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The effects of calorie restriction on aging: a brief review

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Abstract. – Calorie restriction (CR) without malnutrition slows aging and increase average and maximal lifespan in model organisms and rodents. In human and non-human primates, CR has beneficial effects on human longevity and reduces the incidence of age-related diseases such as obesity, diabetes mellitus hypertension, cardiovascular disease and cancer. CR exerts its anti-aging effects through different mechanisms including small noncoding RNA molecules (sncRNAs), the composition of diet and IGF-1 signaling. The aim of this review was to discuss recent developments to understand the consequences and mechanisms of CR on longevity.

Key Words:
Aging, Dietary restriction, Small non-coding RNAs.

Introduction

Calorie restriction (CR) defined as a reduction in calorie intake below usual *ad libitum* without malnutrition, slows aging, extend maximal and average life span in animals of diverse origin[1,2]. The role of CR to enhance life span dates back to early 20th century when Osborne et al[3] reported that female rats having retarded growth and delayed sexual maturity lived longer than those that grew rapidly. McCay et al[4] published the first scientific paper to report that restricting food intake at or soon after weaning in rats, extended median and maximum life span and decreased the onset and severity of chronic diseases. Subsequently, 30-60% reduction in calorie intake below usual *ad libitum* had increased the average and maximal life span in many species of mouse and rat strains[2]. As cancer accounts for major cause for mortality in rodents (70-80%), CR has been shown to inhibit the development of different types of tumors in mice and delayed or prevented the occurrence of chronic nephropathies and cardiomyopathies and diabetes, autoimmune and respiratory diseases[5]. In nonhuman primates, two independent studies have shown mixed results. One[6] carried out in two age groups young (1-14 years old) and old (16-23 years old) of rhesus monkeys at National Institute on Aging (NIA) in Bethesda, Maryland, found no difference in the longevity of treated versus untreated animals following 30% calorie restriction. Another study[7] at the Wisconsin National Primate Research Centre (WNPRC) from the University of Wisconsin, Madison, had found a positive effect of CR on longevity, reporting 13% mortality for the CR group compared to 37% in the control group, although not statistically significant. A detailed analysis of these two studies highlighted the difference in the genetic background, the age of onset and diet composition. However, these two reports suggest a beneficial effect of CR on health span in nonhuman primates which can serve as a good aging model to understand different longevity factors that can be translated to human aging and human health.

Although a wealth of data is available in mice and primates, the beneficial effects CR in humans has been observed in many settings. Data from natural and controlled investigations suggest that CR with adequate nutrition has useful effects on human longevity and protects against the development of obesity, cardiovascular disease, hypertension and cancer. Individuals from Okinawa, Japan, who usually have low calorie intake compared to the residents of the main Japanese Island, had very low mortality from coronary heart disease and cancer[8]. A controlled study[9], Biosphere 2 (carried out in normal weight individuals) reported that CR with high levels of physical activity, reduced insulin levels, blood pressure, body weight, cholesterol and other physiological and anthropometric parameters.

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Data obtained from Calorie Restriction Society (CRS) members, taking 30% fewer calories than age- and sex-matched controls, exhibited positive effects of CR on various physiologic and metabolic parameters such as a decrease in BMI, total body fat, serum cholesterol, triglycerides, fasting glucose and insulin levels[10]. CRS members also had lower levels of blood pressure and inflammatory markers (C-reactive proteins, tumor necrosis factor α and interleukin-6) than age- and sex-matched healthy controls. No adverse effects of CR such as eating disorder, cognitive impairment, depressed mood or change in spontaneous physical activity has been observed in humans practicing CR, making it a useful intervention[11]. Experimental evidence suggested that CR improved memory in elderly[12].

The composition of diet is important while considering the role of CR on longevity. Restriction of macronutrients such as proteins, carbohydrates or fats has beneficial effects. Limiting the consumption of certain amino acids such as methionine and tryptophan extend longevity and prevent multiple age-related diseases. In mice, methionine restriction (MR) had reduced levels of serum IGF-1, insulin, glucose and thyroid hormone and visceral fat deposition[13]. An analysis of diet regimen of Okinawa inhabitants indicates the presence of plant-based low protein diets consist mostly of vegetables, fruits and grains[14]. A recent cross-sectional study[15] based on US NHANES III database which includes dietary intake data, reported an association of age with protein consumption and mortality. A comparative analysis of individuals from two age groups 50-65 and ≥ 65 years of age revealed that younger population having consumption of > 20% of calories from proteins had a 4-fould increase in risk of developing cancer and a 75% increase in mortality compared to subjects taking <10% of calories from proteins. However, when the protein source was plant-based, this association was eliminated and there was a decrease in cancer mortality. On the other hand, older population on high protein intake had reduced cancer and overall mortality. These findings suggest that reduced protein intake during middle age and moderate to high protein consumption in older individuals may help in longevity and healthy life span[15]. Variations in the protein consumption reflect the requirements of different age groups, if high protein consumption has negative effects in younger individuals, it is equally essential in older individuals for providing essential amino acids to maintain healthy muscle mass and positive nitrogen balance. Thus, restricting certain amino acids, not complete diet has beneficial effects on longevity.

Many cellular, metabolic and physiological mechanisms have been implicated for life extending and anti-aging effects of CR[10]. Evolutionary conserved nutrient- and energy- sensing pathways which include, IGF-1/insulin signaling, mTOR, sirtuins, AMPK and GCN2 have been suggested in the regulation of aging by CR[16]. In mice, mutations that suppress insulin/IGF-1/mTOR pathways increase health and life span[17]. Ames dwarf mice (*df/df*) deficient in growth hormone (GH), prolactin and thyroid stimulating hormone (TSH), had significantly longer life span than their normal siblings[18]. Other mutant mice, deficient in GH/GH receptor or GH resistant, lived longer (median and maximal life span), exhibited delayed symptoms of aging and had reduced circulating IGF-1 levels[19]. These mutant mice had reduced mTOR signaling, resulting in reduced cell size and protein synthesis which possibly contributed to delayed aging and extended longevity[20].

Increased resistance to oxidative stress is another possible mechanism for longevity and CR. In Ames dwarf mice, protection form oxidative damage has been associated with reduced production of reactive oxygen species (ROS) and increased activity of anti-oxidant enzymes[18]. However, accumulating data does not support a major role of oxidative stress in modulating aging in mammals[21]. Mutant mice deficient in several antioxidant enzymes or overexpressing the major antioxidant enzymes (CuZnSOD, Mn superoxide dismutase, and catalase) did not have extended life span or decreased age-related diseases[22,23]. Hence, whether the reduction in oxidative damage by CR has any role in life extension is still an open question.

Summing up, accumulated evidence indicates that moderate CR associated with adequate nutrition protects against many age-related anomalies in many species including humans. However, much work is needed to understand the cellular and molecular mechanisms responsible for beneficial effects of CR.

Small Noncoding RNAs, Calorie Restriction and Aging

Small noncoding RNAs (sncRNAs) are a complex category of RNA molecules, less than 400 nucleotides in length, which are not translated into proteins but they control a variety of cellular

functions and biological processes by interacting with target genes[24]. The sncRNAs circulate in the bloodstream and extracellular spaces and can modify gene expression of target cells and are also part of cell-to-cell communication and signaling system involved in disease and normal biological conditions[24]. Advances in sequencing methods such as deep sequencing have identified another class of sncRNAs which are shorter in length, initially taken as degraded products, are reported to have certain functions[25]. These short sncRNAs include transfer RNA-derived RNA of size 30-33 nt, micro RNA of 20-24 nt, and YRNA-derived RNA of two sizes, 27 and 30-33 nt in length. Informations regarding the role of tRNA is scarce. Therefore, the role of miRNA in the context of aging and CR will be discussed in the following section.

MicroRNA (miRNA) are evolutionary conserved, single-stranded noncoding RNA molecules that bind target mRNA to induce its degradation or inhibit protein translation. It has been reported earlier that approximately 60% of human genome is regulated by miRNAs reflecting their importance in many biological functions. MicroRNA is also present in many species including nematode *Caenorhabditis* (*C.) elegans,* fruit fly, mice and humans[26]. Many miRNAs have been identified having a role in aging, with levels either up- or down- regulated[27]. Genome-wide analysis of miRNA expression in humans revealed a significant decrease in miRNA levels in older individuals, some of them having a role in cancer pathogenesis[28]. In a study[29], using next generation sequencing technology and real-time quantitative PCR, three serum miRNAs (miR-151a-3p, miR-181a-5p and miR-1248) were found to be lower in older individuals compared to younger ones. These miRNAs have been involved in the expression of genes responsible for inflammatory pathways and proinflammatory cytokines IL-6 and IL-8 and DNA repair pathways. Therefore, it is possible that decreased levels of these miRNAs may potentiate inflammation and other developmental stages during aging and can be taken as biological markers of aging.

In aging, age-related changes occur in gene expressions which are important in cell division, senescene and apoptosis, and prone to dysregulation and oncogenesis. Most of these changes are tissue-specific such as skin, brain and adipose tissue. CR delays age-related changes in gene expression and decreases the occurrence of age-related dysfunction[30]. Recent data have shown a relation among miRNA, aging and CR. Mercken et al[34] have reported differential miRNA expression in the skeletal muscle of young and aged rhesus monkeys and CR had reversed these alterations. In mice, CR had prevented the elevation of age-dependent miR181a-1, miR-30e and miR-34a levels with a reciprocal increase in their target gene Bcl-2 in the brain of aged CR-animals, which resulted in the loss of pro-apoptotic signaling and gain of neural survival to achieve cognitive robustness[31]. Another aspect of CR is to reduce age-induced changes by acting on key metabolic enzymes and genes involved in energy metabolism[32]. Therefore, changes in circulating miRNA may help to maintain the beneficial effects of CR. Victoria et al[33] have shown the involvement of circulating mRNAs to regulate extended life span of long-lived Ames dwarf mice compared to controls which act via CR-like and/or CR-independent mechanisms. Thus, it is possible that sncRNAs take part in the progression of age-related harmful effects and at the same time have the prospective to implement beneficial effects of CR[34]. Therefore, changes in circulating miRNA help to maintain the beneficial effects of CR.

It is evident that sncRNA circulate in the human blood and can influence cellular changes, although there are still many areas which need attention for future research, such as source and target tissues, mechanism of secretion and the altered levels during aging. However, an association of sncRNA in aging and antagonizing effects of CR are potential areas of future research to better understand mechanisms of aging and CR.

CR, IGF-1 Signalling and Aging

The evolutionary conserved signaling pathways, insulin and the insulin-like growth factors (IGFs) play important roles in growth, metabolism, development and aging in various organisms from yeast to mammals[35,36]. The insulin and IGF-1 signaling contribute to regulation of life span and its dysregulation leads to common and potentially life-threatening malignancies[37]. Mutations in the genes coding for IGF-1 in different species such as yeast, nematode, Drosophila, mice and rat are accompanied by delayed aging and increased life span which are the characteristics of CR[38]. Mutant mice genetically lacking GH activity have very low levels of circulating IGF-1 and live longer than wild type animals. Ames dwarf mice and Snell dwarf mice which are deficient in GH, prolactin and

TSH, and secondary suppression of peripheral IGF-1 lived more than 40% longer than their wild type counterparts and CR further extended the life span in these animals indicating the presence of a distinct mechanism to extend longevity[18]. However, the role of IGF-1 in humans is inconclusive. Lower occurrence of cardiovascular disease is associated with higher GH-IGF1 levels, while on the other hand, lower IGF-1 levels are linked to reduced incidence of cancer[39]. Mutations in IGF-1R are associated with exceptional longevity in centenarians, and this gene polymorphism resulted in high IGF-1 levels and reduced activity of IGF-1R[40]. Similarly, studies from growth hormone receptor-deficient and IGF-1 deficient patients provide evidence for reduced occurrence of cancer and diabetes mellitus and are protected from age-related pathologies, possible to increase longevity[41]. Milman et al[42] suggested that individuals with exceptional longevity and history of cancer had lower IGF-1 levels, a possible predictor of longevity.

As mentioned earlier, CR is a reproducible and robust intervention to extend life span and delay onset of age-related diseases and cancer[5]. Many of the characteristics relevant to CR such as reduced plasma insulin, IGF-1 signaling and glucose levels and increased insulin sensitivity are also observed in animals with reduced IGF-1 signalling[17].

CR reduces the occurrence of different types of cancer, partly due to a reduction in IGF-1 levels and increase in corticosteroid levels[5]. Moderate CR had reduced the occurrence of cancer by more than 50% in, Rhesus monkeys[6,7]. Furthermore, it has been shown by us and others that animals with reduced GH/IGF-1 signaling exhibit increased corticosterone levels, possibly to compensate for the absent metabolic effects of GH and to increase the animal's ability to cope with stress[43].

Another beneficial effect of CR is to increase IGF-1 binding protein (IGFBP-1) levels which have a negative effect on IGF-1 activity and downstream signaling. In rodents, CR decreases plasma IGF-1 levels by 25% and increases serum corticosteroids[44]. However, in humans, short- and long-term randomized clinical trials suggest that CR does not reduce serum IGF-1 concentration and IGF-1: IGFBP-3 ratio unless protein intake is reduced[45]. In another randomized clinical trial, long-term CR did not reduce serum IGF-1 levels but a significant and persistent increase in IGFBP-1 levels in young and middle-aged lean or slightly overweight men and women, with a mild increase in serum cortisol levels[46]. These findings in mice and humans need further elaboration to better understand the discrepancy in the effect of CR on healthy aging.

Conclusions

The observations from animal and human studies suggest that CR is a useful intervention to promote healthy aging. However, it is still an open question, how CR extends longevity in humans because it is difficult to conduct diet-controlled, randomized, long-term survival studies due to compliance, cost, logistic and ethical issues. Further studies are required to explore cellular and molecular mechanisms including the potential of sncRNA molecules mediating beneficial effects of CR, which would help to develop new markers of aging and interventions for healthy aging.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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