Caloric Restriction and Antiaging Effects

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Key Words

Caloric restriction • Aging • Lifespan • Energy metabolism

Abstract

Caloric restriction (CR) is widely used to study aging processes. It is a simple and highly reproducible method for delaying the aging process, preventing the onset of aging-related diseases and extending average or maximum lifespan. However, the mechanism underlying these effects of CR is still not clear. CR can inhibit growth, reduce body size and maintain a low body temperature. At the same time, there is a measurable decrease in the volume of adipose tissue, hyperglycemia and hyperinsulinemia, accompanied by modifications of lipid and energy metabolism and increased resistance to endogenous and extraneous stress. The metabolic changes induced by dietary restriction, the inhibition of fat deposition in nonadipose tissue and the effects on signal transduction are considered the most likely candidates for mechanisms underlying the effects of CR.

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Introduction

In 1935, McCay et al. [1] first reported that caloric restriction (CR) could extend the lifespan of ablated rats. During the following 70 years of research, CR was also termed dietary restriction, food restriction and energy restriction. Applying CR to mammals is designed to induce undernutrition without malnutrition, with food in-

take reduced by usually 30–40% compared to ad libitum (AL) levels. It is a simple method for inducing a highly reproducible extension of lifespan that has been widely used in research on aging [1].

CR extends the average and maximum lifespan and delays the onset of age-associated alterations in diverse species [2, 3]. Lifespan extension by CR has been observed in a number of organisms ranging from yeast, *Cae-norhabditis elegans* and *Drosophila melanogaster* to rats [4, 5]. CR is thought to exert antiaging effects including an increased resistance to endogenous stresses such as hyperglycemia and hyperinsulinemia, to changes in energy metabolism and oxidative stress, or to extraneous environmental stresses. However, a detailed mechanism for CR responses has not yet been established.

In 1989, Holliday [6] introduced the compatibility hypothesis as an explanatory mechanism for the antiaging and lifespan extension effects of CR. The importance of neuroendocrine and metabolic changes effected by food insufficiency was explained from an evolutionary viewpoint. In periods when abundant food is available, body size would grow quickly and reproduction would be vigorous. Excess energy would be converted into fat and stored as adipose tissue. In subsequent periods of food shortage, body growth and reproduction would be inhibited and the energy stored in adipose tissue used to extend the lifespan until the diet again became sufficient. The animals that had this ability to adapt to an insufficient diet were therefore selected during the course of evolution. CR could extend the lifespan through reactions occurring in response to insufficient diet. Therefore, in this

review, we have generalized CR to include metabolism, responses of the neuroendocrine system, signal transduction changes and molecular mechanisms of antiaging.

Effects of CR on Metabolic Changes

CR is different from starvation. The levels of plasma leptin, insulin, growth hormone (GH) and luteinizing hormone (LH) were shown to be low, while corticosterone was very high in CR and fasting groups [7, 8]. However, the respiratory quotient (RQ) is low and the source of energy shifts from glucose to lipids in starvation. Fats from white adipose tissue are channeled into mitochondrial β -oxidation to provide ketones as a fuel source for the brain [9, 10]. Fasting can ultimately activate the transcription factor of peroxisome proliferator-activated receptor- α (PPAR- α), which induces expression of many genes related to mitochondrial β -oxidation, peroxisome β -oxidation and microsome ω -oxidation. The activity of oxidative processes could protect against fatty liver [11, 12].

In turn, in a CR rat group, the RQ was high after food intake, but was low before food intake. Daily food intake brought about large changes in RQ, whereas the daily RQ changes seen in AL rats were relatively small [13]. The AL rats could simultaneously utilize glucose and fats as energy sources according to a fixed ratio. However, the CR rats mainly utilized glucose after food intake and essentially used fat metabolism before food intake due to exhaustion of glycogen stores. The CR rats reduced the fuel for mitochondrial β -oxidation before food intake. PPAR- α and gene expression related to mitochondrial β -oxidation were increased. Free fatty acids and ketone bodies in blood were higher prior to feeding than after food intake. In contrast, the total fats, triglycerides, total cholesterol and free cholesterol, phospholipids and free fatty acids were decreased in the CR rats after food intake [13]. However, the gene expression of ADD1/SREBP1, the synthesis of fatty acids and the activity of acyl-coenzyme A carboxylase in liver were significantly increased. Furthermore, CR activated the gene expression related to glucose, amino acid, lipid and energy metabolism without an effect of food intake [14].

Consequently, CR rats with a low RQ before food intake utilized endogenous fatty acids from white adipose tissue for basic mitochondrial β -oxidation to produce ketones. In contrast, CR rats with a higher RQ primarily used sugar from food as fuel, with surplus fuel used to synthesize fatty acids in the liver, which were then stored

in white adipose tissue as triglycerides. The end result is that CR can induce fatty acid synthesis in the liver and activate mitochondrial β -oxidation through PPAR- α . Lastly, CR improves the efficiency of lipid metabolism and inhibits lipid deposition in nonadipose tissue [14].

Effects of CR on the Neuroendocrine System

The gonadal, thyroid and GH-insulin-like growth factor 1 (GH-IGF-1) axis is suppressed by long-term CR. However, CR activates the adrenal glucocorticoid system. CR upregulates orexigenic peptide and neuropeptide Y while downregulating anorexigenic peptide and proopiomelanocortin [15–17]. The plasma IGF-1 level is significantly reduced in CR rats [18] and the plasma leptin concentration in CR rats is also lower than that in AL rats [19]. The hormonal profiles are the same in CR and fasting animals. However, since fasting is a short-term intervention to modulate energy homeostasis, it is necessary to distinguish the short-term and the long-term signals in the hypothalamus that regulate energy expenditure.

Effects of CR on Rodents

Small Body Size, Delay of Growth and Maturation, and Inhibition of Reproduction

CR inhibits growth and body size after maturation and reduces body weight [2, 3]. GH and hormones such as IGF are considered to play important roles in these responses. GH in the pituitary gland is reportedly secreted as a pulse in an AL-fed group, whereas this secretion pattern is not apparent in a CR group [20]. The IGF-1 level in blood is decreased by about 25% [18].

CR also inhibits sexual maturation of both males and females. The inhibition by CR is stronger for females than for males. A reduction in LH by CR maintains a low follicle-stimulating hormone (FSH) level and decreases the size of the sex organs while maintaining reproductive ability [21, 22]. Restriction (50%) in daily food intake for 20 days significantly reduced plasma prolactin and testosterone levels without affecting basal LH and FSH concentrations and testicular weight in male rats [23]. In females, CR maintains the estrous cycle during aging. A female group fed AL after a 7- or 8-month period of CR showed an increased frequency of estrous cycles and numbers of progeny compared with a control group without CR. CR does not delay the aging of sex organs in males [23]. The levels of LH and FSH in blood are differ-

ent from those seen during starvation. Circulating LH, thyroid-stimulating hormone, GH and prolactin were significantly depressed in acutely and chronically starved rats, and FSH was lowered only in acutely starved rats. After 7 days of refeeding, serum levels of LH and FSH were significantly higher than in AL-fed controls [24]. In addition, starvation markedly decreased plasma insulin and testosterone levels in male rats. The weight of the testes was maintained, whereas the testicular interstitial fluid volume decreased [25].

Hypercorticonsteronemia

In mammalian species, the hypothalamic-adenohypophyseal-adrenal cortical glucocorticoid system takes part in successfully coping with stressors. CR enhances the daily peak levels of plasma free corticosterone in rats. The free corticosterone in a CR group was 2- to 8-fold higher than in an AL group [26]. Corticosterone as a stress hormone and CR provided light stress hormones over a long period. This hormesis of light stress is considered to be the most important reason for the antiaging effects of and lifespan extension by CR [5].

Modification of Hyperglycemia and Hyperinsulinemia

CR reduces the glucose and insulin levels in the blood when the stomach is empty [27]. Glucose tests show that in CR rats, glucose disposal after glucose load is facilitated without any significant surge of serum insulin, and insulin tolerance tests also indicate an increase in insulin sensitivity. Similar effects are seen after glucose and insulin load in dwarf transgenic rats, in which the GH-IGF-1 axis has been selectively suppressed by overexpression of antisense GH transgene. CR in these tg/- rats further facilitates glucose disposal during glucose and insulin tolerance tests [28]. These findings suggest that both shared and separate mechanisms exist for regulating the glucose-insulin system between CR and the reduced GH-IGF-1 axis.

Reduction in Metabolic Rate and Hypothermia

The importance of metabolic rate during the aging process has been apparent since ancient times. CR before food intake transiently decreases the unit lean body mass from birth to 6 weeks of rats, but this effect is lost after 12 weeks and large differences are not observed until 18 months [29, 30]. Based on this, it is very difficult to consider that metabolic rate plays any important role in the antiaging effects of and lifespan extension by CR. On the other hand, CR induces low body temperature [31, 32], but this effect is lost when mice are fed at 30°C. Moreover,

calorie-restricted monkeys have lower body temperatures and insulin concentrations than do control monkeys [33], and both these variables are biomarkers for longevity in rodents. These findings suggest that low body temperature induced by CR might contribute to antiaging and extension of lifespan by CR.

Reduction in Adipose Tissue and Phenotype Changes

The reduction in body size and body weight by CR also decreases the volume of adipose tissue. CR strongly affects fat volume in internal organs more than body weight or adipose tissue levels [29, 34]. Under normal conditions, with increased age, the volume of adipose tissue also is increased. CR inhibits this change in response to aging [29, 30, 34] and significantly decreases the size of fat cells in adipose tissue [14]. Some evidence indicates that CR induces changes in gene expression related to metabolism of sugars, amino acids and fats, and to mitochondrial function. On the other hand, gene expression related to inflammation, blood vessel regeneration, extracellular matrix and the cytoskeleton-related genes are inhibited by CR [14]. These changes in gene expression are not affected by the pattern of food intake.

Some of these gene changes are also seen for experimental in vitro fat cell differentiation. There is a regressive correlation with gene expression in adipose tissue of obese animals [14]. Furthermore, the changes in metabolism are in part induced by gene expression of transcription factors that activate β_3 -adrenergic receptors, reduce leptin and increase SREBP1/ADD1 gene expression [14].

The cytokines and hormones derived from adipose tissue are referred to as 'adipokines'. Leptin is an adipokine that can control food intake and energy consumption. Animals reportedly increase food intake and become obese when the leptin control mechanism is unbalanced [35]. Leptin levels in the blood are dependent on the volume of adipose tissue and can also be increased with increases in food intake [19]. CR significantly inhibits the expression of leptin mRNA [14]. The plasma leptin shows the same circadian change as seen for increased ingestion behavior. CR can reduce plasma leptin levels by 50% [19].

Adiponectin, another main adipokine, is derived from special small fatty cells and controls the energy balance and metabolism of glucose and fats [36]. Plasma adiponectin is reduced in patients with type 2 diabetes and obesity. It induces an increase in triglyceride deposition in skeletal muscle and liver [37, 38]. Adiponectin activates 5'-AMP-activated protein kinase (AMPK) and inhibits synthesis of glucose in the liver. It may also take part in

activities involving glucose utilization and fatty acid oxidation [39]. CR can increase the plasma adiponectin levels 2- or 3-fold [40]. Therefore, CR not only reduces the volume of adipose tissue but also strongly induces secretion patterns and gene expression patterns that change the nature of the fatty cell.

Involvement of CR in Cell Damage and Antiaging

Lower insulin sensitivity is related to obesity, but also to excessive triglyceride deposition in nonadipose tissue [41]. Furthermore, steatosis induces lipotoxicity or lipopapoptosis [42]. This lipotoxicity is apparent in the β -cell of pancreas, in cardiac muscle and in skeletal muscle in type 2 diabetes and in insulin tolerance. In addition, excessive triglycerides promote lipid peroxidation [41]. A contribution of reactions involving free radicals and hyperglycemia to aging has been proposed [43]. Steatosis of nonadipose tissue in the liver induces tumor necrosis factor- α and cytokine production in cytoskeleton and promotes inflammation [44]. However, CR inhibits inflammation processes by increasing activity of nuclear factor- κ B, tumor necrosis factor- α and inducible nitric oxide synthase [45]. At the same time, CR also differentially inhibits and lowers insulin sensitivity, lipotoxicity, free radicals and other reactions. Under normal conditions, leptin, adiponectin and activity of PPAR- α in nonadipose tissue play important roles in the inhibition of steatosis [46–48]. Furthermore, CR can reduce the triglyceride content in hepatocytes and affect hepatic insulin sensitivity and glucose production. In addition, short-term CR resulted in increases in insulin sensitivity, in both non-diabetic and type 2 diabetic subjects [49].

Changes in Signal Transduction by CR

Recent studies on long-lived mutants of lower organisms and rodents have also indicated that reduced insulin-like signaling or GH-cIGF-1 signaling can extend the lifespan of these animals [50]. Since CR diminishes GH-IGF-1 signaling as serum insulin levels are lowered, it thereby acts on aging and lifespan in animals [51]. The energy-sensing AMPK is a candidate for mediating CR responses in prolonging lifespan. AMPK is activated in response to decreased energy or glucose levels, which are consequences of CR [52], and in specific cases is induced by CR itself [53]. FOXO factors are activated and decrease the activity of the insulin signaling pathway [54, 55]. In

C. elegans, the FOXO transcription factor DAF-16 mediates longevity due to a mutation in the insulin receptor *daf-2* [56, 57]. Although the lifespan extension due to the *eat-2* mutation and to several CR regimens has been shown to be independent of FOXO/DAF-16, FOXO might still mediate lifespan extension under other CR experimental conditions. FOXO/DAF-16 is a direct in vitro substrate of AMPK and AMPK increases FOXO-dependent transcription. Together, these results point to the AMPK-FOXO module as a central pathway in the regulation of lifespan by CR.

Molecular Mechanism of CR Antiaging Responses

The molecular mechanisms underlying CR responses that extend the lifespan in many species remain unclear. Some specific signals such as IGF have been implicated in lifespan extension in lower organisms [58]. In the following, we discuss the antiaging effects of CR, focusing on the mechanism of sirtuins, target of rapamycin (TOR)/AMPK and *pha-4/SKN-1*.

The sirtuin family is a critical regulator of lifespan. Both deletion and overexpression of sirtuins affect the lifespan of lower organisms [59]. Sirtuins are NAD⁺-dependent protein deacetylases and exert regulatory functions through deacetylation of a wide range of protein targets. There are 5 sirtuins found in budding yeast [60], 4 sirtuins in *C. elegans* and 7 sirtuins in mammals [61]. After *Sir2* of yeast as the first gene discovered in this family, much evidence indicated that *Sir2* protein was involved in transcriptional silencing at cell-mating-type loci and telomeres in yeast, and suppressed recombination of yeast ribosomal DNA [62, 63]. Kaerberlein et al. [64] first indicated that extra copies of *Sir2* extended lifespan by up to 30%. Similar antiaging effects of *Sir2* were subsequently observed in *C. elegans* and the fruit fly [4, 64–67]. Human sirtuins (*SIRT1*–7) share the catalytic domain with *Sir2* [61]; *SIRT1*–3 and 5 show NAD⁺-dependent protein deacetylase activities, while *SIRT4* and 6 have mono-ADP-ribosyl transferase activities. NAD⁺ required by sirtuins as a cosubstrate suggests that sirtuins might act as sensors of cellular energy and redox states. *Sir2* in yeast and its mammalian homologue *SIRT1* can be upregulated by CR [68].

The amino acid-sensing TOR is a conserved serine/threonine protein kinase and regulates protein synthesis and growth in response to nutrient intake [69]. Recently, the TOR pathway has been suggested as another mechanism through which CR may act. TOR comprises a rapa-

mycin-sensitive and an insensitive complex and both of them can regulate downstream targets [70, 71]. A reduction in amino acid intake extends lifespan in yeast [72], *Drosophila* [73] and rodents [74, 75]. Therefore, TOR signaling is thought of as the mechanism whereby altered nutritional intake could result in organism-wide changes in aging processes.

AMPK is another important potential mediator of effects of CR. It is a key sensor of cellular energy levels and regulates the TOR and insulin/insulin-like signaling pathways under low-nutrient conditions [76]. Under low-energy conditions, AMPK inhibits energy-consuming processes through the inhibition of TOR signaling [77, 78]. Therefore, AMPK like TOR plays a role in aging and stress resistance.

To understand the molecular regulating mechanisms of longevity and survival in animals, many mechanism-based experiments were conducted. Recent reports identified two transcription factors, PHA-4 and SKN-1, which could regulate the extension of lifespan by CR in *C. elegans* [79, 80]. Pha-4 was found to be the ortholog of the mammalian Foxa family of forkhead transcription factors (Foxa1, 2 and 3) in the worm [81]. It is bifunctional in early development and in maintaining metabolic homeostasis. The role for PHA-4 in CR-mediated longevity in *C. elegans* was revealed by an RNAi-based screen testing [82]. The pha-4 gene produces three PHA-4 protein isoforms called PHA-4 A, B and C that differ only at their N-termini [76]. Panowski et al. [80] indicated that PHA-4 affected lifespan independently of its developmental function. CR induces FoxA [83, 84] and PHA-4 expression. Therefore, like the Foxa family members, PHA-4 has a role in lifespan extension by CR and early development.

SKN-1 is the worm ortholog of nuclear factor-E2-related transcription factors Nrf1 and Nrf2 [85]. The Nrf proteins can induce expression of phase II detoxification enzymes in the liver and digestive tract in response to

oxidative stress and xenobiotics [86–88]. During adulthood, SKN-1 involves resistance to damage induced by oxidative stress [89]. Therefore, SKN-1 could be a key regulator of the response to CR [76].

Discussion and Conclusion

In this paper, we focused on the effects of CR on metabolic changes, the neuroendocrine system, cell damage and aging to provide an overview and give the latest insights into molecular mechanisms of CR in antiaging effects in lower organisms and rodents. It can offer important information for interdisciplinary research on nutrition metabolism and antiaging. Further, this information can provide a background for the utilization of CR to delay the aging process in humans and as a therapy for aging-related diseases like Alzheimer's and Parkinson's disease. CR is widely used as a novel model for aging research. The latest research report indicated that dietary restriction can increase longevity in humans [89, 90]. CR changes the endocrine and neuroendocrine systems in humans. Long-term severe CR does not reduce the serum IGF-1 concentration or IGF-1:IGF-binding protein 3 ratio. In contrast, total and free IGF-1 concentrations are significantly lower in moderately protein-restricted individuals [91]. This evidence suggests that protein intake is a key determinant of circulating IGF-1 levels in humans. Reduced protein intake may therefore become an important component of anticancer and antiaging dietary interventions. Future studies are necessary to determine the mechanisms behind lifespan prolongation by CR in humans.

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