CALORIC RESTRICTION

PreConference Abstracts

(P) Denotes presenter

CALORIE RESTRICTION IN YEAST AND WORMS: PUBLIC OR PRIVATE MECHANISMS

Matt Kaeberlein (P), University of Washington

We are using two model organisms, the budding yeast, *Saccharomyces cerevisiae*, and the nematode, *Caenorhabditis elegans*, to identify evolutionarily conserved determinants of longevity and to characterize the genetic factors involved in life span extension from calorie restriction. Through genome-wide studies of longevity in yeast, we have determined that calorie restriction slows aging by down-regulation of the TOR, PKA, and Sch9 (Akt) nutrient-responsive kinases, and we are testing the degree to which this mechanism is conserved in *C. elegans*. In addition, we are performing a systematic analysis of the aging properties of ortholog pairs, in which one ortholog is known to regulate longevity in either yeast or *C. elegans*. Thus far, our analysis suggests a significant enrichment of longevity-determining genes among ortholog pairs, providing quantitative evidence that genetic pathways influencing aging are evolutionarily conserved. Future efforts will be aimed at testing candidate conserved longevity determinants in mice and monitoring life span and other phenotypes associated with aging in these animals.

EFFECTS OF LIFELONG EXERCISE AND 8% CALORIC RESTRICTION ON FREE RADICAL BIOLOGY

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Aging causes a dysregulation in redox status due to an enhanced oxidant production and reduced antioxidant protection, which may lead to changes in specific signaling pathways including a pro-inflammatory response and altered tissue morphology. We investigated whether long-term voluntary exercise and mild 8% calorie restriction (CR) could attenuate these changes in the heart, liver and skeletal muscle. Four groups of male Fischer-344 rats were compared: young (6 mo) *ad libitum* fed (YAL), old (24 mo) *ad libitum* fed (OAL), old lifelong calorie restricted (8%CR: OCR) and age-matched old lifelong 8% CR with daily voluntary wheel running exercise (OExCR). After the age of 6-mos, running activity was maintained at an average of 1145 $\pm$ 248 meters/day until all animals were sacrificed at 24-mos of age. Daily energy expenditure, as determined by the doubly-labeled water technique, was approximately 70% higher in runners compared to sedentary rats. In general, we found that oxidant production (hydrogen peroxide (H_2O_2), nitric oxide ($NO^{\bullet}$), and peroxynitrite ($ONOO^{-}$)) was increased with age in several tissues, and moderate CR and wheel running exercise were able to attenuate the age associated increases. In addition, antioxidant status in tissues and in plasma (sulfhydryl (-SH), glutathione (GSH) and total antioxidant status) was decreased with age, and exercise and CR were also able to attenuate the age-associated decline. Moreover, old rats had significantly increased nuclear presence of NF-κB and in connection, increased levels of regulatory cytosolic phosphorylated I-κBα and decreased dephosphorylated I-κBα in the liver, suggesting an increased inflammatory response. Interestingly, a significant increase in liver RNA oxidation (8-oxo-7,8-dihydroguanosine) in the old *ad libitum* fed rats was detected and DNA oxidation (8-oxo-7,8-dihydro-2'-deoxyguanosine) also tended to increase. The age-associated increase in oxidative stress and upregulation of pro-inflammatory proteins was attenuated in the tissues from both the CR and the

exercise + CR groups. Moreover, lifelong exercise + 8% CR showed a marked decrease in plasma CRP levels compared to 8% CR, while levels of CRP in 8% CR were also markedly lower as compared to the *ad libitum* fed animals. We also showed that lifelong voluntary exercise and mild caloric restriction preserve fast-twitch muscle morphology in the aging plantaris. In addition, both 8%CR and exercise protected against the large increase (+373%) in connective tissue found in OAL plantaris when compared with YAL. Furthermore, exercise protects skeletal muscle mass and muscle morphology against the effects of aging, while mild caloric restriction lessens age-induced changes in muscle morphology. In summary, lifelong exercise and 8% caloric restriction reduce oxidative stress and pro-inflammatory effects of aging in rats. This research was supported by grants to CL from the National Institute on Aging (AG17994 and AG21042) and an American Heart Association Fellowship to SJ (0215053B) and TH (0525346B). [1-5]

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THE UNIVERSITY OF WISCONSIN DIETARY RESTRICTION AND AGING STUDY

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Dietary restriction (DR) is the only nutritional intervention shown to extend maximal life span and oppose the development of a broad spectrum of age-associated pathophysiological changes in warm-blooded animals. This effect has been observed repeatedly and robustly in laboratory rodents, but not yet in primates. In order to investigate the effects of DR in a nonhuman primate species, we began a study, in 1989, of moderate (30%), adult-onset DR in rhesus monkeys (*Macaca mulatta*). Our study at the Wisconsin National Primate Research Center at the University of Wisconsin, Madison, began with a group of 30 adult males and was expanded in 1994 to include 30 adult females and an additional group of 16 males. In each group, animals were randomized so that half of the animals were maintained *ad libitum* and the remaining half received 70% of their individual baseline food intake values. We have now completed 16 years of study and animals subjected to the restricted diets are showing consistent signs of improved health. Most noteworthy is the reduced body fat and increased insulin sensitivity of DR animals compared to controls.

THE NIA STUDY OF DIETARY RESTRICTION AND AGING

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Dietary caloric restriction (CR) is the only intervention repeatedly demonstrated to retard the onset and incidence of age-related diseases, maintain function, and extend both lifespan and healthspan in mammals. In 70 years of study, such beneficial effects have been demonstrated in rodents and lower animals, but prior to 1987, had never been examined in primates. To determine whether CR might eventually be applied to humans, the NIA initiated a study of CR

and aging in nonhuman primates. After nearly 18 years, approximately 150 monkeys have been involved in the study, mostly rhesus monkeys (*Macaca mulatta*) with a small group (~ 36) squirrel monkeys (*Saimiri sciureus),* aged across their respective lifespans at the time of initiation,. Control monkeys receive two meals per day sufficient to attain apparent satiety, while the CR group receives 30% less, adjusted for age and body weight. The diet is supplemented with extra micronutrients such that the only substantive variable is the amount of calories consumed. Results to date indicate that CR animals are healthier than fully-fed counterparts based on reduced incidence of various chronic diseases, exhibit significantly better indices of predisposition to disease (such as lower insulin levels and greater insulin sensitivity, reduced blood lipids and pressure, and elevated HDL), and may be aging at a slower rate, based on a number of hormonal and functional indices. In addition, CR rhesus monkeys that were juveniles at the onset of the study showed delayed skeletal and sexual maturation, and CR groups have lower body temperatures than controls. Thus far, regarding the health of older monkeys, our results indicate several significant beneficial effects and no negative effects; however, many of these findings remain preliminary and will require additional time to confirm.

DELAY OF T-CELL SENESCENCE BY CALORIC RESTRICTION IN AGED LONG-LIVED NON-HUMAN PRIMATES

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Caloric restriction (CR) has long been known to increase median and maximal life spans and to decreases mortality and morbidity in short-lived animal models likely by altering fundamental biological processes that regulate aging and longevity. In rodents CR was reported to delay aging of the immune system (immune senescence), believed to be largely responsible for a dramatic increase in age-related susceptibility to infectious diseases. However, it is not clear whether CR can exert similar effects in long-lived organisms, because testing of effects of 2-4 year CR treatment on the immune system of long-lived primates failed to find CR effect or reported opposite effects compared to those in CR-treated rodents. Here, we show that long-term CR delays the adverse effects of aging upon primate T-cells. The most remarkable impact of CR was at the level of maintaining and/or increasing production of naïve T-cells and the consequent maintenance of T-cell receptor repertoire diversity. Furthermore, CR also improved T-cell function and reduced production of inflammatory cytokines in memory T-cells. This provides the first evidence that CR can delay immune senescence in non-human primates, potentially contributing to extended lifespan by reducing susceptibility to infectious disease.

FEASIBILITY OF LONG-TERM CALORIC RESTRICTION IN HUMANS

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There remains no consensus over whether long-term caloric restriction (CR) is feasible in humans. As part of the NIA-funded CALERIE (Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy) trial we have examined the effects of dietary macronutrients and level of caloric restriction (CR) over 12 months on adherence to the prescribed regimens, weight and fat loss, and related variables in an randomized controlled trial of high glycemic (HG) vs. low glycemic (LG) load diets fed at 10% and 30% CR. The subjects were 46 healthy overweight adults (mean±SD, age 35±6y; BMI 27.6±1.4 kg/m^2). All food was provided for 6 months in diets controlled for variety, palatability and other variables, and subjects self-administered the plans for 6 additional months. Consistent behavioral support was offered to all groups. Main outcome assessments were energy intake assessed by doubly labeled water, body weight and fatness,

hunger, satiety and dietary satisfaction, and resting metabolic rate. All groups consumed significantly less energy during CR than baseline, ($P<0.001$), and changes in energy intake, body weight, body fat and resting metabolic rate during CR did not differ significantly between groups. However, both 30% CR groups ate more energy than provided (e.g. 21%CR and 28%CR in 30%HG and 30%LG, respectively, at 3 months), while the 10% groups ate less than provided (15%CR and 22%CR in 10%HG and 10%LG). There was no effect of dietary composition on hunger, satiety, or satisfaction with the amount of provided food, but 10%CR subjects were significantly more satisfied with the amount of provided food than 30%CR subjects ($P<0.05$). These findings provide more rigorous support than available previously for the suggestion that a wide range of diets can support long-term CR. Supported by NIH grant U01-AG20480 and USDA Agreement # 58-1950-4-401.

EFFECT OF CALORIE RESTRICTION ON ENERGY METABOLISM WITH EMPHASIS ON MITOCHONDRIAL BIOGENESIS.

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Caloric restriction (CR) extends life span and retards age-related diseases in rodents and lower species. Several biomarkers of longevity (glucose, insulin, core temperature and DHEAS) have been identified in rodents and monkeys but whether CR improves these in humans is unknown. Furthermore, the mechanism through which CR increases life span is unclear. One hypothesis is that metabolic rate is reduced beyond that expected for the reduction in metabolically active mass leading to reduced oxidative damage and possibly improvement in mitochondrial function. 48 healthy (36.8±1.0 y), nonsmoking, overweight (BMI 27.8±0.7) men and women were randomized into one of four groups for 6-mo; Control = 100% of energy requirements; CR = 25% restriction; CREX = 12.5% CR +12.5% increase in energy expenditure by structured exercise; LCD = low calorie diet until 15% weight reduction followed by weight maintenance. Individual diets were provided to subjects for 2 weeks before baseline tests, during the first 3 months of intervention and again for 2 weeks before tests at M6. At baseline and M6, body composition was measured by DEXA, 12-h fasting blood samples were taken and sedentary 24h energy expenditure (24h-EE) and core body temperature were determined in a metabolic chamber and a muscle biopsy was taken. Weight change at M6 was –1.0±1.1% (Control), –10.4±0.9% (CR), –10.0± 0.8% (CREX), -13.9±0.8% (LCD). At M6, fasting insulin levels were significantly reduced from baseline in the CR, CREX and LCD groups (all, $p<0.01$), whereas DHEAS and glucose levels were unchanged. Core temperature was reduced in CR group by 0.2±0.05 °C and by 0.3±0.08 °C in the CREX group (both, $p<0.05$). At baseline, fat-free mass accounted for 86% of the variance in sedentary 24h-EE. After adjustment for changes in body composition, sedentary 24h-EE was unchanged in controls (-18±52 kcal/d; $p>0.05$), but decreased in the CR (-135±42 kcal/d), CREX (-117±52 kcal/d) and LCD (-125±35 kcal/d, groups (all, $p<0.008$). These “metabolic adaptations” (~6% more than expected based on loss of metabolic mass) were statistically different from controls ($p<0.05$). Serum protein carbonyl concentrations and urinary isoprostanes excretion rates were not changed from baseline to M6 in any group, whereas DNA damage was reduced from baseline in the CR, CREX and LCD groups at M6 ($p\leq 0.005$) but not in the Control group. CR and CREX was associated with an increase in the muscle expression of genes involved in mitochondrial biogenesis and mitochondrial fusion including PGC1- α , mitochondrial transcription factor A, endothelial nitric oxide, SIRT1 and PSARL (all, $p<0.05$). In parallel, mitochondrial content increased by 35±5% in the CR group ($p<0.05$) and 21±4% in the CREX group ($p<0.05$), with no change in the control group (2±2%). However, the activity of key mitochondrial enzyme of the TCA cycle (citrate synthase), β-oxidation (β-HAD) and electron transport chain (COX II) was unchanged. This study suggests that 6-mo of CR in non-obese humans was sufficient to improve biomarkers of aging and supports the theory that 24h-EE is reduced beyond that expected due to reduced metabolic size. Whether this metabolic adaptation translates into overall reduced oxidative damage remains to be determined. The increased mitochondrial content in association with a decrease in whole body DNA damage is however an important indication that CR improves mitochondrial function in human skeletal muscle.

AGE PROGRAM SPEAKER ABSTRACTS

Abstract number corresponds to speaker presentation number in program schedule

(P) Denotes presenter **(G)** Denotes Post-doctoral Candidate for Glenn Award
(N) Denotes Pre-doctoral Candidate for Nicolai Award

1. FROM POST-MITOTIC INVERTEBRATES TO LONG-LIVED MICE AND AGE-DEPENDENT DISEASES

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The gene *clk-1*, which affects aging and numerous other physiological rates and rhythms in the nematode *Caenorhabditis elegans*, encodes an enzyme that is necessary for the biosynthesis of ubiquinone (co-enzyme Q), an essential cofactor in numerous redox reactions, including in the mitochondrial respiratory chain, and as a membrane antioxidant. *clk-1* mutants accumulate the biosynthetic intermediate demethoxyubiquinone instead of ubiquinone. *clk-1* mutants have low levels of reactive oxygen species (ROS) at crucial cellular sites, as well as lower levels of oxidative damage to lipoproteins (Shibata *et al*., 2003, Science, 302: 1779-82). In particular, we found that reducing the level of the extracellular Cu/Zn superoxide dismutase, which increases oxidative stress, partially suppressed the *clk-1* mutant phenotype, indicating that these phenotypes are indeed due to low levels of ROS. In addition, we found that *clk-1* mutations could suppress the developmental phenotypes of activated *ras*, a signaling pathway that is modulated by ROS.

Although a complete knockout of murine *clk-1* leads to embryonic lethality, *clk-1 +/-* mice, which have low levels of CLK-1 protein, show a significantly increased lifespan. We are studying how a decrease of CLK-1 activity impacts the physiology and pathophysiology of these mice. In particular, we are determining how the heterozygous condition affects the function of stem cells during aging, as well as how it alters the development of age-dependent diseases. For this we are also using double mutant strains that carry homozygous disease-predisposing mutations (e.g. *sod2*, *sod1, apoE, LDLr*), or transgenes (v-Ha-*ras*) in combination with the heterozygous *clk-1* genotype. Our findings, which suggest that the genetics of aging in short-lived, post-mitotic invertebrates are relevant to mammalian organismal and cellular aging, will be presented at the meeting.

2. OXIDANTS AS SIGNALING MOLECULES

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Originally viewed as toxic metabolites, the last ten years has seen a significant reappraisal of the physiological and pathophysiological role of oxidant species. A large body of evidence suggests a causative role of oxidants in the initiation and progression of a number of age-related diseases including atherosclerosis, neurodegeneration as well as cancer. Although originally viewed as causing random and indiscriminate damage, emerging evidence suggests that the production of reactive oxygen species (ROS) may be tightly regulated and that the intracellular targets of oxidants may in fact be exquisitely specific. Nearly ten years ago we observed that certain cells produce high levels of ROS when stimulated by peptide growth factors. The production of ROS was transient, peaking in the first few minutes following ligand stimulation and returning to baseline within 30 minutes after stimulation. Our initial observation was in vascular smooth muscle cells stimulated with the growth factor PDGF, but subsequently it has become clear that similar events transpire in a wide variety of different cell types stimulated by a host of different ligands. Interestingly we found that inhibiting this rise in ROS blocked the initial signaling events demonstrating an essential role in ROS generation for normal physiological signal transduction. We have also demonstrated that the source of the ligand stimulated ROS-generator in non-phagocytic cells shared certain molecular and biochemical similarities with the phagocytic NADPH oxidase. In particular, we were able to demonstrate a role for the small GTPase Rac1 in the regulation of the intracellular redox state. We have also shown with the help of our

collaborators that the related GTPase Ras also plays an important role in redox regulation within cells. Interestingly, the ability of Ras proteins to induce transformation in the context of immortalized cell, or to induce senescence in the context of primary cells, appears dependent in some fashion on the ability of Ras proteins to induce a change in the level of ROS. I will also discuss recent evidence as to how mitochondria oxidants might signal and regulate both nuclear and cytoplasmic events. Together these observations raise fundamental question as to how oxidants participate in disease processes. In particular, do oxidants contribute to aging or to diseases such as atherosclerosis, neurodegeneration or cancer through random non-specific damage or instead do they produce disease phenotypes through the activation of specific redox-sensitive processes.

3. MITOCHONDRIA, OXIDATIVE STRESS AND AGING

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The hypothesis tested in these studies is that mitochondrial oxidative stress - an imbalance between pro-oxidant production and antioxidant defense/ repair processes - could underlie the aging process of animals. This hypothesis is supported by two major lines of evidence. First, the rate of mitochondrial oxygen consumption during state 3 and maximum (uncoupled) respiration decreases as a function of age, indicating that the capacity for energy production is diminished. Second, the rates of mitochondrial superoxide anion radical and hydrogen peroxide release are elevated with advancing age. Overexpression of various antioxidative enzymes using native regulatory sequences has no effect on the life spans of transgenic fruit flies, Drosophila melanogaster. These enzymes include Cu,Zn and Mn superoxide dismutases (SOD), catalase, and thioredoxin reductase. Studies of human Cu,Zn SOD (SOD1) expression in a Drosophila SOD1-null background showed that only ~5% of the wild type activity is sufficient to sustain a normal, 60-70-day mean life span. In contrast, the life span was significantly diminished by the simultaneous overexpression of Mn SOD and ectopic expression of catalase in mitochondria, but it was extended by overexpression of glutamate cysteine ligase or glucose-6-phosphate dehydrogenase using the binary GAL4-UAS transgenic system. The reasons for contrasting results in studies of antioxidants and life span remain unclear. However, the overall correlative relationships between mitochondrial pro-oxidant generation, decreased energy production and aging are well established.

4. MOLECULAR MECHANISMS IN RELATION TO SARCOPENIA

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As well as the IGF-I gene providing the main anabolic resource for muscle maintenance, it is also apparently involved in the activation of muscle satellite (stem) cells. Following exercise or mechanical damage of muscle the IGF-I gene is first spliced to produce a pulse of a splice variant called MGF (mechano growth factor). In addition to the IGF-I receptor binding domain, the MGF splice variant has a unique C-terminal peptide and one of its function is to replenish the muscle satellite (stem) cell pool. Decreased satellite cell number has been suggested as a reason for loss of muscle mass in disease and during ageing. Our studies on rodents and humans indicate that age-related muscle loss is associated with an inability to express MGF. Using primary human muscle satellite cell cultures from patients with muscle wasting diseases it was found that the yield of these cells was less than for normal muscle. However, addition of MGF peptide to these cultures increased the satellite cell percentage by even a greater amount within 48 hours than was the case for normal muscle. This indicates that the deficiency is not in the satellite cells per se but in the ability to express MGF. This was again confirmed in elderly human subjects but it was also found that administration of hGH significantly increased the expression of MGF and the muscle Xs area but only in combination with exercise training. It seems therefore that sarcopenia may result from a combination of GH deficiency, inability to produce MGF and the lower activity levels of elderly people. Financial support was from the Novartis Alliance programme, the Wellcome Trust, Motor Neurone Disease Association, MRC and an EU Framework grant.

5. Abstract not available at time of printing.
6. Abstract not available at time of printing.

(N)
7. EFFECT OF PROTEIN INTAKE ON CHANGE IN MUSCLE STRENGTH IN OLDER PERSONS: DOES INFLAMMATION MATTER?
Benedetta Bartali (P), Edward Frongillo, Fulvio Lauretani, Stefania Bandinelli, Stefano Volpato, Jack Guralnik, Luigi Ferrucci
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The preventive role of protein intake on the decline in physical function in the older population is still unclear. We assessed the association between protein intake and change in muscle strength in 596 persons, aged 65 years or older, participating in both baseline and 3-year follow-up of InCHIANTI, an epidemiological study on risk factors contributing to the decline of mobility in late life. Since systemic inflammation has been associated with reduced physical function, we also examined whether a synergistic effect exists between protein intake and C-Reactive Protein (CRP) on change in muscle strength. Dietary intake was estimated by a validated food frequency questionnaire, and muscle strength was measured with a hand-held dynamometer. After adjustment for age, sex, energy intake and muscle strength at baseline, the main effect of protein intake on change in muscle strength was not significant ($p= 0.959$), but we found a significant interaction ($p<0.05$) between protein intake and CRP on change in muscle strength. Selectively in persons with high levels of CRP, lower protein intake was associated with a greater decline in muscle strength. This study may help the development of strategies aimed at preventing the decline of physical function in older persons living in the community.

8. TOURNIQUET-INDUCED ISCHEMIA/REPERFUSION DAMAGE IN AGING SKELETAL MUSCLE
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As people age there is an increase in the number orthopedic surgeries requiring tourniquet (TK) application.The attenuation of blood flow for 2 or more hours, followed by a rapid increase in blood flow upon release of the tourniquet I/R, is characterized by edema, loss of vascular integrity and a massive inflammatory response, which induces localization and activation of neutrophils and macrophages, with resulting degradation of damaged tissue. The regeneration of muscle fibers includes proliferation and differentiation of satellite cells leading to fusion of myotubes to form multinucleated muscle fibers. Our data show that muscle IGF-I is increased 40-fold at 7 days of recovery from a 2 hour tourniquet-induced I/R, but that aged musculature has an attenuated response. Subjects were adult, 3-4 mo. and 23-24 mo. old SD male rats. In vivo force production of the plantar flexors about the ankle were activated through an indwelling nerve cuff about the tibial nerve, Walters et al (1990), and was measured in a method similar to that of Ashton-Miller et al (1992). The TK was applied for two hours and the max force generated by the plantar flexor group, was measured longitudinally at 7 &14, and of recovery post-TK. The forces, as a % of the maximum force prior to tourniquet application ±SEM at day 7 & 14, were 24 ± 4% , 56 ± 5%, for 3-4 mo. rats and was 16.4 ± 5% and 41.2±6%. This slower recovery of force was correlated with lower percent areas of the regenerating muscle having viable muscle cells. IGF-I receptor activated response was attenuated in the aged musculature. These data demonstrate that there is either a greater induction of damage and/or a reduced rate of recovery from ischemia/reperfusion injury. This study was funded by DoD grant to RPF.

9. AMYLOID-BETA AND ALZHEIMER'S DISEASE -CENTER RING OR SIDE SHOW?
Benjamin Wolozin[1] (P) and Mark A. Smith[2m] (P)
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Two major investigators in the field of Alzheimer disease (AD), Drs. Benjamin Wolozin and Mark Smith, will debate whether amyloid-β (Aβ) in AD is center ring or side-show regarding the pathophysiology of this disease.

According to Dr. Wolozin, the amyloid cascade hypothesis is built on three pillars of research: 1) Genetic data indicating linking familial AD with proteins required for the processing of amyloid precursor protein (APP) and generation of Aβ; 2) Cellular and physiological data showing that Aβ is harmful to neurons both in cell culture and in transgenic mice; and 3) Pathological data linking Aβ with AD. The amyloid cascade hypothesis convincingly explains the etiological and pathophysiology of early onset AD. Much of the debate in the field centers around the pathophysiology of late onset AD, where amyloid accumulates but the slow development of the disease suggests that the factors driving the illness are weaker than those driving early onset AD and more likely to be multi-factorial. Despite these questions, evidence still suggests that Aβ plays a pivotal role in the pathophysiology of late onset AD. The pathology of the disease is very similar to that of early onset AD. The most significant genetic associations, APP, ubiquilin, insulin degrading enzyme, β-secretase, appear to revolve around Aβ accumulation. The anti-amyloid vaccine, which had to be stopped because of side effects in an early clinical trail, appears more recently to have helped many patients. Ultimately, the only way of testing the amyloid cascade hypothesis is to determine whether therapies that reduce Aβ are therapeutically beneficial. The absence of such clinical trials is not the fault of the amyloid cascade hypothesis but reflects difficulties in pharmaceutical development that are largely unrelated to the amyloid cascade hypothesis. However, medications such as γ-secretase inhibitors and second generation vaccines are moving closer clinical trials and will ultimately allow the amyloid cascade hypothesis to be tested in late onset AD.

According to Dr. Smith, the relationship between Aβ and AD may finally have hit the "irreconcilable differences" stage. Things have been rocky ever since revelations of a spatial, temporal and pathologic separation between the parties such as: 1) the weak relation between Aβ and disease state; 2) the very high Aβ loads often seen in cognitively-intact aged individuals; and 3) that transgenic animal models with supraphysiological Aβ levels show little/no neuronal loss. Now, perhaps the most damning evidence to date: mutations known to cause AD actually REDUCE the overall levels of Aβ. While these findings are at odds with the Amyloid Hypothesis, they are consistent with an Alternate Amyloid Hypothesis (Lee et al., 2006) whereby Aβ serves as a protective molecule produced in response to oxidative, or other, insult.

Is Aβ center ring or side show or is there room for compromise? Maybe Aβ is etiologic in early-onset familial AD but is only part of the degenerative pathway in late onset AD where other factors, such as oxidative stress and cardiovascular disease, initiate the disease, but then Aβ joins the cascade of events.

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10. OSTEOPOROSIS: IN SEARCH OF AN UNDERLYING MECHANISM

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The demographics of aging suggest that unless drastic measures are taken to prevent the development of osteoporosis, the incidence and the costs associated with treating osteoporosis will climb in the coming decades, posing a major socio-economic burden. Consequently, there is an urgent need for suitable preventative strategies. While the identification of genetic determinants of osteoporotic fracture risk is an active area of current research, progress has been slow because of inconsistent associations between candidate genes (or genetic loci) and various bone phenotypes. In addition to possible gene-gene interactions, it is likely that there are interactions between osteoporosis genes and environmental factors, such as nutrients and food composition, which could modify expression of bone-related traits. While these potential interactions add to the complexity of the genetic influences on bone health, the study of genetic variations alone without taking into consideration their interaction with nutrients may do little to advance prevention and treatment of osteoporosis. As opposed to genotype, diet and nutrition can be modified to optimize an individual's bone health. Our group has recently identified a gene (*Alox15*) affecting skeletal development in mice that may have relevance to human osteoporosis. Indeed, genetic variation in *ALOX12* (a human homolog of the mouse *Alox15*) has recently been

reported to account for nearly 3% of the natural variation in peak BMD – the largest genetic effect on this trait thus far identified in normal human populations. The *Alox15* gene encodes 12/15-lipoxygenase (12/15-LO), an enzyme that converts essential polyunsaturated fatty acids (PUFAs) into eicosanoids - lipid mediators with wide ranging influence on inflammation, vasomotor activity, and nuclear transcription. Dietary studies indicate that PUFAs may play a role in osteoporosis risk. In particular, a diet rich in w-3 PUFAs is protective against osteoporosis. Studies of other multifactorial diseases (*e.g.,* atherosclerosis, cancer) have begun to indicate the presence of diet-phenotype-genotype interactions. Our findings suggest the possibility of a targeted dietary approach to osteoporosis prevention based on genotype.

11. OSTEOPOROSIS: LIFE CHOICES FOR PREVENTION

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Prevention of diseases with no cure is an optimal strategy for reducing health care costs. Modifiable factors for increasing bone mass during growth and decreasing bone loss during aging are largely related to diet and physical activity. Adequate dietary calcium and vitamin D are the most studied nutrients related to bone health and are the most often prescribed supplements. Dietary calcium acts both as a nutrient and a suppressor of bone resorption, which may be more important. Vitamin D enhances calcium absorption, suppresses PTH, and reduces falls. Estrogen therapy for menopausal women is no longer recommended for long term bone loss suppression. Phytoestrogens are being explored for their antiresorptive effects. Insights from The Women's Health Initiative on these factors will be discussed. Recent findings of other dietary factors on bone health including salt, protein, and potassium will be discussed. Weight bearing exercise is another means of suppressing bone loss.

12. Abstract not available at time of printing.
13. Abstract not available at time of printing.
14. Abstract not available at time of printing.

15. MECHANISMS AND CONSEQUENCES OF AGE-ASSOCIATED CHANGES IN THE SKIN BARRIER

Peter Elias (P)

Much attention has focused on structural and biochemical changes in the dermis that accompany cutaneous chronologic and photoaging. In contrast, relatively little research is available on aging-related epidermal alterations, perhaps because both epidermal structure and function appear only minimally altered. Yet, when stressed by external insults, such as tape stripping or solvent-treatment, permeability barrier kinetics are remarkably delayed in chronologically-aged epidermis (greater than 75 years), with a further, 20% delay in photoaged skin sites from the same individuals. In addition, the tape stripping experiments revealed that aged humans display a profound abnormality in stratum corneum (SC) cohesion (the amount of protein removed per stripping).

We next assessed the basis for these abnormalities in aged mice, which exhibit comparable age-related declines in barrier function and SC cohesion, beginning at about 15 months. In these moderately-aged mice, epidermal lipid synthesis remains normal, but lipid-processing, a pH-dependent function, declines in 15-18-month old mice, resulting in immature extracelllular membrane structures, accounting for a relatively
minor abnormality in barrier homeostasis. The abnormality in acidification, in turn, could be shown to be due to reduced expression of the epidermal sodium-proton exchange mechanism (NHE1), as well as an age-associated decline the expression of select isoforms of secretory phospholipase A2, which also regulate acidification of the stratum corneum (SC). As a result of inadequate acidification, the activities of two key, pH-dependent lipid processing enzymes (i.e., beta-glucocerebrosidase and acidic sphingomyelinase) are reduced. Both the abnormalities in SC acidification and function could be normalized when moderately-aged mice were treated with topical 'superacids'.

With more advanced aging (20-24 month old mice), the processing abnormality recedes in importance, as it is superceded by a global decline in epidermal lipid synthesis, resulting in a paucity of extracellular lamellar membranes, accounting in turn for the more severe abnormality in barrier homeostasis that accompanies more advanced aging. the principal lipid biochemical

abnormality is a decline in cholesterol synthesis. Accordingly, triple-lipid mixtures of the three key SC lipids (ceramides, cholesterol, and free fatty acids), if formulated with cholesterol as the dominant species (3:1:1 molar ratio), normalize barrier function not only in aged mice, but also in aged humans.
Together, these studies demonstrate a progression of developmental abnormalities that accompany epidermal aging, and point to potential therapeutic approaches that could be deployed to benefit moderately- and extremely-aged skin.

16. AGE AND THE ADAPTIVE IMMUNE SYSTEM

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Morbidity and mortality from viral infection increase after the age of 50 years, documenting age-dependent immune incompetence. Changes in the adaptive and in particular the T cell system appear to contribute to immunosenescence through several mechanisms. Thymic activity logarithmically declines with age and essentially seizes between the age of 40 and 50 years. Homeostatic proliferation of naïve T cells is efficient and apparently unbiased to maintain a naïve T cell compartment with high T cell receptor diversity for 20 more years in the majority of individuals. In the absence of significant influx of newly generated T cells, diversity of the T-cell compartment sooner or later contracts. Fewer T-cell specificities fill the T-cell compartment, which leads to increased oligoclonality and increased clonal sizes. Our studies show that the diversity in the naive and in the memory CD4 T cell compartments is well maintained between the ages of 25 and 65 years despite the rapid decline in thymic function. However, between age 65 and 75 the repertoire rapidly contracts by more than 99%, virtually eliminating the ability to generate T cell responses to novel antigens. Up to the age of 70, naïve human CD4 T cells (contrary to T cells from old mice) are functional and respond quite well to antigenic stimulation. The kinetics of the subsequent clonal expansion - a critical determinant of the antiviral response – is influenced by the avidity of the initial stimulatory interaction, which may be low if the T cell receptor repertoire is contracted or if antigen-presenting functions are impaired and by the degree of telomeric erosion. Telomeres progressively shorten with age; healthy individuals lose approximately 75 base pairs per year corresponding to 1 to 2 cell divisions. Telomeric erosion involves naïve and memory T cells. Functional changes in the memory T cell population in the aging individual are more evident than in naïve T cells. Defective transcription of the costimulatory molecule CD28 is a well established finding, compromising the interaction of T cells and antigen-presenting cells. The frequency of $CD8^{+}CD28^{null}$ T cells is a good biomarker for impaired vaccine responses, even after correction for age. Gene expression profiling of memory T cells yielded a number of additional cell surface molecules that differ between young and old individual in expression including CD57, HLA-DR, CD26, negative regulatory receptors and the chemokine receptor, which dramatically changes the response pattern of aged memory T cells to antigenic stimulation and environmental cues.

17. THE EFFECT OF AGE ON THE COGNATE FUNCTION OF CD4 T CELLS

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With increasing age, the ability to produce protective antibodies in response to immunization declines, resulting in reduced efficacy of vaccination. We have examined how reductions in CD4 T cell function contribute to reduced humoral responses, using a model which allows us to compare identical numbers of antigen-specific naive T cells from young and aged TCR transgenic mice. Naive cells from aged mice exhibit reduced responses, both in vitro and in vivo. In vitro, responses of aged T cells can be enhanced by addition of IL-2. In vivo, using an adoptive transfer model with young hosts, naive cells from aged mice exhibit significant reductions in cognate helper function, leading to reduced B cell expansion and differentiation. These age-related defects could be overcome by prior in vitro effector generation of the aged T cells. This improvement in cognate function of the aged effectors may be related to the enhancement of CD154 expression, which occurs on aged T cells in the presence of exogenous IL-2. We also found no difference in B cell expansion and differentiation when young cells were transferred to

young or aged hosts. Our results indicate that age-related reductions in humoral responses are mainly due to defects in the cognate helper function of naive CD4 T cells from aged individuals.

18. INFLUENZA VACCINATION: T-CELL RESPONSES TRANSLATE TO HEALTH OUTCOMES IN OLDER ADULTS

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Influenza is a serious illness and probably the single most important cause of excess disability and mortality during the winter months. Influenza vaccination programs are cost saving due to the reductions in hospitalisations for pneumonia, exacerbations of heart failure and, surprisingly, heart attacks and strokes. However, rising hospitalisation and death rates due to influenza illness in older adults in spite of widespread vaccination programs call for breakthrough strategies in vaccine development. A major challenge to vaccine development is that the current standard using antibody titers may be limited as a sole measure of vaccine efficacy in the older adult population. Measures of the cell-mediated immune response to influenza vaccination in ex vivo mononuclear cell cultures can be used as an alternate measure of vaccine efficacy and may shed light on the poorly understood mechanism of loss of immunity to influenza and poor vaccine responsiveness with aging.

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19. VITAMIN E (E) SUPPLEMENTATION REVERSES THE AGE ASSOICATED DECLINE IN PHOSPHORYLATION OF THE ADAPTOR PROTEIN LAT IN CD4+ T CELLS OF OLD MICE

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T cell proliferation and interleukin (IL-2) production declines with age. Engagement of the T cell receptor (TCR) by antigen (Ag), known as the immune synapse (IS), in coordination with phosphorylation of key signaling proteins, leads to increased IL-2 synthesis and T cell proliferation. Defects in effective IS formation have been reported with age. We have shown that vitamin E supplementation improves IL-2 secretion and cell proliferation of T cells from old mice. To determine the underlying mechanisms we evaluated the effect of E on IS formation, and the total and phosphorylated expression of key signaling proteins LAT, Lck, and Zap-70. Purified spleen CD4+ T cells from young (4 mo) and old (24 mo) C57BL/6 mice were incubated with E (46mM RRR-a-tocopherol) for 4 hrs and stimulated with anti-CD3/anti-CD28. Effective IS was detected by confocal microscopy and the levels of total and phosphorylated LAT, Lck, and Zap-70 were analyzed by western blot. E supplementation of old T cells significantly increased effective IS formation ($p<0.05$). Further, LAT phosphorylation, but not total expression, was selectively and significantly reduced in the T cells of old mice (2 fold reduction, $p<0.05$). E significantly enhanced LAT phosphorylation in T cells from old mice (1.7 fold increase, $p<0.05$), but had no effect on that of T cells from young mice. This indicates that aging hinders synchronized IS formation and LAT phosphorylation, impairing T cell signaling, IL-2 production, and proliferation. E supplementation restores IS and LAT phosphorylation, leading to improved T cell proliferation and IL-2 production in aged mice. This is the first demonstration of a nutrient-induced reversal of a defective early signaling event in aged T cells. These findings may have significant implications for understanding the underlying mechanisms and development of strategies to prevent age-related T cell dysfunction. Supported by NIA #R01AG009140-10A1, USDA #58-1950-9-001 and Unilever Health Institute fellowship.

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20. REGULATION OF T-CELL RESPONSES TO ACUTE AND CHRONIC VIRUSES IN OLD RODENTS

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T-cell immunity undergoes profound changes in senescence, including, but not limited to, decline in naive T-cell production, increased compensatory proliferation of the remaining naive T-cells, consumption of naive T-cells by repeated and/or persistent pathogenic assaults, and decreased

efficacy of T-cell signaling. We investigated the interplay of acute or chronic viral infection and regulation of homeostasis and immunological memory in old mice. We provide evidence for antigen-independent disturbances in T-cell homeostasis and repertoire that can lead to increased vulnerability to pathogens. Moreover, we show that chronic, but not acute, viral infections directly contribute to additional disturbances in T-cell homeostasis and memory maintenance. Relative importance of antivirals and of restorative T-cell intervention is discussed in light of these results.

21. THE USE OF NIR SPECTOROSCOPY AND IMAGING TO MONITOR FOREBRAIN FUNCTIONAL ACTIVITY

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In order to monitor brain functional activity in a simple and effective manner that does not restrict the subject as do MRI and PET, we have developed an imager pad that can be applied to the PFC and from which NIR optical signals of hemodynamic changes are readily obtained. Specifically, the device has four dual wavelength LED light sources and 16 silicon diode detectors which cover the PFC from nose bridge to hairline and from ear to ear. The system measures hemodynamic changes in response to physiological stresses of functional activity, particularly increased blood volume (analogous to BOLD) and decreased saturation of hemoglobin due to oxygen extraction in neuronal activity. The device can be encased in a plastic envelope so that no contact is made, is readily fastened to the head with a headband, and electronically connected to a laptop computer which calibrates the device and monitors changes in response to functional activation of the prefrontal cortex.

We have used this device over several years to measure skills in anagram solutions where an easy anagram is replaced by a difficult anagram (difficulty matching ability) and back to an easy anagram so that displays of the blood volume and deoxygenation responses due to the transition from easy to difficult problem solving are readily measured and imaged as follows. The signals are converted appropriately to the two metrics of blood volume and tissue oxygenation and are accumulated as histograms from which ρ and σ are obtained and their quotient is compared in the 16 voxels across the forehead giving a "brain print." This image can be followed as a function of time for young and old brain responses. In order to accumulate statistically significant data on brain functional activation, it is appropriate to have at least 4-5 sessions of data recording in which at least 20 anagram tests are applied.

Many other types of tests have been used, for example Stroop test, N-back, etc. as are commonly used in NMR studies. Most interesting is the response of the individual to the stress of prevarication where guilty knowledge is denied to an interviewer and significant localized increases of blood volume are imaged.

It is of great interest to learn how these tests, currently applied to young populations, will be handled by an elderly population, particularly those who may have early losses of cognitive function. The talk will explain how the device can be used for effective monitoring of cognitive function of the elderly.

22. THE MODIFICATION OF IL-7 AND THE REVERSAL OF THYMIC ATROPHY IN AGED MICE

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The role of IL-7 during thymopoiesis has led to it being the focus of a number of therapeutic interventions. However, its small size and pleiotropic nature present problems for thymus-directed therapies. We have created a fusion molecule between the extra-cellular N-terminal domain of CCR9 and IL-7, which has the potential to overcome these difficulties. This novel fusion protein retains the thymopoietic activity of IL-7 and the ligand-binding ability of CCR9. As a thymopoietic agent, compared with IL-7, it shows an enhanced retention in the thymus, increased de novo T cell production, and increased thymic output. Old mice receiving the fusion protein show improved CD8 T cell responses and reduced viral load after infection with influenza virus compared with those receiving IL-7. This chimeric molecule offers a novel therapeutic strategy that may result in the production of an effective immunorestorative agent.

23. FUNCTIONAL EFFECT OF FAILURE OF ADAPTIVE RESPONSES IN SKELETAL MUSCLE DURING AGING

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A significant loss of skeletal muscle mass after the age of 50 is a major factor in frailty of the elderly. In addition to the loss of muscle mass, the remaining muscle is weaker, more susceptible to damage and recovers poorly if damage occurs. The mechanisms responsible for this age-related functional deficit are unclear. In muscles of adults, Reactive Oxygen Species (ROS) production by skeletal muscle increases significantly during certain forms of exercise. Evidence that this increase in ROS mediates damage is sparse, but it is now evident that ROS interact with cell signalling pathways to modulate adaptive changes in gene expression. The initial adaptive responses that occur in skeletal muscle are in the antioxidant defence enzymes and the stress or Heat Shock Proteins (HSPs). Increased content of these proteins in muscles of adult mice has been associated with significant protection against subsequent cellular damage. The mechanism by which changes in ROS result in upregulation of defence systems has been characterised. Nuclear Factor κB (NF-κB) and activator protein-1 (AP1) transcription factors are involved in the upregulation of antioxidant enzymes such as SOD and catalase in response to oxidative stress and HSP expression in response to acute stress in eukaryotic cells is primarily regulated by the transcription factor, Heat Shock Factor 1. Studies have shown that the activation of these transcription factors is dependant on the acute increased production of ROS and/or the presence of oxidized or unfolded cellular proteins. The ability of muscles from old mammals to respond to exercise stress by an increased content of HSPs and an increase in the activity of antioxidant defence enzymes is severely attenuated, primarily due to the lack of activation of the appropriate transcription factors. This age-related inability to produce HSPs plays a critical role in the development of age-related functional deficits in skeletal muscle. Studies using transgenic mice, over-expressing HSP70 in skeletal muscle demonstrated that lifelong increased muscle content of this protein provided protection against the fall in specific force associated with ageing and facilitated rapid and successful regeneration following contraction-induced damage in muscles of old HSP70 transgenic mice, compared with significant deficits in muscles of old wild type mice (McArdle *et al*, 2004). The hypothesis that a chronic increase in the generation of ROS plays a key role in the inability to respond to stress and that this is related to development of age-related muscle dysfunction has received considerable attention. Work from our laboratory and others has provided evidence of an accumulation of oxidative damage in skeletal muscle of old rodents (Broome *et al*, 2006) and further data suggests that lifelong HSP70 overexpression results in prevention of this accumulation of oxidative damage and preserves the ability to activate NFkB and AP-1 following contractile activity Broome et al, 2006), providing a link between accumulation of oxidative damage and muscle dysfunction.

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24. RECONSTRUCTION OF YOUNG AND AGED DERMIS IN CULTURE

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The biology underlying the aging process is only poorly understood. Whilst aging affects both cells and the extracellular environment, little is known about how one affects the other. Therefore,

we have developed a culture model of tissue aging that permits examination of the crosstalk between cells and the extracellular matrix in which they reside. To reconstruct young and aged dermis we trypsin-purified fibrous dermal collagen (FDC) from young or aged rat skin, which provides an acellular matrix. FDC retains the anatomical organisation of native dermal collagen. Moreover, aged FDC retains hallmarks of the native aged dermis from which it is derived. Young or aged FDC was seeded with pre-senescent or senescent dermal fibroblasts. FDC supported the attachment and migration of cells, regardless of the age of the donor skin. Cells migrated more readily through an aged matrix than a young matrix and, overall, senescent cells migrated more efficiently than pre-senescent cells. Significantly, culture on aged FDC caused an increase in matrix metalloproteinase 2 activity in both pre-senescent and senescent cells though the highest increase in level of activity was found when pre-senescent cells were cultured on aged FDC. We show that the aged extracellular matrix is capable of providing signals to young, growth competent cells causing them to upregulate activities associated with matrix degradation and remodelling. Such inappropriate behaviour by young cells may contribute to the imbalance of tissue homeostasis encountered during aging.

25. Abstract not available at time of printing.

26. UNDERSTANDING SOFT TISSUE AGING - THE EFFECT OF CYCLICAL LOADING

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Soft tissue injuries incur a large cost to the health industry, estimated at 30 billion$/year in the USA. The incidence of tendon injury in both man and the equine athlete increases with increasing exercise and age. In the racing thoroughbred, the prevalence of superficial digital flexor tendinopathy can be as high as 43%, while the incidence of Achilles injury in humans has doubled in the past 10 years, especially in the older generation. Increasing longevity and participation in sport are therefore both risk factors in humans, and exercise studies post skeletal maturity in the horse suggest that age-related tissue changes and cyclical loading play important roles in the dysregulation of tendon homeostasis in both species. However, the mechanism(s) by which these risk factors lead to tendon injury and failure are poorly understood. Whether such failure is due to mechanical failure by 'wear-and-tear' or involves more complex biological pathways needs to be resolved if viable strategies for reducing the incidence of tendinopathies are to be identified. This paper will review our experimental evidence to explain the mechanisms behind the cumulative fatigue damage without apparent functional adaptation or repair that appears to occur in tendons post skeletal maturity:

1) Failure of adaptation

Three separate but concurrent mechanisms are implicated – (a) a reduced responsiveness of tenocytes to stimuli - tendon cells recovered from old superficial digital flexor tendon (SDFT) show an ~50% reduction in synthetic response *in vitro* to both exogenous growth factors and mechanical load when compared to tenocytes recovered from young superficial digital flexor tendons; (b) reduced levels of anabolic growth factors - high levels of TGF-β are found throughout the fascicles in young SDFT, while there is decline in TGF-β levels and a change in distribution after skeletal maturity; and (c) reduced intercellular communication to co-ordinate a functional synthetic response - there is marked reduction in the number of gap junctions in old tendon.

2) Mechanisms of degeneration

We have hypothesised that structural changes that predispose the tendon to failure are induced by a biologically active process in response to cyclical loading. In addition, we hypothesise that this cumulative 'damage' results from fragmented matrix protein-mediated induction of matrix metalloproteinase (MMP) expression. We have therefore examined the effect of cyclical loading on equine SDFT explants *in vitro* and measured changes in material properties, matrix protein release, and MMP expression. Our results show that the ultimate tensile strength (UTS) was significantly reduced following 24h of cyclical strain in explants from all ages (by 39.7% ±12%, p=0.0055, n=20). In a more detailed analysis, we also found that the magnitude of the strain-induced reduction in UTS was less pronounced in immature and young mature animals (1-3 yrs, 34%; 4-10 yrs, 35% respectively) than in old animals (11-25 yrs, 54%), demonstrating the increased susceptibility of aged tissue to degeneration. In contrast, in experiments in which cells were killed by freeze-thaw or by culture in 0.2% sodium azide prior to strain, strain application failed to significantly modify the UTS when compared to dead or live non-stretched explants (13%, p=0.128 and 3.8%, p=0.522,

respectively). This confirms that the load-induced loss of mechanical strength was dependent upon the presence of live cells in tendon explants. Using gelatin zymography to assess MMP activity, tissue extracts from cyclically loaded explants were found to express 2-3-fold more active MMP-2 compared to non-strained controls. A proportion of this active enzyme was from the latent pool of the proenzyme, suggesting MMP activity was up-regulated in part via the latent pool and in part from new transcription. To test whether the load-related reduction in UTS was related to this increased MMP activity, we examined load-induced changes in UTS in the presence of a pan-specific MMP inhibitor, Ilomastat. This showed that the load-induced decrease in UTS was reduced to insignificant levels (12% ±3% reduction, p=0.166 compared to unloaded controls) confirming that MMPs had a pivotal role in this process.

These findings indicate that exposure of the equine SDFT to prolonged cyclical loading of physiological magnitude can lead to a decrease in tensile strength. Our results also suggest that this process is an active one, which fails when the sparse cells in the tendon matrix are rendered non-viable. The inhibition of MMP activity during cyclical loading restricted, at least partially, this load-induced tendon failure, which, together with our results indicating a load-induced up-regulation and activation of MMPs, suggest that normal mechanical usage contributes to the loss of functional competence via a proteolytic disruption of the extracellular matrix. This cleaved matrix can contribute to further damage via the action of protein fragments on cells. This hypothesis has been supported by separate experiments on unstrained tendon explants treated with a 40kDa fragment of fibronectin, which induced the loss of a specific component of tendon matrix, cartilage oligomeric matrix protein (COMP). These findings are consistent with our *in vivo* studies demonstrating a deleterious effect of exercise on tendon matrix in mature horses and support our hypothesis that tendon failure is exacerbated by cyclical loading which may explain, in part at least, the increasing incidence of tendinopathy with ageing in both horses and humans.

27. INCLUSIVE DESIGN TOOL BASED ON PSYCHOLOGICAL AND BIOMECHANICAL FUNCTIONAL PERFORMANCE

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The inclusive design movement aims to address the disabling effects of poorly designed products by setting out to design goods that can be used by all. For many elderly people declining strength, flexibility and balance interact to limit their capacity to use many every day products and services. However, while large anthropometric databases provide invaluable aid to designers there is almost no accessible information on the functional capacity of older adults. Without access to this type of data, inclusive design can only have limited success. In this study we have investigated age related changes in the performance of a range of movement tasks for integration into a computer aided design tool for use in inclusive design. Measurements were taken of the biomechanical requirements of older adults during numerous activities of daily living. Supplementary measures of maximal muscular strength, range of motion, together with several psychological parameters were also gathered. This has resulted in the formation of a large database which can provide designers with a wealth of information on the control and regulation of actions and the psychological factors which influence this. Selected data from these measurements are being integrated into the new software package in the form of animations of a human model. These animations provide a highly visualised method for studying how virtual products challenge the functional capacity of different age groups and thereby can assist designers in testing the functionality of new products and devices. The research has also produced detailed psychological profiles on how older adults deal with novelty, learn new tasks and how they think and feel about their movement capabilities: factors which need to be taken into account when designing products for all.

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28. ADIPOCYTES, INFLAMMATION AND THE METABOLIC SYNDROME IN AGING

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Aging is typically associated with increased visceral fat (VF), which is a risk for insulin resistance, diabetes, and arteriosclerosis disease. We examined a cause-effect relationship between VF and

insulin resistance by surgical extraction of VF in aging rats 3-12 mo before studying the animals. We demonstrated that surgical removal of fat significantly improved peripheral and hepatic insulin action similar to the effect obtained by CR. When VF was removed from a pre-diabetic Zucker rats it prevented the appearance of diabetes. VF is biologically distinct from other adipose depot, in particular in the expression of inflammatory cytokines. Finally rats whose fat is removed live significantly longer than rats that are fed ad libitum.

To see if insulin action/metabolic syndrome is associated also with human's longevity we determined the levels of the anti-inflammatory, insulin sensitizer adiponectin in unrelated subjects between ages 65-105 (>200 over 95). Adiponectin levels increase with age by 2 folds and are very high after age 95. This effect is independent of gender and BMI. To test if this may be associated with a genotype that is over-represented with exceptional longevity we tested several genotypes in the gen. The prevalence of homozygosity for the *ADIPOQ* +2019 deletion (del/del), but not other ADIPOQ variants, increased from 12% at age 65 to 31% at age 105, (p=0.35, and p=0.05 respectively) and was associated with significantly higher serum ADIPOQ levels, independent of BMI (p=0.01). Subjects with these genotype were also protected from the metabolic syndrome of aging.

These studies suggests that fat and its peptides have a determinant role in aging and longevity

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31. SIRT1 REGULATES INSULIN SECRETION BY REPRESSING UCP2 IN PANCREATIC BETA-CELLS

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The silent information regulator (Sir) protein Sirt1 is a NAD-dependent deacetylase that connects protein modification, caloric intake, and lifespan. If mammalian Sirt1 links dietary conditions to metabolic changes, for example during calorie restriction (CR), then this gene might regulate processes impacted by CR like insulin secretion.

In this study we showed that Sirt1 positively regulates insulin secretion in pancreatic beta-cells. Sirt1 represses the uncoupling protein gene UCP2 by binding directly to the UCP2 promoter. In beta-cell lines, in which Sirt1 is reduced by shRNA, UCP2 levels are elevated and insulin secretion is blunted. The up-regulation of UCP2 is associated with a failure of cells to increase ATP levels after glucose stimulation. Knockdown of UCP2 restores the ability to secrete insulin in cells with reduced Sirt1, showing that UCP2 causes the defect in glucose-stimulated insulin secretion. Food deprivation decreases NAD levels in the pancreas, thereby inhibiting Sirt1 and up-regulating UCP2. This regulation of UCP2 by starvation does not occur in Sirt1 knockout mice, which display constitutively high UCP2 expression. Our findings suggest that Sirt1 regulates UCP2 to coordinate the level of insulin secretion to the diet.

32. AGING UP-REGULATES EXPRESSION OF INFLAMMATORY MEDIATORS IN MOUSE ADIPOSE TISSUE

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Obesity is a leading cause in development of type 2 diabetes (T2D). Aging is associated with increase in T2D incidence, which is not totally explained by the much lower prevalence of obesity in the elderly. Low-grade inflammation in adipose tissue (AT) is implicated in development of insulin resistance and T2D. We hypothesized that inflammatory response is upregulated in AT as a result of aging, independent of obesity, and may contribute to age-related insulin resistance. We conducted a study to determine if inflammatory responses are up-regulated with age in AT.

Results showed that visceral AT from old mice have significantly higher mRNA expression of IL-1beta, IL-6, TNF-alpha, and COX-2 (263, 208, 165, and 115% higher, respectively) and lower (60%) PPAR-gamma, a nuclear receptor with anti-inflammatory and insulin-sensitizing property, than those of young. In determining the relative contribution of different components of AT to these age-related changes, we further showed that adipocytes (AD), and not stromal vascular cells including macrophages are the cells responsible for this increased inflammatory response of adipose tissue with aging, which is thus distinguished from obesity-related AT inflammation in which macrophages are the main contributor. Macrophages of either age (young or old) produced more IL-6 in response to old AD-conditioned medium (CM) compared to young CM. This suggests that in addition to producing more IL-6, AD from old mice can induce more IL-6 by other cells such as macrophages. Addition of ceramide or sphingomyelinase increased IL-6 production in young AD to a level comparable to that seen in old AD. Inhibiting sphingomyelinase, ceramide synthesis, or NF-kB activation reduced IL-6 production by AD. NF-kB regulates expression of several inflammatory products and ceramide was shown to induce NF-kB activation. Thus, these data suggest a potential role for ceramide and NF-kB in the age-related increase of AT inflammation. Further study is needed to determine the underlying mechanisms of the observed effects and their contribution to T2D in the aged. This work was supported by USDA #58-1950-9-001 and NIA #R01 AG009140-10A1.

33. Abstract not available at time of printing.

34. RELATIONSHIPS AMONG SIGNALING PROTEINS, AGE, AND MEMORY

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Recent evidence shows that non-pathological memory impairment among aged subjects is related to levels and activity of Ca2+-dependent signaling proteins in the hippocampus, a brain structure involved in memory formation. Among many examples, we reported that spatial learning is related to the levels of Ca2+/phopholipid-dependent protein kinases (PKC) among individual subjects and that these relationships differ according to age, isoform, and subcellular fraction. Moreover, metabotropic glutamate receptor-mediated hippocampal phosphoinositide turnover is blunted in aged rats with spatial learning impairments. The decrement in mGluR-mediated signal transduction in the hippocampus that is related to cognitive impairment in aging may be attributable, at least in part, to a deficiency in the enzyme PLCβ-1. Calcium signaling integrates several neuronal functions including modulation of ion channels, neurotransmitter release, and gene expression. Members of the cAMP response element-binding protein (CREB) family of transcription factors interact with Ca2+-dependent signaling proteins to mediate the transition from short-term to long-term memory. In a recent study, levels of CREB protein were measured in the hippocampi of individual 6- and 24-month-old rats trained on a place-learning task. Overall, CREB protein was significantly lower in aged rats in comparison with young rats. Aposteriori analysis showed that this difference was due to a significant decrease in CREB levels among aged rats with memory impairment, whereas aged rats without memory impairment had CREB levels comparable to young rats. These results suggest that alterations of the CREB protein may contribute to age-related memory deficits. Current studies are underway to test the hypotheses that use of HSV vectors in vivo to manipulate levels of CREB can (a) prevent the onset of age-related memory impairments and (b) rescue memory among aged rats with memory impairments. Supported by NSF IBN-0133734 to PJC.

35. THERAPEUTIC TARGETS IN ALZHEIMER DISEASE: SIGNAL TRANSDUCTION MECHANISMS

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There are multiple lines of evidence showing that oxidative stress and aberrant mitogenic alterations play important roles in the pathogenesis of Alzheimer disease. However, while both oxidative stress and cell cycle-related abnormalities are early events, occurring prior to any cytopathology, the relationship between these two events, and their role in pathophysiology was,

until recently, unclear. However, based on the study of mitogenic and oxidative stress signaling pathways in Alzheimer disease, we proposed a “two hit hypothesis” states that while either oxidative stress or abnormalities in mitotic signaling can independently serve as initiators, both processes are necessary to propagate disease pathogenesis (Zhu et al., 2004). This presentation will summarize evidence for oxidative stress and abnormal mitotic alterations in Alzheimer disease and articulate the two hit hypothesis by describing how both mechanisms are necessary and invariant features of disease. Additionally, we will discuss novel therapeutic opportunities afforded by this hypothesis.

Work in the authors’ laboratories is supported by the National Institutes of Health, the Alzheimer’s Association, and Philip Morris USA Inc. and Philip Morris International.

Reference: Zhu X, Raina AK, Perry G, Smith MA (2004) Alzheimer’s disease: the two-hit hypothesis. Lancet Neurol, 3, 219-226.

AGE POSTER ABSTRACTS

Posters are listed alphabetically, by presenter.

(P) Denotes presenter **(G)** Denotes Post-doctoral Candidate for Glenn Award
(N) Denotes Pre-doctoral Candidate for Nicolai Award

36. CALORIE RESTRICTION ENHANCES T CELL MEDIATED IMMUNE RESPONSE IN OVERWEIGHT MEN AND WOMEN.

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It is well known that dietary energy restriction prolongs lifespan and enhances immune responsiveness in a wide range of laboratory animals. However, information on the applicability of these results to humans is limited. In this study we examined the effects of calorie restriction on T cell mediated function in humans. Forty-six overweight (BMI= 27.9+1.5 kg/m2) men and women aged 35 to 40 years (35+5) were randomly assigned to a 30% or 10% (control) calorie restricted groups. Delayed type hypersensitivity skin test (DTH), lymphocyte proliferation, prostaglandin E2 and cytokine productions were determined at baseline after six months of calorie restriction. DTH responses, as well as Con A and PHA stimulated lymphocyte proliferation, were significantly increased in both calorie restricted groups compared to baseline ($p<0.05$). However, proliferative response to anti-CD3 was increased significantly only in the 30% calorie group. LPS stimulated PGE2 production was reduced in both groups, but reached statistical significance only in the 30% calorie restricted group (33% decrease, $p<0.05$). These results, for the first time, show that 6 months calorie restriction in humans improves T cell mediated function. The effect of calorie restriction is, at least in part, due to decrease in PGE2 production, which has been shown to suppress T cell function. Supported by NGA-3U01-AG20478 (NIA), USDA contract #58-1950-9-001 and International Nutrition Foundation-Ellison Medical Foundation Scholarship.

(G)

37. THE ACTIVITIES OF OXIDATIVE PHOSPHORYLATION ENZYMES IN MITOCHONDRIAL DNA DEFICIENT VASCULAR ENDOTHELIAL CELLS

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Mitochondrial DNA (mt-DNA) encodes 13 subunits of oxidative phosphorylation (OX-PHOS) enzyme complexes I, III, IV and V. Mt-DNA is more vulnerable to oxidative damage than nuclear DNA and was proposed to be involved in many pathological processes including atherosclerosis and aging. Ethidium bromide (EB), a DNA interchalating agent, binds more selectively to circular DNA than linear DNA. EB is therefore used for generating mt-DNA deficient cell lines. In this study, as a model of mitochondrial DNA damage, we generated mt-DNA deficient vascular endothelial cell line from an immortal line and analyzed the activities of OX-PHOS enzymes I, II,

III, and IV. In EB (50ng/mL) treated cells, the activities of all the enzymes were found decreased compared with the controls. The activities of the control versus EB treated group were 9.65 vs 5.18, 7.82 vs 4.41, 12.62 vs 1,73, 8.49 vs 3.42nmol/min/mg respectively for complexes I, II, III, and IV. After correction with the decrease of complex II (which has no mt-DNA encoded subunits) activity, the decrease in complex III was still very significant. We propose that the changes in the mt-DNA region encoding the subunit of complex III needs further investigation for its likely role in aging.

38. BODY COMPOSITION BY THE 4C MODEL AND VALIDATION OF AIR-DISPLACEMENT PLETHYSMOGRAPHY IN MEXICAN OLDER PEOPLE

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Nowadays it is controversial if the air displacement plethysmography system (BOD POD) is a valid method to estimate the body composition in older people. Purpose: This study determines body composition by the four-compartment (4C) model and examines the validity of the air displacement plethysmography in 202 Mexican elderly men and women subjects. Methods: Body density and body fat were measured by the BOD POD, total body water by deuterium dilution, and total body bone ash by DXA. Body composition was determined using Baumgartner's equation. Results: Percent body fat by the 4C model was 31.2% and 42.5% in men and women, respectively ($p<0.001$). Group mean accuracy of body fat by BOD POD against that of the 4C model showed an effect of sex ($p<0.00001$), but not the method ($p=0.27$). Results of individual accuracy showed no significant difference with the identity line. Precision assessed by model R2 was high for all subjects and for men and women by separate. SEE was low for all and for men and women by separate. Bland and Altman analysis showed no significant bias. Conclusion: The BOD POD technique is a valid and reliable method compared to the 4C model and it could be applied in subjects with similar physical and anthropometric characteristics to subjects of this study.

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39. IN VITRO CELLULAR RESPONSES TO SERUM COLLECTED FROM HUMANS ON 3-WEEK ALTERNATE DAY FASTING OR ON 3-MONTH CALORIE RESTRICTION

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Prolonged caloric restriction (CR) has been proven to have many beneficial health effects including extension of life span, reduced morbidity, increased function and enhanced stress resistance. Some studies have suggested that other lifestyle changes including alternate-day fasting and regular exercise may result in many of the same benefits as CR. Two studies on non-obese humans were undertaken to examine the effects of 3 weeks of alternate day fasting (FEAST study) and 3 months of 25% daily calorie deficit with or without exercise (CALERIE study) on several markers of longevity. Effects on body weight, body composition, energy metabolism and skeletal muscle gene expression from the FEAST study have already been presented (Heilbronn et al., Obesity Res 13: 574-581, 2005; Heilbronn et al., Am J Clin Nutr 81: 69-73, 2005). Based on an in vitro assay of CR (de Cabo et al., Exp Gerontol 38: 631-639, 2003), we have expanded on these findings by using serum and plasma samples! from the participants of both studies to assess their effects on cultured FaO and HepG2 cells (rat and human hepatoma cells, respectively). We show changes in cell proliferation rates, cell survival after exposure to heat shock and hydrogen peroxide, and oxygen consumption. In addition, western blot analysis and real time RT-PCR will be used to assess changes in the expression of genes hypothesized to modulate CR-induced stress responses, mitochondrial biogenesis and lifespan extension including HSP70, SIRT1, and PGC-1.

40. CALORIC RESTRICTION IN HUMANS: CONSIDERATIONS FOR IMPLEMENTATION

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Humans attempting to use caloric restriction in the hope that it will delay aging in our species as it does in so many others are faced with a challenge: there is no ad-libitum-fed control group upon which to base the diet. Guidelines for ascertaining the appropriate level of restriction are thus much less obvious for humans than they are for animals in the laboratory. The actual caloric intake required by each individual varies widely depending on body mass, body composition, and activity level. In addition, humans, unlike the animal populations in which caloric restriction has been shown to provide benefit, are not a genetically homogenous group, and the genetic contribution to basal metabolic rate is thus also a concern. It is suggested here that if caloric restriction is begun in adulthood, one's own body composition may be used as a gauge for the relative level of restriction that is being experienced. Suggestions for appropriate use of this measurement, and for the implementation of such a program while maximizing physical ability and minimizing discomfort, will be provided. In addition, a brief discussion of the merits and risks of caloric restriction in the naturally lean will also be undertaken. While this author remains somewhat skeptical about the potential for caloric restriction to extend the maximum lifespan and truly slow aging in humans, it is well established that a lean lifestyle promotes health and aids greatly in achieving a vigorous and enjoyable old age, and the extension of mean lifespan in groups undertaking such a regimen seems assured.

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41. INTRAVENOUS ADMINISTRATION OF HUMAN UMBILICAL CORD BLOOD CELLS INCREASES NEUROGENESIS IN THE GRANULE CELL LAYER OF AGED RATS

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An impoverishment in the microenvironment of the aged brain has been postulated to be responsible for the age-related decrease in hippocampal neurogenesis. In particular, reduction in the availability of trophic factors in the aged hippocampus has been proposed as one of the most critical elements involved in age-related decreases in hippocampal neurogenesis. Human umbilical cord blood cells (HUCBC) are rich in mesenchymal progenitor cells, growth factors, and endothelial cell precursors. It has been shown that intravenous transplantation of HUCBC can provide therapeutic benefits including, behavioral recovery, increased angiogenesis and neurogenesis, in an animal model of stroke by delivery of neurotrophins, cytokines, and growth factors. The present study analyzed the effect of intravenously administered HUCBC on hippocampal neurogenesis in aged rats (24 months: n=18) and young rats (3 months: n=12). On day zero, aged rats were randomly selected to receive a single injection of one of the following: 10^6 HUCBC (n=6), or peripheral blood cells (n=6), or media (n=6), whereas young rats received either 10^6 HUCBC (n=6) or peripheral blood cells (n=6). All animals were anesthetized before a single injection was delivered (500µm total volume) through the isolated penile vein. The following day all the animals received a daily injection of bromo-deoxy-uridine (BrdU, 50mg/kg) once a day for 5 consecutive days. Rats were sacrificed 10 days after the last injection of BrdU. Unbiased stereology, using the optical fractionator, was utilized to estimate the number of BrdU+ cells in the granule cell layer. Immunofluorescence was conducted to identify human cells and neural phenotype. The results demonstrate that intravenous administration of HUCBC in aged rats increased migration and differentiation of newborn cells in the granule cell layer when compared to controls. This suggests HUCBC can provide a microenvironment in the aged brain that enhances neurogenesis, most likely through trophic support

42. ANVITA: A REFERENCE AND TEACHING TOOL FOR GERIATRICS AND THE PATHOLOGY OF AGING.

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Anvita is a computer-based reference integrating geriatrics with the pathology of aging. It includes information and tools for interpretation of data that span internal medicine, molecular pathology, biochemistry, laboratory medicine, radiology, pharmacology, anatomy, and statistics. It is used as a reference during patient encounters in a geriatric clinic and as a tool for teaching geriatrics and the pathology of aging to medical students and residents.
Clinical and molecular information is needed from laboratory bench to patient bedside, during contact between physician and patient, educator and student, scientist and technician. The information needs to be structured so that specific and real-time retrieval is achievable and it should be presented in a manner that is easy to understand. Additionally, an information system should display a certain degree of intelligence, including flexibility in accepting input from the user, the capacity to reason with structured information, and the esthetic display of context-specific information.
We have been building an information system, Anvita (formerly Senex) for the past 18 years with these objectives for the purpose of understanding human aging and diseases of old age. Anvita is both a computer-based manual for Geriatric Medicine and an engine for in silico experiments investigating the molecular basis of diseases of old age. Anvita is a large application with: 2686 organisms, 3198 anatomic structures, 226 cells, 131 cellular compartments, 15,814 molecules, 12,140 proteins, 659 genes, 959 motifs, 68 molecular pathways, 6445 diseases, 595 clinical laboratory tests and 21,032 database links (8888 UniProt, 8036 OMIM, 986 Prosite, 4444 Entrez Gene, PubChem 5865, Kegg 165, Enzyme 648). Anvita allows a user to add proprietary information to the core Anvita knowledge base, thus customizing the application for personal information needs. Anvita runs on Macintosh and Windows computers. The Macintosh version has a microarray data analysis and data mining module. website: www.anvita.info

43. THE EFFECT OF VIATMIN E SUPPLEMENTATION AT DIFFERENT AGES ON PREVENTION OF ATHEROSCLEROSIS AND MORTALITY IN LDLR-/-MICE

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Epidemiological and experimental evidence have indicated potential health benefits of vitamin E supplementation (+E) on cardiovascular disease (CVD), but recent clinical trials showed no benefit of vitamin E supplementation in subjects with diagnosed CVD. We hypothesized that +E from an early age may prevent or retard development and progression of atherosclerosis and CVD mortality. To test this hypothesis, a total of 250 LDLR-/- mice were divided into groups receiving high fat/chol diet (HFHC), moderate fat/chol (MFMC), or low fat/chol (LFLC) diets. The HFHC and MFMC, groups were further subdivided into groups (N=25) receiving respective diets with +E (500 IU E/kg diet) from the age of 5wk, 6 mo, and 12 mo to the age of18 mo. The LFLC groups received either +E or not (N=25) for 18 mo. After 18 mo, plasma cholesterol was high in all dietary groups and plasma E was high in +E groups. Body weight was highest in HFHC groups and lowest in LFLC groups. Higher mortality was observed among mice treated with HFHC (28 to 64 %) or MFMC (36-60 %) than with the LFLC group (8-36 %). +E diets had no significant effect on mortality and extent of aortic lesions in HFHC and MFMC mice regardless of duration of E supplementation. However, mortality was significantly ($p=0.04$) lower among the LFLC mice receiving +E diet from early life than with those that did not receive +E diet (8% vs.36%). This was associated with reduced aortic lesion areas in the LFLC mice, which received +E diet for 18 mo, compared to LFLC mice fed non-supplemented diet (50% vs. 65%, $p=0.03$). In conclusion, a relatively low dose and long-term E supplementation from early life is effective in reducing mortality and atherosclerotic lesions when the diet of mice, genetically prone to CVD, contains low fat and low cholesterol.
Supported by NIH grant HL069897-01A1.

44. RESVERATROL IMPROVES PHYSIOLOGY AND EXTENDS THE LONGEVITY OF MICE ON A HIGH-FAT DIET

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Resveratrol, a putative mimetic of caloric restriction (CR), has been shown to extend the lifespans of S. cerevisiae, C. elegans, D. melanogaster in a SIR2-dependent manner. Recently, this small molecule was shown to extend the lifespan of a short-lived species of fish up to 59%. In rodents, resveratrol prevents or slows the progression of a wide variety of illnesses including cancer, cardiovascular disease, and ischemic injuries, but its ability to mimic CR and extend lifespan has not been tested. Here we present the first set of data from ongoing studies, in which we are examining the effect of resveratrol treatment on mouse longevity as well as biomarkers of CR on normal and high-fat diets. We show that resveratrol, provided in the chow at 100 or 400 mg/kg, ameliorates many negative consequences of a high fat diet in C57BL/6 mice. Notably, resveratrol restores insulin sensitivity, as reflected by an oral glucose tolerance test, and significantly increases average lifespan. This extension of lifespan is not due to a decrease in the amount of food consumed or the body weight of the animals since no change in body weight was observed in the 100 mg/kg group, and only a minor decrease in the 400 mg/kg group. Further studies are needed to distinguish whether resveratrol acts on specific disease processes in obese animals, or is truly able to slow the intrinsic rate of aging and increase maximal lifespan, as does CR. The present data is particularly relevant to individuals in Westernized countries who are obese and/or consume a diet that is high in fat.

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45. EFFECTS OF LONG-TERM CALORIC RESTRICTION ON ANTERIOR PITUITARY AND HYPOTHALAMIC GENE REGULATION

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Long-term moderate caloric restriction (CR) is a powerful intervention to prevent or delay age-related diseases and to maintain physiological functions. In aged rats, CR is associated with the maintenance of a youthful level of functional pituitary growth hormone-releasing hormone receptors (GHRH-R). To assess the effects of long-term dietary interventions on the anterior pituitary and hypothalamus, gene expression profiling (cDNA microarray) was performed. Male rats were fed ad libitum or submitted to CR from 8 to 20 months of age. High quality total RNA was analyzed with Rat Genome GeneChip 230 2.0 from Affymetrix. The results indicate that GHRH-R mRNA levels decreased with aging and increased with CR in the pituitary while they were not altered in the hypothalamus. Growth hormone secretagogue (ghrelin) receptor mRNA levels were not modified by CR and aging. Hypothalamus preproGHRH mRNA levels declined with aging but were not altered by CR. Pituitary genes coding for enzymes playing a role in controlling oxidative stress, such as glutathione peroxidase, glutathione S-transferase, and heme oxygenase 1, were regulated by aging and CR. This pattern was not observed in the hypothalamus. Finally, CR regulated the expression of hypothalamic neuropeptide Y, orexin and orexin-2 receptor as well as galanin receptor type 2 genes, all involved in the control of food intake. Altogether, these results indicate new avenues for studying hypothalamo-pituitary functions, in conjunction with oxidative stress in the course of aging, and may help to design new dietary interventions. Supported by the Canadian Institutes of Health Research and Fonds de la Recherche en Santé du Québec.

46. THE MECHANISM OF THE ANTIAGING ACTION OF CALORIE RESTRICTION: EVIDENCE FOR THE INVOLVEMENT OF MACROAUTOPHAGY AND SELECTIVE MITOPHAGY

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Autophagy is the major catabolic pathway of long lived proteins and cell organelles, including mitochondria and peroxisomes. Vacuoles are formed around a little island of cytoplasm, are enriched in lysosomal enzymes and imprison and digest whatever organelles happen to be within. The autophagic process is basally active in cells, is stimulated by starvation and is inhibited by nutrients and insulin. Evidence was obtained that autophagy is stimulated by caloricrestriction and has a major role in cell maintenance and in the antiaging mechanism of caloric restriction. To support this hypothesis, data will be presented shiowing i) that in ad libitum fed animals the in vivo and in vitro function of autophagy decline with increasing age; ii) that the temporal pattern of the decline parallels changes in biomarkers of membrane aging and in signal transduction; iii) that the age-depemdent changes in autophagy are prevented by caloric restriction; iv) that the prevention of the age-dependent changes in autophagy by caloric restriction co-vary with the known effects of calorie restriction on life-span; v) that a long-lasting inhibition of autophagy accelerates the process of aging; vi) that a long-lasting stimulation of autophagy retards the process of aging and might help to counteract aging in humans; vii) that the stimulation of autophagy by an injection of ACIPIMOX rescues older cells from accumulation of altered mtDNA by selective mitophagy in less than 6 hours.

47. CHARACTERIZATION OF THE SIRT1 MOUSE: ENERGY METABOLISM AND OXIDATIVE STRESS

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In yeast, worms and flies, the class III histone deacetylase Sir2 modulates aging. It is still not known whether the Sir2 mammalian closest homologue, SirT1, also affects aging. To address this question, our lab has generated SirT1 mutant mice. These mice are grossly normal but their weight is about 20-25 % lower than those expressing SirT1, suggesting differences in energy metabolism. In order to understand this phenotype, we measured different parameters related to energy metabolism. Our results show that SirT1 mutant mice eat similar amount of food (about 10-15 % less than control mice), are less active and are hypermetabolic (consume more oxygen per body weight). Interestingly, state 3-respiration rate of isolated liver mitochondria from mutants was lower and proton leak was higher, suggesting a lower efficiency of ATP production per unit of mitochondria. Since glucocorticoids and thyroid hormones are known regulators of energy metabolism, we measured plasma corticosterone and serum thyroxine (T4) levels in our mice. Corticosterone levels were not different but T4 levels were about 25 % lower in mutants. We also measured levels of the oxidative metabolite malondialdehyde (MDA) in the liver of our mice. Our data show no difference in 8-12 month old mice whereas levels tend to be higher in 18-23 month old mutant mice. In conclusion, our data suggest that mitochondria of SirT1 mutant mice are less efficient in producing ATP, which would lead to a compensatory increase in energy expenditure at the cellular and organismal levels as well as to a decrease in energy storage. This increase in mitochondrial proton leak and overall energy expenditure are not likely regulated by T4 since we observe lower serum levels. The increase in liver mitochondrial proton leak and the increased liver MDA levels are consistent with the hypothesis that SirT1 mutant mice age prematurely.

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48. STRESS SENSITIVITY OF MITOCHONDRIAL DNA POLYMERASE EXONUCLEASE-DEFICIENT CELLS IN CULTURE

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Mice engineered to have a mutation in the exonuclease proofreading domain of mitochondrial polymerase gamma accumulate mitochondrial DNA mutations with age and show a phenotype

consistent with many signs of premature aging. These mice have increased numbers of apoptotic cells especially in tissues where the cells are actively dividing. We have transformed embryonic fibroblasts from these animals and wild type controls to establish a culture model to study this process. Cells were assayed for the ability to divide and form colonies in the presence of mitochondrial inhibitors, peroxides, and other cell stressors. Polymerase gamma exonuclease-deficient cells showed an altered sensitivity to some but not all of the stressors assayed. These results yield insight into possible molecular mechanisms for the increased levels of apoptosis caused by mitochondrial DNA mutations that accumulate with age.

49. DECLINES IN BRAIN MITOCHONDRIAL FUNCTION IN OLD AGE PREDISPOSE TO STRESSOR TOXICITY

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Appearances aside, the aging brain and its inability to tolerate stressors may be critical to aging pathology. The age-dependence of both acute and chronic neurodegenerative diseases raises the question, what is it about aging that is essential for the neuronal loss? Since glutamate toxicity increases with age and mitochondria are critical to glutamate toxicity we wanted to determine the mechanism of age-related perturbation of mitochondrial function that contributes to neuronal death. As an environmentally controlled model, we isolated and cultured hippocampal neurons from rat brains at embryonic day 18, middle-age (9 month) or old-age (24 month). We studied redox levels by measuring fluorescence of NAD(P)H and FAD simultaneously from single neurons. In neurons, individual mitochondrial membrane potentials (MMP) were measured simultaneously with reactive oxygen species (ROS) production. At rest, MMP was most depolarized in old neurons. At rest with increasing age, mitochondrial ROS levels were higher, NAD(P)H and redox ratios (NAD(P)H / FAD) were lower. Glutamate exposure resulted in age-related declines in MMP and increases in ROS. After glutamate exposure, NAD(P)H levels and the redox ratio declined in all age groups with the largest decline in the old neurons. This age-related decline in NAD(P)H and redox ratio correlated well with the decline in glutathione and MMP and increases in ROS. In the respiratory chain, we also found evidence for age-related deficits in complexes I (NADH dehydrogenase), III (ubiquinol-cytochrome C oxidoreductase) and IV (cytochrome C oxidase), cardiolipin and cytochrome C and glutamate-stimulated respiration, without decreased mitochondrial number. Some of these changes can be reversed by pre-treatment with estrogen, blue-berry extract or neuron multiplication. These results support a mechanism in which age-related declines in MMP, NAD(P)H, redox state, complexes I, III and IV, glutathione and increased ROS production predispose old neurons to a catastrophic response to glutamate.

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50. CALORIE RESTRICTION EFFECTS ON OXIDATIVE PHOSPHORYLATION COMPONENTS IN LONG-LIVING AMES DWARF MICE

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Ames dwarf mice live 49-68% longer than their wild type siblings (males and females, respectively). Calorie restriction has been shown to extend the life span of Ames dwarf and wild type mice over that of ad libitum fed counterparts. A deficiency of growth hormone (GH) and thus, reduced insulin-like growth factor 1 (IGF-1) and insulin signaling in Ames mice are significant contributors to the observed longevous phenotype. Previous data showed that several components of oxidative phosphorylation are increased both in activity and protein levels in Ames mice. Therefore, it was of interest to examine the effects of calorie restriction on mitochondrial proteins in long-living mice to identify potential differences between dwarf and wild type mouse adaptation to reduced caloric intake. Six month old mice were subjected to every other day feeding for a six week period. Liver tissue activity of complexes I+III, II+III and IV (cytochrome c oxidase) were determined spectrophotometrically. Expression of liver mRNA for complexes I, II, III, IV, V, adenine nucleotide translocase and PGC-1 were determined by real time RT-PCR.

Protein levels were determined by immunoblotting. Liver complex II+III activity was higher in dwarf mice compared to wild type. Gene expression for complexes II and III was also significantly elevated in dwarf mice. Protein levels of complexes II and IV were greater in dwarf compared to wild type mice. Calorie restriction decreased the coupled activities of complexes I+III (78 and 76%) and II+III (24 and 21%) in both dwarf and wild type mice, respectively, suggesting a reduction in potential energy production. Similar increases in mitochondrially-encoded complex IV mRNA expression were observed following calorie restriction in both genotypes. However, several other OXPHOS genes were differentially expressed between dwarf and wild type mice following calorie restriction. These data contribute to the evidence suggesting that different mechanisms are at play in the delayed aging and life span

51. PAIN AND DECLINING PHYSICAL PERFORMANCE: AN ANIMAL MODEL FOR INTERVENTIONAL TESTING

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This presentation describes a pain assessment procedure, using an operant escape-based method, and its adaptation for use in older animals. Female Brown Norway X Fisher 344 rats (12, 20 and 30-months of age) were tested in a two-chambered apparatus. One chamber was dark (preferred environment) and the other brightly lit (aversive environment). The dark chamber was fitted with a floor which could either be cooled or heated. The brightly lit chamber contained an escape platform. Therefore, when the temperature of the floor in the preferred environment became aversive, the animal could escape to the platform in the brightly lit environment. Standardized physical performance measures (inclined plane and grip strength) were used to assess whether older animals' potential inability to perform in this task might be related to declining muscle strength and stamina. Our results demonstrate that use of the operant escape procedure results in temperature-dependent increases in escape duration which do not differ as a function of age, although physical performance measures decreased in an age-dependent fashion. These data suggest that older animals can be studied using operant escape procedures and that physical performance decrements observed with increasing age do not confound performance in this task. Implications of this task for the study of pain in older animals include the preclinical assessment of pain sensitivity following experimentally induced pain manipulations, the screening of initial sensitivity to and the effects of chronic administration of opioids and other analgesics, and relationships between chronic pain conditions and physical disability.

52. AGE CHANGES AND SEX DIFFERENCES IN SERUM DHEAS CONCENTRATIONS AND ITS RELATED FACTORS

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Background: There is no epidemiologic data of dehydroepiandrosterone (DHEAS) in Korea. It has been known that the levels of serum DHEAS were influenced by many factors. We studied age changes and sex differences in serum DHEAS throughout adulthood and its related factors.
Methods: Serum DHEAS and several biochemical markers (total cholesterol, triglycerides, HDL-cholesterol, glucose, etc.) were measured and structured questionnaires were administered in 1,710 healthy men (857) and women (853), aged 17-76 years who visited health promotion center of a community hospital. We also measured their height, weight, waist and hip circumferences and body fat contents with bioimpedance method.
Results: The DHEA concentrations showed a peak at age below 30 years in both sexes. The mean values of DHEAS declined steadily according to the ages in both men ($R=-0.38$, $p<.001$)and women ($R=-0.46$, $p<.001$). DHEAS concentrations were significantly higher in men than women at all age group except age group above 70 years. DHEAS levels in men were significantly higher with smoking, alcohol drinking, high BMI after adjusting age. On the other

hand, DHEAS levels in women were higher with alcohol drinking and regular exercise after adjusting age. However, there were no significant association between DHEAS and lipid profile (total cholesterol, triglycerides, HDL-cholesterol), and fasting blood glucose level in both sexes. Conclusion: Our data suggest that serum DHEAS levels were influenced by several sociodemographic factors and body mass index, differently in men and women.

53. PARKINSON'S DISEASE IN RELATION TO TOXIN EXPOSURE AND MITOCHONDRIAL COMPLEX I GENE VARIANTS

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Parkinson's disease is an age-related neurodegenerative condition. Pathologically, PD results from the loss of pigmented dopamine-producing neurons in the substantia nigra pars compacta region of the brain. Tremor, rigidity, slowness of movement and postural instability are the predominant symptoms. One clue to it's molecular etiology is that mitochondrial complex I compromise is found in both rare familial forms of PD (alpha-synuclein, parkin, DJ-1, PINK1, and LRRK2), experimental models where mutant genes are expressed, and parkinsonism associated with environmental toxins. We investigated variants (n=22) in nuclear genes encoding mitochondrial complex I proteins and environmental exposures together to predict risk in a case (n=306) and control sample (n=321) using grade-of-membership analysis. Five extreme pure type latent groups were identified implying G x E interaction: “I: Toxin exposure, low risk” “II: Toxin exposure, onset < age 60,” “III: Limited exposure, onset < age 60,” “IV: Limited exposure, onset at ages 60 to 69,” and “V: No exposure, low risk.” Few subjects exactly matched these groups. Nonetheless, each 20% increment in resemblance to groups II to IV multiplied the odds of PD: II: 3-fold at ages < 60, III: 4-fold at ages < 60 and IV: 3-fold at ages 60 to 69. No one polymorphism dominated the results. We conclude that toxin exposure and complex I variants together determine, in part, the risk for PD. Even partial resemblance to identified sets of variants carried appreciable risk for PD. This form of analysis may prove a particularly useful way for hypothesis generation and subsequent investigation of specific gene x gene and gene x environment interaction in relation to common sporadic PD.

54. HIGHER EXPRESSION OF AN HSP-16::GFP REPORTER IS A STRONG PREDICTOR OF INCREASED SURVIVAL IN C. ELEGANS; BUT WHY?

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Higher expression of the HSP-16 heat shock protein is observed in life extension mutants of C. elegans. Moreover, greater expression of an hsp-16::GFP transcriptional fusion transgene is a strong predictor of improved survival (Rea et al, 2005). Here we examine other characteristics of animals displaying strong vs. weak expression of GFP and whether those characteristics are associated with improved survival. Fertility, motility and microarray data are presented but offer little ability to explain the dramatic differences in survival seen in bright worms as compared with dim. We are also investigating inheritance of these effects and whether environmental perturbations, such as a sub-lethal heat stress, influences the inheritance of expression.

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55. REDOX-ACTIVATION OF MITOGEN ACTIVATED PROTEIN- AND PHOSPHOINOSITOL-3-KINASE SIGNALING REGULATES SENESCENCE-ASSOCIATED INCREASES IN MATRIX METALLOPROTEINASE-1 PRODUCTION

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Matrix metalloproteinases (MMPs) are a class of zinc-containing endopeptidases important for both physiologic and pathologic tissue remodeling. Matrix metalloproteinase-1 (MMP-1) is a prototypical interstitial collagenase and its aberrant production is implicated in a many age-related degenerative diseases including cancer, rheumatoid arthritis, pulmonary emphysema and fibrotic disorders. Our laboratory has identified MMP-1 as a redox-responsive gene whose expression can be controlled by efficient and targeted antioxidant delivery systems. In this study we set out evaluate whether the age-associated increases in MMP-1 expression are also redox-sensitive.

Using an in vitro cell culture model of replicative senescence (RS) we demonstrate that age-associated increases in MMP-1 expression can be prevented by either short or long-term exposure to low oxygen (3%). Acute treatments of senescent IMR-90 fibroblasts with either N-acetyl cysteine or antioxidant metalloporphyrins prevent the senescence-dependent increase in MMP-1 production. RS-dependent increases in MEK/ERK and Phosphoinositol-3-Kinase (PI3K) signaling were also observed and their pharmacologic inhibition attenuated senescence-dependent MMP-1 expression. These findings indicate that age related increases in MMP-1 expression are attributed to the redox-dependent activation of both Mitogen Activated Protein Kinase (MAPK) and PI3K signaling. The development of efficient and targeted antioxidant therapies may prove useful in restricting age-associated alterations in matrix homeostasis.

56. A MODEL OF AGING AS ACCUMULATED DAMAGE PREDICTS OBSERVED MORTALITY PATTERNS AND THE LIFE-EXTENDING EFFECTS OF PROSPECTIVE INTERVENTIONS

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The physiological decline associated with aging derives ultimately from the accumulation of side-effects of metabolism. Here we model that accumulation starting from a biologically intuitive interpretation of the way in which those side-effects interact; we validate this model by showing that it accurately predicts the distribution of ages at death seen in typical populations that are protected from age-independent causes of death. We then exploit the mechanistic basis of this model to explore the impact on lifespans of interventions that combat aging, with an emphasis on interventions that repair the direct molecular or cellular consequences of metabolism and thus prevent them from translating into pathology. We establish that a dramatic extension of healthy and total life expectancy can be achieved by a plausible rate of progress in the development of such therapies, once a threshold level of efficacy of those therapies has been reached.

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57. METABOLIC RATES ARE NOT ASSOCIATED WITH LONGEVITY IN EUTHERIAN SPECIES

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For decades, it has been argued that metabolic rates are associated with longevity and aging. According to the rate of living theory, animals with a faster metabolic rate tend to have a shorter lifespan and a faster aging process, and thus differences in metabolic rate can be used to explain the huge diversity of lifespans between different mammalian species. In this work, we took advantage of AnAge (http://genomics.senescence.info/species/), an aging-oriented database featuring over 1,000 mammalian species, to test whether basal metabolic rate (BMR) is associated with maximum longevity in mammals. Contrary to many previous such studies, we were rigorous in our data selection, and we employed modern statistical methods to correct for the potential bias due to body mass and phylogeny by using residuals that remove the effects of body mass and by deriving phylogenetically independent contrasts. When the effects of body mass and phylogeny are corrected, our results show that BMR does not correlate with maximum longevity in mammals. We then tried to relate BMR to maximum longevity in mammalian orders. In marsupials, there was a weak, statistically significant correlation between BMR and maximum longevity. There was no significant correlation between BMR and maximum longevity in eutherians as a whole or within any eutherian order. We conclude that in eutherian species, including primates, animals with a higher metabolic rate for their body size do not tend to be shorter-lived and vice-versa. It appears that, contrary to the rate of living theory, the evolution of longevity in eutherians is not associated with metabolic rates. These results are also discussed in the context of theories of aging. For example, many formulations of the free radical theory of

aging are based on the assumption that BMR, which is estimated from oxygen consumption at rest, correlates with longevity.

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58. CHRONIC GINSENG SUPPLEMENTATION ALTERED HEMODYNAMIC AND ANTIOXIDANT RESPONSE TO EXERCISE IN OLDER WOMEN

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The effects of North American ginseng (Panax Quinquefolium) supplementation on blood oxidative stress and antioxidant markers were investigated in older women at rest and after a mild bout of treadmill walking. In this double-blinded study, 25 female subjects (age range 55-75) took two capsules containing either placebo or 500 mg ginseng everyday for four months. Blood and urine samples were collected immediately before and after 30 minutes of treadmill walking on a 5% grade incline at 60% of VO_2max (estimated from heart rate response to submaximal workload) both prior to and after the supplementation regimen. Resting and exercising heart rates and estimated VO2max were not significantly different before and after ginseng supplementation. The ginseng group showed higher mean arterial pressure after supplementation compared to the placebo group, due to an elevated diastolic pressure ($P<0.05$). Plasma glutathione disulfide (GSSG) content was decreased, whereas the glutathione (GSH):GSSG ratio was increased after exercise in all subjects ($P<0.01$). The ginseng group displayed a lower GSH:GSSG ratio after supplementation compared to the placebo group ($P<0.07$). While erythrocyte GSH peroxidase activity was unaltered, superoxide dismutase ($P<0.05$) and GSH reductase ($P<0.06$) activities were increased after ginseng supplementation. This data suggests that chronic ginseng supplementation, at the given dose (1,000 mg/day), changed vasomotor behavior in the older women. Plasma redox status was also altered as a result of ginseng treatment, which may explain the increased erythrocyte antioxidant enzyme activity.

59. IMPLICATIONS OF SMOKING ON OCULAR SURFACE AND TEAR FUNCTIONS

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Purpose: Smoking , a well known risk factor for aging, has been reported to induce inflammation in several tissues and shortening of telomere length in human body.
We aimed to investigate the effects of smoking on ocular surface health and tear functions in this prospective controlled clinical trial and report our preliminary findings.
Methods: Fourteen of 10 heavy smokers(5 males, 2 females; age range: 36-47 years) who smoke 20 cigarettes for 20 years and 14 eyes of 7 healthy control non smokers(3 males, 4 females; age range: 38-43 years) were included in this study. None of the subjects had a history of Sjogren's syndrome, collagen diseases, other systemic or ocular diseases, history of ocular surgery, contact lens or drug use. All subjects underwent measurements of hemoglobin CO concentration, tear film lipid layer interferometry, tear evaporimetry, tear film break up time (BUT), Schirmer test-I, ocular surface fluorescein staining, conjunctival impression and brush cytology. Samples from brush cytology were also evaluated for MUC5AC mRNA expression in Real Time PCR.
Results: The mean Hb CO level was significantly higher in smokers compared to nonsmokers. Tear film break up time was also significantly shorter in smokers . Tear lipid layer showed significant slowing in spread over the tear film. Tear quantity was significantly reduced in smokers

with a concomitant significant increase in tear evaporation rate. Conjunctival impression cytology revealed significant loss of goblet cells and advanced squamous metaplasia in smokers with a decrease in MUC5AC mRNA expression. Brush cytology showed significant conjunctival neutrophil infiltration in smokers .
Conclusion: Tear film and the ocular surface epitheliae showed distinctive quantitative and qualitative disturbances in heavy smokers which we attributed to the marked ocular surface inflammation associated with chemicals in cigarette smoke.

60. THE EFFECT OF LATITUDE, CLIMATE AND EXERCISE ON THYROID HORMONE PRODUCTION IN SLED DOGS

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Thyroid function in recent years has become a topic of interest and concern in racing sled dogs. Many variables afflicting racing sled dogs, such as exposure to cold climate and extreme exercise, are variables that have been shown to influence thyroid hormone production. Aside from the difficulty in assessing thyroid function, sled dogs may have lower normal ranges, similar to other working canine athletes. In an attempt to reconcile some of this unease, thyroid hormone levels were measured in both exercising and non-exercising sled dogs from two different locations (Fairbanks, Alaska and North Creek, New York) throughout a 6 month period at various times within a given day. This protocol was used in an attempt to determine the effects that seasonal variations as well as light exposure and climate may have on thyroid production. Preliminary data analysis reveals definite differences in respect to season, exercise, and latitude.

61. A NEW POLYPHENOLICS WITH STRONG RADICAL SCAVENGING ACTIVITY

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Oxidative stress and antioxidant are believed to be involved in aging process. Dietary antioxidant may delay the onset of aging and progression of chronic disease associated with aging. Herbal medicines are still the mainstay of about 75–80% of the world population. We have identified and isolated two new prenylated polyphenolic anthronoids, harunmadagascarins A and B, (Figure1) along with two known compounds from the stem bark of praying hands (*Harungana madagascariensis*), a common herbal medicine used in Europe and Africa for its antidiabetic and anti diarrhetic effects. The pure compounds have strong antioxidant activity in *in vitro* system as determined by scavenging of superoxide, DPPH and nitric oxide radicals as well as using TAS (total antioxidant status) and xanthine/ xanthine oxidase system. Our results indicate that these compounds possess significant activity. We found that harunmadagascarin A which is new compound has ten fold higher superoxide scavenging activity compared to synthetic antioxidant (3- t-B utyl-4-hydroxyanisloe) and can quench NO and DPPH by more than 93-95 percent compared to di-hydroxyflavone. The *in vitro* evidence strongly suggests variety of health benefit such as cancer, inflammation and heart disease. These and other biological activity of these polyphenolic compounds are currently under investigation in our laboratory. This project was funded by HEC Pakistan.

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62. EFFECTS OF AGING AND NADH HYPEROXIDATION ON NEURONAL RECOVERY FOLLOWING HYPOXIA/ISCHEMIA IN RAT HIPPOCAMPAL SLICES

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Mitochondrial dysfunction and susceptibility to hypoxia/ischemia (H/I) both increase with age. Therefore we investigated the relationship between neuronal recovery and NADH hyperoxidation following H/I across the lifespan. Hippocampal slices (400mm, 95% O2, 36°C, interface) were prepared from adult F344 rats (1->22 months). Tissue pO2, extracellular neuronal responses (fEPSP) and NADH fluorescence images were recorded from the CA1 region during induced H/I episodes (95% N2). Rapid reoxygenation (within 15 s following hypoxic spreading depression:

hSD) was reversible in all age groups, with 100% neuronal recovery (at 50 min following reoxygenation). However, the rate of neuronal recovery (to 50% of baseline) was slower in slices from older rats (>22 months; 11 min) compared to all younger rats (<22 months; 6.5 min). Delayed reoxygenation (2.5 min post-hSD) was less well tolerated by older animals (50% recovery in 12-18 month old rats, 45% recovery in >22 month old rats) compared to younger animals (91% in 1-2 months old, 116% in 3-6 months old). However, the elimination of Ca2+ from the ACSF in slices from aged rats improved neuronal recovery (110% versus 45%). The recovery in slices from 1-2 month old rats following prolonged H/I (5 min post-hSD) was reduced to 52% and 20% or less in all older age groups. The percentage of NADH hyperoxidation (measured at 15 min following reoxygenation) increased with the length of hypoxia and was also age dependent. At five min of H/I post-hSD, NADH hyperoxidation was significantly higher in >22-month-old rats compared to 1-2 month old rats (-8% vs -2%, respectively). In conclusion, hyperoxidation, which is moderately correlated with neuronal recovery, may result from cellular damage caused by radical oxygen species following reoxygenation. Hyperoxidation may contribute to the increased susceptibility of aged rats to H/I and is indicative of mitochondrial dysfunction. Supported by VAMC, NS 045304 and NS51856.

63. OMEGA-3 PUFA SUPPRESS CHEMOKINE PRODUCTION FROM HUMAN CONJUNCTIVAL FIBROBLASTS

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Purpose: Omega-3 polyunsaturated fatty acids (PUFA), such as docosahexaen acid (DHA) and eicosapentaen acid (EPA), have been found to suppress inflammatory diseases as well as cardiovascular diseases. To study the suppressive effects of omega-3 PUFA on ocular inflammation, we investigated chemokine production in conjunctival fibroblasts cultured with omega-3 PUFA. Human conjunctival fibroblasts were cultured with DHA (100micro-g/ml) for 24 hrs, and consequently stimulated with TNF-alpha (30ng/ml) and IL-4 (30 ng/ml). Eotaxin, RANTES and ICAM-1 mRNA expressions in cultured cells were investigated by using realtime-PCR (n=3). Eotaxin and RANTES production in culture supernatants were measured by ELISA (n=3). Results;Pre-incubation with DHA increased omega-3 PUFA in cultured fibroblasts by 2-3 folds. Eotaxin, RANTES and ICAM-1 expression in fibroblasts were suppressed by DHA pre-incubation (59%, 64% and 43% suppression). Eotaxin and RANTES productions were also suppressed by DHA pre-incubation (42% and 38% suppression). The regulatory effect of omega-3 PUFA chemokine production from conjunctival fibroblasts may be a factor that affects the severity of ocular inflammation.

64. A NETWORK MODEL OF BIOLOGICAL INTERACTIONS IN HUMAN AGING

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The many observable signs of human senescence have been hypothesized by various researchers to result from several primary causes. Close inspection of the biochemical and physiological pathways associated with age-related diseases and with the hypothesized causes reveals several parallel cascades of events that involve multiple interactions and feedback loops. We have constructed a network diagram to aid in visualizing the many processes and interactions among them, including promising intervention points for therapy development. This network diagram refers to both intracellular and extracellular processes, and it ranges in scale from the molecular to the whole-body level. Important pathways include: Glycation, oxidation, and crosslinking damage extracellular proteins; Aggregates clog proteasomes and lysosomes; Repair and turnover of macromolecules and organelles is impaired; Reactive, crosslinked material accumulates in lysosomes and leaks into cytoplasm; Oxidized aggregates in cytoplasm crosslink and increase redox poise; Increased redox poise alters signaling and enzyme activities, and erodes telomeres; Stem cells stop dividing or die; Chromatin alterations change gene expression; Stiffer blood vessels promote stroke and heart disease; Cell death leads to tissue wasting, neurodegeneration, and organ malfunction; Damaged molecules and sick cells promote

inflammatory cascades; Mitochondrial DNA mutates; Neuroendocrine and immune systems degrade. This diagram is continuously maintained on the Web [www.LegendaryPharma.com/senescence.html/Mechanisms] as a reference for researchers, with the content updated as new information comes to light. In addition, we are formalizing the network diagram's contents in first-order logic and using a probabilistic framework known as Markov logic to evaluate the completeness and consistency of this diagram.

65. EXTRACELLUAR GLYCATION AND CROSSLINKING: A REVIEW

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Extracellular aging -- accumulating molecular damage by glycation, oxidation, and crosslinking of long-lived extracellular proteins, mainly collagen and elastin -- is a major cause of several important human aging pathologies. Crosslinking increases mechanical stiffness of blood vessels and urinary bladder. Crosslinking impairs functioning of kidney, heart, retina, and other tissues and organs. Glycation adducts trigger inflammatory signaling, provoking tissue damage and cancers. Crosslinking tightens up the extracellular matrix (ECM), hardening it against natural turnover processes. Known crosslink breakers are only partly effective because they break only a subset of AGE crosslink structures. So far, no agent has been found which breaks the prevalent glucosepane and K2P crosslink structures. The chemistry of glycation and crosslink formation is reviewed, along with mechanisms leading to pathologies of aging.

66. REGULAR TREADMILL EXERCISE REDUCES OXIDATIVE DAMAGE OF THE NUCLEAR AND MITOCHONDRIAL DNA AND MODULATES THE DNA REPAIR ACTIVITY IN OLD RAT LIVERS

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Aging is associated with oxidative modifications of proteins, DNA and phospholipids that can impair cellular functions and/or result in the death or malignant transformation of cells. A number of antioxidant intervention strategies have been tested to retard the aging process, but no robust reproducible result has been reported. While exercise is often stated to increase the generation of reactive oxygen species (ROS) that are potentially harmful, it is shown to have a number of beneficial outcomes on health even late in life. We report here that 2 months of regular treadmill running of aged rats (21 month-old) significantly reduced the increased 8-oxo dG content to the level of young animals (11 month-old) in both nuclear and mitochondrial DNA of the liver. The activity of the major repair enzyme OGG1 for this oxidative lesion was upregulated in the nucleus but downregulated in mitochondria by the exercise regimen. Thus, the activity of the repair enzyme is differentially regulated in the nucleus and mitochondria, although the oxidative damage of DNA is reduced in both organelles by the exercise. This apparently beneficial outcome is discussed in terms of hormetic effect of moderate oxidative stress and the adaptation to the stress in regular exercise (see Radak et al. Biogerontology 6:71-75, 2005).

67. ALTERATION OF THE ENDOCYTIC RECEPTORS, CUBILIN AND MEGALIN IN THE KIDNEY OF RATS WITH AGE

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Cubilin and megalin are endocytic receptors located in the renal proximal tubule. Both receptors play an important role for uptake of albumin passed through the glomeruli into the primary urine. The amount of protein, mostly albumin, is increased in urine with age in rats (F344/DuCrlCrlj). We hypothesized that the age-related increase in the incidence of albuminuria is at least partly due to decreased reabsorption of albumin by cubilin and/or megalin. To examine this possibility, we investigated age-related change of the amount and cellular localization of both receptors in rat kidney. We used normal young and old rats that exhibited proteinuria. Although there was no

difference in the amount of cubilin between the young and old, the amount of cubilin fragments increased with age. The amount of megalin significantly decreased with age. The immunohistochemical study revealed that cubilin and megalin were predominantly localized in brush border membrane of proximal tubular cells in young animals, but both receptors tended to diffuse into the cytoplasm of the cells in the old. The altered cellular distribution of cubilin and megalin in old rats may be due to an activation of cubilin/megalin mediated endocytosis of albumin associated with an increased amount of glomerular filtrated albumin. It is also possibly due to decrease in recycling rate of the receptor by the alteration of receptor itself with age. Thus, age-related increase in the amount of albumin in urine might partly be due to the direct and/or indirect effects of quantitative and qualitative alteration of both receptors with age.

68. PERCEIVED AGE AND SKIN AGEING

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Perceived age is a measure of ageing which can be generated by assessors via visual examination of individuals in photographs. By taking high quality standardised facial images and cropping the photographs to remove hair and clothing visual cues, we have generated a skin targeted perceived age. When compared to dermatological skin scores, this perceived age was found to have a higher correlation with these scores than actual age. Thus, by tailoring human's perception of age towards the skin, we have found that perceived age can be an indication of the biological age of skin. We have also investigated the reproducibility of this perceived age by using over 100 assessments per photograph and have found that when using over 50 assessments per photograph the score becomes reproducible. Subsequently, investigations in the U.K. and Spain with over 400 subjects (over 20,000 perceived age assessments) have demonstrated self-reported lifestyle factors such as oral-care, diet and smoking are associated with the measure. These lifestyle factors also correlate with skin replica measures which support the evidence that this perceived age is a measure of the biological age of skin.

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69. METHYL ESTER OF AVENANTHRAMIDE-C INHIBITS TNF-ALPHA- AND IL-1 BETA-INDUCED NF-KAPPA B ACTIVATION IN ENDOTHELIAL CELLS

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Atherosclerosis is an age-associated chronic inflammatory disease of arteries accompanied by the expression of endothelial pro-inflammatory molecules. Avenanthramides (Avns) are polyphenols found exclusively in oats. We have reported that avenanthramide-enriched mixtures extracted from oats significantly suppressed interleukin (IL)-1 beta-stimulated secretion of pro-inflammatory cytokines IL-6, IL-8 and monocyte chemoattractant protein (MCP)-1 in human aortic endothelial cells (HAEC). Here we report that these effects could be mediated by Avn interference with nuclear factor-kappaB (NF-kappaB) dependent transcription. We used a synthetically prepared methyl ester of Avn-c (CH3-Avn-c), which shows a higher potency of inhibition of human smooth muscle cell proliferation than that of non-methylated form. CH3-Avn-c significantly and dose-dependently decreased IL-6, IL-8 and MCP-1 secretion in HAEC as determined by ELISA, and it inhibited TNF-alpha- and IL-1 beta-stimulated NF-kappaB activation as determined by a NF-kappaB DNA binding assay and a NF-kappaB luciferase reporter assay. CH3-Avn-c also significantly and dose-dependently decreased phosphorylation levels of IkappaB kinase (IKK) and IkappaB, thus prevented IkappaB degradation as measured by Western blotting. CH3-Avn-c markedly increased the overall levels of high mass ubiquitin-conjugated proteins. The proteasome activity was also mildly inhibited by CH3-Avn-c. These results suggest that the Avns decreased the expression of endothelial pro-inflammatory cytokines at least in part through inhibition of NF-kappaB activation by inhibiting the phosphorylation of IKK and IkappaB, and suppressing proteasome activity.

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70. PARAOXONASE ACTIVITY AND LIPID PARAMETERS IN PATIENTS WITH ALZHEIMER'S DISEASE.

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Background: It is becoming clear that the common sporadic late onset form of Alzheimer's disease is not caused by a single pathogenic factor but by many genetic and environmental factors operating together.

Paraoxonase is an arylesterase enzyme that is expressed in the liver and found in the circulation in association with the high-density lipoprotein, and prevents the accumulation of oxidized lipids in low-density lipoproteins in vitro. Common polymorphisms in genes encoding paraoxonase are established risk factors in a variety of vascular disorders including coronary artery disease and carotid artery stenosis, but their association with Alzheimer disease (AD) is controversial. Paraoxonase has antioxidative potential. Paraoxonase 1 (PON1) decreases the peroxidation of LDL. Serum PON1 activity decreases with aging and in disorders associated with a high risk of adverse cardiovascular events

Objectives: The implication of vascular factors in Alzheimer-type dementia (ATD) is strongly suspected. Subjects with AD may however have less active enzyme (lower plasma paraoxonase levels) as an independent risk factor for the occurrence of Alzheimer's pathology. In our study, the serum paraoxonase activity, cognitive impairment and lipid parameters in patients with late-onset Alzheimer's disease (AD) were investigated to investigate correlation between their levels.

Methods: A group of 40 patients with sporadic late-onset Alzheimer's disease and relevant amount of control (40) were included in the study. The DSM IV criteria were used as diagnostic criteria for dementia Alzheimer's type. MMSE was used to assess cognitive impairment in examined groups (mean 16 +/- 6).

Paraoxonase activity was measured spectrophotometrically using phenyl acetate as the substrate. The enzyme activity was expressed in the international units per ml of serum (U/ml). HDL, LDL and triglycerides levels were measured in human's sera and expressed as mg/dl and mmol/l.

Results: In AD group the level was significantly different as compared to control group (70.78±58.15 U/ml vs. 89.78±22.38 U/ml). The results were positively correlated with MMSE and HDL level. We didn't found any correlation with level of LDL cholesterol and triglycerides. LDL levels however were or higher then norm or they were at the high and of a normal range.

Conclusion: Despite the fact implication of vascular factors in Alzheimer-type dementia (ATD) is strongly suspected, the activity of paraoxonase may be an independent risk factor for late-onset AD.

71. DYSARTHRIA AS A SYMPTOM OF STRIATONIGRAL DEGENERATION

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First coined by Graham and Oppenheimer in 1969, the term 'multiple system atrophy' (MSA) describes a syndrome with features overlapping with Shy-Drager syndrome, striatonigral degeneration, and olivopontocerebellar atrophy. MSA is characterized clinically by the combination of Parkinsonian, pyramidal, cerebellar, and autonomic symptoms.

In a recent case report we described, a 67 years old women with a clinical diagnosis of striatonigral degeneration and five year history of the symptoms. She was initially misdiagnosed as Parkinson's disease, largely because of tremor and rigidity. She did not, however, respond well to levodopa and within a few months she developed severe dysarthria. Features (helpful in differentiating SND from other extrapiramidal disorders) included early-onset falling, severe dysarthria and dysphonia, respiratory stridor, hyperreflexia.and ataxia. Cerebellar signs were present in as well, whereas autonomic symptoms were less severe. Besides of neurological and imaging study a specially designed phonetics test for dysarthria was performed.

In the speech of the patient studied, numerous deficiencies can be observed. At the suprasegmental level, speech is monotonous, with a flat Fo contour and without normal pitch variability. The speech pace is slow and there is a tendency to divide words into syllables of equal prominence. At the syllabic level, vowels (especially peripheral /i/, /u/ and /a/) are centralized, and word final non-front consonants are reduced to a schwa. Consonants, especially the apical obstruents, are articulated with impeded precision. There is a tendency to use incomplete closures in plosives and affricates both in and outside clusters. In stop clusters, the first plosive has only closure and no release. Glottal activity is implemented in an untypical way: Fo is lowered and the phonation is breathy. The nasal consonants are produced with irregular soft palate timing.

72. ASSOCIATION BETWEEN METABOLIC SYNDROME AND BRACHIAL-ANKLE PULSE WAVE VELOCITY IN KOREAN ADULTS

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Many studies have shown that MetS is associated with increased risk of developing cardiovascular disease and related mortality. Pulse wave velocity (PWV) is known to be an indicator of arterial stiffness and to predict future cardiovascular events. We hypothesized the metabolic syndrome, defined using the NCEP-ATP Ⅲ criteria, has been related with increased baPWV. We examined a total of 400 participants (120 males and 280 females) who did not have a past history of either coronary heart disease or peripheral vascular disease. All subjects underwent physical examination, blood chemistry, and baPWV. Mean value of baPWV (adjusted for age, BMI, and blood pressure) is significantly higher in subjects with MetS(P=0.002). baPWV was positively correlated with age, BMI, systolic and diastolic blood pressure in subjects with MetS. In group without MetS, baPWV was associated with age, waist circumference, blood pressure, total cholesterol, LDL cholesterol, triglyceride, fasting blood sugar, AST, ALT, homocysteine, CRP, and ferritin. Mean values of baPWV with 0, 1, 2, 3, 4, 5 components of MetS were 1362.3±182.2cm/s, 1531.3±267.0cm/s, 1694.1±315.8cm/s, 1777.0±334.0cm/s, 2087.7±192.3cm/s respectively. We concluded that the MetS is associated with increased ba PWV

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73. MITOCHONDRIAL DNA MUTATIONS INDUCE SKELETAL MUSCLE LOSS ASSOCIATED WITH MITOCHONDRIAL DYSFUNCTION WITHOUT OXIDATIVE STRESS.

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The aging process can activate stress-associated signal transduction pathways that result in mitochondrial dysfunction and apoptosis. Because the mitochondrion contains its own circular DNA, a central role for mitochondrial DNA (mtDNA) mutations in mammalian aging has been postulated. In order to determine whether mtDNA mutations underlie the aging process in skeletal muscle, we constructed a mouse model that expresses a proofreading-deficient version of the mitochondrial DNA polymerase gamma (PolgD257A), resulting in increased spontaneous mutation rates in mitochondrial DNA. PolgD257A mice exhibited significant skeletal muscle loss (-23.5 %) compared to WT, by 10-mo of age. We measured oxygen consumption, H2O2 production and DNA oxidative damage in isolated mitochondria from skeletal muscle of 10-mo-old wild type (WT) and PolgD257A mice. State-3 oxygen consumption decreased significantly (-43 %) in PolgD257A compared to WT, which led to a significantly lower respiratory co! ntrol ratio (-43 %) in PolgD257A mice. H2O2 production in isolated mitochondria was also significantly decreased (-36 %) in PolgD257A mice. Furthermore, we did not detect any

differences in oxidative damage to mtDNA between WT and PolgD257A mice, as measured by 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo). Importantly, we previously showed significantly elevated levels of caspase-3, in PolgD257A mice, the final effector caspase involved in the induction of apoptosis. All together, our findings suggest that the accumulation of mtDNA mutations does not lead to increases in reactive oxygen species (ROS) production or oxidative stress in mitochondria with age, in contrast to previous beliefs. Instead, mtDNA mutations may be associated with induction of apoptosis irrespective of elevations in ROS production and oxidative stress in skeletal muscle. Hence, loss of critical, irreplaceable cells through apoptosis may be a central mechanism of skeletal muscle loss associated with mtDNA mutations. In future studies, we will investigate the possibili

74. Trx2 (+/-) KO MICE ARE MORE SENSITIVE TO OXIDATIVE STRESS INDUCED BY DIQUAT

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Thioredoxin 2 (Trx2) is the mitochondrial form of thioredoxin, which plays an important role in redox control and protection against ROS -induced damage in mitochondria. In this study we evaluated the effect of reduced levels of Trx-2 on oxidative damage and mitochondria function using mice heterozygous for the Trx2 gene (Trx2+/-). The Trx2+/- mice showed approximately 50 % decrease in the Trx2 protein expression without upregulation of other major antioxidant enzymes. To test the response of the Trx2+/- mice to oxidative stress, we treated the mice with diquat, a superoxide anion generator, and measured mitochondrial function and oxidative damage to macromolecules such as DNA, lipids and proteins. Mitochondrial function was assessed measuring ATP production, ETC activity, and apoptosis, as measured by cytochrome c release and caspase-3 activation. Our results demonstrated that reduced levels of Trx2 induce a decrease in mitochondria function, i.e., a reduction in ATP production and ETCs activity, that is associated with an increase in the oxidative damage to DNA, Lipids and proteins relative to wild type animals. Moreover, we observed an increase in apoptosis, the release of cytochrome c from mitochondria and caspase-3 activation in the livers of diquat treated Trx2+/- mice compared to treated wild type mice. Our results suggest that Trx2 plays an important role in protecting the mitochondria against oxidative stress, sensitizing the cells to ROS-induced apoptosis and the maintenance of ATP generation by the mitochondria. (Supported VA Merit Review and NIH grant R01 AG23843)

75. OVEREXPRESSION OF THIOREDOXIN 1 PROTECTS AGAINST OXIDATIVE DAMAGE *IN VIVO* AND ALTERS AGING

Ikeno, Y. (P), Lew, C. M., Cortez, L. A. Webb, C. R., Chaudhuri, A., Qi, W., Yodoi, J., Lee, S., and Richardson, A.

We examined the effect of increased levels of thioredoxin 1 (Trx1) on resistance to oxidative stress and on aging, using transgenic mice overexpressing Trx1 [Tg(*hTRX1*)$^{+/0}$]. This study was conducted because substantial evidence suggests that oxidative stress affects aging, and Trx1 has antioxidant properties. Our results showed that Tg(*hTRX1*)$^{+/0}$ mice had significantly higher Trx1 expression (protein and biological activity) compared to wild-type mice, which was associated with reduced oxidative damage to lipid and protein. Tg(*hTRX1*)$^{+/0}$ mice also showed increased resistance to oxidative stress *in vivo* (diquat treatment). More importantly, the Tg(*hTRX1*)$^{+/0}$ mice extended lifespan compared to wild-type mice during the first half of lifespan, i.e., a 25% increase in the 75% survival and a 13% increase in 50% survival; however, only a 5.5% increase in 25% survival and no increase in 10% survival. We propose that the Tg(*hTRX1*)$^{+/0}$ mice did not show an increase in maximum survival for two reasons: 1) the overexpression of the Trx1 in the Tg(*hTRX1*)$^{+/0}$ mice is significantly reduced with age and/or 2) it

is necessary to have increased expression of Trx in the mitochondria (Trx2) to have maximum impact on aging. (Supported by grant from the VA Merit Review)

76. EXTENDED LIFESPAN AND REDUCED AGE-RELATED PATHOLOGY BY OVEREXPRESSION OF CU/ZN SOD IN RATS

Ikeno, Y.(P), Cortez, L. A., Lew, C.M., Webb, C.R., Qi, W., Chaudhuri, A., Lee, S., and Richardson, A.

We examined the effect of increased levels of Cu/Zn superoxide dismutase (SOD) on oxidative stress and on aging in transgenic rats overexpressing Cu/ZnSOD [Tg(*hSOD1*)$^{+/0}$]. This is the first study to use transgenic rats to test the oxidative stress theory of aging. A previous study using transgenic mice showed that overexpressing Cu/ZnSOD had no effect on lifespan. Our results showed that Tg(*hSOD1*)$^{+/0}$ rats had significantly higher Cu/ZnSOD activity compared to wild-type rats without a down-regulation of other major antioxidant enzymes. The increase in Cu/ZnSOD activity was associated with lower levels of oxidative damage to DNA, lipid, and protein. More importantly, Tg(*hSOD1*)$^{+/0}$ rats showed a significant increase in lifespan (12%) and reduced age-related pathology compared to wild-type littermates. These data demonstrate that overexpressing Cu/ZnSOD has a different effect on lifespan in rats and mice. Our data would be consistent with the view that oxidative stress might play a greater role in the lifespan of rats than mice. (Supported by grant from the VA Merit Review)

77. CHANGES IN THE RELEASE OF NITRIC OXIDE AND PROSTAGLANDINS FROM AGED CORONARY ARTERIES STIMULATED BY UROTENSIN II.

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Endothelial function is modulated by aging. The objective of this study was to elucidate whether aging influences urotensin II-induced coronary vasodilatation, and whether aging influences the production of endothelial factors in response to urotensin II. We examined the effects of urotensin II on coronary flow in Langendorff-perfused rat hearts. The production of nitric oxide (NO), prostacyclin and prostaglandin (PG) E_2 were determined in the coronary effluent of both young and aged rats. Urotensin II increased coronary flow in Langendorff-perfused hearts in both young and aged rats and vasodilatation did not differ between young and aged rats. N^G-nitro-L-arginine (a NO synthase inhibitor), significantly inhibited urotensin II-induced vasodilatation in young rats, but not in aged rats. In addition, urotensin II increased the production of NO only in young rats. On the other hand, the cyclooxygenase inhibitor diclofenac significantly attenuated the urotensin II-induced coronary vasodilatation in both young and aged rats. Urotensin II markedly increased the release of the vasodilating prostacyclin and PGE_2 into the coronary effluent. Production of these prostanoids was maintained even in the aged coronary arteries. These results indicate that the production of NO in the coronary endothelium is impaired in aged rats, and that prostacyclin and PGE_2 may play an important role in regulating urotensin II-induced coronary vasodilatation.

78. COMPARING COLONOSCOPIC RESULTS IN ASYMPTOMATIC AND SYMPTOMATIC PATIENTS

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Study: We examine and compare data for a group of patients referred for colonoscopy in an open-access system with specific indications for endoscopy with a group of patients referred for screening colonoscopy.

Methods: We examine charts for two hundred and twenty-two patients who are referred for colonoscopy in a busy academic center with open-access referral from primary-care physicians. Of those patients 110 were referred only for screening colonoscopy, with their only indication being age greater than fifty. One hundred and twelve have specific indications including gastrointestinal bleeding (34%), previous history of polyps (29%), anemia (13%), change in bowel habits (11%), family history (3%), mass on examination (3%), and other (4%).

Results: Of those patients with specific indications, 42 (38%) had a normal examination, with other results including tubular adenoma, tubulovillous adenoma, and hyperplastic polyps (33%), adenocarcinoma (2%), colitis (2%), internal hemorrhoids (7%), and arteriovenous malformation

(2%). Of the 110 patients referred without specific signs or symptoms, 55% have normal examinations, with other results including tubular adenoma, tubulovillous adenoma, or hyperplastic polyp (38%), arteriovenous malformation (3%), internal hemorrhoids (1%) and severe diverticulosis (1%). Seven percent of patients in the indications group have indications not adherent to guidelines of the American Society of Gastrointestinal Endoscopy including chronic pain (3%), weight loss (3%), and history of hemorrhoids (1%). Conclusions: Patients with specific symptoms are more likely to have positive results on colonoscopy and have similar results in percentage of polyps found compared to the screening population. The prevalence of adenocarcinoma, colitis, internal hemorrhoids, and severe diverticulosis is greater for patients with significant indications for colonoscopy. The presence of an equivocal percentage of polyps for patients without a significant history or symptoms supports the efficacy of the appropriate use of colonoscopy as a screening tool.

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79. CALORIE RESTRICTION PREVENTS AGE-RELATED CHANGES IN THE LIVER SINUSOIDAL ENDOTHELIUM

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Aging is associated with thickening and defenestration of the liver sinusoidal endothelium called pseudocapillarization. These changes impede the exchange of substrates, such as neurotoxins and lipoproteins, across the endothelium, contributing to the pathogenesis of a wide range of age-related diseases including cardiovascular disease. In this study we assessed the effect of CR on the age-related changes in the liver sinusoidal endothelium. Biopsies of livers from young (6 months, 5 CR, 5 AL) and old (24 months, 5 CR, 11 AL) rats were processed for immunohistochemistry and electron microscopy. Young CR rats had a higher fenestrae frequency than young AL rats (10.7 ± 3.7 vs 8.0 ± 3.5 fenestrae / mm2 liver sinusoidal endothelium). Old CR rats also had a higher fenestrae frequency than old AL rats (10.8 ± 3.8 vs 7 ± 3.7 fenestrae / mm2 liver sinusoidal endothelium, $p<0.001$). Endothelial porosity was increased in the young CR compared to the young AL rats (4.3 +/- 1.4 vs 3.4 +/- 1.5%) and in the old CR compared to the old AL rats (3.9 +/- 1.7 vs 2.4 +/- 1.0%). Hepatic endothelial thickness was also increased in the old AL rats compared to the young AL rats (236 ± 106nm vs 180 ± 136nm, $p<0.001$). However, CR prevented this increase (old CR 190 ± 143 vs old AL 236 ± 106nm, $p<0.05$). Immunohistochemistry showed that CR prevented both the age-related decrease in caveolin-1 expression and age-related increase in peri-sinusoidal collagen IV staining. In conclusion, CR attenuates age-related pseudocapillarization. Understanding the mechanisms underlying pseudocapillarization and how they are prevented by calorie restriction. may lead to the development of novel treatments for the prevention of age-related diseases.

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80. MODELING EARLY AGE-RELATED MACULAR DEGENERATION BY INDUCING OXIDATIVE STRESS

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Age-related macular degeneration (AMD) is a degenerative eye disease that accounts for the majority of irreversible severe visual loss in the elderly population of industrialized countries. We tested the theory that oxidative damage plays a role in the development of AMD by using Adeno-Associated Virus (AAV) to deliver ribozymes to the retinas of mice to knock down the levels of manganese superoxide dismutase (MnSOD). This enzyme mitigates the levels of reactive oxygen species (ROS). An active and an inactive version of a ribozyme (Rz432) that targets MnSOD mRNA (SOD2) were cloned in a plasmid containing the CMV-beta actin promoter and a GFP marker gene. These constructs were packaged in AAV1. Adult wild type C57BL/6 mice were

injected in the subretinal space in the right eye with active ribozyme, control ribozyme or GFP only. At 1, 2 and 4 months post injection, retinal response to light was measured, and changes in retinal structure were examined by histology. We also assayed for levels of MnSOD, markers of oxidative damage and cell death. The mice showed a progressive loss of light response due to active ribozyme. In the retinas treated with active ribozyme, we also observed shortening and disorganization of photoreceptors, condensed chromatin, thinning of the outer nuclear layer, and loss of pigmentation and vacuolization of the retinal pigment epithelium (RPE). SOD2 knockdown caused a decrease in MnSOD protein levels, increased oxidative damage to proteins and the formation of apoptotic cells. Since AAV1 leads to transduction of the RPE, our results suggest that the primary effect of SOD2 Rz432 is on the RPE and the retinal changes we observed were the consequences of RPE damage caused by an increased ROS burden. Thus oxidative damage to the RPE may contribute to pathogenesis similar to that occurring in early AMD.

81. SIRT1 FAILS TO AFFECT p53-MEDIATED BIOLOGICAL ACTIVITY

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The SirT1 gene encodes a protein deacetylase that acts on a number of nuclear substrates. p53 was identified as a SirT1 substrate whose transcriptional activity was reported to be negatively regulated by SirT1-dependent deacetylation. It has been suggested that mice with hyperactive p53 age prematurely. These mice share some characteristics with mice lacking SirT1, indicating that SirT1 knockout mice may represent an early-aging phenotype. We set out to determine whether developmental defects and perinatal lethality observed in SirT1-null mice were caused by p53 hyperactivity by creating mice deficient for both SirT1 and p53. Animals null for both proteins were smaller than normal at birth, had eyelid opening defects and died during the late prenatal and early postnatal periods, a phenotype indistinguishable from mice deficient for SirT1 alone. Upon re-examination of the role of SirT1 in modulating p53 activity, we found that while SirT1 interacts with p53, the SirT1 protein had little effect on p53-dependent transcription of transfected or endogenous genes and did not affect the sensitivity of thymocytes and splenocytes to radiation-induced apoptosis. These findings suggest that SirT1 does not affect p53-mediated biological activities despite the fact that acetylated p53 has been shown to be a substrate for SirT1.

82. SEX, AGE AND DIET-SPECIFIC EFFECTS OF S6 KINASE GENE ON ENERGY METABOLISM AND INNATE IMMUNE RESPONSE IN *DROSOPHILA MELANOGASTER*

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An accumulating body of evidence has revealed a tight link between obesity and inflammation which in turn predisposes individuals to insulin resistance and type 2-diabetes. The *S6 kinase* (*S6k*) gene codes for a ribosomal protein that has been shown to play an evolutionarily conserved role in cell growth and ribosome biogenesis. We are using *D. melanogaster* as a model system to examine the potential effect of variation in the *S6k* gene on energy metabolism and innate immune response. We used virgin offspring of the cross between females of two laboratory fly strains, Oregon R and 2b, to male heterozygotes with one deficient copy of the *S6k* gene to examine the allelic effects of *S6k* on glycogen levels, fat storage and the ability to clear an artificially induced bacterial infection. To examine the influence of age, diet and genetic variation in this gene we measured each of these traits at one and four weeks of age under regular and restricted diets. Our results provide compelling evidence that genetic variation in the *S6k* gene affects both energy storage and immune function, but these effects are dependent on the sex and age of the flies as well as on nutritional conditions.

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83. EFFECT OF CALORIE RESTRICTION ON THE AGE-RELATED DECLINE OF CHAPERONE-MEDIATED AUTOPHAGY

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Autophagy is a conserved catabolic process responsible for the turnover of intracellular components in lysosomes, and critical for the maintenance of cellular homeostasis. We have previously reported decreased activity with age of a particular form of autophagy, chaperone-mediated autophagy (CMA). During CMA, selective cytosolic proteins are targeted to the lysosome-associated membrane protein type 2A (LAMP-2A), the receptor for this pathway, subsequent to their binding to a cytosolic chaperone/co-chaperone complex. At the lysosome membrane, the substrate protein unfolds and translocates into the matrix for degradation. The age-related decline in CMA is caused by a decrease in lysosomal levels of LAMP-2A with age. In this work, we investigate the effect of caloric restriction (CR) on CMA activity both, in livers of CR rodents and in an in vitro model of CR with cultured fibroblasts. In both models, we have found that CR results in constitutive activation of CMA by preventing the age-related decrease in the levels of the lysosomal receptor. In contrast to the slow turnover observed for LAMP-2A in the lysosomal membrane of old rodents, CR maintained LAMP-2A degradation rates similar to the ones observed in the younger animals. We have recently shown sequestration of LAMP-2A in lysosomal membrane microdomains during CMA inactivation, but exclusion of the receptor from these cholesterol, glycosphingolipid-rich regions upon activation of this pathway (i.e. starvation and mild oxidative stress). We now demonstrate an age-related decrease in the levels of LAMP-2A in membrane microdomains and the reversal of this trend upon CR. Interestingly, these microdomains are enriched in the protease responsible for LAMP-2A cleavage, becoming the specific site where LAMP-2A turnover initiates. Age-related changes in the lipid composition of the lysosomal membrane could thus be behind the impaired degradation of LAMP-2A in lysosomes in old organisms.

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84. EFFECT OF EXERCISE AND DRUG INTERVENTIONS ON HEAT SHOCK PROTEIN PRODUCTION (HSP) AND SKELETAL MUSCLE FUNCTION DURING AGING.

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Muscle strength decreases by 30-40% by the age of 70 leading to increased risk of falls. Muscle of aged mammals is more susceptible to exercise-induced damage and has an impaired ability to recover. Muscles from young individuals adapt to prevent damage following exercise by increased production of HSPs (1). Induction of HSPs is impaired in older individuals (1). Transgenic studies demonstrated that increased content of HSP70 throughout life provided protection against exercise-induced damage and facilitated a full recovery (2). This study examined the effect of treadmill training or pharmacological interventions on the stress response and susceptibility to exercise-induced damage in aged mice. Twelve month old mice were either subjected to 10 weeks of treadmill running or treadmill trained for 12 months. Maximum tetanic force of EDL muscles was determined. EDL muscles were subjected to lengthening contractions. Force deficit was assessed 3 hrs and 28 days following the contractions to determine susceptibility to and recovery from damage. The 10-week protocol resulted in a significant increase in HSP70 content of hindlimb muscles compared with muscles of untrained mice. The 12-month protocol had no significant effect on maximum tetanic force or force deficit of the EDL muscle 28 days following lengthening contractions; control and trained mice had a mean 41% and 30% force deficit respectively. Investigations into pharmacological elevation of HSPs, identified 17-(allylamino)-17-demethoxygeldanamycin (17AAG), which increased HSP70, HSC70 and HSP25 in C2C12 myotubes. In-vivo, treatment by I.P. injection of 1.2mg per day for 2 days resulted in a significant increase in the HSP70 in hindlimb muscles. The effect of longer-term 17AAG treatment on ability of muscles to increase HSPs following non-damaging exercise will be

presented.
This work is funded by Research into Ageing.
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85. MITOCHONDRIAL DNA DELETIONS ARE ABUNDANT AND CAUSE FUNCTIONAL IMPAIRMENT IN AGED PIGMENTED NEURONS OF HUMAN SUBSTANTIA NIGRA.

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Involvement of somatic mitochondrial DNA (mtDNA) mutations in aging has been controversial for many years, primarily because of the presumably low abundance of mutations in aged tissues. The total burden of deleted mtDNA is difficult to measure, however, and has not been determined in most tissues including brain. We developed a novel single molecule PCR approach to measure mtDNA deletions in individual cells and used it to study the substantia nigra, a prominent site of neurodegeneration in Parkinson's disease. We show that pigmented neurons of the aged but not the young human substantia nigra contain very high levels of deleted mtDNA. Deleted mtDNA molecules are largely clonal within each cell, i.e. most originate from a single initial mutational event. A large proportion of pigmented neurons in the aged substantia nigra are deficient in cytochrome c oxidase (COX), a mtDNA-encoded enzyme. The fractions of mtDNA deletions in COX-deficient neurons are significantly higher than those in COX-positive cells. The dopaminergic neurons of the human substantia nigra may thus be an example of a cell type where somatic mtDNA mutations cause degenerative age-related alterations. Reference: Y. Kraytsberg, E. Kudryavtseva, A. C. McKee, C. Geula, N. W. Kowall, and K. Khrapko. Nature Genetics (in press; e-publication date: 9 April, 2006)

86. AGE-RELATED CHANGES IN ANTIOXIDANT ENZYME ACTIVITIES IN THE BRAIN AND LIVER OF BN/BI RATS: STRIKING DIFFERENCES FROM THOSE IN F344 RATS

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Past reports on age-related changes in antioxidant enzyme activities in animals are controversial. The present study aimed to clarify possible sex and strain related differences in changes in antioxidant enzyme activities caused by aging in rats. Antioxidant enzyme activities such as superoxide dismutase (SOD) and catalase(CAT) were compared in several brain regions and the liver between young (8-9 months) and old (27-29 months) BN/BiRijHsd rats of both sexes obtained from Harlan Sprague Dawley (Indianapolis, IN). CAT activities in brain regions were quite comparable between young and old rats of both sexes. SOD activity changes with age also were not remarkable, with the exception of significantly lower Mn-SOD activities in Substantia nigra (S. nigra) of old male rats and significantly higher activities of Cu,Zn-SOD in the same region of old female rats in comparison with respective values in young rats. CAT activities in the liver tended to be lower in old male rats in comparison with young counterparts, while in females the opposite was observed. SOD activities in the liver stayed essentially unchanged with age in males, while in females total as well as Cu,Zn-SOD activities were more than 2 fold higher in old animals. In contrast with these new findings, we previously reported marked (2-3 fold) increases in SOD activities in old F344/DuCrj male rats compared with the young in most brain regions studied, while in female rats SOD activities remained essentially unchanged with age1). Further, other authors reported an age dependent-decline in SOD and CAT activities in the brain and liver in F344/N male rats2,3). These data taken together indicate that no generalization can be made in terms of age-related changes in antioxidant enzyme activities, even in the same species, sex and organs. Accordingly, much reservation is needed in any attempt to explain mechanisms of aging based on these enzyme activity changes with age.

References

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87. SOY ISOFLAVONE EXTRACT FAILS TO IMPROVE MEMORY IN POSTMENOPAUSAL WOMEN

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There have been mixed findings in human studies investigating the effects of soy-derived isoflavones on neurocognitive function. Isoflavones have weak estrogenic effects and have been thought to have the potential to enhance memory ability in women. Further, they have antioxidant and anti-inflammatory properties, which would confer additional benefit for neural function and cognition in aging adults. However, factors such as menopausal status and the nature of the matrix in which isoflavones are delivered may affect whether and to what extent benefit is realized. We report data from an ongoing double blind, placebo controlled trial in postmenopausal women with mild memory changes. Ninety-two women were randomly assigned to receive either 150 mg/day soy isoflavone extract or placebo. Measures of memory ability were obtained prior to and at the end of the 16-week intervention. There was no difference between the groups in age (64 v 62), years of education (14.2 v 14.3), IQ (102 v 101), or time since menopause (204 v 175 months). Analysis of variance indicated no difference in memory performance on either paired associate learning (11.2 v 13, $F=1.77$, $p=.18$) or list learning (11.5 v 12.1, $F=.43$, $p=.51$) tasks. These negative findings are not inconsistent with results from a number of studies using estrogen therapy in older postmenopausal women and support the notion that estrogenic agents may be ineffective with respect to brain function when administered after a critical period following menopause. Also, it has become apparent that the antioxidant and anti-inflammatory effects of polyphenols tend to be more efficacious when administered in whole food matrices or in combinations that induce synergistic increments in potency. These factors suggest that future investigation of soy isoflavone effects on neurocognition in women might be focused on the perimenopausal period and on the use of whole soy isoflavone preparations.

88. MICROARRAY-BASED MAPPING OF QTLs AFFECTING LIFESPAN IN DROSOPHILA

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Understanding of the mechanisms underlying the process of aging is crucial to develop effective strategies to prevent and treat age-related diseases. This requires both identifying genes involved in the aging process and understanding how their activities are modulated by environmental factors, such as dietary intake and life style. Drosophila has long been considered an ideal model organism to study aging because of its short lifespan, powerful genetic tools, and small and well-characterized genome with 60% of genes shared with humans. More importantly, Drosophila has adipose-like tissues and a lipid transport system, making it a suitable model for human aging studies. To map QTLs to small chromosomal regions, we crossed two inbred strains, Oregon and 2b, that had previously been characterized for QTLs affecting lifespan, and generated approximately 11,000 F2 males and 11,000 F2 females, which were aged in 3 cohorts. About 15% of young and old flies for each sex were collected from each cohort. We then isolated DNA from each pool. To map QTLs affecting lifespan, we developed a two-color hybridization system with Affymetrix Drosophila expression microarrays to identify single feature polymorphisms (SFPs) between the parental lines, from which about 8000 SFPs were identified at a false discovery rate of 5%. DNA pools of young and old flies from each cohort were labeled by two different dyes and pooled together before each pooled sample was hybridized to a single microarray. The hybridization signal differences of 8000 SFPs between two dyes (young and old)was computed using a linear model fit of log2-transformed expression ratios. We computed moderated /t/ statistics to detect QTLs affecting lifespan. Here, we report the locations of the QTLs identified using this method and compare them with those that had been previously inferred from high resolution recombination and deficiency complementation mapping.

(N)

89. SIR2 HOMOLOGUES REGULATE AGING IN YEAST

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The NAD+-dependent histone deacetylase Sir2 has been shown to regulate both replicative and chronological aging in yeast. Sir2 homologues have been implicated in aging in a variety of higher organisms, including worms, flies, and mice. In yeast, there are four homologues of Sir2 (HSTs), and the roles of these genes in regulating lifespan is generally unclear. Recently, we showed that the Sir2 homologue Hst2 plays a role in replicative lifespan. Overexpression of Hst2 extends replicative lifespan, and Hst2 is required for Sir2-indpendent lifespan extension by calorie restriction (CR). We have now further investigated the role of the HSTs in regulating replicative and chronological lifespan.

(G)

90. INHIBITORY EFFECT OF BLUEBERRY EXTRACT ON THE PRODUCTION OF INFLAMMATORY MEDIATORS IN LPS-ACTIVATED BV2 MICROGLIA

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Microglial activation in the central nervous system (CNS) has been extensively investigated in age-related neurodegenerative diseases. Recent studies have shown that anti-inflammatory drugs and dietary antioxidants may suppress microglial activation and thus protect against these diseases. Blueberry extract is enriched with antioxidants. Our previous studies showed that aged rats on a blueberry supplemented diet significantly improved their cognitive and motor behaviors as compared to that of controls. The current study investigated the effect of blueberry extract on activated microglia. We found that treatments with blueberry extract significantly and dose-dependently inhibited the production of inflammatory mediator nitric oxide (NO) as well as cytokines interleukin-1 beta and tumor necrosis factor alpha in cell conditioned media from lipopolysaccharide (LPS) activated BV2 microglia. Also, mRNA and protein levels of inducible NO synthase and cyclo-oxygenase 2 in LPS-activated BV2 cells were significantly reduced by treatments with blueberry extract. The results suggest that blueberry polyphenols attenuate inflammatory responses of brain microglia and could be potentially useful in the modulation of inflammatory conditions in the CNS.

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91. THE STRONG LIVING PROGRAM: DISSEMINATION OF A COMMUNITY-BASED GROUP EXERCISE PROGRAM FOR OLDER ADULTS THROUGH EXERCISE LEADERSHIP TRAINING AND CERTIFICATION.

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Purpose: Reducing the prevalence of physical inactivity is a leading national health objective. This is particularly important for older adults who have the highest prevalence of no physical activity participation and the lowest participation rate in all other fitness categories. **Methods:** The Strong Living Program is a model for establishing self-sustaining, community-based group exercise classes for older adults. The model has two main components: 1) a moderate intensity, progressive exercise program including strength training, balance and flexibility exercises; and 2) a training curriculum and certification process for health care professionals and laypersons to disseminate the program in their communities. Women and men ≥40 yr of age who obtain medical clearance for exercise are eligible to participate. **Results:** Ninety-one (91) Strong Living Program classes have been established in three New England states. Exercise classes meet 2x/wk for 1 hr at convenient community locations. Certified leaders (n=224) who instruct the classes are both laypersons (63%) and health care professionals (37%). Professional leaders

include nurses (30%), physical therapists (25%), health educators (24%), and fitness instructors (17%). Professional leaders are younger than lay leaders 55.1±14.0 yr vs. 62.8±12.1 yr. Participants (n=1,759) are primarily older women (n=1,553 or 88%) with an average age of 72.0±9.7 yr (range 40-97). Men (n=206 or 12%) are 75.3±8.7 yr of age (range 50-94). The average participant has 3.7±2.0 chronic medical conditions. The most prevalent conditions are hypertension (48%), hyperlipidemia (38%), osteoarthritis (37%), osteoporosis (27%), and depression (15%). **Conclusions:** Training community leaders to implement a multi-dimensional exercise program is a feasible and effective model for increasing exercise participation among older adults with multiple chronic health conditions. The long-term goal of this research is to develop the Strong Living Program into a nation-w wide public health initiative to increase physical activity and improve health-related quality of life with aging.

92. ASSOCIATION BETWEEN SERUM INSULIN-LIKE GROWTH FACTOR-1 AND BRACHIAL-ANKLE PULSE WAVE VELOCITY IN HEALTHY KOREAN ADULTS.

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Background: The activity of the growth hormone(GH)-IGF axis declines in midlife, leading to relative GH and IGF-1 deficiency. Several previous studies have suggested that the decline in IGF-1 and/or GH secretion may play a role in the development of cardiovascular disease in adult GH deficiency. Brachial-ankle pulse wave velocity (baPWV) is a good marker of arterial stiffness which is an independent and strong predictor of cardiovascular disease, and reflects aging of arterial system. The aim of the present study was to investigate whether serum IGF-1 concentrations were associated with baPWV in healthy adults. Methods: A total of 306 healthy participants (84 Males and 222 females) aged more than 40 years were included. We measured serum IGF-1, lipid profiles, homocystein, c-reactive protein (CRP) and fasting blood sugar(FBS). As a estimate of arterial stiffness we measured baPWV, and also anthropometric measurements was checked Results: Mean ages of study population were 51.9±8.1 in men, and 55.2±6.3 in women. In men, baPWV was positively correlated with age ($r=0.41$, $P<0.001$), systolic and diastolic blood pressure ($r=0.42$, $P<0.001$ and $r=0.43$, $P<0.001$ respectively), homocysteine ($r=0.33$, $P<0.005$), C-reactive protein ($r=0.23$, $P<0.05$) and negatively associated with IGF-1($r=-0.22$, $P<0.05$). In women baPWV was positively associated with age ($r=0.49$, $P<0.001$), waist and hip ratio ($r=0.26$, $P<0.001$), systolic and diastolic blood pressure ($r=0.53$, $P<0.001$ and $r=0.43$, $P<0.001$ respectively), LDL cholesterol ($r=0.23$, $P<0.001$), and homocystein (0.14, $P<0.05$), but was not associated with serum IGF-1 levels. On regression analysis, IGF-1 was found to be an independent factor for baPWV in men, but not in women after adjustment was made for possible confounders. Conclusions: IGF-1 concentrations were negatively correlated with baPWV in men aged more than 40 years. This suggests that low IGF-1 may be associated with arterial stiffness in men.

93. AGE REALTED INCREASE IN PLATELET ACTIVATION

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Objective: Platelets clearly play an important role in inflammatory responses. In this cross sectional study, we aimed to evaluate the relationship between aging and platelet activation. Participants: A total number of 799 persons (383 males and 416 females), who were apparently healthy and aged more than 20 years were recruited by a health promotion center in a community-based hospital in Seoul, Korea. We collected material data about their medical history and health behavior. Measurements: For evaluating platelet activation, we collected 5 ml blood in a vacuum tube containing EDTA as the anticoagulant. Platelet parameters including mean platelet component (MPC), mean platelet volume (MPV), and platelet component distribution width (PCDW) were determined within 1 hour after blood collection using the ADVIA 120

automated hematology analyzer (Bayer, Tarrytown, NY, USA). Results: The mean MPC of the women (27.2±1.2) was significantly lower than that of the men (27.5±1.3). The mean MPC of all participants was found to decrease with increasing age (P<0.001). Study participants in their twenties had the highest mean MPC (27.7±1.1), followed by those in their thirties (27.6±1.1), forties (27.4±1.3), fifties (27.2±1.3), sixties (27.2±1.2) and seventies (27.1±1.2). Multiple regression analysis showed that aging and sex were related with MPC after adjusting for confounding factors, including age, sex, smoking habit, hypertension, diabetes, body mass index and total cholesterol level. Conclusion: The present study shows that aging is related to platelet activation. Future research will need to determine the implications of increased platelet activation with aging, especially regarding the increased incidence of cardiovascular diseases and related mortalities that occur in older age groups.

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94. SIRT1 REGULATES LXR AND REVERSE CHOLESTEROL TRANSPORT

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The NAD+-dependent deacetylase Sir2 and its homologues regulate lifespan in lower eukaryotes. The mammalian orthologue, SIRT1, regulates a number of ageing-related processes including apoptosis, fat metabolism, glucose homeostasis, and neurodegeneration. Here we show that SIRT1 regulates Liver X Receptors (LXRs) mediated cholesterol homeostasis. SIRT1 deficiency in mice reduced plasma HDL and elevated tissue cholesterol, suggesting that SIRT1 regulates reverse cholesterol transport. SIRT1 formed a complex with LXRs, nuclear receptors that function as cholesterol sensors, and thus promoted the expression of ABCA1, an ATP-binding cassette (ABC) transporter that mediates one of the first and rate-limiting steps of reverse cholesterol transport. Loss of SIRT1 reduced expression of ABCA1 and a variety of LXR targets involved in lipid metabolism, blunted the normal response to a LXR agonist, and compromised cholesterol efflux in mouse embryonic fibroblasts and human monocytes. SIRT1 deacetylated LXR to promote its turnover and up-regulate its activity. Our findings suggest that SIRT1 may affect ageing and age-associated diseases by modulating cholesterol homeostasis.

(N)

95. GLUTATHIONE PEROXIDASE 4 KNOCKOUT MICE ARE SENSITIVE TO OXIDATIVE STRESS INDUCED APOPTOSIS AND SHOW EXTENDED LIFESPAN

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Glutathione peroxidase 4 (Gpx4) is a unique enzyme that detoxifies oxidative damage of biomembranes. Experiments using cell lines show that overexpression of Gpx4 protects against oxidative stress induced apoptosis. In this study, we used Gpx4 knockout mice (*Gpx4*$^{+/-}$ mice) to investigate the underling mechanism by which Gpx4 regulates oxidative stress induced apoptosis *in vivo* using diquat injection as a model of oxidative stress. We found that diquat strongly induced apoptosis in the liver; the level of apoptosis was further increased in the *Gpx4*$^{+/-}$ mice. The induction of apoptosis by diquat occurred through mitochondria, as evidenced by cytochrome c (cyt. c) release from the mitochondria; the level of cyt. c release was increased in the *Gpx4*$^{+/-}$ mice as well. Diquat induced cyt. c release was paralleled by cardiolipin (CL) peroxidation, and *Gpx4*$^{+/-}$ mice showed more CL peroxidation. These data suggest that Gpx4 plays a critical role in the regulation of apoptosis *in vivo* by suppressing CL peroxidation mediated cyt. c release. We also conducted the lifespan measurement of the *Gpx4*$^{+/-}$ mice. Surprisingly, *Gpx4*$^{+/-}$ mice had a significantly increased median lifespan compared to wildtype controls. Because *Gpx4*$^{+/-}$ mice are sensitive to the induction of apoptosis, we hypothesized that *Gpx4*$^{+/-}$ mice are more efficient in

eliminating damage cells that contribute to tumor/loss of tissue functions and therefore show extended lifespan.

96. SERUM FROM CALORIE RESTRICTED RATS INCREASE SIRT1 AND EXTENDS LIFESPAN OF NORMAL HUMAN FIBROBLAST CELLS

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Calorie restriction (CR) is the only reliable and reproducible method for increasing lifespan and slowing the rate of aging in a wide range of species from yeast to mammals. In yeast and worms, CR extends lifespan by increasing the expression of the silence information regulator-2 (Sir2) gene. In a recent study (Cohen et al. Science, 305: 390, 2004), we showed that the mammalian SIRT1 protein, an analogue of Sir2, was increased in most tissues from rats on 40% CR, but the molecular and cellular mechanisms by which Sir2 acts remain unknown. In the present study, we used again the in vitro model of CR (de Cabo et al., Exp. Gerontol 38: 631, 2003) to assess the effects of serum from rats on ad libitum (AL) or CR (40%) diets on SIRT1 protein expression and the in vitro life span of normal human fibroblasts cells (IMR-90 and WI-38). CR remarkably delayed the replicative senescence and decreased expression of the senescence-associated beta-galactosidase activity (SA-beta-gal) in the fibroblast cultures. Levels of the endogenous nuclear SIRT1 protein in fibroblasts decreased with cellular senescence. The level of SIRT1 protein expression was much higher in fibroblasts grown in CR serum compared to AL serum treated cells. Notably, CR treatment increased fibroblast lifespan by 1.4-fold compared to AL serum treatment ($P<0.001$). These results suggest that CR serum is able to upregulate SIRT1 levels in normal human fibroblasts and delay cellular senescence in vitro.

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97. THE PRODUCTION OF REACTIVE OXYGEN SPECIES BY MITOCHONDRIA IN LIVING CELLS: MODULATION BY PATHOPHYSIOLOGICAL FACTORS AND THE SITES OF GENERATION

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The production of reactive oxygen species (ROS) by mitochondria in living cells was studied in normal cells as well as in cells under three pathophysiologically relevant conditions where mitochondrial ROS is directly related to cell death: oxidative glutamate toxicity, the ATP synthase inhibitor oligomycin-induced cytotoxicity and tumor necrosis factor-induced cell death. It was found that mitochondrial ROS production in living cells is much more complex than what has been revealed by in vitro studies. There are at least four ROS-generating sites in the mitochondrial electron transport chain in living cells: the flavin mononucleotide (FMN) group of complex I and the three ubiquinone-binding sites in complexes I, II and III. These sites are differentially accessible to commonly used antioxidants. Most of the existing antioxidants are not effective at scavenging ROS around these sites. Therefore new drugs that can inhibit ROS production around the ubiquinone-binding sites within complexes I to III may prove to be the most useful in delaying aging and mitochondrial ROS–related diseases. In addition, vitamin E may be a useful treatment for patients with ATP synthase deficiency because it protected cells from oligomycin-induced cell death.

98. DEFICIENCY OF LON PROTEASE IN *ESCHERICHIA COLI* SHORTENS ITS LIFE-SPAN DURING ANAEROBIC CARBON STARVATION

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Lon is one of the most evolutionarily conserved ATP-dependent proteases, with homologues identified in bacteria, yeast, and mammals, suggesting Lon may perform essential functions in

cells. Downregulation of human Lon impairs mitochondrial function and causes cell death. In *Escherichia coli*, the Lon protease, together with the Clp proteases, is responsible for approximately 70% of protein degradation. Lon degrades abnormal proteins and a number of rapidly degraded regulatory proteins. During amino acid starvation, accumulated inorganic polyphosphate forms a complex with Lon and free ribosomal proteins, facilitating degradation of the ribosomal proteins to supply amino acids for the synthesis of starvation proteins. Here we report that deficiency of Lon protease in *E. coli* K-12 strain caused a more rapid loss of viability during prolonged anaerobic carbon starvation (99.3% vs. 81% for wild type after 7 days carbon starvation). In contrast, during aerobic starvation, there was no difference between the two strains. DNA microarray measurement of cells starved for one day demonstrated that the expression of clpA, clpP, clpS, and clpX were all lower than the wild type under anaerobic condition, while all were higher than the wild type when starved aerobically. It has been reported that clpP or clpX mutants die more rapidly than the wild type during aerobic carbon starvation. Thus, the Clp proteases appear to compensate for loss of Lon during aerobic carbon starvation. While the mechanism remains to be studied, our results demonstrate that the *E. coli* Lon protease is required for prolonged anaerobic survival under the stress of starvation.

(# Both authors contributed equally to this work.)

99. 3-DEOXYGLUCOSONE- A NEW TARGET FOR ANTI-AGING TREATMENTS

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Proteins that have a relatively low turnover rate, such as collagen and elastin can be especially sensitive to non-enzymatic protein glycation. One type of glycation, a glucose modification of amines (Amadori product), is removed by fructosamine-3-kinase (F3K), a novel enzyme that phosphorylates fructoselysine. Although the non-modified amino acid is ultimately restored by this process, a byproduct of the reaction is 3-deoxyglucosone (3DG), a highly reactive and toxic molecule. 3DG mediates numerous downstream effects, including inflammation, oxidative stress and formation of advanced glycation endproducts (AGEs). AGEs contribute to the stiffness and loss of elasticity associated with aging tissue. The activity of 3DG directly or its formation of AGEs are thought to have a role in diabetic complications, aging and atherosclerosis. As therapies for these and other diseases, Dynamis has been working to identify mechanisms to decrease 3DG, including inhibiting the F3K enzyme and inactivating 3DG. While pursuing this research, we noticed that animals treated with a F3K inhibitor showed improved skin elasticity. Following this reasoning, our scientists were the first to document that 3DG is present in skin and that the F3K gene is expressed in skin tissue. Results from three independent clinical trials have shown that the combination of a 3DG inactivator and an alternative F3K substrate in a topical cream have beneficial properties for aging skin. Tests on 75 volunteers with photo-aged facial skin showed a statistically significant, quantitative decrease in fine facial lines, an increase in moisturization and an increase in skin firmness after 2 weeks of twice daily application. There was a further benefit in all of these measurements after 4 weeks of treatment. These results indicate that the 3DG pathway is a viable target for anti-aging therapies, including treatments for aged skin.

100. HEALTH STRATEGY RECOMMENDATIONS OF 2003-2005 AMERICAN AGING ASSOCIATION ANNUAL MEETING ATTENDEES

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Data collection and initial analysis took place at the 2003, 2004 and 2005 annual meetings of the American Aging Association in Baltimore, Maryland, St Petersburg, Florida and Oakland, California. Subsequent data analysis was conducted at the MMT Corporation, 1 Barlow Farm Road, Sherman, CT 06784.

Scientists attending American Aging Association annual meetings have been informally polled each year since 2003 to obtain a health strategy list suitable for participants of the 2002-2006 Blueberry Health Study. Results of this survey have been provided to study participants as a reward or thank you for their weekly health reports and online memory and decision speed measurements, to increase satisfaction with the study and boost compliance and retention.

Recommendations provided by highly-informed scientists serve as an option list that study participants can discuss with their physicians and other professional health advisors. These recommendations can also enable scientists to learn whether current evidence for any health strategy is sufficiently compelling to attract widespread support among those attending AAA annual meetings. The 2005 survey included questions about respondent age (mean age=50.4 range 22-80) and number of refereed publications (mean=43.9, range 0-400+). Among over 200 health strategy recommendations, the most commonly mentioned were fruits and vegetables (listed by 38 respondents), exercise (22 respondents), multivitamins (22), fish, fish oil and omega 3 fatty acids (17), berries (10), vitamin C (9), vitamin E (6) and green and black tea (6). The diversity of recommendations received is noteworthy: over 76 separate health strategies were listed by 5 or fewer respondents. A password-verified web page and associated database table have been posted online to enable American Aging Association members to contribute to and comment on this list of health strategies during 2006 (Blueberrystudy.com/healthstrategies). Our thanks to respondents for their responses and recommendations. Health strategies on this list are suggestions by individual conference attendees and are not endorsed by the American Aging Association.

101. ONLINE STUDY RESOURCES FOR GERONTOLOGISTS.

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MMT Corporation, 1 Barlow Farm Road, Sherman, CT 06784. Residents of Sherman, New Fairfield, Danbury, Bethel and Mansfield, Connecticut and surrounding towns are participating in the Blueberry Study.

The following resources have been posted for use by gerontologists: (1) Cognitive performance measurement pages including word recall, decision speed, arithmetic, reaction time and general vocabulary assessment. Measurement precision is sufficient to detect a 0.5%-1% change at 95% confidence during a 2-year study period. These data collection pages now generate their own "back end" database tables so that entering a new study name is all that is required to form necessary primary and real-time backup tables. Terms-of-use restrictions apply. (2) Free radical-antioxidant reaction rate constants from the NDRL/NIST database sorted so that the most kinetically active nutrient and pharmaceutical antioxidants, including several antioxidant amino acids, are listed first (vitamins C and E are relatively slow-acting by this measure). (3) Antioxidant amino acid analysis and mapping software that creates maps of cysteine, tyrosine, histidine, tryptophan and methionine resides and identifies and quantifies antioxidant amino acid clusters. It can process up to 500 protein sequences at a time and is designed to enable investigators to determine if changes in expression of large gene sets can have a significant impact on the intracellular location and concentration of kinetically-active antioxidant amino acids. (4) Antioxidant amino acid composition tables containing each peptide in the human and chimpanzee genomes – to enable comparison of antioxidant-rich and poor peptides in these species. (5) Links to study design and data set analysis tools, including a coefficient of variation calculator for large, heterogeneous data sets. Properly “powered” studies will only rarely miss the 95% confidence mark. (6) Blueberry Study methods for working with local senior centers and centenarians that can enable other investigators to conduct similar studies with high statistical power and low cost. For further information, please visit Blueberrystudy.com/resources.

102. OCULAR FUNCTION AND PATHOLOGY IN RHESUS MONKEYS: PRELIMINARY FINDINGS

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Age-related macular degeneration (AMD) is the leading cause of blindness in adults over 60 years of age and affects 30% of those over 75. The macula is a specialized area of the retina critical for central vision and acuity which is compromised by the accumulation of drusen. The objective of the current study was to assess age-associated changes in macular structure and function and the effect of a calorie restricted (CR) diet, an intervention known to slow several aging processes. Monkeys housed at the NIH Animal Center, Poolesville and Oregon National Primate Research Center, Beaverton, OR have been examined and evaluated for the presence of drusen and macular degeneration. Preliminary data suggest that consistent with human studies, female rhesus monkeys may be at slightly greater risk but that CR may slow this process. An age regression was not evident in the males or a cohort of younger monkeys. The remainder of the Poolesville colony will be tested over the next year and blood samples are being evaluated to identify genetic markers of AMD to better understand the etiology of this disease.

103. THE EFFECT OF CALORIC RESTRICTION AND GLYCEMIC LOAD ON MEASURES OF OXIDATIVE STRESS AND ANTIOXIDANTS IN HUMANS.

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Jean Mayer, USDA Human Nutrition Research Center on Aging at Tufts University.

It has been suggested that reduction in oxidative stress and increase in antioxidant defense is one potential mechanism by which caloric restriction (CR) increases longevity in several animal models. To determine whether a short-term CR modulates indices of oxidative stress and antioxidants defense in moderately overweight subjects, a total of 46 subjects, age 20-42 yr with BMI of 25-30 kg/m^2 were recruited and randomized to one of 2 dietary groups, i.e., high glycemic (HG) load (60% CHO, 20% protein, 20% fat) or low glycemic (LG) load (40% CHO, 30% protein, 30% fat) with food provided at a level of CR, which was either 10% (n=12) or 30% of their basal caloric intake (n=34) for 6 mo. Blood and urine samples were collected at baseline and after 6 mo of CR. CR intervention for 6 mo significantly (p=0. 002) increased glutathione peroxidase (GPX) activity in plasma compared to baseline (82.53 ±2.87 vs. 75.01 ±2.35 nmol/min/mL), which was more prominent in the 30% CR group on the HG diet (86.60 ±3.53vs.73.69 ±3.00, p=0.004). However, the activity of other indigenous antioxidants such as SOD and catalase were not affected by CR. The level of plasma protein carbonyls was significantly reduced with CR (57.60 ± 1.86 vs.62.23 ± 1.65 nmol/mL, p=0.005). The HG diet for 6 mo significantly reduced plasma protein carbonyl levels in the 30% CR group (54.38 ±3.18 vs. 60.74 ±2.89, p=0.01), but the LG diet marginally (p=0.096) decreased this index of oxidative stress in 30% CR (59.89 ±2.24 vs.64.18 ±2.53). CR was effective in reducing the plasma 8-isoprostane levels (141.21 ± 21.92vs.165.11±23.95 pg/mL, p=0.026); however, the reduction in this index did not reach a statistical significance in each of the diet groups. The urinary 8-OHdG level was not affected by CR or dietary glycemic loads. In conclusion, short term CR either by 10% or 30% in moderately overweight subjects reduces some but not all measures of oxidative stress and antioxidants. HG load in CR may favorably increase plasma GPX, an antioxidant defense enzyme, and decrease oxidative stress to body proteins. Funded by NIA grant: NGA-3U01-AG20480

104. 2-DEOXY-D-GLUCOSE EFFECTS ON SIRT1 PROTEIN LEVELS AND STRESS RESISTANCE IN VITRO

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In anticipation of positive effects on long-term health and lifespan conferred by calorie restriction (CR) to long-lived species--including humans--the hunt is on for viable mimetics of CR. In addition to its beneficial effects on multiple health parameters, CR is known to alter gene expression pathways thought to affect longevity including an enhanced production of proteins involved in cellular stress responses. 2-deoxy-D-glucose (2DG) can act as a glycolytic inhibitor and, thus, a metabolic effector. Treatment of rats with 2DG in the diet has been shown to confer some effects similar to CR and has been proposed a potential CR mimetic. The current experiments were undertaken to further explore the extent to which 2DG can elicit CR-like changes in vitro.

Exposure of FaO and HepG2 cells (rat and human hepatoma cell lines, respectively) to 2DG increased SIRT1 protein expression. Cells pretreated with 2DG were assessed for alterations in survivorship following heat shock or oxidative stress. These changes in cell viability correlate with changes in protein levels of HSP70 and GRP78. 2DG is also an effective antiproliferative agent at low millimolar concentrations on these cancer cells, and 2DG in combination with capsaicin or doxorubicin was observed to be more suppressive than the compounds singularly. The present results continue to suggest avenues through which 2DG could be an effective CR mimetic and warrant further study. A longitudinal in vivo study of the effects of 2DG on health parameters and longevity in the rat has also been initiated.

105. PLASMA MEMBRANE COENZYME Q REDUCTASE PARTICIPATES IN LONGEVITY PATHWAY

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Most aging processes are characterized by the accumulation of damaged macromolecules induced by endogenous oxidative stress. Antioxidants such as coenzyme Q (Q) and its reductases are involved in the prevention of oxidative damage through free radical removal and antioxidant recycling. This system is particularly present in the plasma membrane and is induced in those models that extend life span such as calorie restriction. *YML125c* is a yeast gene encoding for a *cytochrome b_5* reductase mostly concentrated in the plasma membrane. Deletion of this gene is lethal. Over-expression of *YML125c* in *Saccharomyces cerevisiae* increased Q reduction in the plasma membrane and Q-dependent reductase activities. Functional analysis of this protein has shown to increase both replicative and chronological lifespan. Like calorie restriction also increases life span in yeast, we have used this intervention in yeasts and observed that *YML125c* was higher expressed with lower concentrations of glucose indicating then its relationship to longevity. The increase of life span induced by *YML125c* over-expression was independent on sirtuins indicating its possible participation in a Sir2-independent longevity pathway. Respiration is required to improve *YML125c*-dependent extension of lifespan, because yeast strains harbouring defective mitochondria can not growth in over-expression conditions. Preliminary results on *Drosophila melanogaster* over-expressing the homologous Q-reductase showed similar effect on lifespan extension. We provide results that support the requirements of a *cytochrome b_5* reductase at the plasma membrane of yeasts to regulate life span through an aerobic growth that is sirtuin-independent.

106. MECHANISM BY WHICH AVENANTHRAMIDE-C, a POLYPHENOL OF OATS, BLOCKS CELL CYCLE PROGRESSION IN VASCULAR SMOOTH MUSCLE CELLS

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Atherosclerosis is a chronic inflammatory disease which manifests its clinical symptom at a later age. Abnormal growth of smooth muscle cell (SMC) contributes to the initiation and progression of this chronic disease; therefore, nutritional inhibition of the proliferation of SMC is considered to be important for the prevention of atherogenesis or reduction the risk this aging related disease. Avenanthramides (Avn) are unique polyphenols present in oats. We have reported that Avn-c, one of the major forms of Avn with the most antioxidant activity, inhibited the serum-induced cell proliferation of SMC. In the present study, we investigated the mechanism by which Avn-c inhibits proliferation of SMC. Rat embryonic aortic smooth muscle cell line A10 was used in this study. Flow cytometry analysis revealed that treatment of A10 cell with 80 uM Avn-c arrested the cell cycle in G1 phase as indicated by an increase in the G1 cell population (from 49.7% to 66.9%) and a decrease in the number of cells in S phase (from 40% to 25.7%). This cell cycle arrest was associated with a decreased in the phosphorylation of retinoblastoma protein (pRb), whose hyperphosphorylation is a hallmark of the G1-S transition in the cell cycle. The inhibition of pRb phosphorylation with Avn-c was accompanied by a decrease in cyclin D1 expression and an increase in cyclin-dependent kinase inhibitor p21cip1 expression, without significant changes in p27kip1 expression. Avn-c treatment also increased p53 protein expression level and its stability,

which could account for the increase of p21cip1 expression. Our results demonstrate that Avn-c inhibits SMC proliferation and arrests the cell cycle at G1 phase by up-regulating p53-p21cip1 pathway and by inhibiting pRB phosphorylation. This inhibitory effect of Avn-c on SMC proliferation is another indication of the potential health benefit of oats consumption for the prevention of cardiovascular disease. Supported by 58-1950-9-001 CRIS.

107. USE OF TWO-DIMENTIONAL IMMUNO-ASSAY AND MASS SPECTROPHOTOMETRY FOR PROTEOMIC IDENTIFICATION OF CARBONYLATED PROTEINS

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Aging, oxidative stress, and certain nutritional factors are associated with accumulation of oxidatively modified enzymes or proteins: increased levels of protein carbonyls. A quantitative proteomic analysis procedure was used to identify enzymes and proteins that are susceptible to oxidative modification under various conditions of starvation. The whole cell extracts of E. coli K12, grown in aerobic condition under carbon, nitrogen, or phosphate starvation, were examined after dialysis with streptomycin sulfate treatment. 2, 4-Dinitrophenylhydrazine-derivatized proteins were separated in the pH range (4-7) or narrow pH range (4.5-5.5) or (5-6) followed by SDS-PAGE two-dimensional electrophoresis system. The carbonylated protein spots were detected by immuno-Western blot using a fluorescent dye in a secondary antibody, and double stained by chromophore to visualize the protein spots. Specifically targeted protein spots were treated for direct in-membrane tryptic digestion and mass spectrometry. To confirm the identification and approach for quantification, protein spots in SDS-PAGE gel were labeled using fluorescent cyanine dyes: Cy2, Cy3 and/or Cy5, or stained using Coomassie G-250. Proteins were identified by peptide mass finger printing and by tandem mass spectrometry sequencing using MALDI-TOF/TOF MS and LC-MS/MS. The advantage of using this highly sensitive detection method will be discussed.

108. DOES TWO ATMOSPHERIC (ATM) PRESSURE PROMOTE CELLULAR SENESCENCE?

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There have been two separate areas of biological research related with pressure research for deep sea bacteria with very high, atmospheric pressure absolute (ATA) (200-1,000 ATA) and for hypertension with moderate pressure (about 1.2 ATA). In hyperbaric oxygen therapy, 2 ATA is used with 100% oxygen, but no study has reported the biological effect of 2 ATA. Therefore, we performed this unique study about the biological effect of 2 ATA in a pressurized cell incubator with 5% CO2. Human diploid fibroblasts (HDF) were cultured at 2 ATA to evaluate growth rates and viability of cells. HDF population proliferation rates and viability were determined by using visual cell counting, incorporation of [H3] thymidine and 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium and lactate dehydrogenase (MTS) assay for over a week. Cell cycle distribution and quantity of reactive oxygen species (ROS) were determined by flow cytometry analysis. Light microscopy and digital imaging were used to evaluate cell morphology. To observe the pressure-induced DNA damage, comet assay was performed. Senescence-associated β-galactosidase activity was determined using the X-Gal stain. Our result demonstrate that cell populations grown under an increased pressure of 2ATA showed reduced growth rates and viability ($p<0.001$). Morphologies of cells grown under pressure had increased cytoplasm to nuclear ratios with senescent shapes. In the comet assay data, cells at 2ATA had more DNA damage ($p<0.05$) but no dead cells were detected. Cells grown at 2ATA had a higher rate (1ATA=20%, 2ATA=95%) of cells staining positive for SA-β-Gal. In conclusion, HDF grown at 2ATA demonstrated a premature aging phenomenon similar to replicative senescence. Further studies will focus on the similarity or difference of pressure-induced cellular senescence with H2O2-induced cellular senescence.

109. SELECTION OF AGING BIOMARKERS IN PRIMATES: CALORIC RESTRICTION OF RHESUS MONKEYS

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Human aging and oxidation biomarkers are a subject of controversy. Possible aging biomarkers include telomere length, cell division potential, and cellular concentrations of lipoic acid, N-acetyl-carnitine, and S-adenosyl-methionine. Biomarkers for oxidative stress include plasma antioxidant concentrations, LDL oxidizability, urinary isoprostanes, oxidized nucleotides, malondialdehyde, protein carbonyls, and Heinz bodies. Macroscopic biomarkers like memory ability, sensory function, muscle strength and endurance can be measured.

Caloric restriction (CR) has been proven to delay physiologic aging and extend lifespan in all animals tested. Comparison of CR and age-matched controls may distinguish chronologic, physiologic, and useless markers of aging and oxidative stress. Started in 1989, the University of Wisconsin caloric restriction project with rhesus monkeys can provide preserved and fresh samples to evaluate proposed biomarkers. Based on individually tracked intake, restricted primates are fed a chow diet containing 30% fewer calories than matched controls. A 70 kg human on a similar diet would consume approx. 940 mg vitamin C and 110 iu vitamin E per day. Taken at least annually are samples of whole serum, plasma, urine, and biopsies of fat, muscle and skin. Biomarkers of aging currently being measured include advanced glycosylation endproducts, lipid peroxidation, mitochondrial DNA deletions, and glucose tolerance testing. Gene expression profiling and proteomic analysis are conducted. However, overall individual health profiles are now preferred as an indication of physiological age compared to any available biomarker.

Discussion of candidate biomarkers to test and collaboration for sample evaluation are invited. Many collaborations are underway and samples may be obtained under specific conditions. (Supported by NIH Grants P01 AG11915 and P51 RR000167)

110. CHEMICAL ANALYSIS OF AGING IN DROSOPHILA MELANOGASTER

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We are using chemical analysis to examine the relationship between the rate of formation of advanced glycation endproducts (AGEs) and longevity in Drosophila melanogaster. Our aim is to determine whether glycation plays a causal or merely a correlative role in aging. We have established analytical techniques that allow us to quantify changes in AGE status with age. Whilst the chemical species known collectively as AGEs have been subjected to detailed characterisation within the context of mammalian aging, their precise role (ie causal agents of aging or simply correlative markers of pathology) still remains unclear. A major barrier to establishing (or disproving) causality is the cost and time required to manipulate lifespan in mammals (whether by environmental or genetic modifications). In contrast, populations of Drosophila combine low maintenance costs with a short and easily manipulated lifespan. Despite this, there is only one publication describing the chemical analysis of Drosophila over their lifespan, and this only described simple fluorimetry conducted at a single wavelength (as a marker of the level of common AGEs). We are undertaking quantitative and qualitative analysis of a broad spectrum of AGEs in Drosophila in order to determine directly the role that AGE formation plays in longevity. Accurate quantification of the effects of a) lifespan-modifying factors on AGE status, and b) AGE-reducing therapies on lifespan, will allow us to determine the true role of AGEs in this system. Understanding the chemistry of such basic aging mechanisms will bring long-term benefits to older people.

(N)

111. LEARNING IS IMPROVED WHEN TNF-ALPHA IS INACTIVATED IN AGED RATS WHEREAS IT IS DEPLETED WHEN TNF-ALPHA IS ACTIVATED IN YOUNG RATS: EYEBLINK-CONDITIONING STUDY COUPLE TO INTRACEREBELLAR MICRODIALYSIS

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The proinflammatory cytokine tumor necrosis factor alpha (TNF-α) is a multifunctional mediator of inflammation involved in aging and neurodegenerative diseases of aging. Previous work from our lab has shown that diets enriched with antioxidants reduce levels of the proinflammatory cytokine TNF- α in the central nervous system of aged rats. These diets have also been shown to improve classical eyeblink conditioning performance in aged rats. Therefore we tested the hypothesis that inflammation and more specifically TNF- α may be a critical factor that modulates classical conditioning behavior during the aging process by administering TNF- α to young rats and blocking TNF- α in aged rats. In experiment one young (3 month old) F344 rats were pretreated (via infusions into the cerebellar cortex lobus simplex) with 2 uL of recombinant rat (rr)TNF- α (50 ng) one day prior to training and then 3 h prior to eyeblink conditioning coupled to microdialysis for 5 consecutive training sessions with one session per day. The control group received rrTNF- α heated at 90 ° C. The results show that young rats treated with rrTN- α have a decreased rate of learning compared to the control group. The neurotransmitter release measured with microdialysis on day 1 of training from young rats resembles that observed in aged rats. In a second experiment aged (22 month old) F344 rats were pretreated with intracerebellar microinjection a 2 uL of anti-rat TNF- α 0.3 pg/ml three times a week for 4 weeks prior to eyeblink conditioning training with microdialysis. Aged rats showed increased conditioned responses compared to controls which received only IgG. The neurochemical profile of this group reached basal levels sooner than the control group but not as early as the young rats. The results of these experiments demonstrate a critical correlation between TNF- α and age and the rate of conditioned learning acquisition.

(G)

112. RESVERATROL ATTENUATES LIVER DAMAGE IN MICE FED A HIGH FAT DIET

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Resveratrol, a polyphenolic compound acknowledged for its cardioprotective and anti-cancer effects, has previously been shown to significantly extend lifespan in yeast, worms, flies, and recently, a short-lived species of fish. Years of research in the field of gerontology have focused on caloric restriction (CR) as the most dynamic method to increase both mean and maximum lifespan across a variety of species. In lower organisms, resveratrol seems to accomplish the same results, thus mimicking CR without limiting food intake. During ongoing studies focused on further investigations of the effects of low and high resveratrol treatments on mouse longevity, we noted a dramatic protective effect on liver morphology as well. Currently, only data comparing mice fed normal chow or high fat diets ad libitum are presented. The data obtained thus far have confirmed the CR-like protective effects of resveratrol. The high resveratrol treatment had a protective effect against hepatomegaly in high fat fed mice, and decreased lipid deposition as determined by oil red O staining. Previous findings have shown that resveratrol treatment had a protective effect against the formation of fatty streaks in hamsters and atherosclerotic lesions in apoE/LDL receptor KO mice. The formation of lesions in the aortas of the high fat, resveratrol-treated mice will be assessed to determine the extent of atherosclerosis present. These results are particularly relevant to individuals in Western nations, where the prevalence of unhealthy diets and subsequent obesity is high.

113. TRANSCRIPTOME WIDE INFLUENCES OF MENOPAUSE WITH HORMONE DEFICIENCY OR WITH HORMONE REPLACEMENT ON SKELETAL MUSCLE OF EARLY POSTMENOPAUSAL WOMEN

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Aging is associated with decrease in muscle mass and strength (sarcopenia). In women, the risk of sarcopenia may increase already after the age of 50 due to menopause related hormonal changes. Hormone replacement therapies (HRT) have been shown to have beneficial effects on muscle mass and performance although contradictory result exists. The mechanisms behind sarcopenia and HRT responses are far from being understood and deserve extensive molecular investigations. Postmenopausal state with estrogen deficiency offers a powerful tool to study the role of estrogen in regulating/maintaining muscle mass and function. The purpose of this study is to recognize genes and signaling routes/networks directly or indirectly associated with menopause or HRT. This study utilizes samples and data collected in a 12-month randomized controlled double-blinded trial in which the effects of HRT on muscle structure and function were studied in early postmenopausal 50-57-yr-old women. Muscle biopsies were obtained from the *vastus lateralis* muscle. For the overall view of menopause and HRT induced transcriptional changes, the microarrays were performed from baseline and follow-up muscle samples (HRT n=10, menopause n=5). Additionally, total lean body mass (LBM) and *quadriceps* muscle cross-sectional area (CSA) were measured. Microarray results showed that in the absence of estrogen muscle gene expression profile changes, while one year HRT appears to balance this phenomenon and further up- or downregulate some specific genes. Menopause-effected genes include e.g. those involved in protein synthesis and degradation machineries indicating changes in protein homeostasis. During the intervention mean LBM decreased in the menopause group and increased in HRT women (ANOVA interaction p=0.004). *Quadriceps* CSA remained unchanged in postmenopausal women whereas it increased in HRT group (p=0.010). These results suggest that HRT may be a beneficial agent in sarcopenia prevention while menopause might predispose to increased risk of sarcopenia through changes in muscle protein homeostasis.

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114. NEURONAL APOPTOSIS IN ADULT DROSOPHILA MELANOGASTER

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Abnormal regulation of neuronal apoptosis has been proposed to play a role in the pathology of several neurodegenerative diseases, such as Alzheimers disease and Parkinsons Disease . The fruit fly Drosophila melanogaster, is being used as model for the study of these diseases. However, apoptosis is generally studied in the context of development of juveniles or larvae. Apoptosis in fully differentiated adult neurons has not yet been well characterized. Studies have shown that apoptotic machinery has been conserved in all known metazoans.. By studying the over-expression of regulators of apoptosis in the nervous system of the adult fly, we intend to investigate the effects of activation of apoptotic genes in adult post-mitotic neurons . By using an inducible system, we are able to use a molecular genetic approach to conditionally activate apoptosis. Here we present the results of over-expression of different apoptotic genes in the nervous system of the adult fly. Preliminary studies suggest that the proapoptotic genes grim and reaper may play differential roles in modifying the post-mitotic nervous system of a young adult fly.

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115. REDUCTION IN BENEFICIAL EFFECTS OF CALORIC RESTRICTION ON DMBA-INDUCED SKIN TUMORS IN NRF2 KNOCKOUT MICE

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Caloric restriction (CR) has long been established as an effective system of tumor prevention and has been shown to significantly decrease tumor formation in a wide variety of spontaneous and

induced forms of cancer, including chemically induced skin tumors. The involvement of detoxification and antioxidant genes in the prevention of carcinogenesis induced by oxyradicals is receiving increased research attention. Nuclear factor E2-related factor 2 (Nrf2), the primary transcription factor responsible for mediating these antioxidant cellular signaling cascades, has been shown to play a pivotal role in tumor initiation through its disassociation from Keap-1 and its nuclear localization in response to chemopreventative phytochemicals, and through the ineffectiveness of some of these anti-carcinogenic compounds in Nrf2 knockout mice. This preliminary study was designed to determine the role of the Nrf2 pathway in the anticarcinogenic effects of CR by evaluating the effects of 40% CR on reducing the formation of 7,12-dimethylbenz[a]anthracene (DMBA) induced skin tumors in Nrf2 knockout mice. CR in the Nrf2 knockout mice did not result in a significant reduction in the number or size of squamous papillomas. Mice homozygous for the Nrf2 knockout showed a greater increase in SIRT1 levels in the liver than did heterozygotes, which corresponded with a more substantial reduction in tumors. These results show that the protective effects of CR against tumor formation are at least partially dependent upon Nrf2 and suggest that transcriptional regulation by Nrf2 may be the primary mechanism by which CR exerts its anti-cancer effects. Further studies are underway to more firmly establish how the effects of CR on tumor formation vary between Nrf2 knockout and wildtype CD1 mice.

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116. SERUM FROM CALORICALLY RESTRICTED RATS APPLIED TO HIPPOCAMPAL PRIMARY NEURONS PROVIDES NEUROPROTECTION AGAINST KAINIC ACID AND CORTICOSTERONE

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Caloric restriction (CR) can extend life span, promote neuronal survival and attenuate stress-induced cell death. Studies using an *in vitro* model of CR have demonstrated that cells cultured with medium containing serum from animals subjected to CR can protect them against neurotoxic insults. This suggests that certain constituents in CR serum can increase stress resistance. However, the mechanisms of CR-induced protection have not been identified to date. Studies have indicated that CR elevates plasma corticosterone (CORT) levels in animals; however, high levels of CORT have been shown to enhance the toxicity of certain neurotoxins, such as kainic acid (KA). Using primary hippocampal cell culture, the present study investigated the role of CORT in CR-induced protection. Specifically, we applied serum to the culture medium obtained from rabbits that were fed ad libitum (AL) or CR diets (40%). We found that CR, but not AL, serum induced protection against the excitotoxic effects of KA. We added the glucocorticosterone receptor (GR) antagonist, RU486, to the culture medium. Results showed that CR serum and RU486 combined further enhanced the protective effect against KA toxicity, which likely resulted from the blockade of GR receptors on hippocampal neurons. In an additional experiment, CR was found to directly block the cell death induced by CORT. These results suggest that 1) the elevated CORT in CR-treated animals may have no direct influence on CR-induced neuroprotection. In fact, elevated CORT may essentially weaken the CR-induced protective effect, and 2) the observed CR-induced protection is likely provided by other protective elements in CR serum that may compensate for CORT-produced deleterious effects.

117. HIGH INTERLEUKIN-10 PRODUCTION IS ASSOCIATED WITH LOW ANTIBODY RESPONSE TO INFLUENZA VACCINATION IN THE ELDERLY

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Influenza is an acute respiratory illness of global importance that causes considerable morbidity and mortality every year. While the influenza virus can cause substantial morbidity across the age spectrum, the elderly are, however, especially vulnerable to the serious complications of influenza, with higher rates of hospitalization or death that exceed by several-fold the rates seen among most other age groups. Aging is associated with a number of impaired immune responsiveness such as reduction in response to recall antigen, alterations in T cell functions, which contribute to increased vulnerability to infectious disease and malignancy, and reduced responses to preventive vaccination in the elderly. We designed the present study to determine the correlation between dehydroepiandrosterone (DHEA) and immune functionality at the moment of vaccination with antibody response to influenza vaccination in young and old healthy volunteers.

Fifty elderly subjects age 63-85 y and fourteen young subjects age 26-41 y entered the study. Plasma levels of dehydroepiandrosterone (DHEA) and cortisol, and in vitro cytokine production in response to lipopolysaccharide (LPS) and phytohaemagglutinin (PHA) by peripheral blood leukocytes were assessed at the time of vaccination, while antibody response to the vaccine was measured before and eighteen days after Influenza virus vaccination.

Elderly subjects were characterized by statistically significant increase in the cortisol/DHEA ratio, mainly due to a significant decrease in DHEA levels and slight increase the level of cortisol. The immune functionality, as assessed by measuring cytokines (TNF-alpha, gamma-IFN and IL-10) with central role in regulating the immune response, was characterized by a statistically significant decrease in LPS-induced tumor necrosis factor-alpha (TNF-alpha), increase PHA-induced interleukin-10 (IL-10) release and similar PHA-induced gamma-interferon (gamma-IFN) production compared to young volunteers. Lower antibody titer to influenza A virus was observed in aged individuals and the sieroconversion factor was 1.08 (SD0.10) in the elderly versus 2.25 (SD0.35) in the young. The sieroconversion factor was inversely correlated with IL-10 production (linear correlation $r=-0.484$, $p<0.0001$), directly correlated with TNF-alpha production ($r=0.3997$, $p=0.0012$) and, to a lesser extent, with plasma level of DHEA ($r=0.363$, $p=0.0034$). The decreased TNF-alpha and increased IL-10 production were both correlated to plasma level of DHEA ($r=0.326$, $p=0.0076$ and $r=-0.490$, $p<0.0001$ for TNF-alpha and IL-10, respectively). These results suggest that altered cytokine production in elderly subjects at the moment of vaccination can be predictive of a low response to Influenza vaccination. Strategies to improve protection afforded by the use of vaccine should include strategies directed to restore altered immune responses associated with aging.

(G)

118. OXIDATION IN THE NUCLEOTIDE POOL: AN "ACHILLES HEEL" MODEL OF CELLULAR SENESCENCE

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Reactive oxygen species (ROS), generated as byproducts of aerobic metabolism or chemical oxidants, are responsible for limiting both organismal as well as cellular lifespan. However, due to their pleiotropic effects, it has been difficult to pinpoint the initiating role of ROS in the process of cellular senescence. A characteristic oxidative DNA damage lesion associated with cellular senescence, oxidative stress and organismal aging is 8-oxoguanine (8-oxo-dG). In order to mimic the damaging effects of ROS on DNA without concommitantly increasing ROS levels, we aimed at increasing endogenous 8-oxo-dG levels. In order to accomplish this, we utilized shRNA-mediated knockdown of Mth1, an 8-oxo-dGTPase, which hydrolyzes 8-oxo-dGTP in the nucleotide pool thus preventing its incorporation into genomic DNA. Stable knockdown of Mth1 using a lentiviral shRNA expression system causes early-passage primary as well as telomerase-immortalized skin fibroblasts to undergo senescence as assessed by increased acidic beta-galactosidase activity, flattened morphology and lack of further population doubling (PD). Loss of Mth1 expression is accompanied by increased cellular 8-oxoguanine levels. The siMTH1-induced senescent phenotype can be partially rescued by culturing the cells under low oxygen (3%) for several PDs prior to shRNA infection. The senescent phenotype induced by Mth1 knockdown fully recapitulates the salient downstream features of replicative senescence such as unrepairable genomic DNA breaks, upregulated DNA damage signaling pathway proteins, altered

telomere structure and elevation of both p21/p53 as well as p16 levels. Overexpression of MTH1 alleviates the effects of chronic oxidative stress on cell proliferation but not that of acute oxidative stress. These results imply that oxidized products in the relatively unprotected nucleotide pool are constantly accumulating and, if not eliminated, are sufficient to initiate the senescence program. This is most likely due to the increased genomic DSBs that can arise through

119. WHAT ABOUT OBESITY MARKERS AND HYPERTENSION IN THE OLDEST OLD?

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Obesity and increased salt intake are linked to hypertension which is the most important risk factor for stroke, cardiovascular disease and vascular dementia. The inter-relationship between blood pressure, salt/sodium and anthropometric-related factors has not been well researched in very old people.

Anthropometric and sodium-related variables were measured and analyzed by tertiles of systolic blood pressure in a cross-sectional study of 590 elderly and very elderly persons (mean age 87 years) from the Belfast Elderly Longitudinal Free-living Aging STudy (BELFAST). After adjustment for age and sex, subjects in the highest tertile of systolic blood pressure had higher serum sodium (odds ratio [0R] 1.42, 95% confidence interval [CI] 1.14-1.86), higher waist hip ratio (OR 1.68, 95% C1 1.09-2.58), waist (OR 1.47, 95% CI 1.05-2.02), weight (OR 1.40, 95% CI 1.09-1.79) and skin fold thickness (OR 1.30, 95% CI 1.05-1.61) compared with those in the lowest tertile. BMI >25 was associated with the highest tertile of diastolic blood pressure (OR 1.35, 95%CI 1.00-1.82). These findings were replicated in subjects characterized as clinically hypertensive (= or >140/90 mmHg) versus those with reference blood pressure (= or<120/80 mmHg).

The BELFAST study of elderly people shows consistent associations between blood pressure in the hypertensive range and higher BMI, waist-hip-ratio, weight and serum sodium. These results suggest that maintaining an ideal body weight could have important effects on hypertension control even in the oldest age groups.

120. ERYTHROCYTE MEMBRANE FATTY ACIDS AND IMMUNE ACTIVATION IN VERY ELDERLY SUBJECTS

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Ageing is associated with changes in lymphocyte and natural killer cell subsets together with a non-specific activation of the immune system. In this pilot study we measured the fatty acid content of red cell membranes together with lymphocyte subsets and activation markers in apparently healthy elderly subjects to see if there was membrane stiffness might be related to activation status.

Subjects (19 male and 33 female) were recruited as part of ongoing Belfast Elderly Longitudinal Free-living Ageing STudy (BELFAST) study and were apparently well, mentally competent and of mean age 83 years. Chromographic separation of erythrocyte membrane fatty acids was achieved using gas-liquid chromatographic assay. Lymphocyte subsets were enumerated using FACS Scan, routine monoclonal antibodies and a panel of activation antigen.
T cells, CD3/4+ and CD3+CD56CD16+ subset numbers were associated with saturated fatty acid (SAFA) but not polysaturated acid (PUFA) or monosaturated fatty acid (MUFA) content of the cell membranes while Natural Killer cells were associated with 18.1 and 22.6 fatty acids. CD3/4+ cells were highly associated with serum Vitamin A. The early activation marker CD69 was associated with the PUFA/SAFA ration and the n3/n6 ration. PUFAs, SAFAs and MUFAs and individual fatty acids showed no age or sex-related change although there was a trend for increased PUFA in >90 year olds. Vitamins A and E were normal but Vit C status in serum was sub-optimal in a majority of subjects.

The erythrocyte membrane reflects general fatty acid status within the lifetime off the red cell. In this pilot study the saturated and polyunsaturated erythrocyte fatty acid profile appeared to be associated with changes in the lymphocyte subsets numbers and activation in elderly people.

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121. SUSCEPTIBILITY TO SALMONELLA TYPHIMURIUM INFECTION INCREASES WITH AGE IN C57BL/6 MICE

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Enterocolitis due to Salmonella infection is the most common cause of death from food borne illnesses in the U.S. Salmonella typhimurium (ST) is most frequently associated with this disease. ST infection can be particularly severe in the elderly. However, a well-defined animal model to study the effect of age on ST-induced enterocolitis, and its underlying mechanisms, has not been established. Young and old streptomycin-pre-treated C57BL/6 male mice were inoculated with high dose (3×10^8 colony forming units [cfu]) or low dose (5×10^5 cfu) of ST or PBS. ST counts were determined by cfu recovered from the gut contents of the ileum, cecum, and colon, and mesenteric lymph nodes, peyer's patches, liver, and spleen of mice at days 1, 2, and 4 post infection (pi).

On day 2 pi, old low dose infected mice had significantly higher ST cfu than young low dose infected mice in the spleen ($p<0.05$). By day 4 pi, old infected mice had higher cfu than young infected mice in the ileum, colon, peyer's patches, and liver ($p<0.01$). Both young and old mice exhibited weight loss following the infection, however, old mice had significantly higher weight loss than young infected mice on days 1 and 2 pi ($p<0.05$). Thus, old mice exhibit significantly greater ST colonization of the ileum, colon, peyer's patches, and liver, and lost more weight compared to infected young mice. This animal model maybe useful for future studies of the aged host response to ST induced enterocolitis. Support by USDA #58 1950 9 001.

122. UNCOUPLING OF THE WEIGHT-LOSS AND BODY TEMPERATURE RESPONSES TO DIETARY RESTRICTION

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Theory strongly suggests that the life-extending benefits of dietary restriction (DR) are the byproduct of a survival response to food shortage. This response is often described as a shift of energy resources from "growth and reproduction to maintenance and repair". Such reallocation would seem to imply a major, coordinated metabolic response. We hypothesized that such coordination would be reflected in how resources are reallocated for maintaining body temperature and body weight. Indeed, previous studies have shown that the amount of energy utilized by mice for maintaining body temperature affects body weight. For example, when less energy is required, by housing mice under thermoneutral conditions, mice gain weight without eating more, and DR mice under thermoneutral conditions lose markedly less weight. We were surprised, therefore, to find that weight loss during DR (60% of *ad libitum* food intake) showed no correlation whatsoever with the response of body temperature, even though both responses showed highly significant strain variation (unrelated to differences in absolute food intake, activity, feces calorimetry, or *ad libitum* fat). The strain means for body temperature decreased by 1.5 to 5 ºC (housing at ~23 ºC) and weight loss ranged from 15% to 40% across 81 strains (temperatures and weights measured in the same mice). The results suggest that these two major physiological hallmarks of DR in mammals are not coordinately specified, implying that there is not a single physiologic process coupling these effects with life-extension. Such uncoupling can be used to distinguish causality of these responses (and others) with the beneficial effects of DR.

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123. ACTIVITY PATTERNS IN NORMAL AGING RHESUS MONKEYS: A COMPARISON OF FEMALE AND MALE PATTERNS.

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Changes in sleep-wake and activity patterns are known to occur in humans, but have not been well studied in the rhesus monkey model of normal aging, where the brains can also be examined. While polysomnography is the ideal way to assess sleep-wake and activity cycles, it is not practical for the investigation of large samples, whereas actigraphic recordings offer a reasonable alternative. To characterize age-related activity changes between the sexes, we studied male (n=28) and female (n=23) rhesus monkeys (Macaca mulatta) between 2.8 and 29.6 years of age. All animals were individually caged in a common room, within visual and auditory range of other monkeys. They were maintained on a 12:12 light:dark cycle. Activity was monitored using an Actiwatch-64 (Mini Mitter, Bend OR) attached to a primate collar with a plastic housing. Data were collected in 1-minute epochs for 21 days, after which they were analyzed using Actiware Sleep Software V3.4 (Mini Mitter). An analysis of total activity for the 24hr period demonstrated a significant inverse correlation between age and activity score in both males ($p<0.0001$) and females ($p<0.02$). Also, the total number of minutes spent moving declined with age in males ($p<0.0001$) and females ($p<0.01$). The females display a more sustained activity pattern, while males show periods of high and low activity throughout the day. While both sexes show reduced activity with age, the patterns of sleep and wakefulness differ between them. Further studies will determine if varying hormone levels and/or brain changes are responsible for these activity differences and if they are associated with age-related cognitive decline. (Supported by NIH grants P01-AG00001, P51-RR00165, & R01-AG017636).

124. EFFECTS OF PVUII GENOTYPE IN ESR1 GENE ON SKELETAL MUSCLE CHARACTERISTICS IN OLDER WOMEN

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Estrogen receptor alpha (ESR1) mediates the effects of estrogen, an anabolic female sex hormone, in target tissues. PvuII polymorphism in the first intron of ESR1 gene is reported to be associated with bone loss rate after menopause, vertebral fractures, fall risk and possibly with bone mineral density. Although ESR1 was recently shown to be expressed in skeletal muscle, the effects of estrogen on skeletal muscle remain poorly understood. At phenotype level, however, hormone replacement therapy (HRT) has been reported to exert positive effects on skeletal muscle. Since menopausal state and the years thereafter are characterized by minor estrogen levels with concomitant loss of muscle power, a connection between ESR1 and muscle variables may exist. The aim of this study was to determine the association of ESR1 PvuII polymorphism with muscle performance characteristics in older women. Subjects of the study were drawn from the Finnish Twin Study on Aging and included 434 women aged 63-76 years (103 monozygotic and 114 dizygotic twins). Maximal isometric knee extension, ankle plantar flexion and hand grip strengths, leg extensor power, and lower leg muscle cross-sectional area were measured. Genomic DNA was extracted from whole blood samples and PvuII variants of ESR1 determined using polymerase chain reaction and restriction fragment length polymorphism methods. Statistical analyses were performed in SAS using general estimating equations with Bonferroni correction for testing multiple means. Models were adjusted by zygosity, age, height, physical activity and HRT. Possible codominant, dominant and recessive effects were explored. No association of separate PvuII genotypes with muscle mass or density, leg extensor power, hand grip, knee extensor or ankle flexor strength was found. Moreover, no dominant or recessive effect of P or p allele was present, respectively. In conclusion, PvuII polymorphism in ESR1 gene is not associated with skeletal muscle characteristics among older Finnish women.

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125. CALORIC RESTRICTION (CR)-INDUCED ALTERATIONS IN THE PROGRESSIVE PATTERN OF AGING-RELATED CHANGES IN HIPPOCAMPAL GLUTAMATE REEPTORS ARE ASSOCIATED WITH CHANGES IN SPATIAL LEARNING

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The aging process is associated with declines in hippocampal levels of NMDA and AMPA receptor subunits and with impairments in spatial learning and memory. The present study investigated the effect of caloric restriction (CR) on these aging-related alterations in young (10-11 months), middle aged (18-20 months), and old (28-32 months) Fischer 344 X Brown Norway rats fed ad libitum (AL) or that were fed a CR diet from 4 months of age. One cohort (N=8) was decapitated and the dorsal 2/3 of hippocampus was sub-dissected into CA1, CA3, and dentate gyrus (DG) for Western blot analysis of subunits of NMDA (NR1, NR2A, NR2B) and AMPA (GluR1 and GluR2) types of glutamate receptors. Those subunits declined with age in AL rats, particularly in CA1 and CA3. Similar aging-related declines in NMDA and AMPA receptor subunits were not seen in CR animals: levels of most subunits were significantly lower at 10 months in CR compared to AL animals and then remained stable across life span. A second cohort (N=12) was evaluated on the Morris water maze test of spatial learning and reference memory. Five training trials per day were given for 4 days followed by a probe trial on the 5th day. In AL rats, there was a progressive aging-associated decline in spatial learning demonstrated in training trial performance, but not in reference memory as evaluated in the probe trial. CR animals exhibited a similar spatial learning impairment between young and middle age, but no further impairment was detected after middle age, resulting in significantly better spatial learning in old CR than in old AL rats. In conclusion, the present study suggests that CR alters the progressive pattern of aging-related decline in NMDA and AMPA receptor subunits in the hippocampus and results in improved spatial learning in old rats. Supported by NIA grants AG11370 and AG019886

126. LIFESPAN AND GLUCOSE METABOLISM IN INSULIN RECEPTOR MUTANT MICE

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Insulin/insulin-like growth factor type 1 signaling regulates lifespan and resistance to oxidative stress in worms, flies, and mammals. To investigate the biological significance of a longevity mutation found in daf-2 of Caenorhabditis elegans, we generated a homologous murine model by replacing Pro-1195 of insulin receptors (IR) with Leu using a targeted knock-in strategy. Homozygous mice died in the neonatal stage from diabetic ketoacidosis while heterozygous mice showed the suppressed kinase activity of the IR, but grew normally without spontaneously developing diabetes mellitus (DM) during adulthood. We revealed that heterozygous IR mutant mice showed enhanced resistance to oxidative stress cooperatively modulated by sex hormone and dietary signals. Manganese superoxide dismutase activity in mutant mice was significantly up-regulated, suggesting that the suppressed insulin signaling leads to an enhanced antioxidant defense. Next, we investigated the lifespan of IR mutant mice. Under normoxia, mutant male mice survived the comparable lifespan as wild-type male mice while the mutant male mice showed decreased fatality in later stage of life. IR mutant female mice also showed the comparable lifespan as wild-type female mice in spite of the fact that IR mutant female mice acquired more resistance to oxidative stress than IR mutant male mice. On the other hand, IR mutant male and female mice showed insulin resistance with hyperinsulinemia but the majority of mutant mice did not develop DM throughout the entire lifespan. These data suggested that the beneficial effects of insulin signaling such as the extension of lifespan and the resistance to oxidative stress might be canceled by the deleterious effects of insulin resistance in mammals.

127. EFFECTS OF A DIOXIN ON AGING IN WISTAR RATS

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The dioxins represent a family of ubiquitous environmental pollutants and are well known as environmental endocrine disruptors. One of the most potent toxicants is 2,3,7,8-

tetrachlorodebenzo-p-dioxin (TCDD) and it changes multiple endocrine systems in the living body. Moreover, it has been reported that exposure to TCDD causes oxidative stress in a variety of animal models and there is a suggestive consideration that the dioxins may have influence on aging.
In order to investigate the lifespan associated with the dioxins, we gave a single subcutaneous injection of TCDD (100ng/kg body weight) to pregnant Wistar rats on gestational day 19 and studied the effects of a relatively low dose of TCDD on mortality, body weight and morphological changes in male and female offspring. The mean lifespan of the TCDD treated animals were 92.5+-36.9 weeks in male and 119.9+-15.4 weeks in female, on the other hand the mean lifespan of control were 115.7+-20.1weeks in male and 132+-22.4 weeks in female. These results indicate that TCDD which was transferred into offspring through the placenta and the mammary gland caused the lifespan-shortening. A statistically significant decrease in body weight was observed in TCDD treated female rats but there was no effect on body weight of treated male rats. Furthermore, exposure of low dose of TCDD increased apoptotic cells in corpus striatum, bed nucleus of the stria terminalis and basal nuclei of Meynert of neonatal rat brains. It is known that neuron density has

128. THE EFFECTS OF GRAPE JUICE ON COGNITIVE AND MOTOR DEFICITS IN AGING
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Animals and humans show increased motor and cognitive declines with aging, thought to be due to increased susceptibility to the long-term effects of oxidative stress and inflammation. Previous findings have suggested that improvements in these age-related declines might be accomplished by increasing the dietary intake of polyphenolics found in fruits and vegetables, especially those identified as being high in antioxidant and anti-inflammatory activity. Therefore, we investigated the beneficial effects of two concentrations of Welch's Concord grape juice (10% and 50%) compared to a calorically-matched placebo for their effectiveness in reversing age-related deficits in behavioral and neuronal function in aged Fischer 344 rats. Results showed that rats which drank the 10% grape juice from age 19 to 21 months had improvements in oxotremorine-enhancement of K+-evoked release of dopamine from striatal slices as well as cognitive performance on the Morris water maze, while the 50% grape juice produced improvements in motor function. These findings suggest that, in addition to their known beneficial effects on cancer and heart disease, polyphenolics present in foods may be beneficial in reversing the course of neuronal and behavioral aging, possibly through a multiplicity of direct and indirect effects that can affect a variety of neuronal parameters.

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129. THE EFFECT OF 2DG ON DROSOPHILA MELANOGASTER
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Calorie restriction (CR) has been shown to extend lifespan and retard aging processes in a wide range of species from yeast, worms, fruit flies to rodents and possibly nonhuman primates. These robust findings have spawned a new research area focused on identifying compounds that mimic CR effects (Ingram et al. Ann N YAcad Sci. 1019: 412, 2004). One such CR mimetic proposed is 2-deoxyglucose (2-DG). 2--DG is a glucose analogue that blocks glycolysis early in its intracellular metabolic cycle. To further evaluate the effect of this compound on aging, we administered 2-DG to the fruit fly, Drosophila melanogaster, and measured lifespan. Diets with combinations of different amounts of 2-DG and sugar / yeast were tested. We found that 2DG extended mean lifespan of the flies reared on a rich medium with a high concentration of sugar / yeast (4.95% yeast, 15% sucrose), but reduced mean lifespan of flies that were on CR. This result suggests that effect of 2-DG on lifespan extension depends on diet composition. The data to date indicate that 2DG is a possible CR mimetic, but it may be only beneficial to animals that are not already on the CR regimen. We are currently investigating effects of 2-DG on reproduction, stress response and behavior in the flies to further assess its effectiveness as a CR mimetic.

130. LIFESPAN MODIFICATION BY METHIONINE AND GLUCOSE IN DROSOPHILA MELANOGASTER FED A CHEMICALLY DEFINED DIET

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Experimental restriction of dietary calories while maintaining adequate nutrient content has been found to extend lifespan in phylogenetically diverse species, pointing to a conserved pathway that can modify lifespan in response to energy intake. However, in some cases the impact on longevity may depend on the quality of the energy source. In Drosophila, restriction of dietary yeast yields considerable lifespan extension whereas isocaloric dietary sugar restriction yields only modest extension, indicating that other diet-responsive pathways can modify lifespan in this species. In rodents, restricting intake of a single amino acid – methionine – extends lifespan. Here we show that dietary methionine can modify lifespan in Drosophila. We fed a chemically defined media containing 15% glucose and 0.045%, 0.135% or 0.405% L-methionine to adult, mated female Oregon-R strain flies. Compared to a diet containing 0.135% methionine, high (0.405%) methionine shortened maximum lifespan by 2.33% from 86 to 84 days and mean lifespan by 9.55% from 71.7 to 64.9 days. Further restriction of methionine to 0.045% did not extend maximum lifespan and shortened mean lifespan by 1.95% from 71.1 to 70.3 days. Restricting dietary glucose from 15 to 5% while maintaining optimal methionine at 0.135%, modestly extended maximum lifespan by 5.8% from 86 to 91 days, without extending mean lifespan. Differences in survivorship were highly statistically significant (log-rank test statistic =1162.74, $p<0.0001$). Notably, all four diets resulted in considerably longer life spans than those that are typically reported for flies fed conventional yeast and sugar based diets. We will present data from microarray studies aimed at determining the influence of these diets on age-related gene expression. Our findings demonstrate that methionine can modify lifespan in Drosophila. Furthermore, the food media that we have developed provides a useful tool for studying lifespan-modifying pathways and specific nutrient-gene interactions in Drosophila.

131. DROSOPHILAS DEFICIENT IN TUMOR SUPPRESSOR SURVIVE BETTER STRESSFUL CONDITIONS: POSSIBLE INVOLVEMENT OF APOPTOSIS

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Recent studies suggest that downregulation of a tumor suppressor (TS) might not only favor cancer development but also have an anti-aging effect on cells and organisms. However, studies demonstrating significant increase in survival for TS deficient mutants together with a postponement of physiological aging in experimental animals are still lacking. Since the aging is accompanied by a general decline in stress resistance, the better resistance to stresses would constitute an important feature of a postponed aging phenotype. Hence, if the reduced dosage of TS someway favors the aging postponement, then one should expect a higher resistance to environmental stresses (such as abnormal temperature) in TS deficient animals as compared with their control age-peers. In this paper we show that the loss of one dose of the lgl tumor suppressor gene increases longevity of Drosophila flies in stressful conditions (high temperature) and suggest possible mechanisms underlying this effect. METHODS. We performed a series of cohort survival experiments under normal (25o C) and high (29o C) temperature. Each experiment involved about 300 flies kept in 5-6 cages. The Canton S line has been used as +/+ control for the mutant lgl/+ flies that are TS deficient. RESULTS. Mutant flies did not show a survival advantage at the normal temperature 25o C. However, at the temperature 29o C, after slightly worse survival at earlier ages, the heterozygous lgl mutants demonstrated clearly better survival in advanced years of life, so that the proportion of longest living flies was higher among the TS mutants. Mutant flies thus demonstrated a late life survival advantage in stressful conditions compared to the wild type genotype. We suggest an explanation of this phenomenon by a better resistance of mutant cells to a stress induced apoptosis. The age-associated increase in apoptotic activity in muscle and fat cells of Drosophila was shown to result in the loss of muscle mass, strength and function, and the decline of locomotor activity (Zheng

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132. A LACK OF SOD1 RESULTS IN ACTIVATION OF REDOX-REGULATED ADAPTIVE RESPONSES IN TRANSGENIC MICE

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Increased production of reactive oxygen species (ROS) can cause lipid peroxidation, inactivation of enzymes and DNA damage. Skeletal muscle cells have mechanisms to protect against an increased production of ROS, including the superoxide dismutases SOD1 and SOD2, catalase and glutathione peroxidase enzymes. Aging is hypothesized to be caused by ROS products accumulating throughout lifespan (1). Thus, cellular antioxidant defenses may play a more crucial role at advanced age. The cellular response to oxidative stress becomes defective with advancing age. Many researchers have examined the possibility that mitochondrial generation of ROS is the major contributor to the increased oxidative damage seen in aging, however the role of cytosolic ROS generation remains unclear. This study examined whether a lack of SOD1 influenced adaptive responses to ROS in adult SOD1 knockout mice ($Sod1^{-/-}$) since these mice have reduced lifespan (2). Male adult wild type (WT) and $Sod1^{-/-}$ mice were killed by an overdose of anesthetic and gastrocnemius muscles were analyzed for heat shock protein (HSP) content (3), NF-κB and AP-1 DNA binding activity. DNA binding activity of NF-κB and AP-1 transcription factors increased in muscles of quiescent $Sod1^{-/-}$ mice compared with that of WT mice. The content of HSP25 and HSP60 in skeletal muscles from $Sod1^{-/-}$ mice was also increased compared with that of quiescent WT mice (e.g. % increase of HSP25 content: 78.2 ±22). These changes are characteristic of skeletal muscle in old WT mice and support the possibility that $Sod1^{-/-}$ mice provide a model of accelerated muscle aging.

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133. EFFECT OF LONG-TERM SPIRULINA ADMINISTRATION ON HIPPOCAMPAL NEUROGENESIS IN THE AGED RAT

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Research on the inflammatory process has gained a substantial amount of interest in the past years. Key pro-inflammatory cytokines responsible for the inflammatory process have been shown to have a strong association with diseases like Parkinson's disease (PD), Alzheimer's disease (AD) as well as naturally accompanying the process of aging. This elevation in pro-inflammatory cytokines during aging may be responsible for the decrease in neurogenesis within the hippocampus observed during aging. Due to adverse side effects observed with long-term administration of NSAIDs, natural compounds high in antioxidants and other anti-inflammatory agents are currently being investigated in this lab as a way to regulate the inflammatory process. Data from this lab has shown that spirulina, a blue-green algae rich in antioxidants and fatty acids, decreases pro-inflammatory cytokines in the aged rat brain (Gemma, 2001). Additionally, data from this lab has been presented demonstrating that a long term spirulina diet can decrease the inflammatory response in vivo after LPS stimulation in young animals, restoring neurogenesis to control levels. This study investigates the therapeutic benefit of spirulina on neurogenesis in the aged Fisher rat. 22 month old animals were either fed a diet infused with 0.1% spirulina or an NIH-31 control diet for a period of 30 days. On days 31-35 bromodeoxyuridine (BrdU) injections (50mg/kg i.p.) were administered to the animals. Following injections, animals were maintained on the diet for an additional 10 days then sacrificed. Immunohistochemistry was performed on

sectioned hippocampal slices to label BrdU positive cells. Using microbrightfield software for stereology an estimation for total number of BrdU positive cells was determined. Additionally, BrdU neuronal labels were verified using double immunofluorescence with neuronal markers.. Results indicate the potential for spirulina to attenuate the loss of neuronal cells in the hippocampus of aged animals.

134. ROLE OF C. ELEGANS SIR-2.1 AND PRION-LIKE GLUTAMINE/ASPARAGINE-RICH PROTEINS IN ER-STRESS AND LIFE SPAN REGULATION

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Members of the silent information regulatory 2 (sir2) family of NAD+-dependent protein deacetylases regulate life span in several model organisms including yeast, nematode, and fruit fly. Overexpression of C. elegans sir-2.1 extends nematode life span and requires the function of the forkhead transcription factor daf-16, a component of the insulin-like signaling pathway. Treatment of C. elegans with resveratrol, a naturally occurring compound found in grapes and red wine, extends nematode life span in a manner dependent upon sir-2.1, but independent of daf-16. Microarray analysis of resveratrol treated worms reveals the transcriptional induction of a number genes belonging to the pqn gene family, which encode prion-like glutamine/ asparagine rich proteins. A subset of these genes, designated as abu for activated in blocked unfolded protein response, are involved in endoplasmic reticulum (ER) stress response to unfolded proteins. RNAi of abu-11 abolishes resveratrol-mediated life span extension, while abu-11 transgenic animals demonstrate dosage-dependent increases in life span. Northern analysis of sir-2.1 mutants and microarray analysis of sir-2.1 transgenic worms both demonstrate that multiple pqn genes, including abu-11, are transcriptionally repressed by sir-2.1, suggesting that resveratrol extends C. elegans life span by inhibiting sir-2.1 function. These results demonstrate a novel connection between prion-like Q/N rich proteins involved in ER-stress response and organismal life span and suggest that sir-2.1 may play a role in regulating ER homeostasis.

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135. ADIPOCYTOKINES' LEVELS AND FAT METABOLISM IN GROWTH HORMONE TRANSGENIC MICE

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Phosphoenolpyruvate carboxykinase (PEPCK)–bovine Growth Hormone Transgenic (Tg) mice exhibit marked stimulation of somatic growth, shortened lifespan, high levels of bGH and insulin. In order to study the mechanisms of altered insulin sensitivity and lipid metabolism in these mice, we have measured plasma adiponectin, leptin, resistin, TNF-α, and IL-6 levels using ELISA, assayed plasma cholesterol, free fatty acids (FFA) and triglycerides (TG) using enzymatic colorimetric assays, and used real time PCR to examine expressions of liver genes. Finally, we performed the same measurements in normal (N) and Tg animals subjected to 30% caloric restriction (CR).

Compared to their N littermates, Tg mice displayed lower adiponectin and TG, but higher resistin and cholesterol. CR increased adiponectin in both N and Tg mice, increased resistin and decreased TG only in N mice, while it decreased leptin, IL-6 and cholesterol only in Tg mice. Compared to their N littermates, Tg mice had increased expression of LPL and decreased expressions of FAS, ACCα, PGC1α, and SREBP-1 which are related to lipogenesis. Tg mice also had decreased expressions of enzymes linked to lipid mobilization or oxidation including HSL, AMPK, CPT-1α, and ACO1. CR increased the expressions of both lipogenic and lipid oxidation-related enzymes and transcription factors in N mice, except for LPL which was not changed in N mice and decreased in Tg mice. CR also increased the expression of ACCα, PGC-1α and AMPK in Tg mice.

Our results suggest that the decreased plasma adiponectin and increased resistin and cholesterol might be involved in the insulin resistance of Tg mice, and these animals might have reduced hepatic lipid metabolism activity. The present findings also suggest that CR might stimulate liver

lipid metabolism activity in N mice by promoting the expressions of genes involving in both lipid mobilization and lipid synthesis, while it has different effects on Tg liver.
(Supported by NIA)

136. ALZHEIMER'S DISEASE THE FUTURE IMPACT ON FAMILY AND HEALTH CARE COST

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New studies estimate that the number of people affected by degenerative diseases, like Alzheimer's, will rise to 13.2 million by about 2050 - three times the 4.5 million people affected today. In my presentation I will compare not only the drastic effect on the cost of health care but most of all the economic and social impact on the families of patients with Alzheimer's disease in relation to medication and extended cost of treatments. Recent declines in death rates after age 65 mean that more people will survive to the oldest ages, where risk of AD is greatest. Early diagnosis may help patients avoid inappropriate drugs and therapies and aid the patients and family members in specific decisions that include arranging legal, and financial matters. We know that Alzheimer's is one of the most expensive diseases, whatever its exact cost is, exceeded only by heart disease and cancer.

137. RADIATION REVEALS A STEM? CELL RESERVE IN THE LENS

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Delivery of high dose (11Gy), low energy x-radiation to the head-only of 2 month old mice results in the appearance of severe cataracts, but only after a 7-10 month delay, during which time no evidence of lens opacity is seen. In fact, the appearance of the cataracts occurs suddenly, often within a 30 day period after this long delay. This led us to ask whether or not the irradiation may reduce a lens epithelial cell (LEC) stem cell reserve that then eventually fails to supply the replicating LEC on the lens surface. These LEC represent the only cell type present on the lens. They lose their internal organelles and convert to the internal lens fibers only after entering the lens interior following extensive replication and migration on the surface. We found that 35 days after the in vivo irradiation the capacity of the irradiated LEC population did not differ from that of un-irradiated control mice in producing total number of clones or percent of large clones in vitro. Thus, the irradiated LEC population behaves normally in this respect at this time. We expect a defect to be observable 90 days after irradiation and much more so 7 months after irradiation, when severe cataracts suddenly appear in the irradiated mice (and none in controls). We have also instituted sequentially timed BrdU studies to determine post-irradiation cell division sites, and stem cell markers by immunohistochemistry in the separate regions of the lens that normally do not have dividing cells (the central zone), or have continually dividing cells (the proliferative zone and beyond). Confocal examination of LEC nuclear and mitochondrial status and health have also been instituted. No successful studies of a stem cell reserve for LEC have been reported.

138. PROTEASOME ACTIVITY DECLINES IN AGED MACROPHAGES

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The ubiquitin-proteasome pathway is involved in regulation of a variety of biologically important processes including antigen presentation by macrophages (Mf). Age-related decrease in proteasome activity has been reported in other tissues. However, the effect of aging on the ubiquitin-proteasome pathway in Mf has not been systematically studied. Thioglycollate-elicited Mf from young (4-6 mo) and Old (24-26 mo) male C57BL/6NIA mice were collected by lavage. Mf from old mice exhibited significantly lower activity of all three proteasome peptidases [chymotrypsin-like activity (young vs old, Mean ± SE): 100 ± 13 vs 62 ± 4, $p<0.05$; trypsin-like activity: 100 ± 4 vs 56 ± 3, $p<0.01$; acidic activity: 100 ± 7 vs 58 ± 6, $p<0.01$]. The age-related decrease in proteasome activity was not due to decreased expression of proteasome subunits, since levels of C2 and S1 subunits were higher in old Mf than young (C2: 100 ± 20 vs 167 ± 13, $p<0.05$; S1: 100 ± 15 vs 183 ± 20, $p<0.01$) while there was no significant age-related difference in

levels of a5, LMP2 and LMP7. The decrease in proteasome activity was associated with an increase in the levels of ubiquitin conjugates. Furthermore, the levels of 2 ubiquitination-related enzymes, E1 and E2 (Ubc7), were significantly higher in old Mf than young. The age-related decline in proteasome activity and the paralleled increase in levels of ubiquitin conjugates indicate that the function of the ubiquitin-proteasome pathway is impaired in the aged Mf. The decrease in proteasome activity in Mf might contribute to the age-related decline in the ability of Mf to present antigens to T cells, a proteasome dependent process. Supported by USDA #58-1950-9-001 and NIA #R01 AG009140-10A1.

(N)

139. OVARIAN RESERVE TESTS AND THEIR UTILITY IN PREDICTING RESPONSE TO CONTROLLED OVARIAN STIMULATION IN RHESUS MONKEYS

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Controlled ovarian stimulation (COS) is an alternative to natural breeding; however these regimens are costly with no guarantee of success. This study evaluated several clinically used ovarian reserve tests (ORTs): day 3 (d3) follicle-stimulating hormone (FSH), d3 inhibin B (INHB), the clomiphene citrate challenge test (CCCT) and the exogenous FSH Ovarian Reserve Test (EFORT) in predicting COS outcome in rhesus monkeys. Animals were monitored for onset of menses (d1). CCCT was performed by orally administering clomiphene citrate on d5-d9 and measuring either FSH or INHB on d3 and d10. The CCCT was modified by adding fold change in E2. The EFORT was performed by administering recombinant-human FSH and measuring E2, FSH and INHB just prior to and 24 hours after injection. Finally a COS was also performed (Antide, d1-d9; recombinant human FSH (r-hFSH) d1-d6; r-hFSH + r-h luteinizing hormone, d6-d9) and response was classified as either successful (COS+; > three 4mm follicles on each ovary and peak E2 levels > 1000pg/mL) or unsuccessful (COS-; < three 4mm follicles on each ovary and peak E2 levels < 600 pg/mL) and retrospectively compared to ORT predictions. Estradiol (E2), FSH and INHB were assessed for best hormonal index in conjunction with the aforementioned tests. d3 INHB (77%) was a better predictor of COS outcome than d3 FSH (59%). Percent accuracy of d3 hormones was also better than with CCCT alone (FSH, 45%; INHB, 59%). Modifying the CCCT to include fold change in E2 improved the accuracy for both FSH (77%) and INHB (82%). Accuracy using the EFORT was 55% for FSH, 80% for INHB and 65% for E2. The modified CCCT using INHB values yielded the best percentage of correct predictions. This is the first report of ORT evaluation in rhesus monkeys, and may provide a useful diagnostic test prior to costly follicle stimulations.

(G)

140. ANALYSIS OF TRANSCRIPTION FACTORS IN AGING OF DROSOPHILA MELANOGASTER

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Aging and oxidative stress alter the expression levels of many genes and increases or reductions in expression of a specific gene have been related to extended lifespan and to increased tolerance to oxidative stress in many organisms. These findings suggest that it is important to elucidate the regulatory systems of gene expression related to aging and oxidative stress in a wide range of organisms. Previous results indicated that two highly diverged animals, the nematode, Caenorhabditis elegans, and the fruit fly, Drosophila melanogaster, possess a shared adult-onset expression program of many genes. We identified several transcription factors, which are candidates for regulating gene expression involved in mitochondria and proteosome function. To elucidate the roles of these factors in aging of Drosophila melanogaster, we analyzed gene expression and the stress resistance phenotype in S2 cells with reduction or overexpression of these transcription factors. The expression levels of the transcription factors were reduced in S2 cells by double-stranded RNA (dsRNA) treatment. We found that viability of S2 cells treated with

dsRNA was decreased after exposure to hydrogen peroxide compared to control cells for several transcription factors. Furthermore, a transcription factor, whose loss-of-function phenotype was sensitive to hydrogen peroxide, was overexpressed in the GAL4-UAS system, and its expression was increased in S2 cells. Gene expression of the candidate downstream genes of the transcription factors as well as the phenotype of their overexpression in S2 cells is currently being examined. Lifespan of strains with mutations in these transcription factors is also under investigation. This study will lead to identification of a transcription network that modulates lifespan.

(N)

141. MITOCHONDRIAL PERMEABILITY TRANSITION PORE AS A CALORIGENIC TARGET

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Thyroid hormone (TH) modulates metabolic efficiency by controlling the coupling of mitochondrial oxidative phosphorylation. However, its uncoupling mode of action is still enigmatic. Mitochondrial transition pore (MTP) is a nonspecific channel that may present itself in two modes: high conductance, that can result in irreversible extensive uncoupling which may lead to cell death and low conductance (LC), that can result in a milled mitochondrial depolarization leading to calorigenesis rather than cell death. Therefore, LC-MTP can be a good candidate for modulation of metabolic efficiency by TH. We have previously demonstrated that triiodothyronine (T3) uncoupling was accompanied by MTP gating in isolated liver mitochondria. The aim of this study is to investigate the TH effect on LC-MTP gating in cells and to identify mitochondrial MTP components, induced or suppressed by in vivo T3 treatment that may be functionally involved in MTP gating by TH. Treatment of Jurkat or GH3 cells by T3 results in limited Cyclosporin A sensitive mitochondrial depolarization, conforming to LC gating of MTP. TH induced LC-MTP (as verified in rat liver mitochondria as well as in Jurkat cells) is not modulated by inducing changes in the constitutive MTP protein components (Cyclophilin-D, ANT, VDAC), but rather via introduction of changes in the composition of mitochondrial Bcl-2 family members, resulting in an increase of the proapoptotic protein members (Bax, Bak) together with a decrease of the antiapoptotic protein members (Bcl-2). Overexpression of Bcl-2 protected Jurkat cells from T3 induced-depolarization. Moreover, T3 treatment resulted in a decrease of mitochondrial serine70-Bcl-2 phosphorylation, which could be abrogated by cell treatment with FK506, a specific calcineurin inhibitor. Underling the molecular mechanism of MTP as a net consumer of metabolic energy can serve as a molecular target for future drugs that will hopefully increase health span.

(N)

142. ADDITIVE EFFECTS OF CALORIC RESTRICTION, COLD TEMPERATURE, THE INSULIN/IGF PATHWAY AND CLK-1 MUTATION ON LIFESPAN IN C. ELEGANS

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Using C. elegans as a model organism, these experiments attempt to use epistatic analysis combined with Gompertz analysis to determine if there is a common mechanism by which insulin signaling, temperature, dietary intake, and/or mitochondrial changes prevent senescence. Daf-2 worms, which have a mutation in their insulin/IGF receptor that decreases signaling, axenic media, a model for dietary restriction, cold temperature, and the clk-1 mutant, which has a mutation that interferes with ubiquinone synthesis, have been used in these experiments. Epistatic analysis has suggested that each of these manipulations extend lifespan by unique mechanisms. Control worms lived an average of 13 days while those treated with all four manipulations could achieve an average of 94 days with some individuals living 120 days and over. While epistatic analysis suggests independent mechanisms, Gompertz analysis shows a minimal Gompertz variable achieved when any 2 manipulations plus cold temperature are used. The addition of a third manipulation to cold temperature tended to influence the initial mortality rate with no effect on Gompertz variable. These data suggest that cold regulates lifespan through a mechanism distinct from those of dietary restriction or the insulin-like pathway, but do not rule out a possible final pathway.

(N)

143. RESTORATION OF CHAPERONE-MEDIATED AUTOPHAGY IN OLD MICE

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Chaperone-mediated autophagy (CMA) is one of the three major types of autophagy in mammalian cells, in which selective cytosolic proteins are degraded in lysosomes after binding to a lysosomal membrane receptor – LAMP-2A. CMA is mostly activated under stress conditions, and is an essential component of the cellular oxidative stress response. In aging, CMA activity decreases due to a decrease in the levels of LAMP-2A in the lysosomal membrane. The goal of this work was to maintain levels of LAMP-2A constant throughout a mouse's life span and to analyze the consequences of this intervention on CMA activity and on cell and organ function. We have generated a bitransgenic mouse model, which overexpresses LAMP-2A specifically in liver under the control of a tetracycline-regulated promoter. Real-time PCR and immunoblot analysis revealed that the expression of LAMP-2A was efficiently regulated by doxycycline, and this tight regulation was maintained in old animals. Lysosomes from 22m old transgenic mice displayed significantly higher CMA activity than lysosomes from the same age wild type mice, reaching values close to those at 6 months of age. In addition, our 22m old transgenic mice had markedly lower levels of oxidized cytosolic proteins compared with the same age wild type mice. We also found a dramatic decrease in lipofuscin content in the livers of the old transgenic mice and their ultrastructural analysis revealed the absence of mitochondrial condensation, glycogen fragmentation or the abnormal proliferations of ER, normally described in old wild type mice livers. Our preliminary studies also indicate improvement of macroautophagy activity, as well as in the activity of the ubiquitin/proteasome system. Our results support that restoration of CMA activity in old rodents enhances also the activity of other proteolytic systems, thus resulting in improved cell function.

144. SINGLE SKELETAL FIBER PASSIVE CONTRACTILE PROPERTIES DO NOT EXPLAIN AGE-RELATED MUSCLE STIFFNESS

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The objectives of this study were to examine the effect of aging on the (1) passive contractile properties and (2) titin isoforms of single myosin heavy chain type IIB and type I fibers (MHC) in order to identify the cellular mechanisms responsible for the reported increase in muscle stiffness with age. Single fibers were prepared (soleus, MHC type I and semimembranosus, MHC type IIB) from young (9-12 months) and old (27-36 months) rats. Following the assessment of contractile properties, MHC and titin isoforms were determined (SDS-PAGE). With age, there was fiber atrophy (12%), a decline in force-generating capacity (30%) and a decrease in specific tension (14%) in both fiber types. In both MHC type I and type IIB fibers, the resting passive force at optimal length (2.5um sarcomere length, Lo) did not differ between young and old rats. With passive stretching, from 110% to 150% Lo, passive force increased exponentially in both fibers types and age groups. However, the MHC type I and type IIB fibers from the young rats exhibited greater passive force at each stretched length compared with the fibers from old rats. The individual MHC type I and type IIB fibers exhibited specific titin isoforms, however no age-related change in titin isoforms was noted. The results of the present study suggest that changes in passive contractile properties of single fibers with age do not contribute to the observed increase in muscle stiffness. Therefore, the increase in muscle stiffness with age is most likely due to intermyofibrillar changes.

145. MODULATION OF ADIPOGENESIS SIGNALING BY CALORIC RESTRICTION IN RAT WHITE ADIPOSE TISSUE

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Alterations in adipogenesis could have significant impact on several aging processes. These alterations involve a highly regulated and coordinated cascade of transcription factors that leads to the establishment of the differentiated state. We previously reported that caloric restriction (CR) in rats significantly enhances the level of circulating adiponectin (ADP), which leads to a concerted modulation in the expression of key transcription target genes and, as a result, to increased fatty acid oxidation and reduced deleterious lipid accumulation in other tissues. These results suggest that adipogenesis contributes to beneficial effects of CR because ADP is exclusively expressed in differentiated adipocytes. To further characterize the changes induced by CR in white adipose tissue (WAT), we investigated two groups of transcription factors, bZIP family of CCAAT/enhancer binding proteins (C/EBPs) and peroxisome proliferator-activated receptors (PPARs), in WAT from CR rats. CR (both 2mo and 25mo) significantly increased the expression of C/EBPα; and PPARγ; relative to the rats fed ad libitum (AL). C/EBPα; and PPARγ; together promote adipogenesis, as evidenced by activating ADP expression at both mRNA and protein levels, maintaining target genes, acyl-CoA oxidase and fatty acid synthase, at high levels, and activating insulin receptor at both mRNA and protein levels with enhanced tyrosine phosphorylation in response to insulin. In addition, CR decreased the ratio of LAP/LIP (C/EBPß) due to increased LIP rather than induced change in LAP expression. Quantitative morphometrics revealed that the distribution of adipocyte sizes in CR rats had a positive skew with a large value of kurtosis (primarily small adipocytes) compared to AL tissue. Furthermore, histological studies demonstrated smaller adipocytes (<2000um2) were more strongly positive for ADP than were larger ones (>8000um2). Overall the data suggest that an alteration in adipogenesis induced by CR may contribute to optimizing glucose.

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