Dietary restriction and its multifaceted effects

R. Sharma

Department of Biochemistry, North Eastern Hill University, Shillong 793 022, India

Dietary restriction (DR), a reduction in calorie intake without malnutrition, plays an important role in neuroendocrine and immune systems and also in general carbohydrate, protein and fat metabolism. Among various intervention strategies, DR has been shown to be the most powerful modulator of the ageing process in diverse groups of organisms. It is an efficacious means of increasing longevity and reducing age-related pathology. Its multifaceted effects are achieved by various modes primarily by potentiating the immune responses, lowering oxidative stress, acting as a neuroprotector, and attenuating major inflammatory processes. DR thus, has robust effects on delaying mortality, increasing the lifespan and attenuating chronic diseases of old age that may be superior to pharmaceutical intervention. Once its detailed regulatory mechanisms are established, it could be an important tool to prevent diseases of old age and to promote healthy (quality) ageing in humans in near future.

'EAT less and be calm' (kum khao aur gum khao) has been an age-old saying in the Indian society for better health and well-being of the people. Systematic studies on dietary restriction (DR), without malnutrition, have been undertaken with success in various experimental animals and have contributed to an increase in longevity and postponement of age-related diseases. The manner in which DR may help extend human lifespan and reduce age-related diseases has been of interest to the scientific community. Since the early work of McCay and his collaborators[1], many more experimental studies have been undertaken using a range of strategies[2–5]. These studies strongly support the involvement of DR in increasing longevity, enhancing functional capacity and reducing diseases associated with old age in a wide variety of experimental animals[6–8].

Theories of ageing and impact of DR

Ageing or getting old is a universal feature of most organisms and has been a concern of every society. The maximum lifespan of an organism is a constitutional feature of speciation to polygenic controls and to environmental influences. Various theories have been put forward to explain the phenomenon of ageing; however, no single theory has yet accounted for all phenotypes, though many have attempted to explain at least some of the major and most frequent ageing phenomena[9,10]. These have been grouped as molecular, cellular and systemic theories. Molecular theories propose that the genes, by interacting with the environmental factors, govern the lifespan of any species. Ageing may result from changes in DNA template activity, which regulates the formation of the final cellular products. The molecular theories include codon restriction[11], somatic mutation[12], error theory[13], and gene regulation theory[14,15]. They also include antagonistic pleiotropy[16,17], dysdifferentiation[18] and soma disposal hypothesis[19]. Cellular theories, on the other hand, relate to changes that occur in structural and functional elements of cells with the passage of time. These theories include wear and tear[20], age pigments[21], free radicals[22], cross-linking[23] and membrane alterations[24].

Systemic theories include the endocrine and neuroendocrine[25,26], and immunological[27] systems. These theories view that the overall performance of an animal is closely related to the efficacy of a variety of control mechanisms that regulate the interaction between different organs and tissues. The effectiveness of homeostatic adjustments declines with age and leads to consequent failure of adaptive mechanisms, ageing and death. Adaptation to external and/or internal stress depends on the control mechanisms orchestrated by the joint interplay of the nervous and endocrine systems. Complete surveillance to foreign invaders is provided for by the concerted efforts of the immune system. The failure of this system leads to a reduced adaptability of an organism to pathogenic challenges in old age. Healthy ageing and maximum longevity may thus depend on the genetic make-up of an organism, with the proviso of strict control by nature and nurture. The details of these theories have been reviewed extensively[9,10]. The impact of DR has been associated with most of the mechanistic approaches of various theories proposed for causation of ageing.

Epidemiological studies in humans and experimental results reported in several animal species indicate that good nutrition and regular physical exercise not only contribute significantly to health and well-being, but also prolong average and maximum lifespan. In fact, these interventions are now accepted as a natural ways to deal with a number of diseases of old age. One of the major goals of biogerontologists is to prolong health-span part of the lifespan and have healthy old age without disability. In other words, the aim of biomedical gerontology is to postpone old-age diseases and increase health span[28].

e-mail: rsharma@nehu.ac.in

Hormonal and metabolic influences during DR

Multifaceted effects of DR include most of the physiological functions ranging from reproduction to endocrine and neuroendocrine responses, immunological systems, metabolic responses, gene expression profiles, and also in pathophysiological conditions. DR reduces pituitary hormone secretion and mimics a hypophysectomized state in rats[29]. Removal of pituitary has been shown to ameliorate anti-ageing action in rats, which entails that pituitary hormones accelerate ageing. Recent studies on genetic variants of mouse indicate that pituitary growth hormone (GH) may accelerate ageing and shorten lifespan[30]. It has been postulated that DR, by reducing the secretion of pituitary hormones such as GH, lowers the oxidative damage of tissues, thereby reducing the age-related pathologies and extending lifespan[29]. Moderate caloric restriction in rodents delays puberty without loss of fertility and enhances longevity[31,32]. This has been correlated with retarded maturation of the hypothalamo–pituitary–gonadal axis. The anti-ageing property of DR seems to have evolutionary significance and might have evolved in nature in response to food scarcity[33]. During such period, inbuilt resources would be diverted from reproduction to maintenance of the adult body and thereby increase survival until the next period of food availability. Another neuroendocrine axis that appears to be influenced by DR is the hypothalamus–pituitary–adrenal axis. During periods of restriction, the axis is stimulated possibly due to a feeling of stress by underfeeding, leading to an increased level of circulating glucocorticoids. Although transient increase in glucocorticoids improves performance at spatial memory tasks and helps synaptic efficacy, long-term elevations of glucocorticoids are associated with decreased cognitive performance, attenuated synaptic efficacy and neuronal atrophy. Elevation of glucocorticoids during ageing is also associated with mild cognitive impairment and hippocampal atrophy. DR paradoxically increases plasma glucocorticoids and also prolongs lifespan[34]. It has been suggested that the beneficial effects of DR outweigh the deleterious effects of glucocorticoids. It has been reported that three months of alternate days feeding selectively decreases glucocorticoid receptors in the hippocamus and cerebral cortex of rats[35]. These findings suggest that DR can alter the responsiveness of brain cells to increased glucocorticoid during such intervention. This adaptation might contribute to the beneficial effects of DR on neuronal plasticity and survival, as observed in recent studies. It is proposed that the lifespan extension in rodents by DR could be an example of hormesis (achieving beneficial effects in response to a low-intensity stressor) and that sustained, moderate hyperadrenocorticism helps life prolongation. Hormesis thus refers to a phenomenon of beneficial biological action from a factor that is generally viewed as detrimental in nature[36]. The anti-ageing action of DR could be regarded as nutritional stress, which might stimulate metabolic responses for better survivability. During mild stress, the organism regulates its metabolism to allocate more energy for its maintenance and survival, and could be in a better condition after the stress, if it is not too strong[37]. Recently, this laboratory has reported an increased level of hepatic glucocorticoid receptors during three months of alternate days of feeding in mice[38]. Increase in the level of hepatic glucocorticoid receptors could be a contributory factor in controlling glucocorticoid-mediated metabolic responses during long-term DR in mice. DR rodents are found to be more resistant to a variety of other stresses such as trauma, heat shock and drug toxicity[39].

The increased glucocorticoids and their receptors may help maintain the anabolic role of this hormone in the liver during DR. It has been reported earlier that the dietary calorie restriction in mice leads to an increase in mRNA and/or activity of key enzymes (phosphoenolpyruvate carboxykinase and glucose-6-phosphatase) of hepatic gluconeogenesis[40]. The up-regulation of glucocorticoid receptors in the liver of mice might have a functional role in inducing such enzymes for better metabolic regulation during DR. Among the adaptive roles of DR, we have recently reported that a long-term DR (alternate days of feeding for three months) leads to a cumulative adaptation in lowering the adenosine deaminase (ADA) activity in gastrointestinal tract (GIT) of mice[41]. Lowered activity of ADA in GIT of dietary-restricted mice may reflect a biochemical adaptation, wherein no futile synthesis of ADA is done if there is no need to metabolize dietary adenosine or otherwise to compensate the changes required for the hydration of GIT during such interventions. Reduced ADA activity may also give rise to excess cellular adenosine, which may be a signal for inadequate ATP during low-energy situations.

DR extends longevity in many organisms ranging from yeast to mice[36]. In rodents, DR decreases the level of plasma glucose, insulin and insulin-like growth factor (IGF-1) and regresses cancer, immunosenescence, and inflammation without irreversible side effects[8]. Mutations in IGF-1-like signalling pathways extend lifespan, but also cause glycogen or fat accumulation and dwarfism[8]. IGF-1 is a key mediator of GH action in mammals. It acts primarily by binding to its cognate IGF-1 receptor (IGF-1R) in controlling the growth and organ size via mitotic and anti-apoptotic effects. The extension of lifespan in GH receptor knock-out mice that are GH-resistant and IGF-1-deficient, has been a significant finding in search of a similar mechanism in other mammalian species as well[42]. Most of the genetic manipulations that extend lifespan cause some side effects. However, DR could serve as a better option in extending lifespan of an organism without having such side effects. The accumulation of advanced glycosylation end products (generally seen in diabetics due to elevated glucose levels) is lowered during DR in several tissues of tested animals[43]. This could lead to

lower cross-linking of various susceptible proteins such as collagen and crystallin. Such lowering of cross-linking may have beneficial effects on skin wrinkles and cataract, and also help better wound-healing[44]. In a systematic study of long-term DR on mortality and morbidity in laboratory-maintained rhesus monkeys, Bodkin *et al.*[45] examined 117 monkeys over a period of 25 years. They have reported a 2.6-fold increase risk of death in the *ad libitum* (AL) fed monkeys compared to DR ones. From their study, it was evident that hyperinsulinaemia led to a 3.7-fold increase risk of death in AL monkeys who have significant organ pathologies at the time of death. The median survival age has been 25 years for AL compared to 32 years for DR-fed monkeys[45]. These findings suggest that DR leads to an increase in average age of death in primates, with lesser degree of hyperinsulinaemia and other age-related diseases.

Myriad of molecular, cellular, structural and functional changes occur in the brain during senescence. Neural cells might adapt to these changes or succumb to neurodegenerative events that result in diseases like Alzheimer's and Parkinson's. Multiple mechanisms operate to maintain the integrity of nerve-cell circuits. These include production of neurotrophic factors and cytokines, expression of various cell-survival-promoting proteins (molecular chaperones, antioxidant enzymes, Bcl-2 and inhibitor of apoptotic proteins), preservation of genomic integrity by telomerase and DNA repair proteins, and mobilization of neural stem cells to replenish damaged neural and glial cells[46]. Such neuro-protective and restorative mechanisms are compromised during the ageing process, giving rise to neuropathological complications in elderly individuals. DR has been shown to reduce the pace of age-dependent decrease in DNA-repair system and thereby protects the genome integrity in ageing animals[47]. The adaptive responses in brain ageing could be determined by the combined action of genetic and environmental factors. Neuroprotective functions thus are achieved by dietary calorie restriction, and by behavioural (physical and intellectual activity) modifications. A slower brain ageing could take place by activating a hormesis response in which neurons increase production of neurotrophic factors and stress proteins to protect the brain from deleterious effects[46].

Oxidative stress and its quenching by DR

There are extensive reports on the effects of DR on reduction of mitochondrial damage, free radical accumulation and lipid peroxidation[6,48]. Such reduction could be possible partly by the decrease in the energy expenditure due to lower metabolic load in restricted animals and/or by the increased activity of those enzyme systems that are responsible for scavenging the reactive oxygen radicals[48,49]. DR has been shown to lower (17%) total energy expenditure, which was attributed to a 20% decrease in resting energy expenditure (REE) in a pilot study conducted on rhesus monkeys for 11 years[50]. These results suggested that DR might lower REE independent of changes in body composition that might contribute to the life-extending properties of this intervention. One of the possibilities could be of lowering oxidative stress/damage by inducing specific gene expression, particularly of antioxidant enzymes, to produce the beneficial adaptive responses[39,51]. It also lowers metabolic load on the experimental animals due to reduced energy input, leading to an altered energy metabolism[39]. In fact, oxidative damage has been linked to many major degenerative diseases, including atherosclerosis[52], diabetes[53], Parkinson's disease[54], Alzheimer's disease[55] and cancer[36,56]. It is corroborated with the genetic studies on *Caenorhabditis elegans*, wherein reactive oxygen species (ROS) are intimately involved with lifespan determination[57]. Thus, a detailed understanding of the pathways that mediate the benefits of DR and also modulate the levels of ROS may lead to novel therapies for a wide spectrum of age-related diseases. Accumulation of oxidatively damaged proteins is evident during ageing process in many organisms[58]. DR has been shown to reduce age-associated increase in the half-life of proteins, suggesting that the dwelling time of the proteins is reduced in DR animals[58]. The activity of proteosome, responsible for degradation of altered proteins, is found to be increased in the liver of old rats subjected to 3.5 months of DR regimen. Thus, DR can increase the turnover of proteins and thereby attenuate potentially harmful consequences of altered proteins.

It is widely observed that DR retards ageing by its antioxidative potentials. It decreases oxidative stress and attenuates inflammatory processes[59]. The age-dependent increase in ROS, mostly generated at complex III in the electron transport chain in mitochondria, alters the redox state of the cellular components leading to activation of redox-sensitive transcription factors such as activator protein-1 (AP-1), nuclear factor-kB (NF-kB) and hypoxia inducible factor-1 (HIF-1)[60]. In particular, NF-kB is a multimeric nuclear transcription factor that exists in an inactive form in the cytoplasm because of its association with inhibitory kB (IkB) protein. It gets activated by various stimuli, including ROS and translocates to the nucleus upon phosphorylation (by I kappa B kinase and MAPKs) and subsequent degradation of its inhibitory subunit (IkB). In the nucleus, NK-kB binds to promoter/enhancer regions of genes containing kB-responsive elements. There are several pro-inflammatory genes such as interleukin 1β, IL-6, TNF-α, cyclooxygenase-2 and nitric oxide synthase, which contain kB-responsive elements and are therefore induced by activated NF-kB[61]. DR reduces DNA-binding activity of NF-kB leading to a suppression of pro-inflammatory genes and thereby produces anti-inflammatory effects[62]. Recently, DR has also been shown to attenuate an age-dependent increase of nuclear thioredoxin and redox factor-1 (Ref-1) that may in turn

suppress the functions of other redox-sensitive transcription factors such as AP-1, NF-kB and HIF-1, giving rise to suppression of inflammation[62]. Depending on the redox state, thioredoxin makes direct contacts with Ref-1 that is a DNA repair enzyme as well as modulator of other redox-sensitive transcription factors by reversible protein–protein interactions. Such effects are accomplished by changing the redox state of cognate cysteines located in the DNA-binding domain of these transcription factors[62]. Thioredoxin and glutathione, thus, are closely linked to several redox-dependent pathways. They provide reducing equivalents in such reactions and neutralize the oxidative load of the system. Besides, they also regulate various up- and downstream signal transduction pathways in controlling cell growth, inflammation and apoptosis. Thus, their levels during DR could be a potential marker in quenching the oxidative stress through these molecular processes.

Immunological improvements during DR

Age-related decrease in immunologic functions is a major predisposing factor contributing to increased morbidity and mortality with age. Hence, the restoration of immunologic function could have a beneficial effect in reducing pathology and maintaining healthful condition during old age. Among various interventions, DR has been the most powerful modulator of the ageing process. Several mechanisms have been proposed to explain its robust beneficial effects on various physiological systems, including the immune system. Overall immunological status of rodents fed with a caloric restricted diet is superior to the status of AL-fed animals[51]. The expression of IL-2, a T-cell growth factor, has been shown to increase during DR[51]. This could be a beneficial factor in ageing rodents and gives better immunological protection. There are several up- and downstream (MAPK/ras/c-fos/jun) signalling events whose modulation accounts for the anti-immunosenescent role of DR. Lectin-induced proliferation of splenic lymphocytes, which decrease sharply during ageing in AL-fed mice, is increased twofold by DR at all ages[2]. The incidence of autoimmune diseases, which increases during ageing of normal fed animals, is significantly decreased in response to DR. The molecular mechanism(s) of such protective action remain(s) to be elucidated.

Gene expression changes during DR

Ageing is characterized by dynamic changes in the expression of many genes that provide a powerful molecular description of the normal ageing process. DR extends lifespan by slowing the rate of normal ageing. In fruit flies, nearly 23% of the genome shows altered transcript representation with age. DR leads to a slower progression of normal age-related changes in transcript levels, particularly of genes involved in cell growth, metabolism, and reproduction[63]. Studies using high-density oligonucleotide arrays for 6347 genes exhibited differential expression patterns of genes in skeletal muscle of mice during ageing[64]. The detailed analysis of expression patterns revealed greater expression of genes involved in oxidative stress, DNA-damage, neural atrophy and a decrease in expression of genes leading to slower rate of glycolysis and mitochondrial dysfunction in aged animals. Such observations corroborated with the earlier findings of global increase in ROS and mitochondrial dysfunction during the ageing process. Most of these deficits in the expression of age-related genes were either fully or partly reversed by DR in age-matched mice. Significant among them were the genes involved in protein metabolism (synthesis and turnover), carbohydrate metabolism (gluconeogenesis and pentose phosphate pathways) and fatty-acid synthesis, whose compromised expressions otherwise during ageing were increased by DR in mice. However, the expression of inducible detoxification enzymes (cytochrome P-450 isoforms IIIA and Cyp1b1), and DNA-repair enzymes was suppressed during such DR. This was correlated with the observation of lower steady-state amounts of noxious metabolic by-products during DR, that may limit the requirement of such detoxifying and repair enzyme systems in DR animals. Taken together, these observations suggest that DR may retard the ageing process by causing a metabolic shift towards enhanced protein turnover and lower macromolecular damages caused by various toxic agents[39,65].

The effects of health- and lifespan-extending property of DR when examined in young and old mice on a short- and long-term basis, using a genome-wide microarray expression analysis of several liver-specific genes, revealed that ageing was accompanied by altered gene expression associated with increased inflammation, cellular stress, fibrosis and reduced capacity for apoptosis, xenobiotic metabolism, normal cell cycling and DNA replication[66]. It has been observed that both long- and short-term DR reverse many of these changes. Hence, it seems plausible to attain the benefits of DR in ageing animals who otherwise have many functional impairments. This could shift the old-age genomic profile to the slow-ageing profile associated with long-term DR[66]. The longevity-extending property of DR at genetic level has also been well-documented in simple organisms such as budding yeast, *Saccharomyces cerevisiae* and free-living nematode, *C. elegans*[57]. In *S. cerevisiae*, nutrient withdrawal extends longevity through a pathway that requires over-expression of the enzyme Sir2. Similarly, in *C. elegans*, increased expression of Sir2 has been shown to extend lifespan[67]. Sir2 belongs to a large family of evolutionarily conserved proteins called sirtuins. In these lower organisms, they regulate a wide variety of cellular processes that affect lifespan, including packageing of DNA inside cells. Sirtuins act as regulators of apoptosis and differentiation in mammalian cells. These effects of sirtuins are exerted by

deacetylation of specific proteins that depend on the metabolic availability of NAD^+ for their activity[57,67]. Therefore, the NAD^+ requirement of Sir2 may provide a link to the energy status of the cell and gene expression during such interventions.

During ageing in rodents and humans, the blood levels of IL-6 and TNF-α, which are involved in acute phase inflammatory responses, increase markedly[68]. Microarray analyses of gene expression profile from the brain of aged mice indicated an increased expression of those genes that are involved in inflammatory responses, oxidative stress and neural atrophy. DR has been shown to attenuate such induction of inflammatory and oxidative stress responsive genes in the brain of older animals[69]. In contrast, high dietary intake is associated with an increased risk of Alzheimer's disease[70], applauding the role of DR in protecting inflammation and neurodegeneration. Alzheimer's disease exhibits remarkable benefits from a broad range of anti-inflammatory drugs, particularly the nonsteroidal anti-inflammatory drugs (NSAIDs)[71]. Long-term use of NSAIDs may be responsible for 80% reduction in the risk of Alzheimer's disease[72]. NSAIDs also reduce the risk of breast, colon and other cancers, possibly by inhibiting proliferation and decreasing angiogenesis[73]. The effects of DR in cancer cases vary depending on the type of tissue/organ and the animal species/strain. In general, it leads to a decreased incidence of tumours in experimental animals. It confers a certain degree of protection against skin and intestinal tumours that are induced by high degree of exogenous carcinogens. This could be because of the reduced fat depots in DR animals available for the storage of carcinogenic metabolites.

Long-term reduction in blood glucose levels during DR may also contribute to a lower degree of inflammation and related disorders in mice. In humans, acute hyperglycaemia induces Mac-1, a monocyte adhesion molecule, leading to secretion of many proinflammatory signals[74]. *In vitro*, a higher glucose induces IL-6 and TNF-α expression and secretion in isolated monocytes[75]. TNF-α is known to inhibit insulin receptor signalling in adipocytes, hepatocytes and skeletal muscle, and is implicated in insulin resistance during ageing[76]. Hyperglycaemia also alters redox status in muscle cells *in vitro*, by decreasing glutathione levels and repressing γ-glutamylcystein synthetase, a rate-limiting enzyme of glutathione synthesis [77]. Keeping these analogies in mind, one can applaud that DR may extend lifespan in mammals by attenuating the major inflammatory diseases of the elderly.

Human studies related to dietary influences

The results on human studies have started gaining momentum. Among Muslims[78] who fast during the daylight and consume meal after sunset, the high-density lypoprotein (HDL) plasma levels increase by 30%. HDL serves to remove cholesterol from the blood and carry it to the liver. HDL is in fact inversely related to atherosclerosis and other cardiovascular diseases. It seems analogous to the report on HDL increase observed in DR monkeys[5]. In a DR study of non-diabetic, healthy, male individuals, Obata *et al.*[79] reported that R192Q paraoxonase gene variant is associated with a change in HDL-cholesterol level. Paraoxonase, an HDL-associated enzyme, is known to protect against the progression of arteriosclerosis. Single nucleotide polymorphisms of paraoxonase have been implicated with incidences of coronary heart diseases. After DR of 12 weeks, the level of HDL-cholesterol (also called good cholesterol) is increased in individuals with RR genotype, but not in the QR and QQ genotypes. Concomitantly, a decrease in hepatic lipase activity after DR in RR individuals has also been observed. A decrease in hepatic lipase could be a potential link for the increased HDL-cholesterol level in DR individuals[79].

Using a unique set of experimental designs, Rao *et al.*[80] performed a study on naturally occurring South Indian population of three age groups (young, 8–14 years; adult, 20–35 years; old, above 55 years), who were having normal and undernourished diets on the basis of body mass index and history of their dietary intake. The DNA repair capacity was found to be higher in the lymphocytes of age-matched, undernourished individuals. They also observed a slower reduction in DNA repair capacity as a function of age in undernourished individuals compared to normal fed ones. These studies provide a potential link between DNA repair mechanisms and nutritional state in humans. It has been reported that 16 weeks of energy restriction (by 33%) results in substantial reduction in body mass, lean body mass and fat mass in sedentary, free-living, overweight men[81]. Further addition of energy restriction with vigorous exercise did not produce any significant change in these parameters. Hence, it seems reasonable to practice mild energy restriction over vigorous exercise to reduce the body mass and composition, and achieve better health span. It has been appreciated that the people of Okinawa, one of the prefectures of Japan, have highest longevity in the world, even more than the Japanese population on the mainland. Okinawa has the highest prevalence of centenarians in the world at 33.6 per 100,000 population, in contrast to an average of 10 per 100,000 in most developed countries. There is high prevalence of those who live greater than 105 years, called supercentenarians. The major contributory factors to such a higher longevity are mild climate, unique eating habits, and an active lifestyle. Among these, eating habits could be the most crucial factor influencing Okinawan longevity. Japanese living on the island of Okinawa, eat 20% less food than those in Tokyo[82]. There is a strong belief among Okinawans that proper eating is the most efficacious medicine for good health. It is being realized that longevity can be attributed one-third to our genes and two-third to lifestyle, of which nutrition is an important component[83]. Healthful ageing

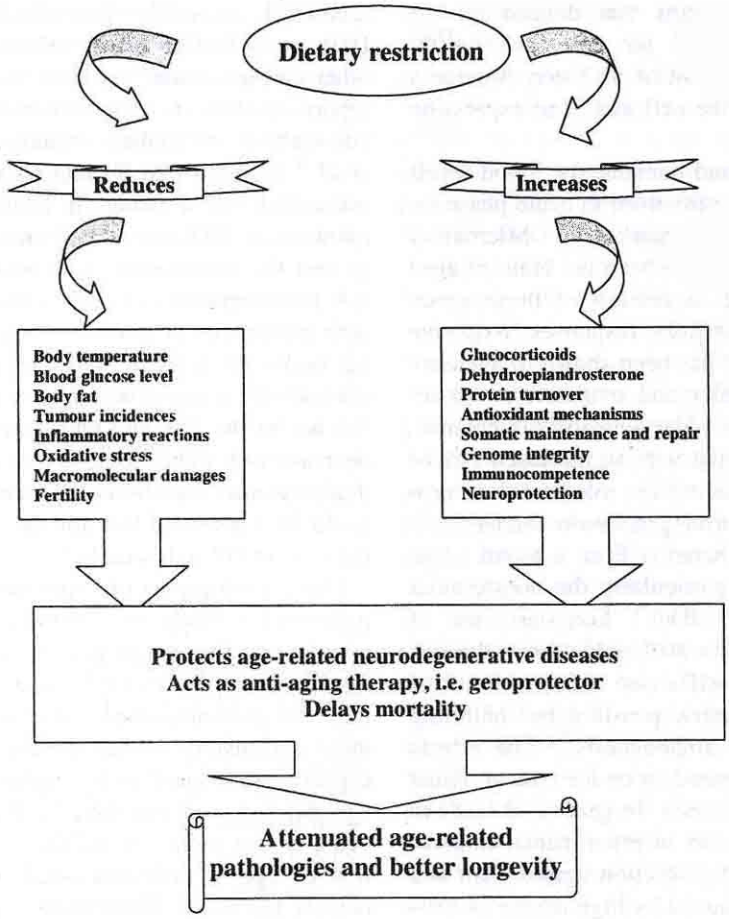

Figure 1. Schematic representation of a variety of effects obtained by dietary restriction leading to attenuation of age-related pathologies and better longevity.

and longevity may thus depend on the genetic makeup within strict control of nature and nurture[84]. The average life expectancy of Okinawans living in Brazil is 17 years shorter than Okinawans who lived in Okinawa[85]. Thus, it is quite reasonable to practice the dietary interventions to achieve better health- and lifespan in humans as well.

However, given that few people would ever reduce their food intake enough to lengthen their lives, gerontologists are now trying for alternatives that could mimic the beneficial effects of DR in people, without forcing them to go for actual restrictions. Such alternative interventions are termed as DR mimetics. Research on DR mimetics includes improvement of mitochondrial function, use of antioxidants, administration of compounds (2-deoxyglucose) known to lower blood glucose levels and increase insulin sensitivity, regular physical exercise and maintenance of body weight over the lifespan[39,86,87]. One of the crucial challenges in ageing studies is the prudent choice of biomarkers depicting the rate of ageing, particularly in long-lived species such as primates, including humans. One of such biomarkers is dehydroepiandrosterone (DHEA) and its sulphated form (DHEAS), whose production declines during ageing and may be a causal factor for many age-related disorders in humans and other animals. Age-associated decline in DHEA/DHEAS levels could be attenuated by DR in experimental animals[4]. It has been reported that dietary supplement of DHEA acts as a geroprotector and helps in promoting longevity. Most of these studies derive conclusions from the rodent model; however, there exist conflicting reports in the literature regarding therapeutic use of DHEA supplements and their beneficial effects in elderly humans which is yet to be ascertained[88]. In search of a DR mimetic, Howitz *et al.*[89] have found a plant polyphenol, resveratrol, present in red wine that prolongs the lifespan of budding yeast *S. cerevisiae*, which is analogous to lifespan prolongation by caloric restriction in the same organism. The probable mechanism proposed for such an extension of lifespan either by DR or by resveratrol is through stimulation of Sir2, a member of NAD^+-dependent protein deacetylases. Hence, in yeast, resveratrol[67] mimics DR by stimulating Sir2, increasing DNA stability and extending lifespan by 70%. Once, the effects of such DR mimetic agents are established in higher organisms, including humans, they may prove a better substitute for

those having lesser compliance of DR on a long-term basis. The DR mimetic drugs, preferably from plant sources, could be a suitable alternative to achieve beneficial effects of actual DR[67] (thereby meaning eating food without having it!).

Such uses of herbal products have been documented in Ayurveda, an ancient Indian system of medicine since 600 BC. In fact, *Sushruta Samitha* (written by Sushruta about 2500 years ago) refers to the use of gugglu (in Sanskrit), a gum resin of the tree Commiphora mukul, in treating obesity and lipid disorders. The extract of resin, now called guggulipid, has been successfully shown to exhibit lipid-lowering activity in normal and hyperlipidimic animals[90]. It is known to elevate HDL levels with concomitant lowering of low density lipoproteins in rabbits on high-fat diet and also to have lipid-lowering effects in humans[91,92]. Among a number of compounds present in the resin, the stereoisomers E- and Z-guggulsterones have been directly implicated in decreasing hepatic cholesterol levels in rodent models. These plant sterols are known to be potent antagonists of the farnesoid-X-receptor (FXR), a nuclear hormone receptor that is activated by bile salts[93]. It has been observed that guggulsterone administration decreases liver cholesterol in wild-type mice on a high-cholesterol diet, but is non-effective in FXR-null mice[94]. Thus, the cholesterol-lowering effect of guggulsterone has been attributed to the inhibition of FXR activation. Among other such natural plant products being investigated, aloe vera (a tropical plant of Liliaceae family) extract supplementation is now a familiar ingredient in a wide range of health care products and cosmetics. A life-long dietary supplementation of aloe vera extract has been found to suppress free-radical-induced oxidative damage and age-related increase in hepatic cholesterol in rats[95]. Use of such natural herbal products may alternatively exert many of the similar beneficial effects of DR, without cutting the food intake.

Conclusion

DR and its mimetics, once critically evaluated, could provide an attractive means to attain good health and increase longevity in various animals, including humans. Despite voluminous data available on the merits of DR (Figure 1), there are many more issues that are yet to be resolved before it could be established as a health care resort for humans. Among these, the most crucial ones are the periods of onset and the duration of such DR and/or the uses of mimetic pharmaceutical and nutraceutical agents, and the genetic variability among individuals. The strategies for such interventions have to wait for some more time before they could be routinely prescribed for healthy ageing.

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