Calorie Restriction and Obesity under the Regulation of SIRT1

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ABSTRACT

Obesity is one of the most important risk factors for various chronic diseases, especially related with environmental life style and eating habits. Obesity is also a risk factor of metabolic diseases, cardiovascular diseases, diabetes, and certain cancers. Numerous studies of calorie restriction (CR) in various organisms have shown several beneficial effects of not only decreased body fat and blood pressure, decreased inflammatory markers in plasma, increased insulin sensitivity, and improved lipid profile but also improved endothelial function, decreased oxidative damage by reducing energy flux and metabolism, and decreased ectopic fat accumulation. Furthermore, CR activates SIRT1, a nutrient-sensing deacetylase, involved in metabolic regulation and longevity. Resveratrol, as a mimic of CR, is one of well-known sirtuin activating compounds. Resveratrol is related with longer lifespan by increasing insulin sensitivity, decreasing insulin-like growth factor-1, and increasing AMP-activated protein kinase activity. Therefore, the present review focuses on CR related with obesity and also the relationship between CR and SIRT1 in metabolic mechanism levels. Furthermore, we will introduce resveratrol, as an activator of SIRT1, and the beneficial effects of resveratrol.

Key words: obesity, calorie restriction, SIRT1, longevity, resveratrol

Introduction

During the last several decades, obesity has dramatically increased worldwide. These trends have been deeply related with environmental life style changes, food product developments, and eating habit changes to the
nutritional transition. The nutritional transition is also associated with large portion sized meals, highly energy dense foods with high fat and sugar, and low fiber of the meal.\(^1,2\) Therefore, obesity caused by these factors is a serious personal, public, and global health concern. Numerous studies have shown that adequate reduction in calorie intake is associated with prevention of chronic diseases, delay in aging process, and extension of lifespan.

One of the surprising data reported by Clive McKay, Mary Crowell, and Leonard Maynard at Cornell University in 1935 was that rodents with reduced amount of food intake lived much longer than their \textit{ad libitum} counterpart.\(^3\) It turned out that calorie restriction (CR) was responsible for health benefits. Similar results have been observed in other various species including yeast, worms, flies, spiders, rabbits, dogs, monkeys and humans. CR is defined as a reduction of energy intake in the absence of malnutrition. Several human studies have shown that CR regimen has various beneficial effects, such as (i) decreased body fat and blood pressure, (ii) decreased inflammatory markers in plasma, (iii) decreased circulating growth factors level, (iv) increased insulin sensitivity, (v) improved lipid profile, and (vi) younger appearance.\(^4-8\) The insulin sensitivity induced from CR regimen is also related with SIRT1 activation. SIRT1, an oxidized nicotinamide adenine dinucleotide (NAD\(^+\))-dependent deacetylase, was shown to promote longevity in various organisms. SIRT1 has diverse effects in metabolically important tissues, such as improved DNA stability, increased repair and defense, coordinated stress response, enhanced energy production and use, and prolonged cell survival.\(^9,10\)

Therefore, the purpose of this review is to summarize some of the literature on CR related with obesity. Then we will focus on the relationship between CR and SIRT-1 in a metabolic mechanism level. We also will try to explain CR mimetic of a resveratrol as an activator of SIRT1 and the beneficial effects of the resveratrol to health and longevity.

\section*{Calorie Restriction (CR) and Obesity}

Numerous studies of CR in humans have presented various beneficial effects such as decreased BMI, body fat, blood pressure, insulin level, leptin level, and others against obesity-related health problems.\(^11,12,13\) In a study of CR on body composition and fat distribution for 6 months, participants (25 \(\leq\) BMI < 30) lost 10\% of BW, 24\% of fat mass, and 27\% of abdominal visceral fat.\(^11\) Furthermore, CR effects for 6 months showed that fasting glucose level and body temperature of participants (25 \(\leq\) BMI < 30) were reduced in CR group and CR with exercise groups compared with the control group.\(^13\) CR may also have alterations and beneficial effects not only on secretory profiles of adipocytes but also on several metabolic factors that are altering insulin sensitivity. Short term (3~6weeks) CR had no effect on circulating adiponectin concentrations\(^14,15\), whereas long term (52 weeks) CR increased the plasma adiponectin levels.\(^16\) Adiponectin is produced white adipose tissue and related to trigger insulin sensitivity by upregulating AMP activated protein kinase (AMPK) in target tissues. In addition to adipocytes, CR reduced circulating proinflammatory factors (C-reactive protein (CRP), TNF\(\alpha\) IL-6, IL-8), in obese DM subjects.\(^17\) It is well known that increased IL-6 and CRP concentrations are predictors of development of myocardial infarction and type 2 diabetes mellitus (T2DM).\(^5\)

CR could minimize oxidative damage by reducing energy flux and metabolism, thereby influencing the aging process.\(^9\) Reduced metabolic rate by using CR may decrease oxygen consumption, which could decrease ROS formation and also decrease protein carbonylation, which is the determining factor of the amount of protein oxidation induced by ROS.\(^18\)

Based on these observations, Guarente L. suggested a hypothesis that metabolic syndrome, such as obesity, T2DM, and cardiovascular disease, and calorie restriction, such as decreasing body fat, metabolic factors and proinflammatory factors, are balanced at opposite ends of the same spectrum on the basis of diet and physical activity. Furthermore, identification of important regulators that mediate the positive effect of CR regimens could offer the hope of new treatments to improve life span.\(^19\)

\section*{Calorie Restriction and SIRT1}

1. Sirtuins Activation by CR

Sirtuins have been known as critical regulators for expansion of life span via CR in model organisms. A role for sirtuins in mediating CR was first demonstrated in yeast (\textit{S. cerevisiae}) when dilution of glucose, the energy
source in growth media, extended the replicative life span of cells while this effect was not seen when silent information regulator 2 (SIR2) gene was deleted. In C. elegans, acquisition of an additional copy of SIR2.1 gene similarly resulted in long life span beyond the wild-type range. Furthermore, an activator of the SIR2, resveratrol, was identified by screening compounds and was shown to extend yeast replicative life span. In mice, resveratrol has also produced changes associated with longer life span. These observations have lent support to the hypothesis that SIRT1, the mammalian SIR2 ortholog, may underlie the beneficial effects of CR in mammal.

SIRT1 belongs to sirtuin gene family with seven members (SIRT1 through 7) that are found in all domains of life from yeast to mammals (Table 1). SIRT1 and SIRT2 exist in the nucleus and cytoplasm. SIRT3, SIRT4, and SIRT5 are mitochondrial sirtuins whereas SIRT6 and SIRT7 are nuclear sirtuins.

Among sirtuins, SIRT1, SIRT2, SIRT3, and SIRT5 have been reported to be up-regulated by CR. Cohen et al reported that expression of SIRT1 was induced in a variety of CR rat tissues as well as in human cells that were treated with serum from these animals. In human, CR increased SIRT1 and SIRT2 expression in the peripheral blood mononuclear cells of obese subjects. Very recently, Geng et al demonstrated that the levels of SIRT1 and SIRT5 protein in cerebral tissues of CR rats were elevated compared to ad libitum rats.

2. NAD⁺-Dependent Deacetylation by SIRT1

Sirtuins was originally defined as class III histone deacetylases that acetylate lysine residues on various proteins and later studies showed that they also participate in non-histone deacetylase reactions. Deacetylase reaction mediated by SIRT1 is specific for acetylated lysines, removing the acetyl group from the lysine residue in a protein substrate and transferring it to the ADP-ribose (ADPR) moiety of NAD⁺. This reaction cleaves the NAD⁺ coenzyme leading to the formation of nicotinamide (NAM) and 2'-O-acetyl-ADPR and the release of deacetylated protein, as shown here:

\[
\text{SIRT1} \quad \text{NAD}^+ + \text{acetylated protein} \quad \rightarrow \quad \text{NAM} + 2' \text{-O-acetyl-ADPR} + \text{deacetylated protein}
\]

SIRT1 mediated deacetylation is NAD⁺-dependent and the requirement of NAD⁺ as a co-substrate supports the view on SIRT1 as sensors of cellular energy and redox states coupled to the metabolic status of the cell.

3. Roles of SIRT1 in Metabolic Regulation

SIRT1, by far the most extensively studied sirtuin, is regulator of protein and genes involved in the regulation of fundamental biological responses to environmental and nutritional perturbations. SIRT1, induced by CR, interacts with and deacetylates a variety of protein in the liver, skeletal muscle, and adipose tissue. In the fasted liver, SIRT1 mediated deacetylation of PGC-1α led to a relative increase in gluconeogenesis and fatty acid β-oxidation. Erion et al showed that SIRT1 knockdown in liver increased forkhead box O1 (FOXO1) acetylation and decreased gluconeogenic gene expression. Indeed, the deacetylated FOXO1 by resveratrol was increased in

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Peroxisome proliferator-activated receptor γ (PPARγ) coactivator-1 α (PGC-1α), Forkhead box O1 (FOXO1), Nuclear factor-kappa B (NF-κB), Sterol regulatory element-binding protein-1c (SREBP-1c), Acetyl-CoA synthetase (AceCS2), Glutamate dehydrogenase (GDH), Superoxide dismutase (SOD2), Carbamoyl phosphate synthetase 1 (CPS1).
the nucleus and activated its target gene involved in gluconeogenesis in hepatocytes.\(^{35}\) Furthermore, SIRT1 regulated cholesterol flux via deacetylation and activation of liver X receptor (LXR) proteins, nuclear receptors that function as cholesterol sensors.\(^{36}\) A recent study demonstrated that increased expression of SIRT1 during fasting led to deacetylation and inactivation of sterol regulatory element-binding protein-1C, resulting in down-regulation of lipogenic gene expression and subsequent decrease of fat storage in the liver.\(^{37}\)

In skeletal muscle, deacetylation of PGC-1\(\alpha\) activated mitochondrial fatty acid oxidation and helped maintain ATP production in response to CR.\(^{38}\) SIRT1 also improved insulin sensitivity by repression of protein-tyrosine phosphatase 1B in skeletal myotube cells.\(^{39}\)

In white adipose tissue, SIRT1-depentent deacetylation and repression of PPAR\(\gamma\) triggered lipolysis and promoted fat mobilization upon fasting.\(^{40}\) SIRT1 also regulated adiponectin gene expression through FOXO1-C/ Enhancer-binding Protein \(\alpha\) transcriptional complex in adipocytes.\(^{41}\) SIRT1 depletion inhibited insulin-stimulated glucose uptake and GLUT4 translocation in 3T3-L1 adipocytes while treatment of cells with specific small molecule SIRT1 activators led to an increase in glucose uptake and insulin signaling, resulting in attenuation of TNF\(\alpha\)-induced insulin resistance.\(^{42}\) Further, SIRT1 deacetylated NF-\(\kappa\)B, a key regulator of inflammation, and inhibited NF-\(\kappa\)B binding to the target gene promoters. Since increasing amount of evidence indicates that chronic, low-grade inflammation can cause insulin resistance, SIRT1 could play a role in protection against proinflammatory responses in adipose tissue. The findings mentioned above reinforce the notion that the identification and development of natural or synthetic agents that mimic some of the protective effects of CR may constitute a new strategy for prevention of age-related complications such as obesity, T2DM, and degenerative disease.

**CR Mimetic Resveratrol**

1. Chemistry and Food Sources of Resveratrol

Resveratrol (3, 5, 4’-trihydroxystilbens) is a phytoalexin and color pigment of some plant species. The resveratrol composed by a molecular formula of C\(_{14}H_{12}O_{3}\) is a polyphenol consisting of two phenol rings connected by a 2-carbon methylene bridge (Fig. 1). Grape and grape skin, red wine, mulberry, cranberry, blueberry, and peanut are the major dietary sources of resveratrol. The concentrations of resveratrol are ranged from 0.05 mg/L to 14.3 mg/L in most grape juice and red wines.\(^{43}\) Furthermore, resveratrol is contained in grape skin (24.06 \(\mu\)g/g), Japanese knot weed (0.524 mg/g), some berries (up to 32 ng/g) and nuts (up to 5.1 \(\mu\)g/g).\(^{44}\) In toxicity studies, increased dose of resveratrol up to 5.0 g/kg was reasonably well tolerated in clinical studies, but the dose of resveratrol to about 3.0 g/kg for 4 weeks appeared to be toxic in rats.\(^{45}\)

2. Health Benefits of Resveratrol

In epidemiological studies, researchers showed that moderate consumption of red wine is related with beneficial effects in cardiovascular diseases with mortality.\(^{45}\) The potential relationship between red wine consumption and cardioprotection has been highlighted in the “French Paradox”, which the beneficial components help to prevent cardiovascular diseases despite the harmful component of alcohol in red wine.\(^{45}\) The main component for cardioprotection of red wine is not only from several flavonoids, such as kaempferol, quercetin, and myricetin, but also from stilbenoids, especially resveratrol. Furthermore, cardioprotection concentration of red wine was observed in up to 300 ml wine per day in contrast to no wine consumption.\(^{46}\) After digestion of resveratrol, it reaches peak concentrations in plasma circulation after 30 to 60 minutes with the metabolite forms of glucuronide and sulfate conjugates. Several forms of metabolites, such as two isomeric glucuronic acid conjugates and one sulfate conjugate were identified in urine.\(^{47}\)

Until 1992, resveratrol was not an interesting compound for biological beneficial effects. After Siemman and Creasy had shown that resveratrol, active compound in wines, was related with decrease of serum lipid, many researchers focused on the beneficial effects of resveratrol against various diseases such as cardiovascular diseases,
neurodegenerative diseases and some cancers. The most important point of resveratrol is a mimic of CR in biochemical mechanism levels. As mentioned above, CR activates SIRT1 which is responsible for various health benefits and longevity-enhancing effects. Resveratrol also activates SIRT1 to mimic the transcriptional response to CR in the same way. Howitz et al. introduced that resveratrol increased cell survival by stimulating SIRT1-dependent deacetylation of p53 and postulated that polyphenols might have similar effects of CR-mimetic response induced by sirtuins. Baur and others in 2006 reported that resveratrol was associated with longer lifespan, attributed to increased insulin sensitivity, decreased insulin-like growth factor-1 (IGF-1), and increased AMPK activity in a comparison study between high calorie (HC) and high calorie diet with resveratrol (HCR) in one year old male C57BL/6NIA mice until 75 weeks of age. HC fed mice manifested onset of diabetes, increased glucose and IGF-1 levels, and shorter lifespan, whereas HCR fed mice showed significantly decreased levels of glucose and IGF-1 with 20% extension of mean lifespan. Furthermore, after a 2 g/kg oral glucose dose, an oral glucose tolerance test presented that HCR fed mice had higher insulin sensitivity than controls. AMPK activation related with promoting insulin sensitivity and fatty acid oxidation was also increased in the HCR group. AMPK, as a key regulator in energy balance, is switched on during metabolic stress and modulated by certain proteins such as leptin, adiponectin, and ghrelin. The increased NAD+/NADH ratio by CR or resveratrol can lead to SIRT1 activation. SIRT1 then deacetylates and activates liver kinase B1 which is necessary for AMPK activation. Both CR and resveratrol are STACs, which can activate sirtuins and can promote cell survival to extended life span. Therefore, CR might help to prevent not only the development of obesity but also several chronic diseases related with obesity, even though further study and clinical studies are needed to ensure the optimal concentration and the safety levels. Furthermore, resveratrol as a CR mimetic may help to prevent the development of chronic diseases by activating SIRT1. Even though there are many data on the beneficial effects of CR and resveratrol as a CR mimetic, additional human studies are needed to clarify the molecular and cellular mechanisms of the reliable and critical markers, identify the safety level, and elucidate the therapeutic effects.

Conclusions

Over the last several decades, obesity related with chronic diseases has emerged as one of the most important health problems. The balance of calorie intake and energy expenditure is a truly important determinant of health and longevity. SIRT1, a nutrient-responsive protein, is related with various beneficial effects, such as increasing insulin sensitivity, inducing hepatic gluconeogenesis and fatty acid oxidation, increasing adiponectin production, and decreasing lipogenesis and inflammation. CR with adequate nutrient intake in humans might have at least two positive effects of enhancing health and extending life span by activated SIRT. Furthermore, resveratrol as a CR mimetic may help to prevent the development of chronic diseases by activating SIRT1. Even though there are many data on the beneficial effects of CR and resveratrol as a CR mimetic, additional human studies are needed to clarify the molecular and cellular mechanisms of the reliable and critical markers, identify the safety level, and elucidate the therapeutic effects.

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