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SIRT3 as a Regulator of Non-alcoholic Fatty Liver Disease

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Non-alcoholic fatty liver disease (NAFLD) is a hepatic presentation of obesity and metabolic syndrome. NAFLD includes a large spectrum of hepatic pathologies that range from simple steatosis and non-alcoholic steatohepatitis (NASH), to liver cirrhosis without an all-encompassing approved therapeutic strategy. Mitochondrial dysfunction is a key component of many metabolic diseases, such as obesity, type 2 diabetes, cancer, NAFLD, and aging. Sirtuin 3 (SIRT3) is a NAD^+-dependent deacetylase that regulates many of the mitochondrial proteins that are involved with metabolic homeostasis, oxidative stress, and cell survival. This review discusses the association between mitochondrial dysfunction and insulin resistance and later explore the possibility that SIRT3 plays a protective role against NAFLD by improving mitochondrial dysfunction.

Key Words: Sirtuin 3, Mitochondrial dysfunction, Insulin resistance, Nonalcoholic fatty liver disease

INTRODUCTION

The combination of easily available high-calorie food and physical inactivity is driving the rising prevalence of obesity and metabolic syndrome. Non-alcoholic fatty liver disease (NAFLD) is more than simply a "hepatic disease." Rather, it is a hepatic presentation of obesity with metabolic impairment by the metabolically active organ triad that comprises the liver, adipose tissue, and skeletal muscle. NAFLD covers a large spectrum of hepatic pathologies, which range from simple steatosis and non-alcoholic steatohepatitis (NASH) to liver cirrhosis. By definition, NAFLD affects 10-24% of the general population in many countries[1]. Because of its association with obesity and insulin resistance, the prevalence of NAFLD is increasing worldwide, including in Korea. However, there is currently no broadly approved therapeutic strategy for treating NAFLD.

There is growing evidence that mitochondrial dysfunction is a common underlying feature of obesity [2], type 2 diabetes [3], NAFLD [4,5], and cancers [6]. Identification of the causes of mitochondrial dysfunction, as well as of potential target molecules that protect cells from mitochondrial dysfunction, will be crucial for treating mitochondria-mediated diseases. Sirtuin 3 (SIRT3) is a NAD^+-dependent protein deacetylase that regulates numerous mitochondrial proteins involved in metabolic homeostasis, oxidative stress, and cell survival. SIRT3 is emerging as a promising therapeutic target against mitochondrial dysfunction.

This review discusses mitochondrial dysfunction as an underlying feature of NAFLD and evaluate the potential for SIRT3 to enhance mitochondrial function and, thus, act as a therapeutic target against NAFLD.

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MITOCHONDRIAL DYSFUNCTION AND INSULIN RESISTANCE

Mitochondria are the main source of cellular energy pro-

duction, mostly in the form of ATP and intracellular reactive oxygen species (ROS); they are encased in a double membrane and contain their own unique DNA [7-9]. Increasingly, mitochondria are being seen as the hearts of cells because they regulate various homeostatic processes, such as cell proliferation, apoptosis, oxidative stress, and calcium homeostasis. They are also capable of inherent morphological and metabolic plasticity and can respond to cellular stresses and nutrient demand [10-13].

A large and growing body of literature has demonstrated that long-chain fatty acids (LCFAs) induce mitochondrial dysfunctions that lead to insulin resistance and type 2 diabetes [11,14-17]. Generally, LCFAs induce excessive mitochondrial ROS production by partial inhibition of mitochondrial respiratory chain activities and depolarization of mitochondrial inner membranes (weak uncouplers) [18]. High LCFA intake may lead to an accumulation of ROS, lipotoxicity, alteration of mitochondrial gene expression, and activation of inflammatory signaling in peripheral tissues, resulting in mitochondrial dysfunction [11]. There are also reports that free fatty acids can induce hepatic lipotoxicity and insulin resistance through mitochondrial dysfunction [19-21].

Another hypothesis is that mitochondrial dysfunction causes insulin resistance; consequently, the defects in mitochondrial beta-oxidation induce an increase in intracellular fatty acid metabolites that disrupt insulin signaling [22-24]. Petersen *et al.* found that skeletal insulin resistance in the insulin-resistant offspring of patients with type 2 diabetes was accompanied by an increase of intramyocellular fatty-acid content, compared with insulin-sensitive control subjects. This may be because of an inherited defect associated with mitochondrial oxidative phosphorylation [3]. Another study showed that intrinsic mitochondrial dysfunction at the level of both electron-transport chain capacity and the oxidative phosphorylation system is implicated as the etiology of type 2 diabetes [25]. There is an impairment of mitochondrial function and structure in the skeletal muscle of patients with type 2 diabetes and obesity [24], suggesting that impaired mitochondrial function in muscle and other tissue can lead to lipid accumulation, which in turn can induce insulin resistance. As a consequence of defective mitochondrial fatty acid oxidation, intracellular levels of lipid metabolites (long-chain fatty acyl-coenzyme A and diacylglycerol) are increased in skeletal muscle, which disrupts insulin signaling [22].

Growing evidence has demonstrated an association between mitochondrial dysfunction and insulin resistance. Nevertheless, the overall picture of insulin resistance remains murky. Herein, mitochondrial dysfunction is postulated as a causal factor for the regulation of insulin resistance.

SIRTUINS IMPROVE MITOCHONDRIAL FUNCTION

The silent information regulation-2 (*SIR2*) gene has been found to promote longevity in *Saccharomyces cerevisiae* [26] and *SIR2* was identified as a NAD^+-dependent deacetylase for histone proteins that induces calorie restriction and life-span extension in yeast [27].

The mammalian sirtuins are a family of NAD^+-dependent deacetylases and/or ADP-ribosyltransferases that are homologous to the *Saccharomyces cerevisiae* gene, *SIR2* [28]. Humans have seven sirtuins, SIRT1-SIRT7 [28,29], that share a catalytic domain with *SIR2* and act as cellular energy sensors that modulate metabolic processes [28].

The mammalian sirtuins all have different subcellular distributions, with a subset of sirtuins residing predominantly in nuclear (SIRT1, SIRT6, and SIRT7), cytosolic (SIRT2), or mitochondrial (SIRT3, SIRT4, and SIRT5) compartments [30,31]. Defects in the pathways controlled by SIRT1 and SIRT3 are known to result in various metabolic and neurodegenerative diseases, such as obesity, type 2 diabetes, nonalcoholic fatty liver disease, Alzheimer disease, and cancer [8,32-35].

SIRT1 and SIRT3 are known to increase mitochondrial biogenesis and improve mitochondrial function [36-39]. SIRT3 is a major regulator of mitochondrial protein acetylation levels and its biological activities [40]. Consistent with this framework, mice lacking *SIRT3* showed increased hyperacetylation of mitochondrial proteins and, in contrast, mice lacking either *SIRT4* or *SIRT5* showed no obvious changes in mitochondrial protein acetylation [41]. SIRT4 regulates ATP homeostasis *via* adenine nucleotide translocator 2 (ANT2) and a feedback loop involving AMP-activated protein kinase (AMPK) [42] and resveratrol induces a mitochondrial NADH oxidation via SIRT3 in hepG2 cells

[43]. Take collectively, these reports suggested that sirtuins may improve mitochondrial dysfunction.

SIRT3 AND MITOCHONDRIAL FUNCTION IN NAFLD

SIRT3 is the most studied member of the mitochondrial sirtuins family; it is nuclear encoded and expressed as a 45-kDa protein containing an N-terminal mitochondrial targeting sequence that is cleaved off after import into the mitochondria, leaving an enzymatically active 28-kDa protein [44].

SIRT3 is highly expressed in the brain, heart, kidney, brown adipose tissue, and liver with high oxidative capacity and is preferentially localized to the mitochondrial matrix [28]. SIRT3 plays an important role in mitochondrial metabolism through a reversible acetylation process of mitochondrial proteins [28,41,45]. SIRT3 expression in the liver increases after fasting [46] and SIRT3 expression in muscle tissue increases after exercise [47], fasting, and caloric restriction and it decreases with chronic high fat eating [48].

The germline knockout of the *Sirt3* mouse model has been utilized extensively to elucidate the physiological role of SIRT3 in metabolism. Heber *et al.* quantified 1,578 mitochondrial acetyl sites during caloric restriction and observed loss of *SIRT3* using mass spectrometry, suggesting that *SIRT3* is an important regulator in caloric restriction adaptation [49]. Additionally, it was recently discovered that *Sirt3* can regulate amino acid metabolism and mitochondrial integrity, including mtDNA transcription and translation, compared to wild-type mice [49].

One particular observational study demonstrated that *Sirt3*-deficient mice are metabolically unremarkable under both fed and fasted conditions and that *Sirt3* increased global hyperacetylation of mitochondrial proteins [41]. Another study demonstrated that mice lacking *Sirt3* had diminished fatty acid oxidation during fasting, hyperacetylated long-chain acyl CoA dehydrogenase (LCAD) [46], reduced basal levels of ATP in the heart and liver and increased acetylation of mitochondrial proteins, including Complex I [50].

Mice fed a chronic high-fat diet had low *Sirt3* activity, impaired mitochondrial function, and hyperacetylation of proteins in their livers [51]. In another study, mice lacking *Sirt3* who were fed a chronic high-fat diet developed accelerated obesity, insulin resistance, and steatohepatitis, compared to wild-type mice [52]. However, mice with liver- or muscle-specific *Sirt3* deficiency showed no significant metabolic differences to wild-type mice, except that, even after being fed a high-fat diet, wild-type mice still experienced hyper-acetylation of mitochondrial proteins [53]. In yet another study of a transgenic mouse with muscle-specific *Sirt3* expression increased oxygen consumption, lipid utilization, and reduced muscle strength were observed compared to wild-type mice [54]. Further studies are needed to clarify the tissue-specific actions of Sirt3.

The study data from *Sirt3*-deficient mice suggest that target mitochondrial proteins, which are in a hyperacetylated form possess sufficient activity to achieve metabolic homeostasis under basal normal conditions, however, under conditions of oxidative stress, such as having a high-fat diet, SIRT3-target enzyme activity required for protection cannot be sufficiently increased to meet the demand resulting from metabolic derangement due to obesity and steatohepatitis [8].

SIRT3 regulates carbohydrate metabolism, ketogenesis [55], β-oxidation [46], and amino-acid metabolism [41] by reversible enzyme deacetylation and activity of specific mitochondrial complexes [50] and stress-related pathways [13].

Overall these studies indicate that SIRT3 acts as a master switch that is an adaptive response to energy shortages

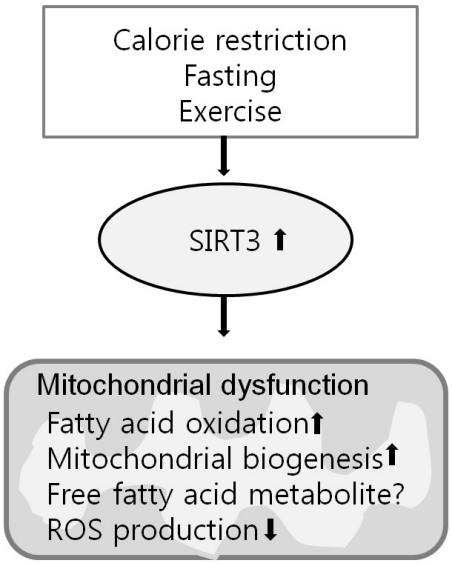

Fig. 1. Schematic representation of SIRT3's primary regulation pathway for mitochondrial dysfunction.

(fasting) in the catabolic pathway and plays a key role in protecting against mitochondrial stress through regulation of acetylation status (Fig. 1). These observations suggest that SIRT3 could be a potential therapeutic target of metabolic syndrome, including NAFLD.

However, there has been little investigation of SIRT3's gain-of-function effect. It is also unclear which specific tissues play a dominant role in mediating the whole-body effects of SIRT3 [13]. In this regard, liver- and muscle-specific *Sirt3*-knockout animals show no detectable changes in their metabolic phenotype in response to a high-fat diet [53], suggesting new questions to explore. During the period during which test animals were fed a high-fat diet (8 weeks or 12 months), the genetic background of the animals and the developmental onset of *SIRT3* deletion may have led to disparate findings [13,53].

Additionally, compared to caloric restriction, relatively little is known about the role of SIRT3 and NAFLD under conditions of caloric excess, such as receiving a high-fat diet. Palmitate modulates oxygen consumption and enhanced ROS levels and apoptosis in the primary hepatocytes of *Sirt3* deficient mice and Sirt3 siRNA-depleted hepatocytes [56].

Overall, these studies demonstrate that there is still much to learn about the metabolic role of SIRT3, including the tissue-specific (liver, muscle, and adipose tissue) and condition-specific (caloric restriction, fasting, and high-fat diet) role of *SIRT3* in model animals, as well as the beneficial effects of sirtuin enhancers that have been shown to treat metabolic diseases.

CONCLUSIONS

SIRT3 has emerged as a pivotal therapeutic target in the regulation of mitochondrial dysfunction and signaling that arise, via protein deacetylation, in response to changes in nutrient flux. However, the exact tissue-specific and condition-specific metabolic role of SIRT3 and the beneficial effects of SIRT3 for treating metabolic diseases are still not completely understood. Additionally, there are still concerns about the long-term effects of chronic SIRT3 activation and the yet undiscovered SIRT3-specific activators.

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