

Carbonyl modification in rat liver histones changes with age and dietary restriction

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Carbonylation of proteins has various implications in health and diseases. The human diseases associated with carbonylated proteins include Alzheimer's disease, chronic lung disease, chronic renal failure, diabetes and sepsis. Protein carbonyls (PCO) are formed as a consequence of the oxidative modifications of proteins by ROS. Many reactions result in the formation of protein carbonyl derivatives such as direct oxidation of arginine or proline to glutamate-semialdehyde or lysine to α -amino adipic semialdehyde. In addition, carbonyl groups may be introduced into proteins by reactions with aldehyde produced during lipid peroxidation or with carbonyl derivatives generated as a consequence of the reaction of reducing sugar or their oxidation products with lysine residues of proteins by glycation and glycoxidation reactions. In order to assess the extent of carbonylation in a group of hitherto unstudied proteins, it was observed that histones, an essential component of nucleosomal chromatin, undergo carbonylation, an oxidative modification, in the liver of rats *in vivo*. It is the H1 (the linker) and H2A, H2B and H3 (the core) histones that are carbonylated, albeit to varying levels in normal conditions. However, under *in vitro* oxidative conditions (using nuclei or isolated histones) all of the histones including H4 were carbonylated, though to a larger extent. Among them, H1 was most highly carbonylated followed by H2B/H2A/H3/H4. Contrary to our expectation, there was a higher carbonylation of H1 and core histones (H2A, H2B and H3) in the liver of young (5-month-old) as compared to old (30-month-old) rats. Dietary restriction (DR, alternate days of feeding) of the older animals for 2 months led to an increase in carbonylation almost comparable to the level in younger animals. Such age-dependent and DR mediated differences in carbonylation of histones are not observed under *in vitro* oxidation conditions. Contrary to the generally accepted view of increased carbonylation of proteins during aging, the modification of histones was low in older than in younger mice. Dietary restriction of older mice resulted in an increase of carbonylation comparable to that at

young age. Our findings for the first time revealed a differential carbonylation of histones during the aging process and by DR, which may have physiological significance in chromatin structure and function during aging and may impart beneficial effects of DR to older animals.

Adrenoceptor mediated central noradrenergic neurotransmission in ageing

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While many evidences indicate that deficits of central noradrenergic neurotransmission are involved in the age-related decline of cognitive functions in animals and man, very little is known about the specific role of central α and β -adrenergic receptors. Interactions between brain α and β -adrenoceptors are of interest in physiological (aging) and pathological (major depression) processes involving both receptors. Biochemical and pathological studies have described abnormalities in the brainstem locus coeruleus noradrenergic neurons in Alzheimer's disease (AD) and in aging. α - and β -adrenergic receptors and their second messengers play an important role in brain neurotransmission. Changes in receptor function with age may be involved in the age-related changes in arousal, mood and memory. The age-related changes in brain function become evident by comparing the responsiveness to drugs stimulants or blocking agents between young and aged rats. Medications directed at the α - and β -adrenergic system are commonly used in the elderly. We report here the biochemical characterization and subtype differentiation of α - and β -adrenoceptors in brain regions of adult rats using radioligand binding studies and compare the differential effect of stimulants (GTP), uptake inhibitors and certain antidepressants. In aging there is not only a reduced density of adrenergic receptors but also a reduced capacity to adapt these receptors to a changing neuronal input or hormonal environment. Changes in the brain α and β -adrenoceptors, correlated with the animal age, are connected to the number of binding sites, rate constants and the affinity of an agonist. The predominance of data indicates decreased α and β -

adrenergic receptors in all areas of the brain. Evidence suggests a decreased rate of receptor synthesis may be contributing to this loss of receptors with age. It is suggested that the specific decrease of high-affinity agonist sites of central α and β -adrenoceptors might represent one of the mechanisms leading to a general impairment of central noradrenergic neurotransmission with aging.

Role of mitochondrial active oxygen metabolism in impairment of testicular function with age

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Reactive oxygen species (ROS) such as superoxide radicals ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^{\cdot}) are generated as a normal byproduct of aerobic life and the accumulation of oxidative damage caused by ROS underlie the fundamental changes found during aging. Mitochondria are the main intracellular source and the target of ROS, which are constantly produced in cells as a consequence of metabolism and incomplete reduction of oxygen in mitochondrial electron transport chain during respiration. Mitochondrial oxidants attack almost all biomolecules and consequently affect the normal physiology of the organism. In the present study, age related effect on testicular mitochondrial oxidant generation and antioxidant defence profile was investigated in male rats of three different age groups (3 months, 12 months and 24 months old). Mitochondrial oxidative stress parameters such as lipid peroxidation (LPx), Protein carbonylation and Hydrogen peroxide (H_2O_2) generation and the activities of antioxidant molecules such as glutathione (GSH) and glutathione dependent enzymes were investigated in rat testis in order to establish the role of antioxidant defence system in reproductive function during aging. An increased level of LPx, H_2O_2 and protein carbonylation and a decreased GSH content followed by decreased activities of antioxidant enzymes (GPx and GR) with advancing age suggest that antioxidant defence profile of testicular mitochondria exhibit age related change which might play a critical role in regulating the physiological function of the testis such as steroidogenesis and spermatogenesis.

Inhibition of mRNA translation extends lifespan in *C. elegans*

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Evolutionary theory proposes that aging is a byproduct of the tradeoff between allocation of resources towards growth and reproduction versus somatic maintenance. Here we test the hypothesis that protein synthesis mediates this tradeoff. Translational control of protein synthesis by regulators such as the cap-binding complex and S6 kinase links nutrients to growth. We observe that inhibition of various genes in the translation initiation complex including ifg-1 (worm homologue of eIF4G), a scaffold protein in the cap binding complex, and rsk-1 (worm homologue of S6K) results in lifespan extension in *C. elegans*. ifg-1 and rsk-1 show phenotypes consistent with antagonistic pleiotropy as their reduction early in life slows development and reduces fecundity in *C. elegans*. Inhibition of ifg-1 and rsk-1 causes a significant increase in resistance to starvation implicating their role in energy balance. A reduction in ifg-1 expression in dauers was also observed suggesting an inhibition of protein translation during the dauer state. Our results demonstrate a key role of growth-dependent protein synthesis in mediating tradeoffs that modulate lifespan. Given the previously reported link between lifespan and protein synthesis in various species, we suggest that differences in lifespan across species may partly be determined by rates of growth-dependent protein synthesis.

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Vitamin E and Exercise on Oxidative Stress in Regions of Aging Rat Brain:

Studies on superoxide dismutase and lipid peroxidation

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A major change with aging of brain is the impact of oxidative stress (OS) and subsequent generation of free radicals that are known to cause degeneration of cellular elements that ultimately lead to several neurodegenerative conditions such as Alzheimer's and Parkinson's diseases. The present study was to understand the combined effects of exercise training and vitamin E (Vit E) supplementation in retarding age-related oxidative modification of lipids and proteins, and analyzing the status of cellular antioxidative defense in the cerebral cortex (CC), hippocampus (HC) and cerebellum (CB) of rat brain.

Animals of 4-(adult), 12- (middle-age) and 18-mos (old) of age were orally supplemented with vitamin E and swim trained at 3% intensity. Vit E increased total SOD activity in the old trainees with Mn-SOD increasing in the middle age and old trainees while Cu-Zn-SOD increased in the supplemented trained adults. Age-specific and region-specific increases in protein carbonyl (PrC) content with decrease in sulphhydryl (P-SH-) content were noticed. Vitamin E reduced protein carbonyl content (PrC) along with AOPP in all ages, and with appreciable changes in the HC and CB. Our study has also indicated a correlation between mitochondrial H₂O₂ generation, Mn-SOD activity and MDA level, revealing in part an age-related increase in lipid peroxidation and protein oxidation, and that may occur under situations such as Vit E deficiency. (UGC Grant: No.F3-196/2001 SR-II)

Possible candidate biomarkers for early diagnosis of Alzheimer's disease

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Originally described by Alois Alzheimer in 1907, Alzheimer's disease (AD) has emerged as the most common type of dementia in the elderly today. In spite of the fact that it is so common, AD often goes unrecognized or is misdiagnosed in its early stages. Through the "process of elimination" probable AD can be diagnosed with almost 90% accuracy but definitive diagnosis is possible only at autopsy. So, biochemical markers for AD would be of great value for its early diagnosis. There are number of possible candidates for the biomarkers in AD that reflect the pathology characterized by two classic changes: plaques and

neurofibrillary Tangles (NFTs). Plaques represent the abnormal deposition of A β produced by APP (Amyloid Precursor Protein). There are two forms of the A β , depending upon the size, 40 & 42. A β 42 is the most consistent biomarker of presence of AD. NFTs consist of abnormal tau protein. Tau protein normally binds cell microtubules. Abnormal (hyperphosphorylated) tau proteins stick together to form tangles. The diagnostic performance of the CSF biomarkers: total tau (T-tau), phosphorylated tau (P-tau) & the A β 42 are of great importance. CSF samples from AD patients participating in a 5-year treatment study were retrospectively analyzed for the aforesaid biomarkers; the study concluded that increased levels of P-tau & T-tau are possible markers for severity & abundance of symptoms in AD. Low levels of A β 42 may indicate a higher risk of early death in AD. Since evaluations of P-tau are highly correlated with reductions in the MRI hippocampal volume hence by using CSF & MRI measures together a better differential diagnosis of AD could be at hand. Rather than a single biomarker, a combination may be more reliable. Measuring A β & tau in the CSF & MRI measures can be proved highly sensitive & specific to the diagnosis but once clinical symptoms have begun.

Age-related differential regulation of tyrosine hydroxylase gene in cerebral and cerebellar cortices of the brain of mice

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Tyrosine hydroxylase (TH) is the first and rate-limiting enzyme for the biosynthesis of three neurotransmitters - dopamine, nor-epinephrine and epinephrine. Dopamine is one of the major modulatory neurotransmitters among the catecholamines in the brain. Catecholamines play a major role in several brain functions such as locomotion, behavior, sleep, emotion, learning and memory. We studied the regulation of TH gene in the cerebral and cerebellar cortices of the brain of mice at birth, post-natal development and old age. RT-PCR study shows that expression of its mRNA is highest at birth (0-day). However, in the old, it is up-regulated as compared to that of the adult in cerebral cortex, and is down-regulated in cerebellar cortex as a function of age. The role of its two *cis*-acting elements, CRE and AP1, in the regulation of its expression was studied by gel-shift and supershift

assays. Gel-shift assay shows one major complex with CRE at 70-week for cerebral cortex. However, for AP1 there is an extra complex at 0-day. Supershift study shows the involvement of Fos-B with AP1 complex at day-15 after birth and onwards in the cerebral cortex. However, there is no such involvement of Fos-B with AP1 complex in the cerebellar cortex at any age. We postulate that cerebral cortex has a compensatory mechanism in old age, whereas there is no such mechanism in the cerebellar cortex. Our studies provide insight into the role that the dopaminergic neurons play in the neuropathological changes that occur in the brain in old age.

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Expression of apolipoprotein E gene in aging mouse brain

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ApolipoproteinE (apoE) plays an important role in plasma cholesterol metabolism as a ligand for specific cell surface receptors. It is predominantly expressed in the liver. In brain, it is expressed by microglia and astrocytes. In human, there are three isoforms of apoE: apoE2, apoE3 and apoE4. The apoE4 allele is associated with increased risk for the development of late-onset form of Alzheimer's disease (AD). ApoE has critical functions in redistributing lipids among central nervous system cells for normal lipid homeostasis, repairing injured neurons, maintaining synaptodendritic connections and scavenging toxins. In multiple pathways affecting neuropathology, including AD, apoE acts directly or in concert with age, head injury, oxidative stress, ischemia, inflammation and excess amyloid b peptide production. In cultured neurons, apoE induces neurite outgrowth and functions as an endogenous antioxidant. Several studies suggest a protective role of sex steroid hormones estrogen and testosterone in neurological diseases. Aged people show improved cognition after treatment with sex steroids. Estrogen increases regional and whole brain levels of apoE mRNA and protein. The present study aims to investigate the effect of age, sex and sex steroid treatment on the expression of apoE by western blot analysis. AKR strain mice of two age groups- adult (24±2 weeks) and old (65±5 weeks) were categorized into four experimental groups- intact, gonadectomized, gonadectomized with

17 α - estradiol treatment and gonadectomized with testosterone treatment. The level of apoE was higher in adult male as compared to old. In adult male, the level was decreased by gonadectomy but increased by 17 β -estradiol treatment. However, in female, the level of apoE was higher in old as compared to adult. These findings reveal that the expression of apoE is modulated by steroid hormones with age and sex, suggesting differential functions of apoE in the brain.

Expression of Fragile X mental retardation (*Fmr1*) gene in mice testis during aging

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Fragile X syndrome is one of the most common inherited mental retardation diseases. It is associated with a fragile site at Xq27.3 in human X chromosome, which affects 1 in 4000 males and 1 in 6000 females. In fragile X syndrome in which FMRP expression is absent due to dynamic mutation attributing to abnormal methylation of CGG repeat in 5'-untranslated region (5'-UTR) of the *Fmr1* gene. Lack of expression of the fragile X mental retardation protein (FMRP) results in mental retardation, macroorchidism, premature menopause and FXTAS as the major pathological symptoms in fragile X patients. The *Fmr1* is highly expression in brain and testis, which are most affected organs in fragile X patients and *Fmr-1* knockout mice. Macroorchidism has been associated with lack of FMRP observed in the majority of post pubescent fragile X patients. The FMRP is a RNA binding protein and possesses two KH domains and an RGG box. It is involved in nuclear localization and transport of its own and other mRNAs and thereby regulates the cellular translational machinery. The process of aging leads to decline in function of various organs of the body in all organisms. Also, since the testicular functions such as testosterone production and spermatogenesis decline in old age, we have studied the expression pattern of the *Fmr1* gene and FMRP in testis in young, adult and old mice using Northern and Western blotting techniques. Our data reveal that the *Fmr1* gene and FMRP are down regulated in testes of young and old mice as compared to that, which are up regulated in adult mice. The data indicate that the *Fmr1* gene is associated with male reproductive functions in addition to its role in brain function. The relevance of the finding will be discussed.

Age associated changes in biomarkers of oxidative stress

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The free radical theory of aging predicts that reactive oxygen species are involved in the decline of functions associated with aging. The susceptibility of an individual to oxidative stress depends on the antioxidants present inside the body. In humans defense mechanism is governed by both by enzymatic and non-enzymatic antioxidants.

The measurement of Ferric Reducing Ability of the Plasma (FRAP) provides a convenient approach to assess total antioxidant status of the body. The present study was undertaken to evaluate the total antioxidant capacity of plasma as a function of human age and how it is correlated with erythrocyte oxidative stress markers: Malondialdehyde (MDA) - a lipid peroxidation product, intracellular reduced glutathione, and membrane -SH groups. We show here a significant negative correlation between the plasma antioxidant potential and human age. MDA has positive correlation with human age, while GSH and membrane -SH groups show significant negative correlation as a function of human age. The results are consistent with interpretation that there is a generalized oxidation associated with aging that may contribute to age related diseases. The observation shows the importance of dietary antioxidants in the human body.

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Expression of S100b, Connexin 43 and GFAP genes in the brain of mice during development and aging

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Brain has two types of cells: neurons and glia. Astrocytes are the largest and most numerous glial cells in the central nervous system. They surround (ensheath) synapses and perform a variety of functions including synaptogenesis, homeostatic regulation, recycling of neurotransmitters, and regulation of energy

supply to neurons, etc. We have studied the expression of genes for S100 b, Connexin 43 and glial fibrillary acidic protein (GFAP) in the cerebral and cerebellar cortices of the brain of new born, 15-day, 45-day, 20-week and 70-week old AKR mice using RT-PCR. S100 b is a cytosolic calcium binding protein involved in growth, structure, energy metabolism, and calcium homeostasis of astrocytes and neurons. Connexin 43 is a gap junction protein, present on astrocyte membrane and is involved in intracellular transport of metabolites between astrocytes and neurons. GFAP is an intermediate filamentous cytoskeletal protein that maintains the structure of astrocytes. The aim of the present study is to find out if there are any age-related changes in the expression of these genes in the astrocytes. Our RT-PCR results show that expression of these genes peak around 15 - 45 day after birth. Their expression is slightly higher in the cerebral and cerebellar cortices of 70-week old mice as compared to that of 20-week old mice. On the basis of the above study we propose that astrocyte functions are fully established around 15- to 45-day of post-natal life. Up regulation of these genes in old age suggests activation of astrocytes that may lead to various pathophysiological conditions in brain.

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Effect of maternal age on lipid peroxidation in follicular fluid of women undergoing ART: relationship to outcome

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A growing body of evidence indicates that pro-oxidant/antioxidant balance inside ovarian follicles plays an important role in folliculogenesis. Intensified peroxidation in the Graafian follicle may be a factor compromising the normal development of the oocyte and the role of reactive oxygen species in ageing process is well established. The impact of age on the intrafollicular markers of oxidative stress has not been fully elucidated. The objective of the present study was to find out the impact of age on the intrafollicular redox milieu and the consequent outcome in women undergoing assisted reproduction. In follicular fluid samples originating from 86 IVF/ICSI patients, lipid peroxidation was assessed by measurement of the thiobarbituric reactive substances (TBARS) along with the

measurement of activities of superoxide dismutase and catalase, the enzymes that offer first line of defence against reactive oxygen species. The data were correlated with no of eggs retrieved, number of embryos formed and 14th Day serum β hCG level. The results indicate that maternal age is inversely correlated with number of eggs retrieved ($Y = 17.46 - 0.33x$, $r = -0.295$, $p < 0.005$) number of embryo formed ($Y = 7.78 - 0.14x$, $r = -0.298$, $p < 0.005$) and positively correlated with level of TBARS ($Y = 0.23x - 5.32$, $r = 0.63$, $p < 5.95 \times 10^{-11}$). Therefore, it is suggested that ageing affects the pro-oxidant/antioxidant balance inside the pre-ovulatory ovarian follicle by inducing intrafollicular oxidative stress. This provides another possible explanation for impaired folliculogenesis in female ageing.

Expression of Alzheimer's disease associated *presenilin* gene and its regulation by sex steroids in ageing mouse cerebral cortex

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by impairment of cognition and memory. The early onset of AD (<60 years) is linked to mutations in amyloid precursor protein (APP), presenilin (PS) 1 and PS2. Presenilins are integral membrane proteins with 7-8 transmembrane domains. Whereas PS1 (43-45kDa) is proteolytically cleaved into stable 27-28kDa N-terminal and 16-17kDa C-terminal fragments, PS2 (54kDa) is proteolytically cleaved into 20kDa C-terminal and ~34kDa N-terminal fragments. Besides its association with AD pathology, PS proteins are involved in regulation of cleavage of type IV transmembrane protein, α -secretase activity, apoptosis, cell cycle of neuronal precursor cells, cell fate decision during development, neuronal differentiation, synaptic function and synaptogenesis. AD is more prevalent in post-menopausal women and sex steroids are used for the treatment of AD related cognitive impairment. However, the effect of sex steroids on PS expression is not known. Therefore, we have examined the expression of presenilin and its regulation by estrogen and testosterone in the mouse cerebral cortex. Presenilin proteins were detected in detergent solubilized membrane enriched preparations, using specific antiserum. Both proteins show significant change in expression with age. PS1 and PS2 showed differential

regulation with aging. Also, the sex-dependent variation in expression of the two proteins which is prominent only in adult is differential. Gonadectomy also lowers the level of presenilin proteins in old age. PS2 protein shows increase in expression with sex steroid treatment in both ages, but estrogen supplementation to ovariectomized old mice lowers PS1 level. The expression of presenilin at mRNA also showed significant variation with age and sex. The effect of gonadectomy and supplementation of sex steroids was also reflected at mRNA level as with PS proteins. Thus, the modulation of presenilin with age, sex and steroid hormones on PS may explain the therapeutic interventions of hormone replacement therapy.