International Journal of Life science & Pharma Research

NUTRITION: A PARADIGM SHIFT IN AGING CONCEPT?

DEVA KUMARI, YASHWANTH RADHAKRISHNAN AND NAVIN SHARMA*

ITC R &D centre, Peenya Industrial Estate, Peenya, Bangalore 560022, India

ABSTRACT

'Ageing' is an outcome of diverse metabolic phenomena like entropy, mitochondrial aging, physiological stress, environmental stress and genetic stress. Antioxidants have long been thought to be involved in delaying ageing processes and a lot of attention has been paid to identifying suitable antioxidants. Cellular studies have also demonstrated that generation of reactive oxygen species in mitochondria due to various stress, results in damage of macro molecules, thus leading to rapid and enhanced cellular ageing. However, recent evidences, at least in higher organisms, suggest that the ageing processes are much beyond single molecule such as anti oxidants. Longevity can also stems from maternal nutrition and fetal nutrition. Further during its life time, the quantity (calorie restriction) and quality of nutrition along with life style impact aging. In addition, the body composition and the ability to regulate energy have been suggested to have a role in metabolic dysregulation leading to diabetes, obesity and cardio vascular disorders, which in turn accelerate aging process. Nutrients either available in the basic food matrix or as single molecules, have been shown to act via several mechanisms, to help the cells to cope with the effects of cellular oxidative stress and prevent rapid aging. Novel concepts such as exercise mimetics seem to hold promise to health and aging since they are being looked up to as a potential target in pharmaceuticals and functional food industry. This review summarizes the contributions of nutrition in understanding the molecular concepts of aging.

Key words: Aging, nutrients, exercise mimetics, epigenetics

INTRODUCTION

Aging is a complex biological phenomenon referred to as cumulative changes during lifetime. While primary aging/chronological is the inevitable, secondary aging/ biological is the deterioration in tissue structure and biological function, independent of disease or harmful lifestyle and environmental factors (Busse EW. 1969). The rapid secondary aging is a potential problem in humans leading to both psycho-somatic and somatic-psychic disorders and is enhanced by several triggers including stress, metabolic disorders (diabetes), chronic illness and other environmental insults. Rapid secondary aging leads to deterioration of human health and results in significant reduction in mean life expectancy and hence the lifespan. In general, aging is slow and gradual process providing us opportunity to delay secondary aging by improving the overall functional capacity through balanced nutrition and exercise.

Nutrition is essential for survival of all living organisms. Culture and ethnicity has an effect on the nutritional intake. Carbohydrates, proteins and fats are essential for managing hunger and satiety. Interestingly, human nutrition is not only for survival, but also, influences the functionality driven by our aspirations, enjoyment and health (Fig 1). Nutrition has been shown to have an effect on the aging process in several organisms. Increasingly the role of diet and nutrition as against individual component/active is becoming important for regulating various health related challenges. Thrifty phenotype or popularly called as Barker's hypothesis states that the poor nutritional status of

the mother during pregnancy might reduce fetal growth and such condition is strongly associated with chronic conditions later in life such as metabolic disorders (Hales CN and Barker DJ. 1992). Researchers have demonstrated the significance of micronutrients in aging (Ames BN. 2006). In addition, the role of calorie restriction in aging is well documented in many experimental models. Now-a-days emphasis is on 'healthy aging', which is marked by well being, appearance, fitness and performance of the individual.

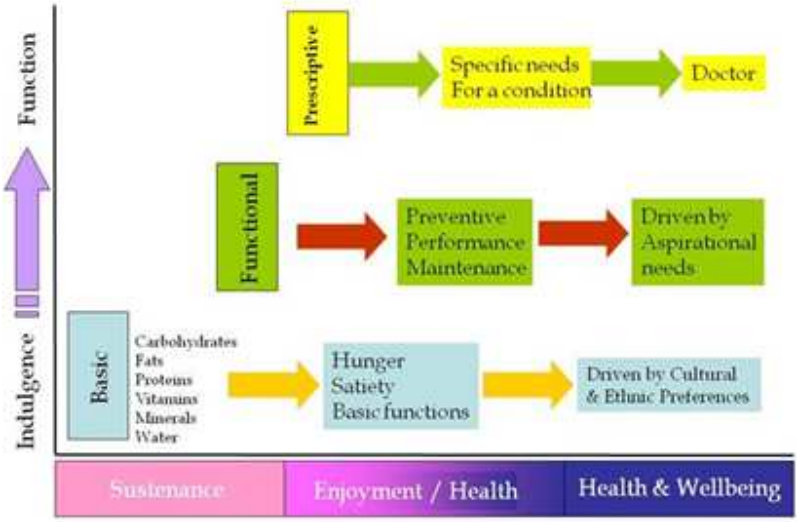

Figure : 1 ***What we eat can be for basic sustenance of life or at times a must to live (prescriptive). In between lies a category, termed functional foods, driven by the aspiration needs of the individual which aids in the prevention of ill health, leading to optimal performance and maintenance of good health.***

A healthy diet contains sufficient calories and balanced nutrients to meet energy requirements and provides growth and maintenance throughout the life time. Lack of healthy diet leads to an imbalance in metabolism; where in excess of calorie leads to several metabolic disorders such as type 2 diabetes, atherosclerosis, cancer, stroke and coronary heart diseases and scarcity of calorie, leads to undernourishment. This review describes the scope of nutrition in understanding certain molecular concepts of aging. Given the abundance of literature in the aging area, we restrict ourselves to the effect of nutritional antioxidants, the effect of calorie restriction and the recent development of exercise mimetics on aging. Increased basic understanding of the molecular mechanisms of cellular aging, especially, based on nutrition and gene interactions (nutrigenomics) and/or the role of nutrition in modulating the gene function (epigenetics) has opened up new vistas in identifying novel targets, paving way to nutrition based interventions for healthy living.

Nutrients: a way to longevity

Several population based observations suggests relationship between the health conditions and nutritional status that include the African malnutrition, French paradox, Mediterranean diet, and Okinawa centenarians. However, when individual components of the food source are analyzed, the ingredients either showed positive results (e.g. Olive oil) or lead to mixed results (e.g. Resverstol). Not only this lead to popularization of these diets world wide, they also paved way to nutritional ingredient based interventional studies. Some of the ingredients are shown to have beneficial effect even at cellular levels.

Nutrients are one of the key environmental factors to which our genes are exposed, from conception throughout life. Nutrients, govern the spatial regulation of proteins in body and function as regulators of gene transcription, protein

translation, nuclear RNA processing, messenger RNA stability and mRNA degradation. Studies designed to identify specific effects of diet on phenotypic expression of biochemical components that determine health have shown tantalizing suggestions for dietary interventions designed to modify gene expression (Petteri Kallio et al. 2007). This new knowledge is opening avenues for many potential nutritional interventions, both in food composition and in food selection. The importance of nutrient management in humans is evident from the fact the some life-threatening errors of metabolism, such as galactosemia and phenylketonuria, is successfully managed by diet modifications.

The state of imbalance between the production and manifestation of Reactive Oxygen Species (ROS) and the ability to detoxify or repair the damage in biological system is termed oxidative stress. ROS has been implicated as putative mediators of the aging process for the last half a century (Dugan LL and Quick KL. 2005). In general, cells defend against ROS damage with several enzymes such as superoxide dismutases (SOD) and earlier studies indicate that maximum life span potential of an organism is dependent on SOD levels in the body (Cutler RG. 1991), albeit some conflicting reports suggest that SODs may have little or no direct role in aging in C. elegans (Ryan Doonan et al. 2008). In general, species which had higher levels of antioxidants and the machinery to detoxify free radicals live longer. There is a positive correlation seen in the concentration of antioxidants in tissues and the maximum life span potential of the organism. In ants, queens live for more than 28 years compared to workers and males who have a very short life and it is pertinent to note that the males have been shown to have a very high level of SOD1 (Parker JD et al. 2004)(Fig. 2). SOD2 null mutation in Drosophila leads to decreased life span (Duttaroy A et al. 2003) (Fig. 3). Interestingly, in humans' significantly higher plasma SOD and other enzyme activity was observed in older compared to younger individuals (Rizvi SI and Maurya PK. 2007).

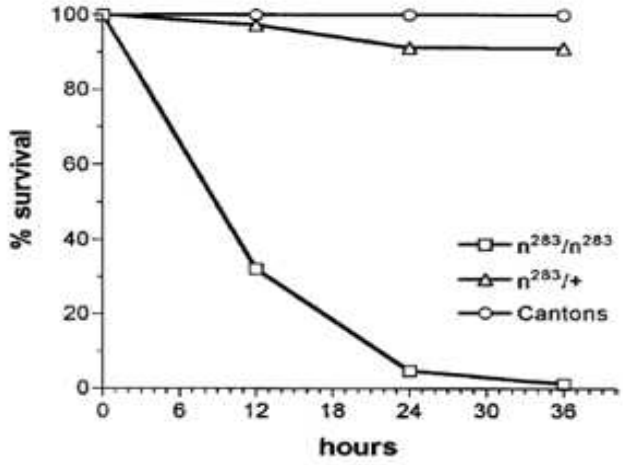

FIGURE 2.—Percentage survivorship among *Sod2*^{n283}/*Sod2*^{n283}, *Sod2*^{n283}/+, and wild-type Canton-S at 25° on normal yeast cornmeal media. A total of 50% of *Sod2*^{n283}/*Sod2*^{n283} flies died within 9 hr, and by 12 hr only 32% survived, compared to the *Sod2*^{n283}/+ or Canton-S, very few of which died during that time.

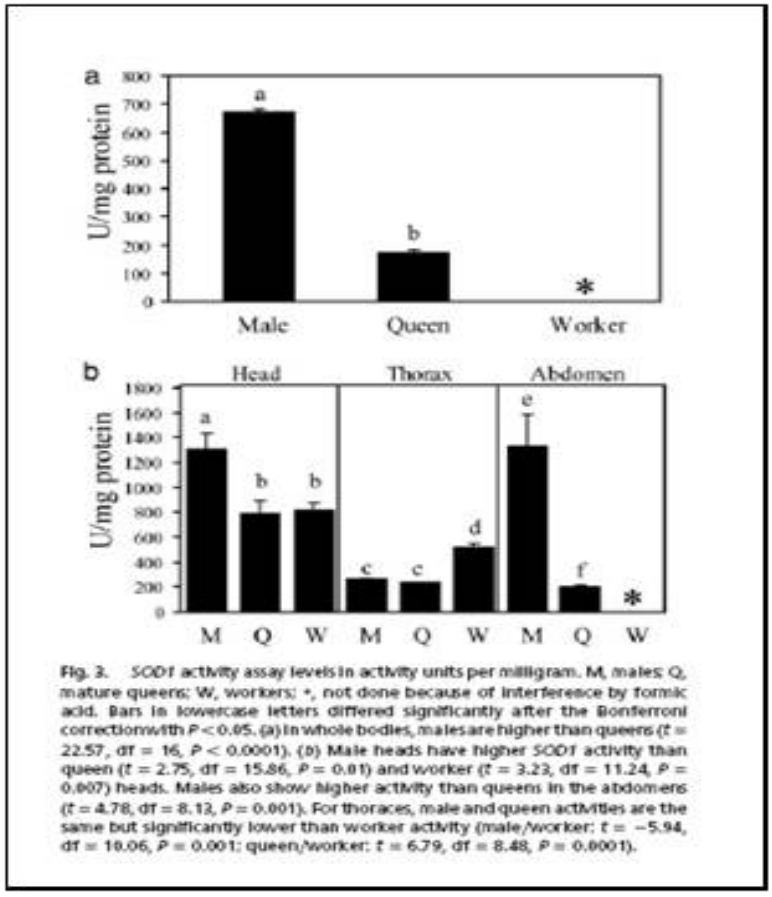

Fig. 3. *SOD1* activity assay levels in activity units per milligram. M, males; Q, mature queens; W, workers; *, not done because of interference by formic acid. Bars in lowercase letters differed significantly after the Bonferroni correction with $P < 0.05$. (a) In whole bodies, males are higher than queens ($t = 22.57$, df = 16, $P < 0.0001$). (b) Male heads have higher *SOD1* activity than queen ($t = 2.75$, df = 15.86, $P = 0.01$) and worker ($t = 3.23$, df = 11.24, $P = 0.007$) heads. Males also show higher activity than queens in the abdomens ($t = 4.78$, df = 8.13, $P = 0.001$). For thoraces, male and queen activities are the same but significantly lower than worker activity (male/worker: $t = -5.94$, df = 10.06, $P = 0.001$; queen/worker: $t = 6.79$, df = 8.48, $P = 0.0001$).

Antioxidants, specifically nutritional antioxidants since long, have been shown to influence the aging process in all organisms. Carotenoids, alpha-tocopherol, ascorbates and urates show higher levels in organisms, with higher lifespan (Cutler RG. 1991). Interestingly, macronutrient intake such as fat and proteins has been shown to stimulate ROS generation (Mohanty P et al. 2002). On the other hand intakes of nutritional antioxidants which include micronutrients and vegetables and fruits, have been shown to modulate metabolic syndromes, aging and cancer (Soory M. 2009). In addition, nutritional defense also plays a pivotal role in reduction of peroxides, sequestration of iron and utilization of dietary lipids (Parke DV. 1999). Dietary vitamin C, E and retinoids act as antioxidants protecting tissues from ROS. Furthermore, food additives such as BHT (butylated hydroxyl toluene) and BHA (butylated hydroxyanisole) prevent the peroxidation of lipids (Parke DV and Lewis DFV. 1992). Iron is a potent generator of ROS and silicic acid in cereals forms complexes with inorganic iron which enables safe sequestration of iron in tissues thereby decreasing the ROS generation (Birchall JD. 1993). The short chain fatty acid, butyrate protects against ROS damage (Rosignoli P et al. 2001). Levels of micronutrients such as copper, zinc, manganese have been shown to have a role in modulating the SOD in several systems (Uauy R et al. 1985, Mocchegiani E et al. 2008). Recently, nutritional zinc concentrations have been implicated in the activity of superoxide dismutase in patients with chronic renal failure undergoing hemodialysis (Magalhaes RC et al. 2011). Albeit certain contrary observations have been made, taken together it is evident that nutritional antioxidant concept sets a paradigm shift in aging however warrants further exploration.

Nutrients effect the epigenetic regulation of genes, leading to longevity

The earlier focus of nutritional science was to identify vitamins and minerals and to defining the

uses, later it was extended to correlate to certain specific disorders based on deficiency. With the rise of metabolic syndromes in the developed world due to over nutrition, the focus of modern medicine and nutritional science changed. This paradigm shifts in parallel to availability of human genome data lead to development of nutrigenomics that refers to the effect of nutrient intake on health via altering the genome, and nutrigenetics that explains the effect of genetic variations on interaction between nutrient intake and health (Ordovas JM and Mooser V. 2004; Mutch DM et al. 2005). In addition, several nutri-omic approaches such as nutrient mediated effects on mRNA synthesis (transcriptomics), protein synthesis (proteomics) and metabolite production (metabolomics) have expanded the understanding and scope of nutrition in health. Of late, one of the best studied aspects of nutrient-gene interaction is the complex epigenetic mechanisms where either DNA methylation or histone modification leads to either over expression or repression of gene expression without any change in the DNA sequence (Fig. 4). Epigenetic mechanisms governing the health and life span in animal models have already been demonstrated (Cooney CA et al. 2002).

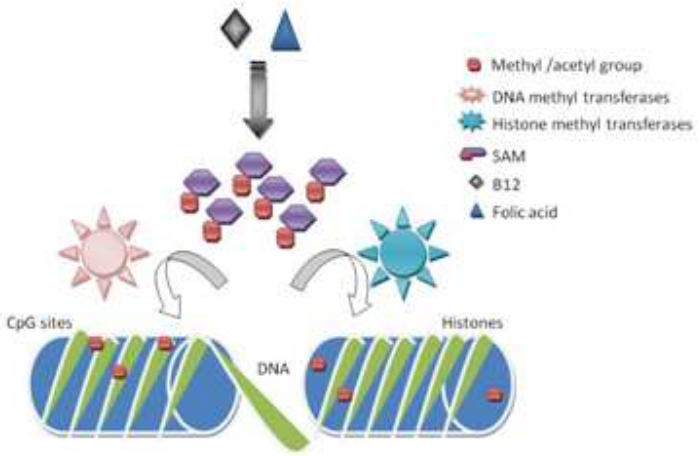

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Fig : 4 ***Methylation or acetylation of CpG islands and histones, leads to suppression or activation of certain genes. DNA methyl transferases are involved in methylation of Cytosine in CpG islands. Acetylation of lysine in histones leads to epigenetic changes. SAM acts a methyl donor and the availability of SAM is turn governed by the nutrients such as vitamin B12 and Folic acid.***

One of the significant fetaures of ageing is hypomethylation of DNA, which is mainly due to the activity of DNA methyl transferase 1(DNMT1). However the exact cause of hypomethylation is still elusive. Hypomethylation of DNA is also an underlying cause of age associated pathologies like cancer, atherosclerosis, Alzheimer's, and other neurodegenerative and autoimmune diseases (Agrawal A et al. 2010; Wierda RJ et al. 2010). In mice, excess expression of agouti gene in all tissues other than hair follicles leads to yellow phenotype, which is prone to metabolic syndromes and has decreased life span. In normal agouti gene, expressed mice, where the expression is restricted to hair follicles, there is no occurrence of diabetes or any metabolic disorders and the mice live their usual life span (Cooney CA et al. 2002, Dolinoy DC. 2008). The role of methyl supplementation in determination of epigenetic phenotype or in DNA methylation has become evident from very early studies (Wolff GL et al 1998). It is necessary to note that vitamins such as B12 from the diet act as methyl group donors and are utilized in one carbon metabolism in the cells. Deficiency in B12 may also reduce the levels of S-Adenosyl Methionine (SAM), a common co-substrate involved in methyl group transfers and SAM supplementation is used in treatment of several age-related problems including Alzheimer. Taken together, it is clear that dysregulation of epigenetic pathways have a major

role in metabolic disorders, autoimmune disease, neurodegenerative disorders, and other longevity-related processes (Hamid A et al. 2009).

In addition to the changes in the DNA methylation enzymes, as individuals' age, the alterations in the activity of histone acetyltransferase (HAT) and histone deacteylases (HDAC) also modulate gene expression. (Garcia SN and Pereira-Smith O. 2008). It has been hypothesized that the modification of the histone acetylation along with DNA methylation profile triggers differential gene expression which contributes to aging and age-related disease progression (Rodríguez-Rodero S et al. 2010). The well characterized sirtuin family belongs to class III HDACs, acts as sensor of nutritional status of cells and cellular energy and distributed from yeast to mammals. Sirtuins have been shown to modulate the calorie restriction pathway and direct implication in regulation of lifespan. Extending longevity and decrease in aging related diseases depends on successful identification and characterization of prolongevity or anti-aging molecules (eg.resveratrol) that alter the epigenetic mechanisms such as methylation, acetylation, non-coding RNAs and polycomb group (Rodríguez-Rodero S et al. 2010).

Calorie restriction results in increased life span

Calorie restriction (CR) or dietary restriction is not scanty eating, but eating the right nutrients, which can help body to undergo all the metabolic processes, leading to healthy aging. Aging rate is related to the metabolic rate or the rate of oxygen consumption per unit weight of tissue (Cutler RG. 1991). Increased metabolic activity in cells leads to accumulation of toxic elements in the body influencing the longevity related genes and metabolic processes in an organism. Therefore, in addition to the maintaining the required nutrition at the appropriate doses for a longer healthier life, restricted eating is considered as an alternative approach for healthy living and inhibition of rapid secondary aging. Calorie restriction is shown to have an effect on longevity (Rogina B and Helfand SL. 2004), ranging from metazoans to mammals. It reduces oxidative damage to proteins, lipids, and DNA (Civitarese AE et al. 2007), caused by the free radical generation and proton leak in mitochondria. CR is shown to increase the median life span, in rats (Heilbronn LK et al. 2006). In rodents, CR results in decrease in oxidative stress, by decreasing the carbonyl content in brain and muscle (Heilbronn LK et al. 2006).

Several biomarkers including core body temperature and insulin have been proposed to be biomarkers of calorie restriction and longevity in rodents and monkeys (Lane MA. et al. 2002). Also, recent reports show that CR significantly reduces fasting insulin levels and body temperature in humans (Roth GS et al. 2002; Heilbronn LK et al. 2006). A study carried out in Wisconsin National Primate Research Center; showed effects of CR on body weight, glucose homeostasis and age related diseases in rhesus monkeys (Colman RJ et al. 2009). CR reduced body weight in primates and improved glucose homeostasis. There was decreased incidence of age related disorders seen in calorie restricted primates. A similar effect is seen when the activity of nutrient-sensing pathways is reduced by mutations or chemical inhibitors.

Nutrient sensing pathways are regulated by CR. The kinase target of rapamycin (TOR), AMP kinase, sirtuins and insulin/insulin-like growth factor are involved in nutrient sensing pathways and influence longevity in an organism (Kenyon CJ. 2010) (Fig. 5). Decrease in activity of daf-2 and Insulin like Growth Factor –I (IGF-I) receptor result in increased life span in C. elegans. The downstream IGF -1 signalling involves transcription factors like DAF-16, a FOXO transcription factor; the heat-shock transcription factor HSF-1, SKN-1, a Nrf-like xenobiotic-response factor (Kenyon CJ. 2010). It is also shown that decreased activity of nutrient sensing pathways has similar effects to that of dietary restriction. Furthermore, dietary restriction has been shown to reduce the incidences of age related occurrence of diseases such as tumors, neurodegenration in rodents and metabolic disorders in primates and humans. Thus, aging might be conserved via nutrient-sensing and dietary restriction pathways (Fontana L et al. 2010), providing evidence to link nutrition and aging.

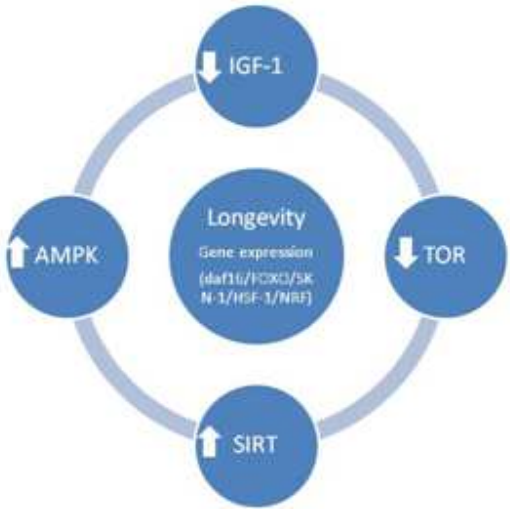

Fig : 5. ***Nutrient sensing mechanisms such as insulin like Growth Factor-I (IGF-I), AMP Kinase, Sirtuins and kinase target of Rapamycin (TOR) effect the downstream gene expression of certain genes like daf16, FOXO variants, that are implicated in longevity in C.elegans.***

In Drosophila, low calorie diet results in increase in Sir2 (sirtuins) and mutants in Sir2, show decreased life span (Rogina B and Helfand SL. 2004). In yeast, CR increases the NAD/NADH ratio by decreasing NADH levels. NADH is a competitive inhibitor of Sir2, implying that a reduction in this dinucleotide activates Sir2 to extend the life span in CR (Lin SJ et al. 2004). Sirt1 mRNA increase is observed in individuals on CR. Sirtuins are also implicated in mitochondrial biogenesis and is marked by decrease in oxygen consumption and ROS production (Civitarese AE et al. 2007). The role of diet in aging is highlighted recently using ant as a model system. In ant, especially in Jerdon's jumping ant, when the queen dies the colony selects an individual as a future queen and is fed with specific diet. This results in the development of queens features in the selected individual along with prolonged life. The ant genome sequencing hopefully, unravels some interesting facts on control of aging by diet.

Exercise and exercise mimetics

Physical activity and exercise is implicated in health and biologic aging (Singh MA. 2004), albeit mechanisms of action and dose-response remain elusive (Bouchard C. 2001). Recent study show that exercise or starvation induced autophagy deficient mice, BCL2AAA, show low levels of endurance, altered glucose metabolism and protection against impaired glucose intolerance due to high fat diet (He C et al. 2012). Regular physical activity or planned exercise is associated with positive responses at physical, physiological and psychological levels imparting health benefits, including increased longevity and decrease in the risk of chronic diseases. An inverse linear dose-response relationship exists between the amount of physical activity and cause mortality rates that are demonstrable for older and younger cohorts (Lee IM and Skerrett PJ, 2001). Mortality reduction by approximately 30 to 50% has been shown for volumes of energy expenditure during exercise of at least 1000 kcal/wk to 2000 kcal/wk respectively (Lee IM and Skerrett PJ, 2001). Albeit the effect of exercise has been associated with several health benefits, many questions still remain to be answered such as what is the duration, intensity, frequency of exercise (Singh MA. 2004) and mechanism by which exercise imparts positive effect against ageing. The etiology of chronic diseases such as cardiovascular disease, stroke, type 2 diabetes, obesity, hypertension, osteoarthritis, and osteoporosis have been shown to be multi-factorial which include physical activity/ exercise, in addition to the genetic component and

environmental insult. Several longitudinal cohort studies, generally have confirmed the cross-sectional data linking exercise to reduced disease risk especially metabolic disorders such as diabetes, obesity and cardiovascular diseases (Singh MA. 2004). Since exercise plays an important role in preventing age related diseases and metabolic syndrome, exercise mimetics may act as a beneficial tool to achieve the same effect as exercise. AMP kinase (AMPK) is one of the well known molecular targets that act as a nutrient and energy sensor and is implicated in longevity. Overexpressing AMPK extends lifespan in *C. elegans* (Kenyon CJ. 2010). Exercise activates AMPK and PGC1 alpha and a number of transcriptional regulators and serine-threonine kinases in skeletal muscles that contribute to metabolic reprogramming (Narkar VA et al. 2008). Type I fibres in skeletal muscle contain high amount of mitochondria and are oxidative in nature (Wang YX et al. 2004).

AMPK and PPARδ agonists result in remodeling of the skeletal tissue by fibre switching and also increase mitochondrial biogenesis (Narkar VA et al. 2008). AMPK and PPAR delta interaction may lead to signalling cascade and gene expression, which is involved in energy metabolism and mitochondrial biogenesis. AMPK and PPARδ agonist are considered as exercise mimetics, which increase endurance without exercise (Narkar VA et al. 2008). Therefore, calorie restriction and 'calorie burning' may act synergistically to enhance healthy aging by effecting the genetics and epigenetics of an organism. Albeit it is attractive for "couch potatoes" to gain all the beneficial effects from taking a pill (Goodyear LJ. 2008), the main criticism remains as to what extent the data can be extrapolated to humans until other organs are studied, besides skeletal muscle (Richter EA et al. 2008) and that these mimetics might not impart any health benefits and might be elusive (Hawley JA and Holloszy JO, 2009).

CONCLUSION

Aging especially mediated by the metabolic diseases are multi-factorial and not a single gene disorders. Hence, it is essential to delineate the role of the environment such as nutrient, CR, exercise and their 'interactome' (genes, proteins etc). Since 'diet induced disease' account for a large portion of the chronic illnesses and health problems worldwide, it is essential to have a sustainable and integrated food policy that encompasses newer technological developments, reducing chemicals and increasing productivity and storage to promote health and prevent diseases. Albeit, it is very early to conclude associations between cause and effect of nutrigenomics and epigenetics in human health and disease, there is a need for systematic study and understanding, rather than proving indirect link via chronic diseases. Since the ultimate aim of nutraceuticals is prevention and management rather cure, it is going to be much tougher and yet challenging.

ACKNOWLEDGEMENT

We would like to extend our sincere thanks to all our colleagues for their suggestions and useful discussions.

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