Molecular Links between Caloric Restriction and Sir2/SIRT1 Activation

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Ageing is the most significant risk factor for a range of prevalent diseases, including cancer, cardiovascular disease, and diabetes. Accordingly, interventions are needed for delaying or preventing disorders associated with the ageing process, i.e., promotion of healthy ageing. Calorie restriction is the only nongenetic and the most robust approach to slow the process of ageing in evolutionarily divergent species, ranging from yeasts, worms, and flies to mammals. Although it has been known for more than 80 years that calorie restriction increases lifespan, a mechanistic understanding of this phenomenon remains elusive. Yeast silent information regulator 2 (Sir2), the founding member of the sirtuin family of protein deacetylases, and its mammalian homologue Sir2-like protein 1 (SIRT1), have been suggested to promote survival and longevity of organisms. SIRT1 exerts protective effects against a number of age-associated disorders. Caloric restriction increases both Sir2 and SIRT1 activity. This review focuses on the mechanistic insights between caloric restriction and Sir2/SIRT1 activation. A number of molecular links, including nicotinamide adenine dinucleotide, nicotinamide, biotin, and related metabolites, are suggested to be the most important conduits mediating caloric restriction-induced Sir2/SIRT1 activation and lifespan extension.

Keywords: Biotin; Dietary restriction; NAD; Nicotinamide; Lifespan; Sirtuins

INTRODUCTION

Life expectancy has remarkably increased during the past century, due mainly to medical and pharmaceutical advances, which help to reduce life-threatening and ageing-associated diseases. For example, the discovery of antimicrobial therapy and vaccines resulted in a huge drop of infectious diseases and a major gain in life expectancy in 1900s. The modern drug development and better treatment contribute to over 50% decrease in death rates for heart disease and stroke since 1972. In 21st century, epidemics of chronic diseases, such as diabetes, obesity, cardiovascular disease, and cancer, are the targets of antiageing therapies. Increased lifespan and ageing-related problems have been brought to the forefront not only because of the financial burden to the health care and government pension system, but also due to the impacts on our society, family, and industry. In fact, many countries are facing the challenges to accommodate older workforce and to extend work lives. Innovations and new conceptions in medicine are inspiring hopes to break and further extend the biological limit of life expectancy, beyond the success achieved for lifespan extension since 1900s, especially to counteract the wide array of contemporary problems in the current century. In order for modern pharmaceutics to break the biological ageing barrier and to reach the ultimate goal of medicine, immortality, a thorough understanding of the biological basis of ageing and lifespan extension is critically urgent and important. Here, the biological pathways mediating caloric restriction-induced lifespan extension will be reviewed and discussed.

CALORIC RESTRICTION AND LIFESPAN EXTENSION

The lower intake of calories, the longer lifespan can be achieved.

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Caloric restriction without malnutrition is a nongenetic intervention that consistently promotes the extension of maximum lifespan in model organisms including yeast, worms, flies, mice, and nonhuman primates [1-3]. The effect can be robustly achieved by restricting up to half of the typical calorie intake in these model organisms, when malnutrition is avoided. The most striking benefit of caloric restriction is to prevent the development of a broad spectrum of ageing-associated pathological changes, such as tumorigenesis, immunosenescence and cardiometabolic disorders. In humans, long life expectancy of Okinawans is attributed to a low caloric intake and negative energy balance at younger ages, a life-long low body mass index, and a low risk of mortality from age-related diseases [4]. Optimal nutrient composition and feeding regimen of the lifespan-extending diets are not yet established. There are also debates on whether the ingested energy, when expressed in per gram body weight of the organisms, is restricted, increased or remain similar during dietary restriction [5]. In fact, the antiageing effect of caloric restriction may be achieved through restriction of certain types of amino acids, carbohydrates, lipids, or vitamins. Thus, the term 'dietary restriction' is increasingly utilized when describing limited food intake in relation to the extension of healthspan and lifespan.

The biological basis of caloric restriction remains poorly understood. The involvement of a single gene and pathway has been investigated in non-mammalian systems [3]. For example, removal of ethanol and/or acetic acid extends the chronological longevity (the survival of a population of nondividing cells) of the model organism yeast, whereas their replicative lifespan (the number of daughter cells generated by a single mother cell) is more sensitive to glucose restriction [6]. Downregulation of Sch9, a serine-threonine kinase that shares high sequence identity with the mammalian Akt/protein kinase B (PKB) and ribosomal protein S6 kinase (S6K), extends the chronological lifespan by up to 2-fold [7]. Reduction of the TOR complex 1 activity leads to an extension of yeast replicative lifespan that cannot be further promoted by caloric restriction [8]. In the fruit fly *Drosophila*, reduction of amino acid consumption, but not sugar intake, extends life span substantially with essential amino acids mediating most of the responses [9]. In mammals, although different nutrient contents are sensed by distinctive pathways; however, it is unlikely that one single pathway is responsible for the effect of caloric restriction. Restricted dietary intake triggers the inactivation or activation of a number of nutrient sensing pathways, including

insulin-like growth factor (IGF)/insulin, mammalian target of rapamycin/S6K, and silent information regulator 2 (Sir2)-like protein 1 (SIRT1) signaling pathways. These pathways are also involved in the antiageing effects of a number of chemical compounds and drugs (Fig. 1).

In rodents, dietary restriction significantly delays the occurrence of many chronic diseases and increases life span by up to 60% [10]. Attenuated IGF-1 signaling mediates some of the antiageing effects. Excess nutrient intake activates the proageing IGF signaling pathway. Mice that are under restricted dietary intake display hypoinsulinemia, enhanced sensitivity to insulin and reduced glucose levels. In humans, dietary restriction provides similar metabolic and cardiovascular benefits as in rodents, but without reducing IGF-1 levels, unless protein intake is also reduced [11], suggesting that restriction of protein intake provides additional antiageing benefit. Older Okinawans consumed a diet with restricted calorie (10% to 15%) and low saturated fat content, but rich in functional foods (e.g., herbs or spices) that may mimic the biological effects of caloric restriction. However, the caloric restriction mimetics and the related nutrient sensing pathways remain to be characterized. In addition to longevity, caloric restriction leads to additional phenotypes, such as increased resistance to oxidative stress, enhanced repairing of DNA and protein damages, improved glucose ho-
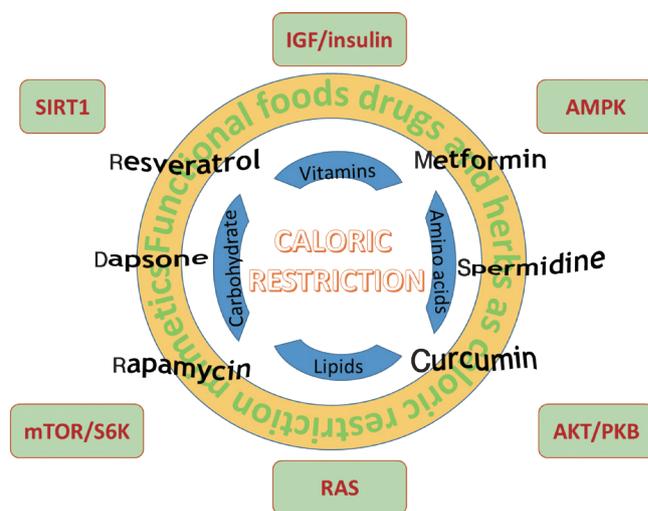

Fig. 1. In response to different dietary intake, a number of nutrient sensing pathways are activated or inactivated to modulate the ageing process. IGF, insulin-like growth factor; SIRT1, Sir2-like protein 1; AMPK, AMP-activated protein kinase; mTOR/S6K, mammalian target of rapamycin/ribosomal protein S6 kinase; ROS, reactive oxygen species; AKT/PKB, AKT/protein kinase B.

meostasis and insulin sensitivity, lowered serum glucose and cholesterol levels, decreased oxygen consumption and body temperature, all of which contribute to delayed onset of age-related diseases [10,12,13]. In the following part of this review, a more specific molecular mechanism involving caloric restriction-evoked activation of SIRT1 will be discussed.

SIRTUINS AND CALORIC RESTRICTION

Sirtuins are a family of nicotinamide adenine dinucleotide (NAD)-dependent protein deacetylases [14]. *Sir2*, the first gene discovered in this family, was originally shown to regulate transcriptional silencing at cell-mating loci, telomeres, and ribosomal DNA (rDNA) in yeast, through deacetylation of the epsilon-amino groups of lysines in the amino-terminal domains of histones [15]. Sir2 cleaves the glycosidic bond between nicotinamide and adenosine diphosphate (ADP)-ribose in NAD and this reaction requires the presence of acetylated lysine [16,17]. Thus, one molecule of NAD and one molecule of acetyl-lysine are catalyzed to one molecule each of deacetylated lysine, nicotinamide and O-acetyl-ADP-ribose. There are seven mammalian sirtuins, termed SIRT1-7, which share the sequence homology of catalytic domain with Sir2 [18]. SIRT1 is the mammalian ortholog most highly related to Sir2. However, unlike the intra-nuclear localization of yeast Sir2, SIRT1 is not tightly bound to chromatin but shuttles between cytoplasm and nucleus [19]. Thus, in addition to histones, SIRT1 interacts with and mediates the deacetylation of a wide range of signaling molecules, including transcription factors, enzymes and tumor suppressors. The dynamic functions of SIRT1 are largely attributed to the additional domains at its NH_2- and COOH-terminus, which allow the regulation of this protein by various post-translational modifications and protein-protein interactions [14,20].

During the past decade, sirtuins have attracted major attention due to their potentials of expanding life span in lower organisms and protecting against age-associated disorders in mammals. In yeast, integration of extra copies of *Sir2* extends lifespan by up to 30% and deletion of this gene shortens life span by about 50% [21]. Calorie restriction by limiting glucose availability in the growth medium of the budding yeast *Saccharomyces cerevisiae* leads to the activation of Sir2 and the extension of replicative lifespan [22]. Sir2 mediates caloric restriction-induced lifespan extension, which requires NAD synthesis. Under conditions of reduced glucose, the metabolism of *S.*

cerevisiae shifts from fermentation to respiration, resulting in elevated NAD or decreased NADH levels. In respiratory deficient yeast cells, on the other hand, caloric restriction could not increase Sir2 activity, but extends the replicative lifespan independent of Sir2 [23]. Moreover, the chronological lifespan of *S. cerevisiae* is not affected by *Sir2* deficiency [24,25]. In some long-lived yeast mutants, deletion of *Sir2* enhances chronological longevity extension by caloric restriction [24]. Thus, Sir2 regulates longevity in yeast through a pathway related to caloric restriction, but a direct link between these two anti-ageing factors has not been firmly demonstrated. The localization of *Sir2* to certain age-related loci, such as the rDNA repeats, promotes its lifespan-sustaining function during caloric restriction [26]. On the other hand, Sir2 function is not a limiting factor for chronological ageing [27].

The role of *Sir2* in caloric restriction-induced lifespan extension has subsequently been confirmed in *Caenorhabditis elegans* and *Drosophila melanogaster* [28,29]. Unlike yeast, most cells in these fully grown and multicellular metazoan organisms are nondividing. Sir2 mediates the beneficial effects of caloric restriction via mechanisms involving metabolic control and stress responses to genotoxicity, heat shock, and oxidative damage [30,31]. Sir2 promotes genomic silencing either by repressing genomic instability or by preventing inappropriate gene expressions. In mammals, increased SIRT1 expression and function contributes to the beneficial effects of caloric restriction on delaying the onset of age-associated diseases, including cancer, atherosclerosis, and diabetes [32,33]. Mice lacking both copies of SIRT1 fail to show an increased activity and extended lifespan in response to caloric restriction, but display a shorter median lifespan than wild type mice [34,35]. Mice with elevated SIRT1 expression exhibit a beneficial phenotype resembling that of caloric restriction: they are leaner, more metabolically active, more glucose tolerant, and have reduced levels of circulating cholesterol, proinflammatory adipokines, insulin, and fasting glucose [36]. However, mice lacking one allele of SIRT1 still show identical lifespan to that observed in wild-type mice, when subjected to caloric restriction [37]. Small molecule activators of SIRT1 replicate signaling pathways triggered by calorie restriction [38]. SIRT1 plays an important role in adjusting the metabolic processes during caloric restriction, thus having been regarded as a metabolic regulator of energy homeostasis [14,39-41]. Importantly, SIRT1 is modulated by caloric restriction in a tissue-specific manner [42]. The activity and expression of SIRT1 in liver is reduced by

caloric restriction, while those of white adipose tissue and skeletal muscle are enhanced. The systemic regulation of mammalian SIRT1 is mediated, in part, by insulin and IGF-1, two serum factors negatively involved in life-span regulation [32]. It is tentative to speculate that in tissues containing rapidly dividing cells, caloric restriction-induced SIRT1 expression shifts the balance away from cell death toward cell survival and/or regeneration, whereas in tissues containing mainly postmitotic cells, SIRT1 promotes longevity by regulating metabolic shift from using different carbon source of nutrients.

MECHANISTIC INSIGHTS OF SIRT1 ACTIVATION BY CALORIC RESTRICTION

When cells have high levels of calories, a substantial portion of the NAD pool is recruited into a high carbon flow of glycolysis by the enzyme glyceraldehyde-3-phosphate dehydrogenase. When calories are restricted, more carbons are oxidized in mitochondria via the electron transport chain-mediated cellular respiration, which produces NAD from NADH [43,44]. Thus, under caloric restriction, the NADH levels are significantly decreased as a result of up-regulated mitochondrial respiration [45,46]. Sir2 depletion does not affect caloric restriction-induced elevation of the intracellular NAD/NADH ratios in yeast. Because the inner membrane of mitochondria is impermeable to NADH and NAD, the malate-aspartate shuttle is used for translocating electrons produced during glycolysis for oxidative phosphorylation. This allows the hydrogen ions of NADH produced in the cytosol to reach the electron transport chain in the mitochondria. Overexpression of the malate-aspartate NADH shuttle components extends yeast replicative life span in a Sir2-dependent manner [47]. Consistently, overexpression of the mitochondrial NADH dehydrogenase specifically lowers NADH levels and extends lifespan [45]. The major modification catalyzed by Sir2/SIRT1 is deacetylation. NADH, nicotinamide adenine dinucleotide phosphate (NADP), or NADPH could not substitute NAD for this reaction. However, NAD levels do not correlate with the lifespan of yeast [46]. During calorie restriction, the NAD levels in yeast are actually decreased, indicating that Sir2 is not primarily regulated by the availability of NAD. In this regard, the deacetylase activity of Sir2 is closely linked to the decreased NADH, as the latter is a competitive inhibitor of Sir2 [45]. However, overexpressing the NADH oxidase or alternative oxidase, both of which increase NADH oxidation, could not alter

the life span of the wild type yeasts [6]. These information suggest that increased respiration plays a major role in lifespan extension by caloric restriction in yeast. Sir2 acts to facilitate this process by detoxifying oxidized macromolecules, including nucleic acids, proteins and lipids. However, the activity of Sir2 and SIRT1 are not affected by physiological alterations in the NAD/NADH ratio [46].

Unlike mitochondria, the nuclear envelope is permeable to a wide variety of small molecules [48], suggesting that cellular perturbations of NAD/NADH affect their levels in cytoplasmic as well as in nuclear compartments. NAD and NADH in nuclei play active roles in regulating gene transcription and genome stability [49]. Genotoxic stress depletes the nuclear and cytosolic pools of NAD, but not the mitochondrial pools, due largely to the extensive use of this substrate by poly (ADP-ribose) polymerases (PARPs) [50]. PARPs catalyze the polymerization of ADP-ribose units from donor NAD molecules on target proteins, resulting in the attachment of linear or branched polymers. There is a strong positive correlation between the longevity of a species and the polymer synthesis capacity of PARPs in mammalian cells [51]. PARP1 knockout mice age much faster than the wild-type control animals [52]. However, hyperactivation of PARP1 results in the depletion of NAD/adenosine triphosphate (ATP) and increases mitochondrial pore formation and cell death [53]. SIRT1 is a consumer of NAD and competes with other NAD-dependent enzymes for this common substrate. Thus, it has been proposed that inhibition of PARPs can increase NAD availability for SIRT1 to elicit the anti-ageing activity [54]. However, only certain types of tissues in PARP1 knockout mice exhibit increased NAD+ content and enhanced SIRT1 activity [55,56]. Moreover, the relationships between PARP and caloric restriction remain uncharacterized.

Alternatively, caloric restriction may activate Sir2 by regulating the level of nicotinamide, a known inhibitor of Sir2 [57-59]. Crystal structures of the conserved sirtuin catalytic domains reveal that NAD and the peptide containing an acetylated lysine residue enter the active site from opposite sides of a cleft between a large Rossmann fold domain and a small Zn-binding domain [60]. During the formation of an alkylimidate intermediate between the ADP-ribose 1' position and the acetyl oxygen, nicotinamide dissociates from NAD and occupies a so-called C-pocket. If nicotinamide binds to the C-site before alkylimidate conversion, it will inhibit the deacetylation reaction. Thus, removal of nicotinamide may be as important for the activation of Sir2/SIRT1 as the production of NAD. The

salvage pathway for NAD biosynthesis begins with either nicotinamide or nicotinic acid, collectively referred to as niacin or vitamin B3 [61]. Nicotinamide is first converted to nicotinamide mononucleotide (NMN) by nicotinamide phosphoribosyltransferase (NAMPT). The production of NAD^+ from NMN and ATP is catalyzed by a family of nicotinamide mononucleotide adenylyltransferases (NMNATs). In lower eukaryotes, including *S. cerevisiae, D. melanogaster*, and *C. elegans*, no NAMPT activity has been found. Nicotinamide is converted to nicotinic acid, which then enters the parallel salvage pathway found in all eukaryotic species. Alternatively, nicotinamide riboside forms a precursor for NAD synthesis, connecting to the nicotinamide salvage pathway through NMN [62]. The predominant form of NMNAT in mammals, NMNAT-1, is a nuclear protein, while other forms, NMNAT-2 and NMNAT-3, are cytoplasmic and mitochondrial, respectively [63]. Overexpression of NMNAT-1 in mammalian cells does not affect total NAD levels, but regulate nuclear NAD-dependent processes [64]. Manipulation of a nuclear NAD salvage pathway delays ageing in yeast, without changing the steady-state levels of NAD [65]. Despite these information, the detailed links between caloric restriction and the NAD salvage pathway in mediating Sir2/SIRT1 activation has not been established. It is also possible that the genes involved in NAD salvage pathway act in a more general manner to promote cell survival [59, 66,67].

We have recently shown that SIRT1 is strongly inhibited by biotin, the water-soluble vitamin B7, and its metabolite biotinyl-5'-AMP [39]. Biotin occupies the binding pocket of nicotinamide, which may affect the conformational change from nonproductive to productive SIRT1 [68,69]. Biotinyl-5'-AMP competitively occupies the NAD binding site and prevents the breakdown of NAD by SIRT1. In addition, biotin may react with NAD to generate biotinyl-5'-AMP, in turn inhibiting the deacetylase activity of SIRT1. Since NAD also acts as a cofactor permitting SIRT1 to interact with protein substrates, by inhibition of NAD binding, biotinyl-5'-AMP prevents the interactions between SIRT1 and acetylated protein substrates. Adipose tissue represents a major reservoir of biotin in mammals. During ageing, biotin is progressively accumulated in adipose tissues. Chronic biotin supplementation mainly increases adipose biotin contents and abolishes adipose SIRT1-mediated beneficial effects on insulin sensitivity, lipid metabolism, and locomotor activity. In fact, caloric restriction prevents biotin accumulation in adipose tissues. Biotin and nicotinamide were

originally discovered as the same class of heat-stable vitamins [70]. However, unlike nicotinamide, nutritional deficiencies of biotin are rare. The role of biotin in metabolism has been established in experimental microorganisms and animals. Biotin functions in mammals as a CO_2 carrier for reactions in which a carboxyl group is transferred to one of four biotin-dependent carboxylases. Consequently, biotin participates as an important cofactor in gluconeogenesis, fatty acid synthesis, and branched-chain amino acid catabolism [71]. Based on these information, we speculate that in mammals, caloric restriction may enhance SIRT1 activity by selective depletion of biotin storage in adipose tissue, in turn preventing ageing-associated metabolic disorders and promoting lifespan extension.

CONCLUSIONS

Caloric restriction has been considered as a robust means of reducing ageing-related diseases and slowing the ageing process. Sir2 and its mammalian homologue SIRT1 are up-regulated by caloric restriction. Thus, Sir2/SIRT1 proteins sense low calories and mediate the beneficial effects of caloric restriction. However, the mechanism underlying caloric restriction-induced Sir2/SIRT1 activation remains elusive. Here, based on the available literature and our own research data, it is postulated that the induction of Sir2/SIRT1 activity by caloric restriction is an evolutionarily conserved response to decreased availability of certain nutrients, such as B vitamins.

The initial breakthrough of identification of Sir2 as a deacetylase with weak ADP-ribosyltransferase activity came along with the identification of the *Salmonella typhimurium* CobB
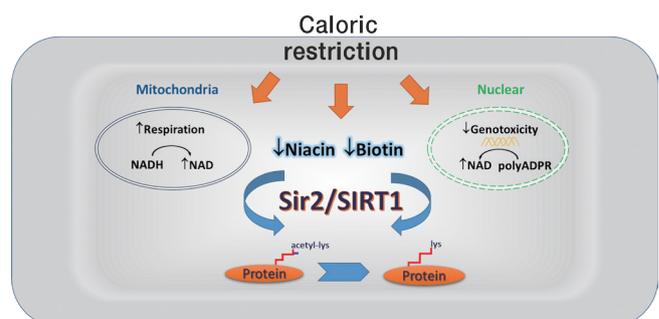

Fig. 2. A number of molecular links, including nicotinamide adenine dinucleotide (NAD), niacin, biotin, and related metabolites, are important conduits mediating caloric restriction-induced Sir2/Sir2-like protein 1 (SIRT1) activation and lifespan extension. polyADPR, poly ADP-ribose.

protein as a Sir2 homolog [72]. CobB compensates for the lack of CobT mutants during vitamin B12 biosynthesis and possesses nicotinate mononucleotide (NaMN)-dependent phosphoribosyltransferase activity. Thus, CobB catalyze the release of nicotinic acid from NaMN, whereas Sir2/SIRT1 removes nicotinamide from NAD. Taken together, Sir2 family of proteins play important roles in modulating the biosynthesis of B vitamins. As a feedback mechanism, increased B vitamins may negatively regulates the enzymatic activities of Sir2/SIRT1. In this regard, caloric restriction-mediated activation of Sir2/SIRT1 may at least partly relate to the nutrient availability of B vitamins, including biotin and niacin (Fig. 2).

Calorically restricted organisms are protected from ageing-induced damages as a result of heightened defensing and repairing capacity. Various caloric restriction mimetics, including caffeine, curcumin, dapsone, metformin, rapamycin, resveratrol, and spermidine, have been developed or under development. However, none of them elicits consistent effects as caloric restriction on extending lifespan across all different organisms. The mechanisms of ageing are more complex than any single type of ageing-related diseases. It is not known which physiological changes elicited by caloric restriction in mammals are most important for longevity. The belief that many benefits of caloric restriction are due to the induction and activation of sirtuins has led to the search for promising sirtuin activators as dietary supplements to promote health and longevity. In the meantime, limiting the negative regulators of Sir2/SIRT1 by restricted diet intake may be alternative or more effective approaches.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENTS

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