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Μηχανισμοί Ελευθέρων Ριζών, Οξειδωτικό Stress και Γήρανση του Ανθρώπου

Διατροφικά συμπληρώματα αντιοξειδωτικών ουσιών ή θερμιδικός περιορισμός για την αναστροφή της γήρανσης;

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Mechanisms of Free Radicals, Oxidative Stress and Ageing in Humans. Dietary Supplements of Antioxidants or Caloric Restriction for Reversing Ageing?

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ΠΕΡΙΛΗΨΗ. Οι μηχανισμοί ελευθέρων ριζών, οι οποίοι έχουν σημαντική φυσιολογική λειτουργία στους αερόβιους ζωντανούς οργανισμούς, μπορούν να καταστούν επιβλαβείς για την υγεία τους κάτω από συνθήκες οξειδωτικού stress. Τις τελευταίες δεκαετίες, πολυάριθμες έρευνες και κλινικά αποτελέσματα έδειξαν ότι το οξειδωτικό stress και η υψηλή συγκέντρωση οξυγονούχων ελευθέρων ριζών προκαλούν βλάβες σε βασικά βιολογικά μόρια. ‘ Όπως, στις πρωτεΐνες και ένζυμα, τα λιπίδια των μεμβρανών των κυττάρων και τα νουκλεϊνικά οξέα (DNA, RNA) του πυρήνα των κυττάρων και των μιτοχονδρίων. Η συσσώρευση των βλαβών αυτών και η μειωμένη δράση των επιδιορθωτικών μηχανισμών και αντιοξειδωτικών ενζύμων θεωρούνται ότι προκαλούν τμηματικά τη γήρανση των αερόβιων οργανισμών.
Η διατροφή αποτελεί σημαντικό παράγοντα υγείας των αερόβιων οργανισμών και οι αντιοξειδωτικές φυτοχημικές και άλλες ουσίες παίζουν ακρογωνιαίο ρόλο στη μείωση του οξειδωτικού stress και ρύθμισης των μηχανισμών ελευθέρων ριζών. Η θεωρία της γήρανσης λόγω μεταβολών στην αντιμετώπιση οξειδωτικών βλαβών, συσσώρευσης οξειδωμένων βιομορίων και θραυσμάτων του DNA έχει επιβεβαιωθεί με πολλές έρευνες. Οι αντιοξειδωτικές ουσίες της διατροφής παίζουν σημαντικό ρόλο, αλλά δεν μπορούν να αναστρέψουν τη φυσιολογική πορεία της γήρανσης, απλώς περιορίζουν την επιτάχυνση των σταδίων της γήρανσης και των κλινικών φαινομένων. Ο θερμιδικός περιορισμός έχει δώσει θετικά αποτελέσματα σε πειράματα γήρανσης σε πειραματόζωα και τις πρώτες ενδείξεις για μείωση των βλαβών στον άνθρωπο. Αλλά είναι νωρίς ακόμη να τεκμηριωθούν οι βασικοί μηχανισμοί με τους οποίους επιτυγχάνεται η αναστροφή των γηραντικών φαινομένων λόγω του πολύπλοκου φυσιολογικού μεταβολισμού και των γενετικών επιπλοκών στον άνθρωπο. Το άρθρο αυτό συνωψίζει τα κυριότερα επιστημονικά επιτεύγματα και τις αποδείξεις της αντιγηραντικής πορείας με αντιοξειδωτικά διατροφικά συμπληρώματα και πειράματα θερμιδικούς περιορισμού.

Abstract

Theories were proposed through the years for the reasons of ageing in aerobic biological systems. Some of these theories lost favor because of lack of scientific support but other remained as potential avenues of biogerontological research. In the 1950s Denham Harman announced his seminal proposal that reactive oxygen species (ROS) are an important cause of ageing. He proposed that free radicals formed endogenously as by-products from normal metabolism played an important role in ageing, as well as the mitochondria because these organelles proved to be a major site of ROS production. Aerobic organisms under normal conditions accumulate oxidative damage to cellular macromolecules (imbalance of pro-oxidants and antioxidants agents) that increases during ageing, contributing progressively to increased oxidative stress and to the decline of cellular processes. In the last decades new clues have been discovered about the role of oxidative stress in the mammalian ageing and a deeper understanding has been developed of age-related diseases in humans. The current research tested the direct involvement of oxidative stress in ageing in mice and other organisms and the data support the oxidative theory of ageing. Supplements with antioxidant vitamins/minerals during randomized clinical trials showed

conflicting efficacy data on improving various oxidative stress diseases. Caloric (or calorie) restriction (CR) in the other hand proved to be beneficial in metabolic, hormonal and functional changes in adult men and women. This review presents the most important advances in scientific research and experiments *in vivo* for the oxidative theory of ageing.

1. Introduction, Theories of ageing and oxidative stress

The origins of the free radical theory of ageing (or aging) go back to the 1950s when it was discovered that oxygen free radicals formed in situ in the cellular compartments of the aerobic organisms in response to radiation and oxygen metabolism are responsible for various oxidative damage.[1,2]

Denham Harman, noting that radiation and oxygen poisoning "induces mutations, cancer and ageing", proposed that oxygen free radicals that are formed endogenously from normal oxygen-utilizing metabolic processes (specifically hydroxyl, $HO^{\bullet}$, and hydroperoxyl radicals, $HO_2^{\bullet}$) play an essential role in the ageing process.[3] Traditionally, chemists thought that free radicals are very limited in biological systems, because of their reactivity and short half-life, despite the reports to the contrary and their detection by various scientists in biological materials.[4,5]

Then, a very important discovery of superoxide dismutase (SOD) by McCord and Fridovich in 1969 and the demonstration of the existence of hydrogen peroxide (H_2O_2) *in vivo* in 1979, gave credibility to the theory of free radicals.[6,7] In 1972, Harman added a modification to his theory, giving a central role to the mitochondria of the aerobic organisms, because these organelles generate a large amount of reactive oxygen species (ROS, the term has been established as meaning oxygen free radicals and highly oxidant oxygen chemicals, such as H_2O_2) in cells.[8] In the following years, the theory was supported by new experimental evidence, namely that oxidative cellular damage, through oxidative stress in aerobic organisms, increases during ageing and that mitochondria are central to ageing. Studies showed that mitochondria DNA deletions and point mutations (mtDNA) are induced by oxidative stress and accumulate with age in aerobic organisms from worms to humans.[9-12]

The hypothesis of free radicals of ageing has been refined in the last decades encompassing also other forms of reactive oxygen substances, such as peroxides, aldehydes, nitrogen oxides and other compounds which contribute to oxidative damage in cells. A chronic state of oxidative stress exists in cells of aerobic organisms even under normal physiological conditions because of an imbalance of pro-oxidant and antioxidant substances and enzymes.[13] This imbalance leads to a steady-state accumulation of oxidative damage for a variety of important macromolecules (proteins, enzymes, lipids, DNA, etc) that increases during ageing. Progressively, these damages lead to loss of functional efficiency of cellular metabolic processes. A "strong" version of oxidative stress determines life span, while a "weaker" version of oxidative stress is associated mainly with age-related diseases.[14]

Reactive oxygen species (ROS) are from one hand very important for the physiological metabolic processes of aerobic organisms, including

humans, but at the same time generate oxidative stress which changes dramatically with age. Also, there is mounting genetic evidence that links oxidants and oxidative stress responsiveness to ageing. In this respect, there are great challenges but also numerous difficulties for the development of anti-ageing therapies (antioxidant supplements, calorie restriction, special diet techniques, etc) or changes to life style in order to reverse the ageing process and increase longevity.

2. Oxidant productions and antioxidant defenses in aerobic organisms

The various reactive oxygen species (ROS), that can be free radicals ($O_2^{\bullet -}$, $HO^{\bullet}$) or oxidative species (H_2O_2) are produced under physiological conditions in all aerobic organisms and play a very important part in energy productions, various metabolic mechanisms and other cellular functions. Most estimates suggest that the majority of intracellular ROS production is derived from mitochondria.[15,16]

The evolutionary process of aerobic organisms for millions of years in an oxygenic air environment on Earth was successful because of the development at the same time of effective antioxidant mechanisms. The burden of ROS production is largely counteracted by an intricate antioxidant defence system that includes enzymatic and non-enzymatic substances. The most important antioxidant enzymatic scavengers are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidases (GPx), In addition to these well characterized antioxidant enzymes, at least five members of a new family of peroxide scavengers termed peroxiredoxins have recently isolated.[17]

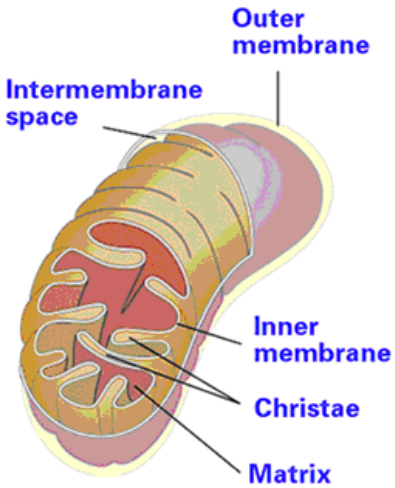

Figure 1. Mitochondria are microscopic bodies in the cytoplasm of every cell. Mitochondria contain many oxidative enzyme systems, producing energy (in the form of ATP) for many cell functions They are the most important locus for the production of reactive oxygen species (ROS), like superoxide anion ($O_2^{\bullet -}$) and other oxygen species capable of generating the oxidant hydrogen peroxide (H_2O_2).

A variety of other non-enzymatic, low molecular mass molecules, such as ascorbic acid, uric acid, flavonoids, carotenoids, glutathione, pyruvate, are present in millimolar concentrations within cells and are effective scavengers of ROS.[18]

The balance between ROS production and antioxidant defences determines the degree of oxidative stress in biological systems. As a consequence of the oxidative stress the "escaped" ROS cause oxidative damage to proteins, enzymes, membrane lipids and nuclear DNA. The most widely studied oxidative stress-induced modifications are to proteins.[19]

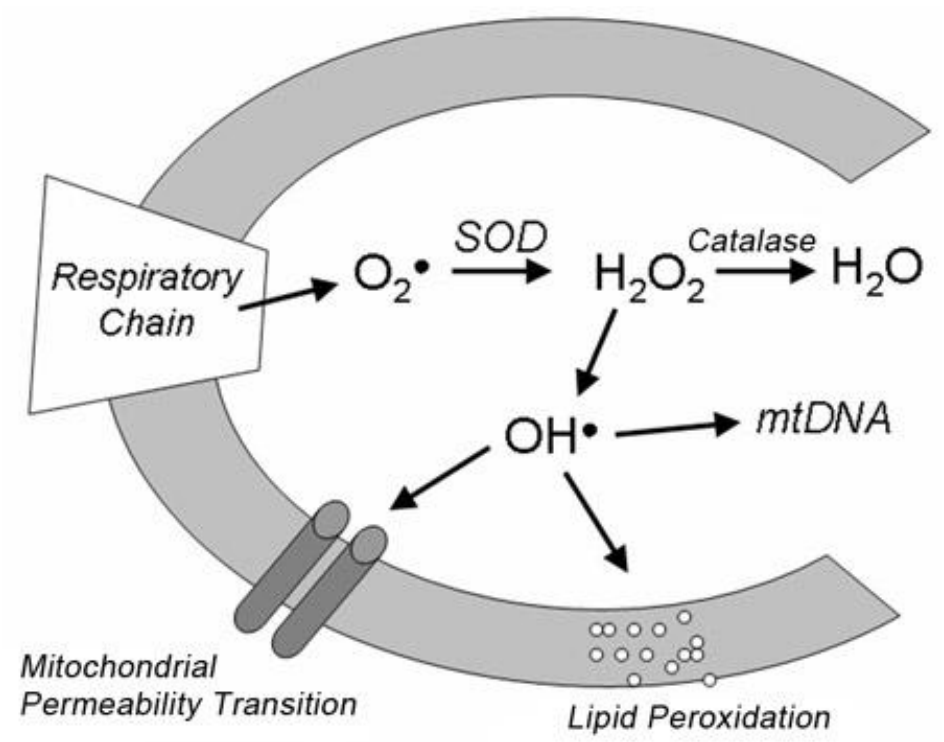

Figure 2. Reactive oxygen species (ROS) are produced by mitochondria respiratory chain reactions (and by some other cellular metabolic functions). The antioxidant enzymes Superoxide dismutase (SOD) can dismutate $O_2^{\bullet -}$ into H_2O_2 and Catalase (CAT) can transform it into water (H_2O). But the antioxidant reactions are not 100% effective and some hydroxyl radicals ($HO^{\bullet}$) can be generated (through the reaction of H_2O_2 with metals, such as Fe^{2+}, Fenton reaction). Hydroxyl radicals are highly reactive and can damage proteins, lipids and DNA (including mitochondrial DNA, mtDNA).

Several studies have shown that ageing cells and organisms accumulate increased levels ox oxidant-damaged nuclear DNA.[20] Also, mitochondrial DNA (mtDNA) because of the proximity to the main source of oxidant generation , or because of a limited DNA repair system, mtDNA is generally considered to be even more sensitive than nuclear DNA to oxidative damage.[21]

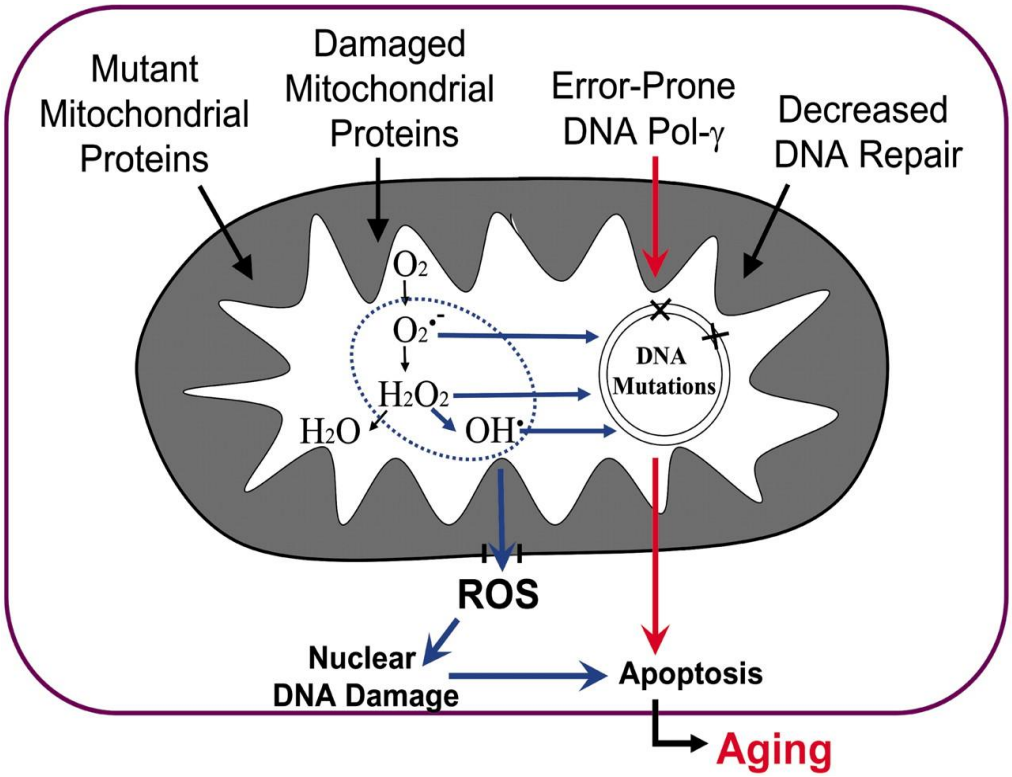

Figure 3. ROS and free radicals such as $HO^{\bullet}$ can cause oxidative damage to mitochondrial proteins, mutations in mtDNA or decrease the DNA repair mechanisms. Also, ROS can escape the mitochondrial membrane causing nuclear DNA damage in cells, or apoptosis because of DNA mutations. These oxidative damages advance ageing of organisms.

3. Oxidative stress, biology of ageing, diseases and cancer

Although maximum life span (longevity) is the most relevant and defined endpoint of with regard to ageing, in large multicellular organisms ageing does not proceed uniformly. This concept of focal ageing or segmented progeria, is particularly important in a variety of age-related human diseases. The two most important age-related diseases are cardiovascular and neurodegenerative disorders that increase exponentially with age. Also, in the last decades there is a growing convergence between the our understanding of the biology of ageing and the basic mechanisms that underlie cancer.[22]

The maintenance of DNA represents a fundamental and continuous problem to every cell. DNA repair and genomic stability in aerobic organisms have been explained in recent years and there are multiple pathways to sense and repair damaged DNA in cells, depending on the nature of damage and on the phase of the cell cycle. Genomic instability is a hallmark of most cancers but also the hallmark of ageing. Age-dependent increase in chromosomal instability has been known for some time to occur in simple organisms (such as yeast) but also in mammals.[23,24]

Increasing evidence from laboratory, animal and clinical studies indicate that ROS may participate in the pathogenesis of these diseases. Experiments showed that the vessel wall of patients with atherosclerotic risk factors, but no overt disease, is characterized by significant increase in vascular ROS production.[25] Mutations in genes that regulate antioxidant enzymes (such as SOD, glutathione, etc) and deficiencies in antioxidant vitamins E or A have been found by *in vivo* experiments to increase the risk for atherosclerosis, retinal degeneration and familial amylotropic lateral sclerosis.[26.-28]

The common feature to many of these diseases of ageing is the recruitment of inflammatory cells. These cells contribute to oxidative stress in large part because they contain the potent NADPH oxidase system. The NADPH (nicotinamide adenine dinucleotide phosphate-oxidase) is a membrane-bound enzyme complex which is present in neutrophils, macrophages, microglia and vascular cells. Once activated, the NADPH oxidase produces large amounts of superoxide anion ($O_2^{\bullet -}$). Although superoxide can be reduced to H_2O_2 by SOD, small amounts escape the enzymatic dismutation and react with nitric oxide ($NO^{\bullet}$) producing endogenously the oxidant peroxynitrite ($NO^{\bullet} + O_2^{\bullet -} \rightarrow ONOO^-$) and other highly damaging radical species. The link between inflammation and ROS seems to provide a useful framework for understanding oxidative stress and disease progression.[29-31]

4. The role of micronutrients (vitamins, minerals, biochemicals), oxidative stress and longevity

For most of human evolution, caloric shortage probably limited population growth. The advent of agriculture made diets rich in calories and nutrients. The introduction of potato in Europe and rice varieties in Asia were major factors enabling high population growth and density. Although caloric shortage is a thing of the past for most of the countries on the global scale, the abundance of carbohydrates, meat, fat-rich foods, inexpensive processed foods and sugary drinks generated a new epidemic of obesity associated with micronutrient malnutrition.[32,33]

Numerous epidemiological and clinical studies in the past decades have demonstrated that regular fruit and vegetable consumption (rich in polyphenols, flavonoids and other antioxidants) reduces cellular oxidative damage, increases plasma antioxidants and reduces the risk for various age-related diseases and mortality.[34-37]

Dietary research and nutritional supplementation with antioxidants advanced in response to these studies. The clinical use of antioxidant vitamins, minerals and biochemicals has gained considerable interest during the last decade.[39-41]

Based on these promising data, supplements of vitamins (E, C, A, D , B_6, B_{12}), minerals (Zn, Ca, Mg, etc) and biochemicals (α-lipoic acid, curcumin, melatonin, resveratrol, isoflavones, etc) were applied through protocols aimed to prevent age-related diseases. Prevention of cardiovascular diseases was an obvious subject (atherosclerosis, hypertension, coronary vasculature, etc) with antioxidants, but the results were conflicting for the efficacy of antioxidants.[42-46]

Chronic Obstructive Pulmonary Diseases (COPD) is connected with oxidative stress. Targeting oxidative stress with antioxidants is obviously a likely goal for beneficial treatment of COPD, but results were very limited.[47] Treatment of amylotropic lateral sclerosis and multiple sclerosis with antioxidants showed conflicting evidence for the efficacy of antioxidants.[48,49] The majority of clinical trials identified positive effects for supplementation with antioxidants for the type 2 diabetes.[50] Ambiguous clinical results have been found in the treatment of cognitive impairment and neurodegenerative diseases (e.g. Alzheimer's disease) with antioxidants.[51,52] Oxidative DNA damage (as measured with the biomarker of the mutagenic adduct 8-OHdG) decreases substantially with supplementation of vitamin D and calcium.[53]

Are concentrations of plasma antioxidants and longevity in centenarians connected? The question was answered by scientists with the measurements of plasma levels of vitamin C, uric acid, vitamin E and A, carotenoids, activity of SOD and GPx in healthy centenarians, elderly aged 80-99 years and over 60 years of age. From the results it is evident that healthy centenarians show a particular profile in which high levels of vitamins seem to be important in guaranteeing their extreme longevity.[54] Antioxidant status in elderly persons is a predictor for health and longevity.[55,56]

Daily human diet intake needs around 40 essential micronutrients. The optimum amount of micronutrients is essential to maximize the protection of important biological macromolecules from oxidative damage and keep the metabolic network. Dietary intake of micronutrients changes with age.[57]

Micronutrient	RDA (recommended dietary allowance)
Minerals	
Iron (Fe)	18,000 (women 20-30 years) µg 8,000 (women 50+ years) µg
Zinc (Zn)	8,000-11,000 (women/men 50+ years) µg
Selenium (Se)	20-65 µg
Vitamins	
Vitamin E (a-tocopherol)	6-10 mg
Vitamin C (ascorbic acid)	40-60 mg
Folic acid	150-200, 400 (pregnant women) µg
B_6 B_{12}	0,3-2,0 mg 0,3-2,0 µg
Biochemicals	Flavonoids, polyphenols, α-lopoic acid, isoflavones, caretonoids, gloutathione, niacin,

5. Caloric restriction can be beneficial for chronic diseases, cancer and ageing

The first research on caloric (or calorie) restriction in experimental animals was contacted in 1935. Calorie restriction in rats (implemented after puberty), extended median and maximum life span and prevented the severity of chronic diseases.[58] Subsequent experiments in different species (rodents, flies, fish, yeast) have shown that calorie restriction, defined as a reduction of food (diet, calories) without malnutrition, slows ageing, increase maximum life span and reduces the extend of diseases and cancer.[59,60]

The age when calorie restriction is started, the severity of diet restriction and the genetic background of the animal determine the extend of

life extension. In rodents (rats or mice) a 30% to 60% reduction in calorie intake below the usual *ad libitum* intake of food (in accordance with desire) caused a proportionate 30% to 60% increase in maximum life span. The experiments showed slow primary ageing and extend of maximum life span for rats and mice.[61,62] Also, experiments with rodents with intermittent fasting (or alternate-day feeding) increases resistance to stress and prolongs maximum life span.[63]

Studies of calorie restriction with rodents found that delayed the occurrence of chronic diseases, including diabetes, atherosclerosis, cardiomyopathy, autoimmune diseases, kidney and respiratory diseases and various cancers.[64-66] In addition, calorie restriction has been proved to decrease neurodegeneration in the brain and enhance neurogenesis in animal models of Alzheimer's and Parkinson diseases, Huntington disease and stroke.[67-69]

Scientists suggest that the mechanisms responsible for calorie restriction-mediated beneficial effects on primary ageing (in rodents) probably involve the metabolic adaptation to restriction itself, including:

(a) calorie restriction (CR) decreased production of reactive oxygen species (ROS) and modulation of the endogenous antioxidant systems, which decrease oxidative stress that induce damages to tissues.[70,71]
(b) decreased circulating triiodothyronine (T_3) (serum thyroid hormone) levels and sympathetic nervous system activity, which cause a decrease in body temperature and whole-body resting energy expenditure from baseline.[72-74]
(c) decrease plasma concentrations of inflammatory cytokines and a mild increase of cortisol, which reduces systemic inflammation.[75,76]
(d) protection of the immune system, which with ageing is becoming weaker.[77]
(e) increased expression of protein chaperons, such as heat shock protein 70, and neurotrophic factors.[78]
(e) CR decreased plasma concentrations of anabolic hormones and growth factors, which are involved in ageing and tumorigenesis.[79,80]
(f) CR enhanced DNA repair processes,[81] increased removal of damaged cellular proteins and oxidized lipids, and decreased glycation end products.[82]

Experimental data suggest that many of the cellular effects of CR are mediated by regulating gene expression, through up-regulation of genes involved in repair and survival mechanism (by protecting against oxidative damage), down-regulation of genes involved in mediating inflammation and prevention of changes in gene expression that occur with ageing.[83,84]

6. Caloric restriction and ageing in experimental animals

Animal models for studies of calorie restriction for many years included yeast, worms, flies and laboratory rodents (mice and rats) and in the last decade nonhuman primates.[85,86]

Experiments of dietary restriction (calorie restriction), i.e. a reduction of food intake by 40-60% without malnutrition, has been proved to have remarkable benefits for health and lifespan in diverse species, as yeast,

roundworms, flies and rodents. The roundworm ***Caenorhabditis elegans*** *(C. elegans)* has been used in a great variety of caloric restriction experiments.[87,88] As in yeast and flies, over-expression of the *Caenorhabditis elegans* sirtuin gene *sir-2.1* leads to extension of lifespan and deletion of the gene shortens lifespan.[89]

Figure 4. Model organisms of ageing studies: yeast (*Saccharomyces cerevisiae*), roundworms (*C*aenorhabditis *elegans*), fruit flies (*Drosophila melanogaster*) and mice. Caloric restriction studies with these model organisms decreased oxidative stress biomarkers and increased substantially their life span.

Scientists were studying for years the budding yeast ***Saccharomyces cerevisiae*** and its replicative ageing. A screen for genes that determine yeast replicative lifespan identified the SIR complex (including the Sir2 histone deacetylase). Yeast Sir2 is a nicotinamide adenine dinucleotide (NAD^+) – dependent histone (protein) deacetylase that has been proposed to mediate effects of life extension under caloric restriction.[90,91] Sir proteins were already known to be involved in gene silencing (SIR stands for Silent Information Regulator). Sir2 is one of several enzymes that remove acetyl tags from the histones, but requires the small molecule of NAD (known as a conduit of many metabolic reactions in cells). This association between Sir2 and NAD is very important because it links Sir2 activity to metabolism and thus potentially to the relation between diet and ageing observed in caloric restriction.[92-94]

Another experimental animal for studying mechanisms of ageing is the fruit fly ***Drosophila melanogaster***. The life span of *Drosophila melanogaster,*

under experimental conditions of caloric or dietary restriction, was extended.[95-98]

Rodents (mice and rats) and primates were used in experiments for the study of caloric restriction on ageing and longevity. It is not yet known whether caloric restriction affects primary ageing and extends maximum life span on long lived mammals. There are ongoing studies that evaluating the effect of such restriction on ageing in mice and rats with positive outcome.[99-101] Experiments with **rhesus monkeys** will probably take another 10 to 15 years before adequate data are available for reliable results. Nonetheless, the data currently available from these studies have shown that many of the metabolic, hormonal, anti-inflammatory, and body compositional changes that occur in caloric-restricted rodents also occur under similar conditions in caloric-restricted monkeys.[102-105]

7. Experiments of caloric restriction and ageing in humans

Studies of caloric restriction and the effects on longevity in humans are very difficult to come to a definite conclusion because there are no validated biomarkers that can serve as surrogate markers of ageing. Also, there are practical difficulties to conduct randomized, diet-controlled, long-term survival studies in humans.[106,107]

There are data from epidemiological studies which suggest that diet restriction can have beneficial effects on the factors involved in the pathogenesis of primary and secondary ageing and life expectancy in humans. During the World War I and II there were food shortages in many European countries that were associated with sharp decrease in coronary heart disease mortality. After the end of the war the cardiovascular diseases increased again.[108,109] In another study it was found that the Japanese in the Okinawa island are consuming 30% less calories than the average Japanese population and had ~35% lower rates of cardiovascular disease and lower cancer mortality than the average Japanese. Also, they had one of the highest number of centenarians in the world.[110]

However, these associations are not enough indicators that can prove a relationship between decreased caloric restriction and improved effects in longevity and decrease in ageing diseases.

There are more than ten recent human studies with voluntary self imposed caloric restriction or accidental induced caloric restriction for short-term randomized controlled trials (6-12 months). These studies examined the connection between caloric restriction and biological adaptations that might be responsible for the slowed ageing process. Findings from these studies were very promising. The caloric restriction group of men and women, compared to the control group, showed decreased body fat and body mass index (BMI), blood pressure, marked reduction in metabolic risk factors for coronary heart disease, decrease levels of insulin, decreased T_3 levels, improved lipid profile, decreased TNF-α/adiponectin ratio and levels of insulin and glucose, lower plasma concentrations of inflammatory markers, and other beneficial markers of reduced oxidative stress and ageing.[111-120]

Some studies found also that CR decreased bone mass as well lower extremity muscle mass and strength. Despite many similarities in the metabolic adaptation to CR observed in rodents and humans, it is not known if

such restriction affects maximum lifespan in humans. Obesity is associated with serious medical diseases and premature mortality. Weight loss induced by CR (negative energy balance) simultaneously improves multiple metabolic risk factors for cardiovascular disease and other medical abnormalities associated with obesity. In the other hand, it must be emphasized that excessive caloric restriction (defined as a decrease in calorie intake that can be dangerous on organ functions and health) that is more than 45% of the energy requirements can cause anemia, muscle wasting, neurologic deficits, lower extremity edema, weakness, dizziness, lethargy and depression.[66,68]

8. Genetics, dietary restriction and mechanisms of ageing in small organisms

Molecular biologists discovered in recent years that the ageing process is subject to regulation by classical signaling pathways and transcription factors in biological systems. Many of these pathways were discovered for the first time in small, short-lived organisms such as yeast, worms, flies and rodents. It was found that many mutations that extend lifespan affect stress-response genes or nutrient sensors. When food is plentiful and stress levels are low, these genes support growth and reproduction, but under harsh conditions their activities change (some up and others are turned down) and as consequence the animal undergoes a global physiological shift towards cell protection and maintenance. These shifts protect the animal from environmental stresses and it also extends lifespan.[121]

Nutrient and stress sensors mediate lifespan extensions that occur in response to many different environmental and physiological signals. The best known of these signals is dietary (or caloric) restriction, which extends lifespan in many species, from yeast to primates.[122]

In worms, flies and mice (as well as yeast), genetic and/or phenotypic analysis suggests that chronic dietary restriction increases lifespan by downregulating TOR activity. The TOR pathway (kinase Target Of Rapamycin, a protein) is known to regulate autophagy and translation (see Figure 5). The small arrows indicate upregulation or downregulation. In worms and flies, lifespan extension by means of TOR inhibition has been shown to require autophagy, and in all three species, inhibiting translation by inactivating the TOR target ribosomal S6 kinase increases lifespan. PHA-4 is required for autophagy in *Caenorhabditis elegans* TOR mutants. PHA-4 also affects expression of stress-response genes in response to dietary restriction; its effect on translation has not been examined. In flies, components of the respiratory electron transport chain escape translational inhibition when TOR activity is reduced, resulting in increased respiration.[121]

Chronic dietary restriction also increases respiration in worms, in response to activity of the SKN-1 transcription factor in neurons (green). In worms and flies, this increase in respiration has been shown to contribute to lifespan extension. Whether TOR affects respiration in worms, or SKN-1 affects respiration in flies, is not known. In flies and mice, chronic dietary restriction may increase lifespan, at least in part, by downregulating insulin/IGF-1 signaling (IIS, red). Sirtuins are required for chronic dietary

restriction to extend lifespan in flies and mice, but whether they act in the insulin/IGF-1 and/or TOR pathways is not known.[123-126]

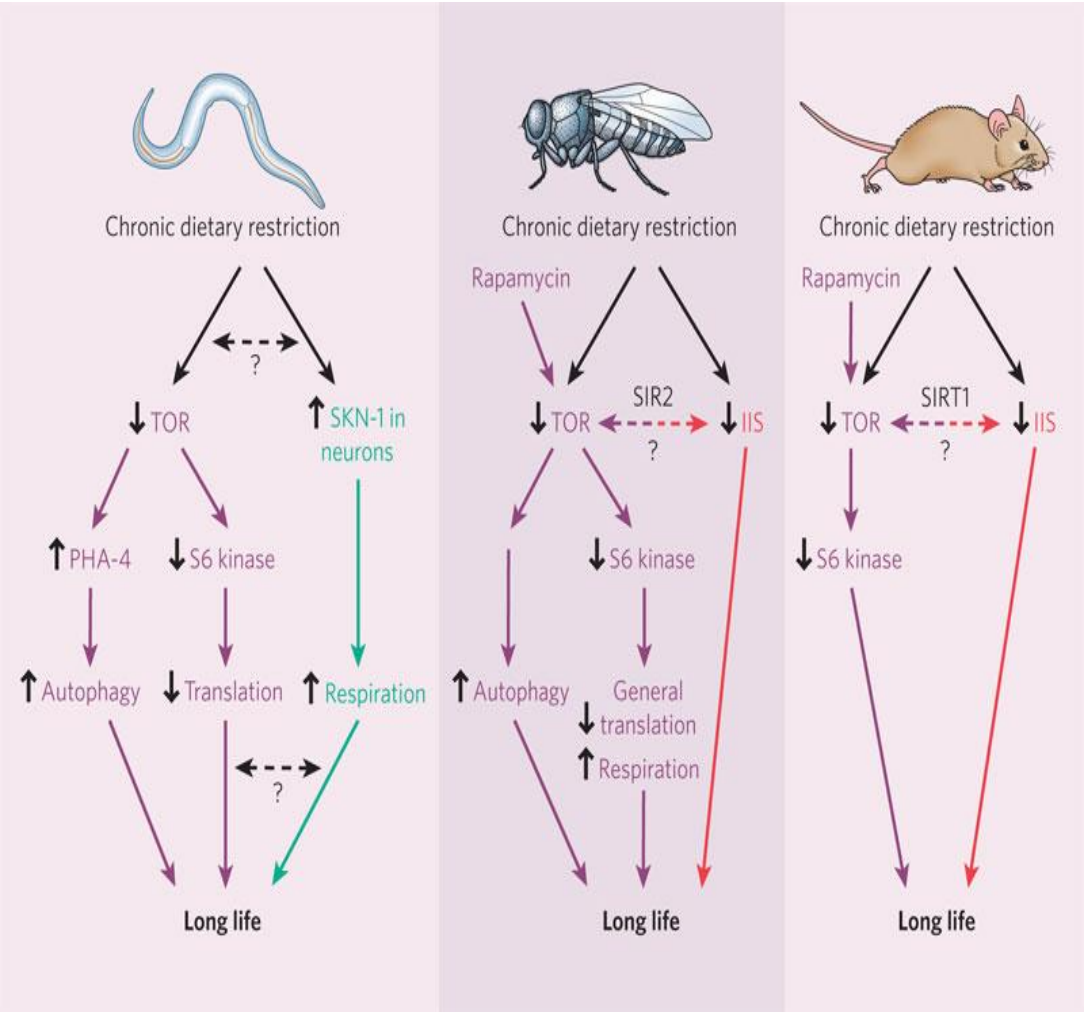

Figure 5. Mechanisms of chronic dietary restriction in experimental animals such as *C. elegans, Drosophila melanogaster* and mouse, with beneficial effects on oxidative stress and prolonged lifespan.[121]

9. Conclusions

One of the most widely accepted theories of ageing is the free radical or oxidative stress theory. Reactive oxygen species (ROS) are byproducts of energy metabolism and oxygen consumption in aerobic organisms, resulting in the slow but steady damage, which accelerates with ageing, of membrane lipids, proteins-enzymes and DNA. These damages progressively lead to degenerative diseases, organ dysfunction, cancer and death. Caloric intake is an important determinant of health. Inadequate or excessive energy intake can have detrimental effects in the body composition, organ functions and ageing. Experiments with animal models showed that caloric restriction (CR) decreased ageing processes, restricted degenerative diseases and prolong maximum lifespan. Experiment with humans showed that caloric restriction

although does not prolong lifespan, decreases degenerative diseases, and hase a beneficial effect on the quality of late life by reducing the burden of chronic diseases. Additional studies are needed to identify the molecular and cellular mechanisms responsible for the CR effects and to discover reliable and sensitive biomarkers of ageing.[127,128]

A series of studies in recent years revealed that dietary antioxidant supplements and mutlivitamins are not protecting older older from higher mortality rate but can be useful in protecting from early signs of degenerative diseases.[129-131]

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