Does SIRT-1 Mediate Calorie Restriction and Prolong Life? – A Mini Review

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Calorie restriction is the only intervention proved to prolong both average and maximum lifespan in yeast, worms, fish, rodents and possibly primates. Not only does the regimen prolong life, but it also reduces the incidence of numerous age-related diseases like diabetes, atherosclerosis or cancer and slows down ageing. Mechanisms by which that is thought to occur have not yet been elucidated, but they probably involve reactive oxygen species signaling, insulin growth factor and transcriptional factors. Here, special emphasis is given to SIRT1 - silent information regulator. There is sound evidence showing that SIRT1 is a key player in mediating physiological response to calorie restriction and that its overexpression is correlated with extended lifespan. The possible mechanism leading to its elevated levels is high NAD/NADH ratio, observed in Sir2 in yeast. SIRT1 increases glucose production, enhances fat mobilization, stimulates angiogenesis, prevents neuronal degeneration and rises insulin sensitivity. Therefore, it seems to be a very beneficial factor activated by such a simple intervention that is calorie restriction.

INTRODUCTION

Calorie restriction (CR) is a dietary regimen decreasing the amount of energy intake without malnutrition. It is the only known mean of intervention increasing lifespan in wide variety of organisms, from unicellular Saccharomyces cerevisiae to possibly mammals [Redman & Ravussin, 2011]. It has been evidenced that calorie restriction delays the onset of numerous age-related diseases like atherosclerosis, diabetes or cancer. Its impact on longevity has been widely studied not only in rodents, but also in yeast, spiders, worms, flies and fish [Canto & Auwerx, 2009]. The molecular mechanisms by which calorie restriction promotes longevity are to be elucidated, but it is known that it has influence on reducing damage caused by reactive oxygen species, improves insulin sensitivity and probably influences neuroendocrine activities [Heilbronn et al., 2006].

MECHANISMS PROLONGING LIFE IN CALORIE RESTRICTION REGIMEN

One of the proposed CR mechanisms of action is “rate of living theory” [Sacher, 1977]. According to this theory, calorie restriction induces lowering of metabolic rate and consequently amount of produced reactive oxygen species and oxidative damage. Heilbronn et al. [2006] have conducted a 6-month randomized controlled trial analyzing calorie regimen influence on various factors including oxidative DNA damage. The results have demonstrated that DNA damage was reduced in CR group in comparison to control. What is more, the quantity of 8-oxo-7,8-dihydroguanine, a common example of single base damage caused by reactive oxygen species, was significantly reduced. Therefore, beneficial effect of CR may be mediated by reduced reactive oxygen species (ROS) and their harmful effect.

Another potential mechanism is reduction of insulin-like growth factor (IGF-1) signalling. Mice with IGF-1 receptor knockout showed 23% increase in both average and max lifespan [Holzenberger et al., 2003]. Low concentrations of IGF-1 increase expression of mitochondrial antioxidants [Page et al., 2010]. As the mitochondria are the major intrinsic source of harmful ROS, elevated levels of its antioxidants are truly beneficial to the cell. In a proposed mechanism weakened IGF signaling enables translocation of gene expression regulator: forkhead transcription factor box 03a (FOXO3a) to the nucleus which in turn facilitates expression of Mn superoxide dismutase (MnSOD) and glutathione peroxidase (GPx). Both enzymes are responsible for ROS clearance. SOD converts superoxide anion to hydrogen peroxide, and GPx further to water and oxygen. In this way weakening IGF signaling may contribute to CR mediated increased lifespan.

Peroxisome proliferator-activated receptor γ-coactivator-1α (PGC-1α) is a transcriptional factor increased by CR. Number of mitochondria declines with age. High AMP:ATP ratio observed in CR leads to inhibition of target of rapamycin (TOR) which in turn facilitates expression of PGC-1α generating mitochondria synthesis [Canto & Auwerx, 2009]. The more mitochondria, the more efficiently the energy is produced and less ROS are generated. In this situation fewer elec-
trons are leaked because they are distributed equally throughout the mitochondria and the same amount of glucose results in more ATP than in the cell with less mitochondria.

**SIRTUIN FAMILY**

Another important player of CR mediated response is SIRT1. It belongs to the sirtuin family, which in mammals includes 7 proteins: SIRT1-SIRT7. SIRT1, SIRT6 and SIRT7 are mainly nuclear, SIRT2 is mainly cytosolic and SIRT3, SIRT4 and SIRT5 are exclusively mitochondrial [Osborne et al., 2014]. They are all nicotinamide adenine dinucleotide (NAD)-dependent deacetylases. Mammalian SIRT1 is the closest homologue to yeast Sir2 and is the best studied member of the sirtuin family [Sack & Finkel 2012]. However, recently more attention has been given to SIRT3 and SIRT6, which are the closest homologues to yeast Sir3 and Sir4. SIRT1, SIRT6 and SIRT7 constitute one of the best studied sirtuin family [Sack & Finkel, 2012]. It is an activator of numerous mitochondrial enzymes regulating cell energy status, involved in electron transport chain, defense against ROS, metabolism of amino acids and fatty acids β-oxidation [Kincaid & Bossy-Wetzel, 2013].

**SIRT1**

The best studied sirtuin, SIRT1 deacetylates numerous important transcription factors like PGC-1α, p53, nuclear factor-κB (NF-κB) and forkhead proteins (FOXO) [Canto & Auwerx, 2009]. Therefore it is involved in many cellular pathways responsible for stress resistance and metabolism [Westphal et al., 2007]. It is induced by increasing NAD: NADH ratio indicating the crucial role of cell energy status on its activity.

SIRT1 can be activated not only by CR but also by natural polyphenols mimicking its effects [Howitz et al., 2003]. The best known is resveratrol found in grapes and red wine. Recently it has been widely investigated due to its antioxidative properties, beneficial effects on cardiovascular system and many others. Natural positive modulators of SIRT1 include curcumin, quercetin, and catechins, also belonging to the polyphenol family [Howitz et al., 2003].

**How do we know that SIRT1 mediates physiological response to calorie restriction?**

There is sound evidence showing that SIRT1 is a key player in mediating physiological response to calorie restriction. Cohen et al. [2004] applied 40% calorie restriction (60% of ad libitum) to rats since weaning and measured their SIRT1 level in different tissues at 12 months of their life. The CR group had higher expression of SIRT1 in many tissues: brain, kidney, visceral fat pads and liver.

Bordone et al. [2007] in turn generated mice overexpressing SIRT1 (SIRT-KI). They reported that this overexpression evoked response similar to calorie restriction. The SIRT-KI mice had decreased insulin plasma level, decreased glucose level, improved glucose tolerance and reduced fat mass.

However in yeast, Sir2 independent pathway has been identified [Kaeberlein et al., 2004]. This in turn supports the thesis that SIRT1 activation is not the only mechanism by which calorie restriction prolongs life. Nevertheless, majority of studies demonstrated that SIRT1 mediates the changes resulting from calorie restriction.

**Does SIRT1 prolongs life?**

Vergnes et al. [2002] revealed that the lifespan was prolonged in yeast overexpressing Sir2. Furthermore, Sir2 seems to play an important protective role against apoptotic cell death.

Boily et al. [2008] revealed that SIRT1 null mice were hypermetabolic and used their energy inefficiently. When 40% calorie restriction diet was applied normal mice showed extended lifespan, whereas SIRT1 null mice did not. Chen et al. [2005] reported that CR mice with SIRT1 exhibited increased physical activity, whereas SIRT1 knockout mice failed to. Therefore, SIRT1 may be essential in normal response to calorie restriction and prolong life.

**How does calorie restriction lead to increasing SIRT1?**

The fact that CR leads to increased NAD concentration suggests its involvement in response to lower energy intake observed in calorie restriction. Lin et al. [2004] indicated that in yeast Sir2 activation depended on NAD/NADH ratio. To confirm this, authors measured Sir2 activity in different NADH concentrations. The results showed that increasing NADH concentration resulted in increased Km (binding constant in Michaelis – Menten formula) without or slightly affecting Vm (maximum velocity). Authors suggested that NADH is a competitive inhibitor of Sir2.

Authors’ another approach was to determine if increase of the concentration of NADH dehydrogenase (Nde 1 and Nde 2) reoxidizing NADH, can itself increase the lifespan. Indeed the results showed increased lifespan in colonies on 2% glucose- not CR but they did not extend it in colony on 0.5% glucose- CR. This effect supports the thesis that calorie restriction acts on the same pathways as NADH dehydrogenase and that NADH is an inhibitor of Sir2.

Another possible mechanism of stimulating SIRT1 is its induction by eNOS (endothelial Nitric Oxide Synthase). Nisoli et al. [2005] have demonstrated that CR led to mitochondrial biogenesis but it did not occur in eNOS deficient mice and that the same relation was observed regarding SIRT1: CR stimulated SIRT1 activity but not in eNOS knockout mice.

**How does SIRT act?**

SIRT1 has influence on multiple pathways in mammals. Among others, it increases glucose production, enhances fat mobilization, stimulates angiogenesis, prevents neuronal degeneration, rises insulin sensitivity and inhibits tumour formation [Haigis & Guarente, 2006]. The role of fat and glucose metabolism and neuroprotection is worth emphasizing.

**FAT MOBILIZATION AND LIPID METABOLISM**

Content of white adipose tissue (WAT) is one of factors which may determine the lifespan in mammals [Bluher et al., 2003]. It may be associated with release of different factors from WAT, proportional to fat mass. SIRT1 affects fat mobil-
zation and the proposed mechanism is repressing peroxisome proliferator-activated receptor gamma (PPAR-γ).

PPAR-γ is responsible for regulating fatty acid storage and glucose metabolism. When activated, it stimulates adipogenesis by fat cells. In calorie restriction overall body weight decreases and fat from white adipose tissue is cleaved. Picard et al. [2004] attempted to determine whether it is mediated by SIRT1. The authors used mouse fibroblasts at 7 days of differentiation, cells overexpressing SIRT1 accumulated less fat, suggesting that SIRT1 decreased fat accumulation. Furthermore, when resveratrol was applied to fully differentiated cells, triglyceride concentration was reduced and free fatty acid (FFA) release was increased. Moreover, the authors checked whether SIRT1 can stimulate fat mobilization in bone fine adipocytes. They applied adrenaline which is known to cause fat mobilization. In presence of resveratrol fat mobilization was significantly higher. In contrast, addition of nicotinamide, which is SIRT1 inhibitor, resulted in decreased concentration of FFA [Picard et al., 2004].

In order to check whether it is mediated by PPAR-γ repression, the protein and messenger RNA amount was measured. Cells with more SIRT1 have shown a reduction in PPAR-γ concentration, whereas cells with down-regulated SIRT1 expressed its higher levels. The chromatin immunoprecipitation (ChiP) assay revealed, that SIRT1 and PPAR-γ bind to the same sequence in DNA. This may suggest that SIRT1 is a co-repressor of factors stimulating adipogenesis, though under calorie restriction conditions it triggers fat mobilization in white adipose tissue.

Another putative pathway through which SIRT1 facilitates fat mobilization is the interaction with AMP-activated protein kinase (AMPK). AMPK is activated by high AMP:ATP ratio facilitating fatty acid oxidation but also SIRT1 can activate AMPK through deacetylation [Price et al., 2012]. Activated AMPK in turn increases NAD+ levels which (as pointed above) is SIRT1 activator. In this way SIRT1 and AMPK cooperate to induce fatty acids mobilization in CR.

Glucose homeostasis is a very important factor of a healthy organism. It has been demonstrated that SIRT1 is involved in maintaining glucose tolerance in the liver and affects insulin release from pancreatic β islets. In a proposed mechanism SIRT1 level is controlled by concentration of glucose and pyruvate. The SIRT1 level rises with pyruvate and decreases when glucose level rises. Probably these changes take place on post-transcriptional level, as the amount of SIRT1 mRNA is not changed. Moreover, SIRT1 may be involved in regulation of gluconeogenesis [Rodgers & Puigserver, 2007]. SIRT1 deacetylates PGC-1α activating two gluconeogenic genes: PEPCK and G6Pase (glucose-6-phosphatase). This indicates that SIRT1, through deacetylation of PGC-1α, triggers gluconeogenesis in fasting conditions.

SIRT1 improves glucose tolerance and increases insulin release in pancreatic β cells. Moynihan et al. [2005] showed that 3-month old mice overexpressing SIRT1 had increased
glucose tolerance and enhanced insulin release in response to glucose. Interestingly, the insulin release in response to KCl was more pronounced that to glucose itself.

The authors also measured if this tendency can be maintained over time. After five months the results were similar, both glucose tolerance and insulin release. SIRT1 downregulated the expression of a few genes but the most interesting was uncoupling protein 2 gene (Ucp2) [Zhang et al., 2001]. UCP2 belongs to the mitochondrial inner membrane carrier family. UCP2 is probably responsible for proton leak into the mitochondrial matrix. Those protons bypass ATP synthase and thus they are not used to produce ATP which results in decreased ATP/ADP ratio. It has been demonstrated that Ucp2 decreases insulin secretion stimulated by glucose and is a very important modulator in β cell glucose response [Zhang et al., 2001]. Decreased level of Ucp2 results in increased mitochondrial production of ATP which consequently facilitates insulin release [Zhang et al., 2001]. Moynihan et al. [2005] showed that SIRT1 overexpressing cells stimulated by 20 nM of glucose produced much more ATP when compared to controls. The results of this study suggest that SIRT1 improves glucose tolerance and enhances insulin secretion, possibly by Ucp2 down-regulation.

However, it has been reported that mice with increased SIRT1 level exhibited lower glucose tolerance and were moderate to slightly hyperglycemic depending on the period of fasting [Rodgers & Puigserver, 2007]. Therefore, SIRT1 influences glucose tolerance and insulin secretion, but the mechanism has not yet been elucidated.

NEUROPROTECTION

Neuronal degeneration is one of the most common features in human ageing. It has been proven that SIRT1 has a protective activity against it. It may also have a role in development of brain and neurons. Sakamoto et al. [2004] reported that in various organs of mouse embryos: brain, spinal cord, heart and dorsal root ganglia the levels of SIRT1 were high.

The results of some study suggested protective SIRT1 role against ischaemia. Raval et al. [2006] used hipocampal slice culture as an in vitro model of cerebral ischaemia. Firstly, the authors showed that resveratrol pretreatment had the same effects as direct SIRT1 activation. But more importantly, the authors applied SIRT1 antagonist sirtinol and demonstrated that in its presence neuroprotection in ischaemia was not observed. This suggests that SIRT1 has a role in neuroprotection in ischaemic conditions. There is also growing evidence that it may be preventive against apoptosis [Cohen et al., 2004].

However, not all studies are consistent. Chong et al. [2005] concluded, that SIRT1 inhibitor-nicotinamide facilitated neuronal survival. Therefore, SIRT1 role in this condition is not fully understood and clear.

Some evidence connects SIRT1 with neurodegenerative diseases like Alzheimer disease (AD), or Huntington disease [Kim et al., 2007]. The hallmarks of AD are extracellular plaques of β-amyloid from cleavage of APP-amyloid precursor protein. Aβ plaques induce NF-κB signaling pathway which in microglia is involved in neuronal cell death [Valerio et al., 2006]. Yeung et al. [2004] demonstrated that expression of SIRT1 led to reduced signaling mediated by NF-κB, which had a highly neuroprotective influence and reduced inflammation.

Araki et al. [2004] checked whether SIRT1 is involved in NADP-NAD-dependent axonal protection. The authors used sirtinol, SIRT1 inhibitor and then performed axonal transection: the cell bodies were removed. The results showed that after 12 to 72 hours sirtinol did not affect uninjured axons but it blocked NADP after transection which indicates SIRT1 involvement in this process. Moreover, resveratrol was used to measure level of neuronal protection and the results indicated neuroprotective SIRT1 activity.

POSSIBLE HARMFUL EFFECTS

Though calorie restriction even intuitively seems beneficial, there are some studies indicating its harmful effects. Ritz et al. [2008] reported that decrease in the number of natural killer (NK) cells increased mortality caused by influenza virus. Moreover, mice overexpressing SIRT1 died earlier due to hypersensitivity in response to lipopolysaccharide (LPS) [Pfluger et al., 2008]. Therefore, it is very important to assess the SIRT1 impact on health and lifespan.

CONCLUSIONS

Calorie restriction is a very beneficial intervention, which delays onset of age related diseases and increases lifespan in numerous organisms from yeast to possibly mammals. Calorie restriction acts via numerous pathways. It decreases amount of reactive oxygen species, decreases IGF signaling, activates PGC-1α and SIRT1 (or Sir2 in lower organisms). SIRT1 affects multiple factors and therefore its activity is not limited to single organ or pathway. There is evidence that SIRT1 can prolong lifespan through neuroprotection, mobilization of fat from WAT, increased glucose tolerance or improved insulin sensitivity. However, not all studies are consistent. Though more research has to be done to understand mechanisms underlying longevity, a conclusion can be made that calorie restriction may prolong life and that SIRT1 is one of key factors regulating its effect.

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REFERENCES


