MOLECULAR ANALYSIS OF DIETARY RESTRICTION AND STARVING

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ABSTRACT

Dietary restriction (DR) is one of the effective ways to regulate the ageing as well as to improve the lifespan of diverse model organisms, which could range from unicellular yeast to primates. As in dietary restriction, there is a restriction of 20-35% in food intake as compared to regular intake. DR requires a strict intervention which has both benefits and adverse effects. However, a certain type of chronic, intermittent, or periodic caloric restrictions has shown significant result which aids in improving the lifespan and minimise the detrimental effects. Enhanced regular caloric dietary restriction approach could be planed as a safe intervention for the benefit of the individuals. Here, in this review, we will discuss the effect of caloric restriction on IGF1 and mTOR, ageing and age-related disease. In this review, the effects of CR on the brain, ageing, age-related disorders and IGF1 are covered. We will also discuss the effect of caloric restriction on IGF1 and mTOR, ageing and age-related disease. Further, it will discuss the salient aspects of intermittent fasting and starvation.

KEYWORDS: Aging, Caloric Restriction, Dietary Restriction, Starvation, Life span.

INTRODUCTION

For several decades the dietary restriction (DR or Caloric restriction: CR) or the caloric intake without reaching the stage of undernutrition has been studied for its advantages such as delay of ageing, increasing youthfulness duration, extending lifespan and their molecular aspects. It is defined as the limiting of food and reducing its intake level than that of ad libitum level without malnutrition. Various neurodegenerative, cardiovascular and ocular diseases can be treated by limiting the dietary intake. Its effects have been observed in flies, yeast, primates, mice as well as rats. In the case of people, self-practising this CR, similar observations have been made like those of model organisms used to study CR [1].

EFFECT OF CALORIC RESTRICTION ON IGF1 AND mTOR

According to the studies, dietary restriction without malnutrition leads to beneficial outcomes such as delayed ageing, increased longevity and better health, in many species. The mechanisms which lead to these outcomes are still unclear, but there are studies which show the link between insulin-like growth factor (IGF1) with phenotypes of caloric restriction. The hallmarks of CR include reduced circulation of insulin and an average decrease in the levels of IGF1. In another conserved pathway, the rapamycin target called mTOR, the intake of nutrients leads to the downregulation of mTOR. This positively affects lifespan and disease vulnerability. mTOR is considered as “Prime candidate” for describing the effects of CR on lifespan. In rodents,

pharmacological or genetic changes lead to the downregulation of the mTOR pathway. In mouse, it was observed that the decrement in mTOR of hindlimb muscle due to CR, which led to the reduction of insulin by up to 40%. The effects of CR are tissue-specific and diverse, as it leads to the phosphorylation of the IGF1 target proteins that are involved in the signalling pathway. One of the critical proteins is pAkt, present in skeletal muscle, gets increased due to CR effect. However, the signalling proteins of the mTOR pathway were not much attenuated by CR. Whole tissue analysis can be beneficial to study the effect of CR on specific cell types [2].

Maternal dietary restriction in the periconceptional period can result in an increased level of adrenal growth and cortisol stress response in the embryo. However, the mechanism behind this is still not clear. The adrenal growth is regulated by IGF activation, suppressors of cytokine signalling (SOCS) and the signal transducer. The adrenal JK/STAT pathway gets activated during the weight loss due to conception. Therefore, the regulation of adrenocortical growth is affected by signals during adverse conditions which are produced at the time of conception. The IGFI receptor (IGF1R) can be occupied by either IGFI or IGFII, which activates the phosphoinositide3 kinase protein kinase B/Akt pathway. This leads to the activation of signals such as eukaryotic translation initiation factor 4E (EIF4E) binding protein 1 (4EBP1), ribosomal protein S6 (RPS6) and the mammalian target of rapamycin (mTOR). Also, maternal obesity during these period results in the increased level of IGF1R and Akt, with mTOR increase in the female child [3].

EFFECT OF CALORIC RESTRICTION ON AGING

Ageing is a complex process which is associated with many physiological changes. Ageing increases the chance of various diseases related to heart, neurodegenerative and cancer. The basic and deep knowledge of ageing and processes related to it can help in delaying or preventing these age-related diseases. Various studies show that the CR slows the ageing process. Also, several proteins are involved in the positive effects of CR. These include AMP-activated protein kinase (AMPK), the sirtuin family of protein deacetylases, ADP ribosyltransferases and FOXO-family transcription factors. Many pharmacological molecules can be used in the process of CR, after understanding the mechanisms followed by it. This could lead to solving many age-related problems [4].

The implementation and maintenance of CR condition are much difficult despite having so many advantages of it. So, the alternatives of CR were investigated and certain chemical compounds found, which have similar effects as that of CR. These compounds are 1) Polyphenols 2) Lactoferrin 3) Astaxanthin 4) Lipoic acid 5) Deoxy-D-glucose (2-DG) 6) Metformin 7) Rimonabant 8) Rapamycin. Among all of these, metformin is used to treat diabetes from last 40 years. It increases the sensitivity of insulin receptors on the fat and muscle cell surface. It also decreases the gene expression of enzymes that increases the oxidation of fatty acids and activates genes that decrease glucose production [5].

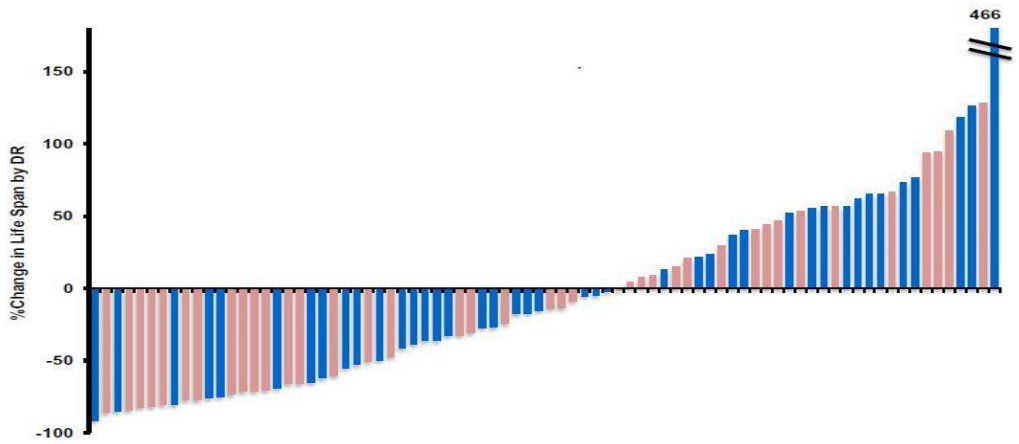

Fig1. Effect of 40% CR on recombinant inbred mouse strains. The difference ranges from an increase in life span more than 400% to a decrease of more than 90% in males. Dark bar- males, Light bars- females [6].

CR mimics are introduced, which are the pharmacological agents and mimic the CR effects on lifespan and health without food intake. Some of these mimics are AMPK activators, TOR Inhibitors and Sirtuin activators. In a study, done on yeast, it was observed that the hypothesis could be developed on it, but important information can be obtained after studying the process in complex organisms. It can be done either by CR practice or by using CR mimic. In the experiment done on life spans of ILS/ISS recombinant inbred mouse strains showed that these strains made up to long life span when kept under CR conditions, i.e. 60% of ad libitum (AL) (Fig 1). In an investigation, the sirtuin histone deacetylase Sir2 was deleted, which prevented the extension of lifespan by CR. However, deletion of Sir2 with Fob1 (replication fork block protein) leads to enhancement of lifespan. It was also observed that the use of the same CR regime, on the various wild type strains, different effects on the extension of life span obtained [6].

Further study shows, during oxidative damage to the skeletal muscle of mice, the level of carbonyl protein and sulfhydryl increases with age in AL mice while there is an increase in CL mice. After the transfer of mice from AL to CR condition, at the age of 18-22 months, lead to the damage of the proteins which are collected with age in the body and were not recovered in the 6 weeks of time duration. These results suggest that ROS/ molecular damage involves in CR related lifespan extension. According to the recent investigations performed on fruit flies, for the effect of CR on them, it was observed that dietary protein is crucial in females for egg production, but not in male flies during the adulthood stage. In females, less carbohydrate content in the diet increases egg production, whereas high carbohydrate content results in long lifespan [7].

DIETARY RESTRICTION IN AGE-RELATED DISEASES

Dietary restriction (DR) extends lifespan in many organisms, including animal and cellular models, where it can delay a range of ageing-related diseases, including Alzheimer's disease (AD). Alzheimer disease (AD) is an

age-related disease, which is very prevalent and is devastating. Patients suffering from this disease show cognitive declination, also function, behaviour and memory defects. Molecular markers of Alzheimer's disease include amyloid-beta (Aβ) and intraneuronal neurofibrillary tangles (NFTs) made of Tau (microtubule-associated) protein. Tau is present in the brain, the regions dealing with memory and learning. Both environmental and genetic factors can be a cause of this disease. Production of Aβ and hyperphosphorylation of tau can be used as tools for examining AD in model organisms. Intermittent fasting did not affect the level of these factors in mice. However, DR decreases the Aβ level, maybe by increasing the activity of the non-amyloidogenic APP (amyloid precursor protein) cleaving enzyme secretase. It also prevents the hyperphosphorylation of tau maybe by decreasing the activity of tau kinase cyclin-dependent kinase-5(CDK-5). This helps in maintaining the cognitive of the model organisms. This whole study well describes that DR leads to a neuroprotective effect on Aβ and tau [8].

As it had been studied earlier that CR reduces age-related dysfunctioning of organs along with the reduction in the ageing process of organs such as the eye. Ageing plays a major role in the various eye diseases, such as diabetic retinopathy, age-related macular degeneration (AMD), cataracts, glaucoma, dry eye disease, pterygium, retinal vein occlusion and conjunctival chalasis. There are many diseases of the eye where CR plays an important role. Some of them are: 1) **Dry eye disease-** it is a disease getting more common in an ageing group of population. This leads to visual disturbances, fatigue and ocular discomfort. Also, as age increases, the functionality of lacrimal gland decreases. Lacrimal glands of CR rats were studied and found to produce more tears and had a better intact structure of mitochondria. Mitochondrial ROS level is highest among all cell organelles, produced during oxidative stress while ageing. It has been found that ROS affect lacrimal gland functioning and thus, the tear formation as well; however, the actual mechanism is still not understood. 2) **Cataracts-** this eye disorder causes blindness in most of the elder age group. In many model organisms, the positive effects of CR are observed. In mouse, CR leads to delay in the formation and progression of cataract. CR retards degeneration of lens by slowing down the process of aggregate formation, it also reduces gamma and alpha crystallins, which are responsible for attenuation of oxidative stress in the lens. 3) **Retinal diseases-** CR reduces lipofuscin accumulation in RPE (retinal pigment epithelial) cells. Lipofuscin is formed after the phagocytosis of degraded photoreceptor cells. It gets accumulated in lysosomes of RPE. The more accumulation of lipofuscin with an increase in age leads to RPE dysfunction and fasten the ageing processes within the retina, resulting in AMD (age-related macular degeneration). It also has neuroprotective effects in the aged retina and decreases the age-related cell-death of the photoreceptors [9].

The dietary restriction also links with brain pathologies. In stroke condition, where the blood supply to the brain is interrupted, CR (40%) and IF lead to an increase in 50% survival rate in stroke-prone rats. In the case of epilepsy, a ketogenic diet is preferred, as ketones can cross the blood-brain barrier and can be used as an energy source instead of glucose. According to a hypothesis, Dietary restriction diet mimics the ketogenic diet. In the

case of Parkinson's disease, administration of rotenone or 1-methyl-4-phenyl- 1,2,3,6-tetrahydropyridine (MPTP) is done, which induces a parkinsonian phenotype by impairing with mitochondrial complex I activity. In a study. It was observed that 30 % CR diet before MPTP administration, improves motor activity, increases the level of neurotrophic factors in the brain and reduces the loss of dopamine and its related metabolites [10].

INTERMITTENT FASTING AND STARVATION

In the case of type 2 diabetes, pancreatic beta cells fail to produce sufficient amount of insulin. This leads to overnutrition, and thus, obesity. In the end, it causes insulin resistance and dysfunctioning of beta cells. The suppression of this insulin resistance involves various active lipid molecules such as acylcarnitines, ceramides, diacylglycerols (DAGs) and long chain fatty acyl-CoA. In a study, it was observed that CR and intermittent fasting (IR) also helps in the reduction of insulin resistance. The investigation was done on obese mice under AL, CR, and IF conditions and results showed 43% diabetes in AL mice while CR and IF mice remained protected from hyperglycemia. Also, the DAG accumulation was less in CR and IF mice. Therefore, it can be concluded that moderate CR and IF lead to prevention of type 2 diabetes [11].

It is the condition of complete when there is no food intake. It was studied in a white shrimp, the effects of starvation and re-feeding on various parameters such as- survival rate, immunity, weight loss, resistance by virus infection and expression of different proteins in it. It was observed that integrin β expression decreased after 0.5-5 days of starvation, but the expression of LGBP, PX, proPO I, proPO II, ppA, and α2-M increased after 0.5–1 day. This study was carried for the benefit of shrimp farming. Immunity of animals decreases as a result of starvation. In the case of mice, the lymphocyte number reduces in liver, spleen and thymus after the 3 days of starvation [12].

CONCLUSION AND FUTURE ASPECTS

The dietary restriction also called as the caloric restriction in some researches, has proved as an important tool to study and treat various diseases at the molecular level. The effect of CR on IGF1 in studied in various investigations. The ageing and age-related diseases such as Alzheimer disease, ocular disorders, brain dysfunctioning, Parkinson’s disease are also found to be affected by CR positively. In all the studies, it had been concluded that CR delays ageing and slows down the processes related to it. Still, the links and basic connections between these diseases and CR are yet to be revealed and explored. The molecular mediators are unknown, but the studies show a remarkable impact of CR on these diseases. The effects include not only the prevention of undesirable conditions but also fast recovery and delay in promotion of diseases. More attention is required in the fields where CR has proven to be inefficient. The identification of minute factors and their role in the processes can aid in understanding the procedure of how this diet restriction works. Much detailed and specificity-oriented work is required to explore more in this field.

REFERENCE

1. Lee, C.; Longo, V. Dietary restriction with and without caloric restriction for healthy aging. *F1000Research* 2016, *5*.

2. Sharma, N.; Castorena, C.M.; Cartee, G.D. Tissue-specific responses of IGF-1/insulin and mTOR signaling in calorie restricted rats. *PLoS One* **2012**, *7*.

3. Zhang, S.; Morrison, J.L.; Gill, A.; Rattanatray, L.; MacLaughlin, S.M.; Kleemann, D.; Walker, S.K.; McMillen, I.C. Maternal dietary restriction during the periconceptional period in normal-weight or obese ewes results in adrenocortical hypertrophy, an up-regulation of the JAK/STAT and down-regulation of the IGF1R signaling pathways in the adrenal of the postnatal lamb. *Endocrinology* **2013**, *154*, 4650–4662.

4. Mair, W.; Dillin, A. Aging and Survival: The Genetics of Life Span Extension by Dietary Restriction. *Annu. Rev. Biochem.* **2008**, *77*, 727–754.

5. Timmers, S.; Konings, E.; Bilet, L.; Houtkooper, R.H.; Van De Weijer, T.; Goossens, G.H.; Hoeks, J.; Van Der Krieken, S.; Ryu, D.; Kersten, S.; et al. Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. *Cell Metab.* **2011**, *14*, 612–622.

6. Schleit, J.; Wasko, B.M.; Kaeberlein, M. Yeast as a model to understand the interaction between genotype and the response to calorie restriction. In Proceedings of the FEBS Letters; NIH Public Access, 2012; Vol. 586, pp. 2868–2873.

7. Sohal, R.S.; Forster, M.J. Caloric restriction and the aging process: A critique. *Free Radic. Biol. Med.* 2014, *73*, 366–382.

8. Kerr, F.; Augustin, H.; Piper, M.D.W.; Gandy, C.; Allen, M.J.; Lovestone, S.; Partridge, L. Dietary restriction delays aging, but not neuronal dysfunction, in Drosophila models of Alzheimer's disease. *Neurobiol. Aging* **2011**, *32*, 1977–1989.

9. Kawashima, M.; Ozawa, Y.; Shinmura, K.; Inaba, T.; Nakamura, S.; Kawakita, T.; Watanabe, M.; Tsubota, K. Calorie restriction (CR) and CR mimetics for the prevention and treatment of age-related eye disorders. *Exp. Gerontol.* **2013**, *48*, 1096–1100.

10. Amigo, I.; Kowaltowski, A.J. Dietary restriction in cerebral bioenergetics and redox state. *Redox Biol.* 2014, *2*, 296–304.

11. Baumeier, C.; Kaiser, D.; Heeren, J.; Scheja, L.; John, C.; Weise, C.; Eravci, M.; Lagerpusch, M.; Schulze, G.; Joost, H.G.; et al. Caloric restriction and intermittent fasting alter hepatic lipid droplet

proteome and diacylglycerol species and prevent diabetes in NZO mice. *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids* **2015**, *1851*, 566–576.

12. Lin, Y.C.; Chen, J.C.; Siti, S.N.; Wan, W.Z.; Awangku, A.S.; Cheng, S.Y.; Hsu, C.H. Modulation of innate immunity and gene expressions in white shrimp Litopenaeus vannamei following long-term starvation and re-feeding. *Results Immunol.* **2012**, *2*, 148–156.