

The concomitant expression of EPC-1 during a quiescent state of early passage HDF cells and our preliminary results suggest that EPC-1 could act as a negative regulator of proliferation in early passage HDFs. To identify the means by which this occurs, we hypothesize that EPC-1 blocks cell cycle progression by interfering with one or more cell cycle-regulated proteins. To examine the molecular mechanism by which EPC-1 may interfere with progression through the cell cycle, we are using two different systems. First, we are using a reversible plasmid expression system to determine the effect of EPC-1 overexpression on cell cycle progression in the context of Balb/c 3T3 stable transfectants. Second, we are using the purified rEPC-1 protein to test the effect on progression through the cell cycle of various populations of synchronized cells.

During the proliferative lifespan of HDF cells, the gradual loss of expression of the G0-specific EPC-1 mRNA occurs as the cells become irreversibly arrested with characteristics of mid-late G1 cells, suggesting that senescent cells arrest just prior to entering S phase. This implies that, during proliferative aging, the ability to enter the normal quiescent or G0 state is bypassed. Our immediate and long- term goals are: 1) to examine the molecular mechanism by which EPC-1 acts as a negative regulator of proliferation, 2) to determine whether EPC-1 expression stimulates entry into G0 or vice versa, and 3) to determine which part of the pathway is bypassed or becomes dysregulated in senescent cells which do not achieve a G0 growth-arrested state.

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PURIFICATION AND CHARACTERIZATION OF EPC-1 PROTEIN.
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EPC-1 (early population doubling level cDNA-1) is a secreted protein that readily accumulates in the growth medium of density-arrested and/or serum-starved early population doubling level (young) cells. EPC-1 does not accumulate in the growth medium of log-phase, low density young cells or of late population doubling level (senescent) cells under any culture conditions tested. Substantial evidence from our laboratory strongly supports the hypothesis that the EPC-1 protein functions as an inhibitor of cell growth.

We presently are purifying the EPC-1 protein from the serum-free growth medium of young, confluent WI-38 cells, using an adaptation of a previously reported method that involves separation through ammonium sulfate saturation, and cation exchange and gel filtration chromatography (Wu and Becerra, 1996). In order to obtain large amounts of the protein, we have also prepared recombinant EPC-1 using a baculovirus expression system. Full length EPC-1 cDNA was inserted into the multicloning site of the polyhedrin locus replacement vector, pVL1392. Trichoplusia ni (High 5) insect cells were then co-transfected with this recombinant transfer vector and baculovirus viral DNA, and the cells were grown in serum-free medium. The growth medium from the infected High 5 insect cells was collected and determined to contain recombinant EPC-1 by Western analysis. We have purified this recombinant protein to near homogeneity. The recombinant protein runs at a slightly lower apparent molecular weight by SDS-PAGE (49 kDa vs. 50 kDa) and does not display the same behavior during cation exchange chromatography as the native protein. These anomalies most likely reflect differences in glycosylation. The effects of both the recombinant and native proteins on cell growth are currently being examined.

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WHAT INFLUENCE DOES CONSANGUINEOUS MATING HAVE ON HUMAN LONGEVITY? Johnsen, P.T.*, AHERF Institute on Aging, 2900 Queen Lane, Philadelphia, PA 19129.

The familial component of human longevity is commonly dismissed as small, although it is statistically significant. Previous studies, however, have not attempted to distinguish between geographically mobile populations with patterns of relatively random mating and other, more restricted populations with high rates of intermarriage. Observations of unusual longevity among many of the author's forebears prompted a systematic plotting of the island-based bilateral kindred shared by a set of seven U.S.-born siblings, six of whom lived beyond age 70 (including two who live(d) well into their tenth decade). Ancestors were identified at 110 of the 126 positions on a chart that extends back six generations. All were from the county of Rogaland in southwestern Norway, and the preponderant majority were born on one or another of a group of small islands near Stavanger (principally Rennesøy, but also Mosterøy, Finnøy and others). Birth and death dates were determined for 84 individuals, eight of whom show up at several positions on the chart because of intermarriage.

First cousin marriage was infrequent during the 18th and 19th centuries but had been quite common in certain lineages during the 16th and 17th centuries (when land that belonged to the Church before the Reformation was granted to favorites of the Crown and thereafter was closely held within those families). First-cousin marriage was one way of ensuring that family holdings of valuable farmland would stay intact. Even when a family's strategy called for widening rather than restricting the network of relatives, there was a definite matrilateral "tilt" to the many marriages that occurred between second and third cousins. This would seem reminiscent of Dumont's observation (a propos of India) that, where patrilyny is strong, one of the things that a man inherits from his

father is the right to go to the same kinship group to which his father (or father's father) had gone in search of a bride. On Rennesøy, an island 14 miles long and no more than 4 miles wide, such a "right" appears to have been invoked rather often. Consanguineous marriage was so common in earlier generations that 36 individuals (or 43% of the sample) can be traced back to a common ancestor, who was reported to be 90 years old in 1631.

By comparison to published longevity data for 8,518 adults from 18th and 19th century European aristocratic families, the mean age at death was greater for the Norwegian individuals (64.8 years vs. 60.8 for males, 67.8 vs. 65.4 for females) despite high incidence of deaths in childbirth and by drowning among the island dwellers. Forty-five percent of the Norwegian sample lived past 70 (the definition of "long-lived" in the comparison studies). Males benefited most from the greater longevity among Norwegians; the sex differential in life span is smaller than that reported for the Continental Europeans.

These preliminary data suggest that the familial, and perhaps genetic, component of human longevity may have been underestimated, particularly among repetitively intermarrying social groups (and, hence, for most of human history). They also raise the possibility that the genetic risks of consanguineous mating may be balanced by some protective mechanisms that result in long life among survivors who are spared the deleterious mutations. At the very least, they show that the assumption of random mating is not warranted among all populations, even if they are associated with modern political and economic institutions.

The next step in analyzing these data is to perform an estimation of narrow-sense heritability based on various parent and offspring characteristics (age at death, number of children surviving past 20, etc.). Concurrently, I plan to gather data on other Norwegian kindreds to help determine whether the findings for Rennesøy are unique or representative of a broader pattern.

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EXPRESSION OF β-GALACTOSIDASE *IN VITRO* AND *IN VIVO*. Balin, S.*, Severino, J. Balin, A. Cristofalo, V. J. Center for Gerontological Research, MCP◆Hahnemann School of Medicine, Allegheny University of the Health Sciences, Philadelphia, PA 19129.

β-galactosidase is a lysosomal enzyme which targets and breaks b-bonds. In vivo levels of the β-galactosidase reporter protein can be determined by using the precipitating substrate X-Gal (5-bromo-4-chloro-3-indolyl D-galactoside). In this reaction the indolyl group of X-Gal is released due to endogenous β-galactosidase activity. This group gets oxidized in the presence of potassium ferrocyanide and ferricyanide, resulting in the formation of a blue insoluble precipitate.

Dimri et al. (1995) have reported that β-galactosidase is a biomarker that identifies senescence in vitro and in vivo. They hypothesized that this marker was expressed by senescent, but not presenescent, normal fibroblasts or immortalized cells. Furthermore, in skin samples from human donors of different ages, there was an age-dependent increase in this marker. Finally, this marker provides in situ evidence that senescent cells may exist and accumulate with age in vivo.

To examine the role ofβ-galactosidase, experiments were carried out in WI-38 fetal lung fibroblast cells, human skin cells, transformed cells, and tissue samples resected from different age donors by Moh's micrographic surgery. Young and old WI-38 cells were cultivated and stained for β-galactosidase under similar conditions. Our results indicate that in young subconfluent cells, there was no blue staining, whereas in old subconfluent cells there was blue staining indicating β-galactosidase activity. When both young and old cells reach confluency, the amount of β-galactosidase staining was greatly increased showing strong blue staining in both young and senescent cells. After harvesting and reseeding the young confluent cells, we found that β-galactosidase activity declined significantly but then reappeared as the cells again become confluent. In human skin cell lines derived from donors of different ages, we saw no significant differences in β-galactosidase activity. In skin samples derived from Moh's surgery, we found only occasional random β-galactosidase stained cells with no apparent difference based on either patient age or site of surgery.

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EXPRESSION OF THE EPC-1 GENE AND ITS ROLE IN THE ETIOLOGY OF OVARIAN CARCINOMA. Severino, J.,* Francis, M. K., Cristofalo, V. J. Center for Gerontological Research, MCP◆Hahnemann School of Medicine, Allegheny University of the Health Sciences, Philadelphia, PA 19129.

EPC-1 (Early Population doubling level cDNA-1) was isolated by subtractive hybridization between young and senescent WI-38 fetal lung fibroblasts. It is a gene expressed in the G0 phase of the cell cycle in young but not senescent cells (Doggett et al., 1992). Furthermore, the gene encodes a 1.4 kb mRNA species and its protein product inhibits cell proliferation. The EPC-1 gene has been localized to chromosome 17p13.1-pter, a site of other tumor suppressor genes such as p53 (Tombran-Tink et al., 1994). Phillips et al. (1996) have reported that, in 57 sporadic ovarian epithelial tumors, 80% showed an allelic deletion on chromosome 17p13.3 and identified EPC-1 as one of the potential targets for this deletion.

To examine the potential role EPC-1 plays in the development of ovarian carcinoma, five ovarian cancer cell lines were obtained from ATCC. Total RNA was isolated from these lines to assay relative EPC-1 mRNA abundance by Northern blot analysis. Since only one of the ovarian cancer lines expressed the message, Western analysis was performed to determine relative protein abundance. The results showed that none of the lines expressed the EPC-1 protein. In addition, Southern analysis was performed to determine if there were any deletions in the EPC-1 gene in these cells, particularly in those lines that did not express EPC-1 mRNA. Our results showed no gross genomic deletions of EPC-1 in the five ovarian carcinoma cell lines that we examined. In future studies we plan to use an inducible system to over-express EPC-1 in order to determine its effect on ovarian cancer cell proliferation.

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EXPRESSION OF PROLYL-4-HYDROXYL-ASE a-SUBUNIT AND hnRNP-K IN ATHEROSCLEROTIC ARTERIAL SMOOTH MUSCLE CELLS: A LINK TO VASCULAR DISEASE IN AGING. Laury-Kleintop, L. D., and Tulenko, T. N.* Department of Physiology, MCP◆Hahnemann School of Medicine, Allegheny University of the Health Sciences, Philadelphia, PA 19129.

Atherosclerosis continues to be the major cause of mortality in the elderly population of Western cultures, accounting for nearly half of all deaths annually. The major characteristics in atherosclerotic lesions are an increased number of smooth muscle cells (SMC) and macrophages and an accumulation of lipid and collagen. Similarly, the age-related changes in the vasculature include increased numbers of SMC and macrophages, as well as alterations in extracellular matrix protein content. Thus, atherosclerosis and aging share, at least, an increase in the SMC population (SMC proliferation) and alterations in extracellular matrix composition. In atherosclerosis the abundance of SMC and collagen is thought to occur because of the phenotypic modulation of medial SMC from the in vivo "contractile" state to the "synthetic" state. In the synthetic state SMC are proliferative, secretory and perhaps also migratory. Using the hypothesis that phenotypic modulation of SMC requires signaling at the nuclear level to alter gene expression, we employed differential display to probe SMC isolated from control and cholesterol-fed (2% for 10 weeks), atherosclerotic rabbits. We have found that genes encoding the a subunit of prolyl-hydroxylase (PH) and hnRNP-K are markedly upregulated in SMC isolated from the aortas of atherosclerotic rabbits compared to SMC isolated from normal rabbits. A 281bp cDNA fragment representing the prolyl-4-hydroxylase a-subunit (PH) mRNA and a 501bp cDNA fragment from the hnRNP-K A isoform were identified and isolated. With semi-quantitative RT-PCR and SMC RNA isolated from the thoracic aorta of cholesterol-fed rabbits, Watanabe and control animals, we confirmed increases in PH and hnRNP-K expression in our in vitro and in vivo models of atherosclerosis. The increased gene expression of the PH a-subunit and hnRNP-K A isoform coincides with the increased production of collagen and proliferation by SMC during atherosclerotic plaque formation. In order to better define the role of hnRNP-K in the proliferation of SMC, SMC were transfected in vitro with a plasmid containing antisense hnRNP-K cDNA after exposure to cholesterol liposomes, a proliferation inducing molecule. The antisense hnRNP-K cDNA inhibited the cholesterol induced proliferation by 35% compared to cells treated with cholesterol liposomes and transfected with the vector alone. These findings shed new light on the cell biology of atherogenesis and potentially open a new avenue for drug therapy for atherosclerotic syndromes. Based on these observations in diseased arteries, we hypothesize that similar disturbances in arterial SMC underlie the age associated changes in the vessel wall. These changes are known to contribute to vascular disease in the aging population. Thus, studies are planned to determine the expression of these genes in aging animals, in order to define their roles in natural vasculature aging.

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PEROXYNITRITE-MEDIATED MODIFICA-TIONS OF TYROSINE HYDROXYLASE IN PC12 CELLS. Ara, J., Horwitz, J., Ischiropoulos, H. Department of Pharmacology, MCP◆Hahneman School of Medicine, Philadelphia, PA 19129; Institute for Environmental Medicine, University of Pennsylvania, Philadelphia, PA 19104.

The neuropathology of degenerative disorders such as Parkinson's, Alzheimer's, and amyotrophic lateral sclerosis is associated with excessive production of reactive species. Nitric oxide, superoxide and peroxynitrite (ONOO-) have been implicated in the pathogenesis of neuronal injury in these disorders and in particular in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity, a model for Parkinson's disease. Using the PC12 cells as a neuronal model for catecholamine producing cells, we have previously shown that peroxynitrite but not nitric oxide or superoxide inhibited the production of DOPA. A direct inactivation of tyrosine hydroxylation, the rate limiting step in the biosynthesis of catecholamines, appears to be the explanation for the ONOO- -mediated inhibition. Exposure of PC12 cells to peroxynitrite resulted in a concentration dependent nitration of tyrosine residues in TH as revealed by the reactivity of anti-nitrotyrosine antibodies with immunoprecipitated enzyme. There was an inverse correlation between the level of nitration and loss of enzymatic activity. Nitration of TH also initiated proteolytic degradation of the protein as indicated by the loss of immunoreactive TH 4-8 hours after nitration. The nitration of TH results in a loss of activity and increased proteolysis, two events that precede the loss of neurons in the MPTP model of the disease. These results may provide a novel mechanism to explain the pathogenesis of Parkinson's disease.

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USE OF IMMUNE PARAMETERS AS BIOMARKERS OF AGING. Murasko, D. M., Gardner, E.* Department of Microbiology and Immunology, MCP◆Hahnemann School of Medicine, Philadelphia, PA 19129.

The purpose of our ongoing project is to determine whether age-associated changes in T cell functions might be appropriate biomarkers of aging. The potential of these parameters as biomarkers was evaluated based on two criteria: 1) an age associated alteration should occur at approximately the same time in the lifespan of multiple strains of the same species and 2) caloric restriction (CR) should postpone onset of this alteration, ideally until the comparable time in the lifespan of the CR animals. Based on these criteria using three genetically diverse strains of rats, we have found that only four of fifteen immune parameters were potential biomarkers of aging: 1) ConA induced proliferation; 2) ConA induced interferon (IFN) production; 3) calcium ionophore (Cal) induced proliferation; and 4) Cal induced IFN production.

In cross-sectional studies, all three strains demonstrate an age associated decrease in ConA-induced lymphoproliferation that is delayed by CR. While the decline in ConA-induced lymphoproliferation is a marker of "late aging", increases in Cal induced proliferation appears to be a marker of late maturation or early aging: All three strains demonstrate increasing Cal induced proliferation of splenic lymphocytes from 6 months to median survival, at which time the level of response plateaus. There is an age related increase in IFN-g production in all three strains. This increase appears to begin early in life (6-12 months), and plateaus about median survival. CR enhances the age associated increased production of ConA induced IFN-g and postpones the plateau/decline in IFN-g production seen at later times.

Currently, we are performing longitudinal assessment of the changes in these four immune parameters. Eighty rats were obtained at 6-9 months of age (20 each of male AL, male CR, female AL, female CR). Peripheral blood mononuclear cells (PBMC) are obtained two months after acclimation to our facility and every three months until median lifespan, and then every two months until moribund. Both F344 and F1 rats demonstrate an obvious decrease in proliferative response to ConA with increasing age. When evaluated as a percent lifespan of each individual rat, there is a consistent decrease in ConA response occuring at 40-50% of lifespan. In both strains there is a continuing downward trend in response after the 50% survival; however, the slope is fairly minimal after 50%. In contrast, there is an early increase in proliferation in response to Cal starting at 20-30% of lifespan. The maximum level is reached at about 50% lifespan and is maintained throughout the lifespan. IFN production is increased with increasing age after both ConA and Cal stimulation. Age-associated increases in IFN induction by either stimulus occurs early, reaching a plateau at about 50% lifespan.

While there is a very consistent age-associated shift in these markers that occurs within a limited time in the lifespan in both strains regardless of diet or sex, this consistency is observed in the mean response of the cohort, not among individual rats. All rats show the same change, but not consistently at the same point in their lifespan. These immune measures, therefore, may be useful to assess changes in lifespan within a population, but appear to have less value when considering the lifespan of an individual.

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STRAIN AND TISSUE DIFFERENCE IN BASAL AND IFNa/ß INDUCED NK ACTIVITY IN YOUNG AND OLD MICE. Plett, P. A.*, Murasko, D. M. Department of Microbiology and Immunology, MCP◆Hahnemann School of Medicine, Philadelphia, PA 19129.

Natural killer (NK) cells are an important component in natural immunity because of their anti-viral and anti-cancer activity. In mice it has been clearly demonstrated that there is a decrease in basal NK activity at ~4 months of age that is maintained through their lifespan. However, the effect of age on the ability of various lymphokines to increase this basal NK activity has received little attention. We have examined both basal and short-term IFNa/ß induced NK activity against YAC-1 cells using a 4 hr 51Cr release assay. Stimulation of splenic NK cells in vitro with IFNa/ß increased cytotoxic NK activity two- to three-fold in young (6 month) C57BL/6 mice. However, this same procedure had little effect on the activity of aged (24 month) mice. This difference was not dose dependent since similar results were observed using 101 to 105 units of IFNa/ß/106 cells. Further, in vivo administration of 103 to 105 u of IFNa/ß or 100 mg of the IFN-inducer Poly I:C ip 24 hr prior to in vitro assessment of NK activity, increase basal NK activity of young 2-3 fold, but had no effect on NK activity of old mice. Similar to C57BL/6 mice, splenic NK cells of aged BALB/C mice showed a reduced NK activity after IFN stimulation compared to young mice (p=.003). Surprisingly, when spleens from young and old (BALB/c x C57BL/6)F1 mice were examined, the old mice did not show a significant reduction in IFN-induced NK activity. The same pattern of reactivity seen in splenic NK cells emerged when NK activity of peripheral blood leukocytes (PBL) of C57BL/6 and (CB6F1) mice were tested: after IFN treatment, NK cells of old C57BL/6 mice was decreased compared to PBL NK cells of young mice while PBL NK cells of (BALB/c x C57BL/6)F1 mice showed comparable IFN inducibility between young and old. The difference cannot be attributed to NK cell number as identified by monoclonal antibodies to the NK1.1 marker, since both strains show comparable percentages of these cells in young as well as in old. These results demonstrate that NK cells from aged C57BL/6 and BALB/c mice have a decreased ability to respond to induction by IFNa/ß in both spleen and PBL. This decrease does not reflect a requirement for increased amounts of IFNa/ß or differences in NK cell number. However, the mechanisms of the decrease in BALB/c and C57BL/6 mice appears to be different since there is no age-associated decrease in IFN-induction of NK activity in (BALB/c x C57BL/6)F1 mice.

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DECREASED EFFICIENCY OF INFLUENZA VACCINE ON THE ELDERLY: REPRODUCIBILITY OF RESPONSES ACROSS SEVERAL YEARS. Bernstein, E.*, Murasko, D.M., Abrutyn, E. Departments of Microbiology/Immunology and Medicine, MCP◆Hahnemann School of Medicine, Philadelphia, PA 19129.

Numerous studies have indicated that influenza is less efficacious in the elderly than in the young. However, several questions arise regarding this general statement: 1) Is this decreased response demonstrable in healthy elderly, as well as nursing home residents? 2) Is this decreased apparent in cell-mediated, as well as humoral responses, to the vaccine? 3) Does lack of response to the influenza vaccine in one year indicate decreased responses to influenza vaccination in subsequent years? To address these questions we recruited subjects from five retirement communities in the Philadelphia area. All subjects were living independently in apartments. Subjects were free of diseases and medication known to alter immune responses. All subjects were >65 with a mean age of 80.2. In the first year, 264 subjects were recruited; 122 remained in the study through three influenza seasons. Although mean antibody titers as determined by hemagglutination were significantly increased from pre to post immunization in each year in the elderly, the young demonstrated significantly higher titers to all three influenza strains than did the elderly (p£.03). Peripheral blood mononuclear cells (PBMC) cultured with influenza vaccine demonstrated significantly in-

creased proliferation (elderly: p£.00005; young: p£.02) one month after vaccination. Again, the mean proliferative response of the elderly to the immunization vaccine was significantly lower than the mean proliferation of the young (p<.05). IFNg production was significantly higher in the young than in the elderly both pre- and post-vaccination (p£.0001). Therefore, healthy elderly do demonstrate decreased response to influenza vaccine relative to young, this decrease is apparent in both cell mediated and humoral immunity.

Evaluation of the 122 subjects who were immunized all three years indicated that in years 1 and 2, approximately 40-50% of the subjects did not achieve levels of antibody that were considered protective (i.e., HI³40). In year 3, however, 80% of the elderly achieved antibody titers of ³40 to the components that were in previous vaccines (H1N1 and B), but only 50% to the new component (H3N2). A four-fold rise in antibody titer traditionally indicates an active response to an antigenic challenge. Only 25-40% of the elderly demonstrated this level of increase to the new vaccine component in each year. In addition, only 31% of subjects in Year 1, 34% in Year 2, and 11% in Year 3 demonstrated a significant increase in proliferative response to influenza vaccine post-vaccination. Interestingly, assessment of individuals across the 3 years indicate that only 9 individuals showed no cell mediated or antibody response to vaccination in all three years. These results suggest that although the mean response of elderly to influenza immunization is consistently lower than the mean response of young, most elderly are not consistently inhibited in their level of response.

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ENHANCED COLON CARCINOGENESIS IN AGING RATS. Komninou, D.,*, Leutzinger,Y., Reddy, B. S. and, Richie, Jr., J. P. American Health Foundation, Valhalla, NY, 10595

The risk for colon cancer, the second leading cause of cancer death in the United States, is increased markedly in elderly individuals. We hypothesize that colon cancer susceptibility is increased during aging, in part, due to a depletion in colonic glutathione (GSH) levels. To test this, we examined the effects of aging on the GSH content and formation of preneoplastic aberrant crypt foci (ACF) in the colon of azoxymethane (AOM) treated rats. Mature (12-mo old) and old (22-mo old) male F-344 rats (n=9 per group) were administered s.c. AOM dissolved in normal saline at a dose of 8 or 15 mg/kg bw/wk for 2 weeks. After 10 weeks, animals were sacrificed and colonic ACF formation was assessed. The 15 mg/kg dose was toxic producing severe body weight loss and early deaths in old but not mature animals. The 8 mg/kg dose-group was less toxic to old animals, but still produced one death and decreased body weight (16%) in three of the animals. After 8-10 weeks, a four-fold increase in ACF containing ³4 crypts per focus, (from 3.1 ± 0.54 to 14.3 ± 3.48 ACF) was observed in the old compared to mature animals (p<0.001). These results suggest for the first time an aging-related increase in colon cancer susceptibility in a well-established aging and colon cancer model. Since an increase in AOM toxicity was also observed in old animals, additional studies were initiated to determine whether enhanced carcinogenicity is due to an increase in initiation by assessing DNA methylation in these animals.

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LONG-TERM DIETARY SUPPLEMENTATION WITH VITAMIN E MODULATES MACROPHAGE PRODUCTION OF PRO-INFLAMMATORY MOLECULES. Beharka, A.,* Wu, D, Martin, K. R., Han S.N, Adolfsson, O. , Smith, D. E., Cao, G,. Prior, R. L, Meydani, M. and Meydani, S. N. Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111.

Macrophage (Mf)-derived proinflammatory cytokines and nitric oxide (NO) play key roles in host defense; however, unregulated production has been implicated in the pathogenesis of several inflammatory diseases. Aging is also associated with dysregulation of immune and inflammatory responses. Dietary supplementation with antioxidants has demonstrated promising potential in improving immune function in the aged. Thus, we determined the effect of dietary antioxidants on unstimulated and induced production of pro- and anti-inflammatory peritoneal and bone-marrow (BM)-derived Mf products. Mf were harvested from 24 mo old C57BL/6NIA mice, that had been fed one of the following semi-synthetic diets for 6 mo: control diet [30 ppm vitamin E] (C); C + 470 ppm E (E); C + 11 ppm melatonin; C + 0.5% glutathione (GSH); C + 1% strawberry extract; C + 470 ppm E + 0.5% GSH. Unstimulated secretion of IL-6 by Mf from mice fed the E (729 ± 311 pg/ml) or melatonin-(674 ± 260 pg/ml) supplemented diets was significantly lower (p<0.05) than that from the controls (1845 ± 301 pg/ml). None of the other treatments affected unstimulated IL-6 production. Vitamin E and melatonin appear to be working, at least in part, through an effect on prostaglandin (PG) E_2 production because, in this experiment, unstimulated PGE_2 production was decreased with E or melatonin supplementation.

In vitro LPS stimulation induced Mf to produce significant amounts of IL-1b, IL-6, IL-10, IL-12, IL-15 and TNF-a; however, production was not affected by dietary treatment. LPS stimulation also induced Mf to produce significant levels of NO. Peritoneal, but not BM, Mf from E-supplemented mice produced less (p<0.05) LPS-stimulated NO than Mf from control mice (30 ± 6 vs 18 ± 3 mM). None of the other antioxidants affected NO production. It is concluded that vitamin E modulates production of at least two molecules produced by Mf which are involved in a variety of chronic and acute inflammatory diseases. These results might of particular interest to the aged, as increased spontaneous production of IL-6 and induced production of NO with age have been reported. Furthermore, IL-6 has been implicated in the pathogenesis of age-associated diseases.

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EFFECT OF LONG-TERM DIETARY ANTIOXIDANT SUPPLEMENTATION ON INFLUENZA INFECTION. Han, S.N. *, Meydani, M. D., Wu, K.R. Martin, B.S. Bender, D.E. Smith, G. Cao, R. Prior, and S.N. Meydani. JM USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111; Veterans' Affairs medical Center, Gainesville, FL 32608.

The aged are more susceptible to influenza infection and have higher mortality from it. Previously, we have shown that short-term vitamin E (vit E) supplementation decreased lung viral titer in influenza-infected old mice. The present study was conducted to compare the effect of vit E on influenza infection and its clinical manifestation with that of other antioxidants. C57BL/6NIA mice were fed one of the following semi-synthetic diets for 6 months: control (C, 30 ppm vit E); vit E supplemented (C + 470 ppm vit E); glutathione supplemented (C + 0.5% GSH); vit E and GSH supplemented (C + 470 ppm vit E + 0.5% GSH); melatonin supplemented (C + 11 ppm melatonin); or strawberry extract supplemented [C + 1% strawberry extract (high in in vitro antioxidant capacity)]. Mice were infected with influenza A/PC/1/73 (H3N2). Lung viral titer, H_2O_2 production by zymosan-stimulated lung cells, serum IL-6 levels, serum TNF-a levels, body weights, and food intakes were determined 5 days after infection. Mice fed the vit E-supplemented diet had significantly lower pulmonary viral titer compared to those fed the C diet ($10^{2.6}$ vs. $10^{4.0}$) and were able to maintain their body weight after infection (1.8±0.9 g weight loss/5 days postinfection in vit E group vs. 6.8±1.4 g weight loss/5 days postinfection in the C group, p<0.05). Other antioxidant did not have a significant effect on viral titer or weight loss. Weight loss showed significant inverse correlation with food intake (r=-0.955) indicating that the observed weight changes were mainly due to decreased food intake. Vit E or melatonin supplementation tended to increase H_2O_2 production by lung cells from non-infected mice (3.6±0.9, 2.5±1.3, and 2.3±0.9± in the C, vit E, and melatonin groups, respectively, p<0.1) but not from infected mice. Serum IL-6 levels increased significantly postinfection. There was, however, no effect of antioxidant supplementation in serum IL-6 levels before or after infection. Thus, among the antioxidants tested, only vit E was effective in reducing pulmonary virus titer and preventing influenza-mediated decreases in food intake and weight. Further studies are needed to determine the mechanisms of this effect of vit E.

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AGE AND CALORIC RESTRICTION EFFECTS ON MOUSE SKELETAL MUSCLE MITOCHONDRIAL VOLUME DENSITY. Utley, S., Manfredi, T., Cosmas, A., Lamont, L., Bronson, R., and Lipman., R. Univ. of Rhode Island, Exercise Science, Kingston, RI 02881; Univ., Connecticut, Allied Health Professions, Storrs, CT. 06269; USDA Human Nutrition Research Center on Aging, Tufts Univ., Boston, MA 02111.

Forty percent diet restriction (DR) has been reported to increase lifespan and lower the incidence of tumors in mice. Earlier, we reported that older B6C3F1 DR mice had lower capillary density and greater skeletal muscle fiber area than ad libidum (AL) fed mice (*AGE*. 17[4], 157, 1994) and that DR appears to lower the aging effect on muscle atrophy. To explore the effect of DR on muscle fiber energy production, we examined the mitochondrial volume density in the nuclear, cell border, and myofibrillar regions of the vastus lateralis muscle of 5 DR (60% NIH-31 diet) and 6 AL B6C3F1 mice, ages 19 and 27 months. Mitochondrial

volume densities were determined from electron micrographs of cell regions taken at 13,500X. Stereology was used to determine volume density on approximately 12 fibers per animal. We found that age increased the mitochondrial volume density in all regions of the AL and DR mice. Diet appeared to have no effect on mitochondrial volume density in 19 and 27 month old mice. However, our qualitative electron microscopic analysis of approximately 15,000 mitochondria suggested that there fewer larger size mitochondria with age in the muscles from DR mice.

These findings agree with an earlier study (*AGE*. S [34], 1996) which demonstrated an increase in mitochondrial size in myocardium with advancing age, even if exercise is used as a potential age retardent. Other studies report that mitochondrial size correlates with susceptibility to membrane and mitDNA damage from free radicals. The result of damage to mitochondria is compromised energy production. Thus the decreased energy production in older muscle fibers may, in large part, be due to mitochondrial damage. Our data suggests that DR tempered the age associated increase in mitochondrial size. We hypothesize that this effect of DR on mitochondria results in enhanced protection from free radical damage and maintenance of energy production.

Mitochondrial Volume Densities of Skeletal Muscle Mitochondria

Region			Age (Months)
19DR		27DR	
19AL		27AL	
CB	6.18+/-0.86		11.12+/-3.58
	7.72+/-1.01		12.42+/-1.81
NR	9.10+/-6.70		11.13+/-2.69
	7.194+/-0.80		9.961+/-1.98
MR	6.72+/-2.10		11.85+/-5.11
	7.51+/-2.77		11.49+/-1.85

CB = cell border; NR = nuclear region; MR = myofibrillar region.

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GENETIC MANIPULATION OF SKELETAL MUSCLE GLUT4 GLUCOSE TRAN-SPORTERS AND PHYSICAL ACTIVITY. Chang, K. S., Kewitt*, K. D., Tsao, T., Charron, M., and McCarter, R. Departments of Physiology, University of Texas Health Science Center, San Antonio, Texas 78284 and Biochemistry, Albert Einstein College of Medicine, Bronx, New York 10461.

Decreased physical activity is commonly associated with advancing age, but factors responsible for this decline are still under investigation. We have investigated the hypothesis that availability of GLUT4 glucose transporters in skeletal muscle fibers influences physical activity. Four groups of male C57BL/6 mice aged 3 months were used in this study. Three of these groups had free access to running wheels in their cages. The fourth group served as sedentary, wild type controls. The three exercising groups consisted of a Control(wild type) group (C-E), an MLC-GLUT4 group (G4-E, Overexpressing the GLUT4 glucose transporter) and a Null group (N-E, ablation of the GLUT4 glucose transporter). The MLC-GLUT4 mice overexpress GLUT4 specifically in skeletal muscle via a myosin light chain promoter ligated to the murine GLUT4 gene. All mice were singly housed and fed ad libitum a diet of standard rodent chow. Voluntary wheel activity was measured continuously for four weeks and spontaneous cage activity was measured over a twenty-four-hour period, immediately before sacrifice. Food consumption, plasma glucose and body weights were recorded. The results show that overexpression of GLUT4 protein in skeletal muscle substantially increases voluntary wheel running (G4-E versus C-E mice), but that ablation of GLUT4 protein has no significant effect of voluntary wheel running (N-E versus C-E mice). Spontaneous movement of the mice in the cages was similar between all groups. Food consumption was significantly greater in the G4-E than in other mice. Body weights of exercising mice were similar between all groups. Plasma glucose levels measured between eight am and ten am in non-fasting mice were not significantly different between all three groups. We conclude that increased availability of GLUT4 glucose transporters in skeletal muscle is associated with increased physical activity. Strikingly, the absence of GLUT4 transporters did not inhibit voluntary wheel running and spontaneous movement around cages (N-E versus C-S mice). In addition, these effects were not associated with altered plasma glucose or decreased food intake.

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GENDER DIFFERENCES IN THE AGE-RELATED DECLINE IN BETA-ADRENERGIC RECEPTOR FUNCTION IN THE RAT HEART. Snyder, D.L.*, Gao, E, Horwitz, J. and Roberts, J., Department of Pharmacology, MCP◆Hahnemann School of Medicine, Allegheny University of the Health Sciences, Philadelphia, PA 19129.

Beta-adrenergic receptor mediated stimulation of adenylyl cyclase activity as well as beta-adrenergic receptor independent activation of adenylyl cyclase activity decline with age. The aim of the present study was to determine if these age-related changes differ in male and female rat hearts. Ventricular membranes were prepared from the hearts of male and female F344 rats of 6, 12, and 24 months of age. 100 uM isoproterenol (ISO), a beta-adrenergic receptor agonist, and 10 uM forskolin (FOR), which stimulates adenylyl cyclase independent of the beta-adrenergic receptor, were used to stimulate cAMP production. These assays are based on the conversion of ^{32}P-ATP to ^{32}P-cAMP. Data were expressed as pmol/mg protein/minute obtained in a 30 minute incubation from 6-8 animals in each age and gender group. All data were analyzed by ANOVA with significance set at $p < .05$. The response to ISO was significantly greater in female rats compared to male rats at all ages. Significant reductions in the response to ISO occur between 6 and 12 months in male rats and between 12 and 24 months in female rats. The response to FOR was comparable in male and female rats at 6 months; however, in male rats there was a greater age-related decline, so that by 24 months female rats had a significantly greater response to FOR than the male. In male rats, ISO and FOR stimulation of cAMP production declined by 42% and 34% by 12 months, respectively and by 58% and 57% by 24 months, respectively. In female rats, the decline was only 11% and 18% by 12 months, respectively and 44% and 34% by 24 months, respectively. These data suggest that the age-related decrease in postsynaptic beta-adrenergic receptor-mediated responses and in adenylyl cyclase activity occurs to a lesser degree in the female than in the male rat heart.

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MODULATION OF OXIDATIVE STATUS IN EXERCISED GLUT-4 OVER-EXPRESSED TRANSGENIC(TG) MICE. Kim, S. H., Kim, J. D., Chang, K. S., Charron, M. J. Mccarter, R.J.M., Yu, B. P And Fernandes, G., Department of Physiology, University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284.

The present study was undertaken to determine the extent of GLUT-4 glucose transporter overexpression with exercise on lipid peroxidation and antioxidant defense modulation. GLUT-4 overexpression with skeletal and cardiac muscle specificity was achieved by the use of a myosin light chain promoter using the murine GLUT-4 gene in C57BL/6 mice. Both control and TG mice were placed in cages equipped with running wheels. The mice were allowed to voluntarily exercise for a 4 week period. Kidney, skeletal muscle, heart, liver and serum were examined for total and unbound malondialdehyde (MDA) levels. Antioxidant enzymes, superoxide dismutase, glutathione peroxidase, glutathione -s-transferase and catalase were measured. The daily running activities were 5414 (1522 meters for TG versus 2153 (359_ meters for control. The results on modulation of the oxidative status were that TG mice exhibited significantly decreased serum and hepatic total MDA levels as compared to controls. In addition, TG mice showed significantly lower unbound MDA levels in skeletal muscle and liver. Glutathione -s- transferase activities in TG groups were higher in the skeletal muscle, while glutathione peroxidase activities were higher only in the liver. No other tissues showed significant differences. These results suggest that overexpression of GLUT-4 coupled with exercise in mice attenuates oxidative stress and enhances levels of antioxidant enzymes. The significance of our finding is that GLUT-4 overexpressed mice can modulate exercise-induced oxidative stress.

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SOURCE OF DIETARY FAT AND TREADMILL EXERCISE MODULATES ANTIOXIDANT ENZYMES AND APOPTOSIS IN ICR MALE MICE. Avula, C.P.R, Yoshida, M., Azar, R. and Fernades, G.*, Department of Medicine, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78284-7874.

We and others have established that feeding corn oil containing Omega-6 (n-6) fatty acids acts as a pro-inflammatory dietary fat in autoimmune-prone B/W mice when compared to a fish oil enriched diet containing Omega-3 (n-3) fatty acids. Mice on n-3 (10% by wt) fatty acids

developed significantly reduced renal disease, an increase in antioxidant enzymes and elevated apoptosis when compared to n-6 fed mice. We have also reported that exercise or calorie restriction decreases blood pressure and increases T cell immune function in hypertensive SHR rats.

The present study was therefore designed to compare the effect of n-6 and n-3 lipids on the development of antioxidant enzymes and Dexamethasone (Dex) induced apoptosis in immune cells of sedentary and treadmill exercised ICR male mice fed semipurified diet containing 10% n-6 or n-3 lipids with equal levels of antioxidant (Vitamin E) supplements. Three-month old ICR male mice were maintained on n-6 and n-3 lipid enriched diets gradually trained to run on a treadmill for approximately 40-50 mins. (1km/day) five days a week. Equal amounts of fresh food was provided daily *ad-libitum* and body weights were recorded weekly.

After 12 weeks of exercise, five mice from each group were sacrificed and both blood and various tissues including spleens were collected in aseptic conditions. Apoptosis was measured in spleen cells incubated with or without 10^{-6}M Dex in RPMI medium for 8 or 24 hours. Apoptosis and necrosis was measured by staining $1x10^{-6}$ cells with Annexin-V and propidium iodide (PI) using a CELLQuest program on a desktop FACScan instrument. Lipid peroxides were measured as thiobarbituric acid reactive species (TBARS) in serum and in a portion of spleen homogenates. The results revealed a significantly higher TBARS (21% and 20%) in n-3 fed sedentary mice compared to the n-6 group, whereas exercise did not alter TBARS levels in n-6 or n-3 fed mice. In the case of antioxidant enzyme levels in spleen homogenates, n-3 fed mice showed significantly higher superoxide dismutase (SOD) (34%), catalase (48%) and glutathione peroxidase (40.7%) activity when compared to n-6 fed mice. Exercise also significantly increased SOD activity (18%) in corn oil and 32.6% gluthathione-S-transferase and 36.7% glutathione peroxidase activity in n-3 feed mice when compared to sedentary mice. Furthermore, n-3 fed mice also showed significantly higher apoptosis by 64% versus 50%, and necrosis 40% versus 22% than that in spleen cells of n-6 fed mice when measured at 8 and 24 hours respectively. Cells from n-3 fed mice, incubated with media alone showed 112% increased apoptosis at 24 hours incubation and necrosis by 82% and 70.6% at 8 and 24 hours incubation, compared to corn oil fed mice. In exercised mice, apoptosis was increased by 193% and 66 % in n-3 and n-6 fed mice and necrosis by 19.6% in corn oil fed mice incubated for 24 hours respectively.

In summary, these results indicate that n-3 PUFA enriched diet, increases apoptosis and antioxidant enzymes activity in the serum and spleen cells, possibly due to the elevated lipid peroxides. Further, treadmill exercise is also found to enhance both antioxidant enzymes and apoptosis both in n-6 and n-3 lipid fed mice. These results suggest that a well balanced n-3 fatty acid supplement, may have beneficial effects relative to n-6 fatty acids, yet, new studies are required to establish either a protective or detrimental effect of n-3 fatty acid supplement on the immune system during endurance exercise.

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EFFECT OF CORN OIL AND FISH OIL ENRICHED DIET ON THE IMMUNE FUNCTION IN SEDENTARY AND TREADMILL EXERCISED FEMALE AND MALE MICE. Yoshida, M., Ernani, F. Azar, R. and Fernandes, G.*. Department of Medicine, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78284-7874.

N-3 lipid (fish oil) enriched diets are known to have a profound effect on cardiovascular and autoimmune disorders in humans and in animal models. The present treadmill exercise study was undertaken in ICR mice to compare the effect of corn oil (n-6) and n-3 enriched diets on physiological, hematological and immunological parameters. *Ad-libitum* three-month-old female and male mice maintained on semipurified diets containing 10% (by wt.) n-6 and/or 10% n-3 mice were trained gradually to run on a treadmill. After two-to-three weeks of training, the mice were run to near exhaustion, 40-50 mins., one km/day for five days for a total period of three months.

Weekly body weights, periodic food and water consumption as well as rectal temperature were recorded on individual mice every four hours for 24 hours. Retroorbital blood was collected for hematological analysis. After three months of exercise, mice were sacrificed and spleen and tissues such as liver and leg muscles were collected.

The results revealed a striking dietary lipid and exercise effect on female and male mice. Female mice maintained on both n-6 and n-3 diets exercised almost equally and showed 10 and 12% decreased body weight, respectively, when compared to sedentary mice. In contrast, male mice maintained on n-3 fatty acids exercised longer and showed 20% less body weight than sedentary (Sed) mice; exercised (Ex) mice maintained on n-6 or corn oil showed only 4% less body weight. Although the body weights of Sed mice maintained on both diets were nearly equal, male mice had significantly higher weights than females. Furthermore, Sed male mice maintained on n-3 diets had gained 13% more body weight than Sed mice maintained on n-6 diets.

Dietary and exercise effects upon hematological parameters were also found to be striking. In the case of white blood cell (WBC) counts, mice maintained on n-3 diets had significantly higher WBC counts than mice maintained on n-6 diets. In addition, body (rectal) temperature was found to be significantly lower in n-3 fed, control and EX male and female mice. Fluorescent Activated Cell Sorter (FACS) analysis of cell surface markers (CD4, CD8, and CD19) did not show marked differences in Sed or Ex (24 hrs rested) animals. Measurement of various serum immunoglobulin isotypes (IgM, IgG1, IgG2a, IgG2b, IgG3, and IgA) in Sed and Ex mice also revealed no significant difference between gender, diet or exercise.

In summary, n-3 or fish oil fatty acid supplement appears to provide higher endurance capacity during exercise to male mice whereas endurance ability to exercise in female mice was not found significantly different between n-6 or n-3 fatty acid supplemented groups. These results may suggest a possible link between dietary fatty acids and physical inactivity in humans as well. Our finding, linking the source of dietary lipids and gender difference to exercise, needs to be confirmed by undertaking various sources of dietary lipids in exercise studies by using both animal models and possibly human subjects.

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OXIDATIVE STRESS INDUCED BY XANTHINE-XANTHINE OXIDASE AND HYPERTHERMIA SUPPRESSES THE INDUCTION OF IL-2 EXPRESSION AND NUCLEAR SIGNAL TRANSDUCTION IN T CELLS FROM YOUNG AND OLD RATS. Pahlavani, M.A and Harris, M. D. GRECC, Audie L. Murphy V.A. Hospital and Department of Physiology, University of Texas Health Science Center, San Antonio, Texas, 78284.

T cells from young (6 month) and old (24 month) Fischer 344 rats were exposed to xanthine-xanthine oxidase (X/XO), hydrogen peroxide (H_2O_2), and heat stress (heat shock at 43°C for 1 h), and viability, mitogen-induced proliferation, IL-2 production, and DNA binding activity of transcription factors NF-kB, AP-1, and NFAT were measured. Before exposure, the induction of proliferation and IL-2 expression was greater in T cells from young rats than that of old rats. Exposure of T cells to H_2O_2 or X/XO resulted in suppression of proliferation and IL-2 expression. The suppressive effect of H_2O_2 and X/XO was greater in T cells from young rats than in T cells from old rats. Heat stress for 1 h at 43°C caused a dramatic decrease in proliferation and IL-2 expression in T cells from young and old rats. Addition of the antioxidants N-acetylcysteine, catalase, or vitamin E to cultured lymphocytes attenuated the suppressive effect of X/XO but not heat stress on mitogen-induced proliferation and IL-2 expression. The most distal step of mitogen-mediated signal transduction is dependent upon transcription factors that regulate a set of genes, including IL-2. We found that X/XO-treated cells exhibited very low DNA binding activity of

NF-$_\kappa$B and NFAT in T cells from young and old rats. In contrast, DNA binding activity of transcription factor AP-1 was enhanced in X/XO-treated cells, suggesting a possible role of AP-1 in T cell stress response.

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ELECTRON TRANSPORT SYSTEM AB-NORMALITIES ARE ASSOCIATED WITH DELETED MITOCHONDRIAL GENOMES IN RHESUS MONKEY SKELETAL MUSCLE. Lee, C. M. Department of Animal Health and Biomedical Sciences,. Lopez, M. E. Department of Animal Health and Biomedical Sciences, Weindruch, R. Medcine and VA Geriatric Research, Education and Clinical Center, and Aiken, J. M Department of Animal Health and Biomedical Sciences. University of Wisconsin-Madison, Madison WI 53706.

We have employed histological approaches to examine the quadriceps of rhesus monkeys for mitochondrial enzymatic activities and the occurrence of mitochondrial DNA deletions. Our investigation of mitochondrial enzymatic activities consisted of both cross-sectional and longitudinal (i.e., multiple cross-sections of individual fibers examining a 350-640 um region) analyses. We first used histochemical techniques for the localization of COX and succinate dehydrogenase (SDH) activities in cross sections from rhesus monkey skeletal muscle. Sections that

were COX^- and hyperreactive for SDH activity (SDH^{++}) were used for the analysis of mitochondrial DNA expression by in situ hybridization. The expression five different mitochondrially-encoded genes (12s rRNA, ND2, Cox I, ND4, and Cyt b) was analyzed to determine if deleted mitochondrial genomes were associated with the enzymatic abnormalities. We report that 88.5% of the combined COX^- and SHD^{++} (COX^-/SDH^{++}) fibers examined contained deleted mitochondrial genomes. When fibers were examined longitudinally, transition regions (i.e., the region where the fiber changed from normal COX or SDH activity to regions of COX^- and/or SDH^{++} activities) were identified. Two distinct phenotypes were observed in the transition regions. In the more predominant one (70% of the fibers examined), the fibers appear first with normal enzymatic activities, and in subsequent sections become COX^- and finally COX^-/SDH^{++}. The second phenotype (30%) included fibers which changed from enzymatically normal regions to SDH^{++} and finally COX^-/SCH^{++}. In situ hybridization studies performed on these transition regions indicate that deleted mitochondrial genomes could be detected prior to the fiber displaying the enzymatic defect. They also indicated that the genotype of the deletion event dictates the phenotype of the enzymatic defect. They also indicated that the genotype of the deletion event dictates the phenotype of the enzymatic defect. Together, these data strongly supports a causal relationship between deletions of the mitochondrial genome and the appearance of age-associated mitochondrial enzymatic abnormalities.

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CHARACTERIZATION OF AGED MUSCLE ATROPHY AND ELECTRON TRANSPORT SYSTEM ABNORMAL FIBERS IN RHESUS MONKEYS AND RATS. Lee, C. M. Department of Animal Health and Biomedical Sciences, Chung S. S.,*, Department of Animal Health and Biomedical Sciences, Lopez, M. E. Department of Animal Health and Biomedical Sciences, Aspnes, L. E. Department of Nutritional Sciences Weindruch, R., Medicine and VA Geriatric Research, Education and Clinical Center and Aiken, J. M. Department of Animal Health and Biomedical Sciences. University of Wisconsin-Madison, Madison, WI 53706. The cause of muscle mass loss with age (sarcopenia) is likely multifactoral. Several hypotheses have been suggested for the etiology of sarcopenia including a decline in satellite cell repair, contraction-induced injury and increased oxidative stress of mitochondrial origin. Studies in our laboratory are focusing on the last of these hypotheses with a special emphasis on mitochondrial enzymatic activities. We have examined skeletal muscle from rhesus monkeys and rats of various ages to determine the degree of muscle fiber loss and the occurrence of electron transport system (ETS) abnormalities. Fiber number was counted from the vastus lateralis muscle mid belly of young (3-4 months) and old (30-32 months) Lobund-Wistar rats. The young rats had significantly greater number of fibers than did the old rats (6619 and 4870), respectively. Two ETS activities were assayed: succinate dehydrogenase (SDH) and cytochrome c oxidase (COX). Fibers were detected which were either negative for COX activity (COX^-), hyperreactive for SDH activity (SDH^{++}), or combined (COX^- and SDH^{++}) In the rhesus monkeys, fibers which stained COX^- but were not SDH^{++} appeared first at 20 years of age while COX^-/SDH^{++} fibers were more often detected in animals 25 years and older. We also observed several ETS abnormal fibers in the rhesus monkeys and rats which displayed considerable atrophy. For example, six of the 44 fibers characterized (14%) with ETS abnormalities in the rhesus monkeys also exhibited atrophy over a 340-640 mm distance examined. These data indicate that atrophy can occur in the same fibers which display ETS abnormalities suggesting the possible contribution of these abnormalities to sarcopenia.

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REDUCED SIGNS OF AGING IN SUBJECTS USING THE MAHARISHI VEDIC APPROACH TO HEALTH (MVAH): IMPROVEMENTS IN THE HYPOTHALAMIC-PITUITARY AD-RENO-CORTICAL (HPA) AXIS AS A LIKELY MECHANISM. Walton, KG, Fields, JZ Harris, DA Pugh, NDC, Alexander, CN. Center for Health and Aging Studies, Maharishi University of Management, Fairfield, IA 52557; & Center for Healthy Aging, Saint Joseph Hospital, Chicago, IL 60657.

Both cross-sectional and longitudinal studies suggest that the Transcendental Mediation (TM) technique reduces biological aging. This includes reversing aging-related trends such as high blood pressure (BP), hearing loss, loss of visual accommodation, cognitive decline, even mortality. The TM program is a principal component of MVAH, an ancient system of natural medicine. Recently, it was reported (Psychosomatic Med 1987; 49:493-507; Am J Man Care 1997;3:135-144) that MVAH reduces the frequency of most diseases, especially chronic diseases, associated with aging. Although the molecular mechanisms are not fully established, the TM technique has been shown to be an effective stress-reduction technique (e.g., reducing anxiety and cortisol (CORT). The idea that the anti-stress effect of the technique is responsible for its anti-aging effect is consistent with the Stress Theory of Aging. That theory suggest that HPA activation, including increases in glucocorticoid and mineralocorticoid activity, are critical factors contributing to senescence. To further investigate a possible causal or contributory role of alterations in the HPA axis in MVAH-induced reductions in the diseases and disabilities of old age, we carried out a cross-sectional study measuring dysfunctions and diseases often associated with chronically high CORT. We compared BP, history of all disease (HIST), history of cardiac disease (CARD) and other health parameters with indicators of HPA axis function. The latter included [1] salivary CORT (including the response to an acute stressor), [2] urinary excretion of CORT, and [3] urinary excretion of the electrolytes Na, K,Ca, MG, Zn. Subjects were 25 long-term users of MVAH (mean age=74) and 26 age-matched non-users (mean age = 76). MVAH users had significantly lower CORT excretion (-61%) and BP (-16mmHG systolic, -7 mmHg diastolic), and greater mental health (+81%). CORT excretion and CORT response to acute stress correlated with BP, HIST & CARD in women. In men, a significant association was found only between high CORT and high systolic BP. MVAH users had lower excretion rates of Na (-45%), K(24%), Ca(-33%), and Zn (-60%) but not Mg (-8%) and had a lower Na/K excretion ratio(-27%). These data suggest that non-users had higher levels of chronic stress and high salt appetite, a well-known correlate of chronic stress and of high BP, and that MVAH users had reversed the trends in these parameters normally seen with aging. High Na and Ca excretion have been associated with high BP and high Zn excretion has been associated with increased risk of certain cancers as well as with high BP. We conclude that 1) elevated HPA axis activity in later years is a major health risk, particularly in women; 2) MVAH reduces HPA activity, stress and chronic aging-related disease; 3) MVAH improves health; 4) all these effects of MVAH appear to be due, at least in part, to improvements in the HPA axis. The conclusions of this study are consistent with the Stress Theory of Aging, especially if stress is defined broadly as any enduring physical abnormality (including such things as the oxidative damage caused by free radicals) resulting from an overload of the system.

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EXCRETION OF VITAMIN C BY CIGARETTE SMOKERS. Matz, M.L. Schwantes, L. G. Thorp, S. C. and Ordman, A. B.Biochemistry Program, Beloit College, Beloit, WI 53511.

Our purpose was to determine a suitable dosage of vitamin C supplementation for cigarette smokers. Cigarette smoking causes increased free radical production. In previous studies, a vitamin C dosage of 500 mg twice a day has been shown to cause increased urinary excretion and saturating blood levels of vitamin C in non-smokers.

In our first study of 9 college students eating normal diets, 500 mg of vitamin C were taken twice daily, all urine for 24 hour periods was collected and assayed by the dichlorophenol-indophenol method. No correlation of urinary excretion of vitamin C was found to age, weight, or exercise level. However, cigarette smokers had substantially lower levels of vitamin C.

In another study with 7 students smoking 10-20 cigarettes per day and eating normal diets, vitamin C was not detectable in most urine samples until vitamin C supplements were taken. When 1000 mg of vitamin C were taken twice daily, vitamin C levels rose to detectable levels. When 500 mg were taken twice a day for eight days, vitamin C was detectable in each micturition, with average excretion of approximately 1,000 mg per day. When 500 mg of vitamin C twice a day has previously been established as a safe dosage, this dosage should be sufficient to provide saturating levels of vitamin C in smokers as well as non-smokers.

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THE RELATIONSHIPS OF APPRAISAL OF DISRUPTIVE BEHAVIORS, LEARNED RESOURCEFULNESS AND ANXIETY AMONG FAMILY CAREGIVERS OF RELATIVES WITH ALZHEIMER'S DISEASE. Gonzales, E. W.,School of Nursing, Allegheny University of the Health Sciences, Philadelphia, PA 19102.

One of the major reasons for institutionalization of Alzheimer's patients is decline in caregiver's health. Despite evidence suggesting that there is considerable variability in the ability of family caregivers to deal with caregiving situations, individual differences in dealing with the caregiving experience are not well understood. This descriptive study used the cognitive transactional theory as a framework to examine caregivers' appraisals of disruptive behaviors, learned resourcefulness, and anxiety in caring for relatives with Alzheimer's disease. The research questions that guided this study were 1) What is the relationship between learned resourcefulness and anxiety among family caregivers of relatives with Alzheimer's disease?; 2) What is the relationship between learned resourcefulness and appraisal of disruptive behaviors among family caregivers of relatives with Alzheimer's disease?

A non-probability, convenience sample of thirty-five women family caregivers who were living with and caring for a relative diagnosed with probable, moderate Alzheimer's disease were recruited for the study. The appraisals of stressfulness of disruptive behavior problems was measured with the Revised Memory and Behavior Problem Checklist subscale (RMBPC). The Cronbach's alpha was .93. Learned resourcefulness was measured with the Self-Control Schedule (SCS). The Cronbach's alpha was .76 Anxiety was measured with the Brief Symptom Inventory, anxiety subscale (BSI). The Cronbach's alpha was .84. A Pearson Product-moment correlation was used to examine the relationships among the study variables and variables such as age, socioeconomic status, length of caregiving time and the number of persons living in the same household. Caregivers age ranged from 49 to 81 years with a mean age of 60 years (SD = 5.4). Most of the caregivers (71%) were Anglo Americans and (29%) were African Americans. The majority (75%) had completed high school. Most were married and caring for their spouses. The average length of time spent in caregiving was 4 years. The median household income of family caregivers was $24,000 annually. The average age of care recipients was 71 year (range = 68-97; SD = 8.4).

The result showed that learned resourcefulness was significantly related to appraisal of stressfulness of disruptive behavior (r= .34). Family caregivers who were more resourceful reported benign appraisals of disruptive behaviors in caring for relatives with Alzheimer's disease than those who were less resourceful. The results also showed that resourcefulness was significantly related to anxiety (r=—.38). Specifically, family caregivers who were more resourceful reported less anxiety in caring for relative with Alzheimer's disease than those who were less resourceful. The results indicate that learned resourcefulness may be an important variable in understanding caregiver stress and may help explain why some caregivers cope better with the demands of their role than others.