Brief Report

SIRT3 Weighs Heavily in the Metabolic Balance: A New Role for SIRT3 in Metabolic Syndrome

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Eating a “Western diet” high in fat and sugars is associated with accelerated development of age-related metabolic diseases such as obesity, insulin resistance, and diabetes while incidences of these diseases are decreased on a low-calorie diet. The mitochondrial NAD(+) -dependent protein deacetylase SIRT3 has previously been shown to be important in adapting to metabolic stress brought on by fasting and calorie restriction. During times of metabolic stress, SIRT3 is upregulated and maintains homeostasis following nutrient deprivation by turning on pathways such as fatty acid oxidation, antioxidant production, and the urea cycle. New studies now demonstrate that SIRT3 is regulated during nutrient excess. During high-fat diet feeding, SIRT3 is downregulated leading to mitochondrial protein hyperacetylation. The consequence of this hyperacetylation is the accelerated development of metabolic syndrome. Thus, SIRT3 is emerging as an important metabolic sensor working to restore metabolic homeostasis during times of stress.

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LOWER incidences of age-related diseases such as hypertension, cardiovascular disease, and diabetes are among the benefits of a low-calorie diet (1). Conversely eating a “western diet”—high in fat and sugars—is associated with accelerated development of age-related metabolic diseases including metabolic syndrome defined by central obesity, insulin resistance, hyperlipidemia, hyperglycemia, and hypertension (2). Although the association among diet, disease, and age has been observed in many model organisms, the underlying molecular mechanisms remain to be elucidated (3). Mitochondria are an important site of intermediary metabolism within the cell, and mitochondrial dysfunction is associated with a number of metabolic diseases. Protein lysine acetylation is emerging as an important regulatory mechanism of metabolic pathways in the mitochondria. Global acetylation levels are controlled in part by the sirtuin family of NAD(+) -dependent protein deacylases (4), with SIRT3 being the primary mitochondrial deacetylase (5). Previous work has established a role for SIRT3 in adapting to metabolic stress brought on by nutrient deprivation (6–9). A pair of recent publications from Hirschey and colleagues (10) and Jing and coworkers (11) now demonstrates that SIRT3 activity is also regulated during high-fat feeding and plays a role in preventing age-related metabolic diseases, including obesity, insulin resistance, and diabetes—all components of the metabolic syndrome.

SIRT3 REGULATES MITOCHONDRIAL METABOLISM

The sirtuin family of NAD(+) -dependent deacetylases is evolutionarily conserved from bacteria to humans and catalyzes a deacetylase reaction that uses NAD(+) as a co-substrate yielding O-acetyl-ADP-ribose, the deacetylated protein substrate and nicotinamide. Seven members of the sirtuin family (SIRT1-7) are present in mammals and differ in their subcellular localizations. SIRT1 is found in the nucleus and has been well studied in the context of longevity due to its ability to integrate metabolic inputs with transcriptional outcomes (reviewed in Cantó and Auwerx [12]). SIRT3, SIRT4, and SIRT5 are located in the mitochondria, but only SIRT3 has robust deacetylase activity, whereas SIRT4 has no deacetylase activity and SIRT5 has recently been characterized as a desuccinylase and demalonylase (13, 14). In addition, SIRT3 KO mice have high levels of mitochondrial protein acetylation, whereas SIRT4 and SIRT5 KO mice show no change. Thus, SIRT3 is believed to be the primary deacetylase in mitochondria where it regulates acetylation levels of several proteins. Several proteomic studies have shown that acetylation is a common posttranslational modification in mitochondria (15–18), and over one third of all proteins are acetylated, with proteins involved in metabolism being preferentially acetylated (19). In fact, every major metabolic pathway including glycolysis, the tricarboxylic acid cycle, the urea cycle, fatty acid metabolism, and...
glycogen metabolism contain acetylated enzymes (16,17). Although a large number of metabolic enzymes are acetylated, little is known about how acetylation changes the activities of these enzymes. It is likely that the effects of acetylation will be substrate specific, enhancing the activity of some enzymes while inhibiting the activities of others. Much additional work is required to fully understand the influence acetylation has on regulating metabolism. Furthermore, acetylation levels change depending on the nutritional status of the cell and are sensitive to fasting (15), calorie restriction (20), and ethanol diet feeding (21). During the conditions of nutrient deprivation brought on by fasting and calorie restriction levels of SIRT3 increase in the liver leading to protein hypacetylation of some SIRT3 substrates. A handful of SIRT3 targets have been characterized including long-chain acyl-CoA dehydrogenase (LCAD) involved in β-oxidation (6), superoxide dismutase 2 (SOD2), an antioxidant enzyme (7), 3-hydroxy-3-methylglutaryl CoA synthase 2 (HMGCS2) involved in ketone body formation (9), and ornithine transcarbamoylase (OTC) in the urea cycle (8). In all the examples listed earlier, deacetylation leads to increased enzyme activity and in these cases helps restore homeostasis following the stress of nutrient deprivation.

**SIRT3 and Metabolic Syndrome**

In two recent articles, Hirschey and coworkers (10) and Jing and coworkers (11) now examine the changes in SIRT3 expression during models of nutrient excess. In the first study, Hirschey and colleagues (10) show that levels of SIRT3 mRNA and protein in the liver are reduced when mice are chronically fed a high-fat diet (HFD) leading to hyperacetylation of proteins in the mitochondria, similar to earlier studies (12,22). Furthermore, Jing and coworkers show a similar finding in skeletal muscle, as well as showing that SIRT3 is also decreased in the muscles of STZ-induced diabetic mice (11). In a previous work on SIRT3, Hirschey and colleagues examined the acetylation status of LCAD, an enzyme involved in regulating fatty acid oxidation (6). They found LCAD is hyperacetylated in SIRT3 KO mice leading to a decrease in its activity and less fatty acid oxidation. In the present work, Hirschey and colleagues showed that LCAD is hyperacetylated during HFD feeding and that this decreases its activity (10). These data strengthen the connection between SIRT3 levels, mitochondrial protein acetylation, and activity of important metabolic enzymes.

To better understand the consequences of mitochondrial hyperacetylation during conditions of nutrient excess, Hirschey and colleagues examined SIRT3 KO mice fed an HFD (10). SIRT3 KO mice fed an HFD develop obesity at an accelerated rate, which correlated with developing insulin resistance and glucose intolerance with age. In addition, SIRT3 KO mice display well-established signs of metabolic syndrome including steatohepatitis, hyperlipidemias, and increased circulating inflammatory cytokines, which are all more severe than found in wild-type mice fed the same HFD. Hypertension was not measured in these mice but is a component of metabolic syndrome that could be addressed in future studies. Most interestingly, when Hirschey and colleagues examined aged SIRT3 KO mice (12 months old) fed a standard diet, they found these mice were also glucose intolerant and insulin resistant (10). To look at the progression of insulin resistance, Hirschey and colleagues examined 3-month-old nonobese mice fed an HFD (10). Although the control mice showed no changes in insulin sensitivity, the SIRT3 KO mice required more insulin to effectively regulate glucose levels in a glucose tolerance test. Taken together, these data indicate that a lack of SIRT3 activity and subsequent mitochondrial protein hyperacetylation contribute to the onset of age-related insulin resistance.

Jing and associates also observed that SIRT3 KO mice are glucose intolerant (11). Because skeletal muscle is an important site of glucose uptake following insulin action, Jing and coworkers studied the role of SIRT3 and mitochondrial protein acetylation in this tissue (11). They found that insulin signaling was blunted in the SIRT3 KO mice as indicated by decreased phosphorylation of insulin receptor substrate-1 (IRS-1), AKT, and extracellular signal-related kinase (ERK) but with no changes in insulin receptor autophosphorylation. The mitochondria are a major site of reactive oxygen species (ROS) production as well as substrate metabolism. High levels of ROS can lead to activation of stress kinases such as c-jun N-terminal kinase (JNK) that negatively regulate insulin signaling. Jing and coworkers measure ROS levels and p-JNK levels in SIRT3 knockout mice and find both to be elevated (11). Thus, SIRT3 KO correlates with decreased insulin sensitivity and increased ROS production and stress kinase activation. Jing and coworkers found a similar blunting of insulin signaling and increase in ROS in a myocyte cell line and show that these cells have defects in mitochondrial respiration capacity (11). A potential mechanism for this observation may be

Figure 1. SIRT3 restores balance following metabolic stress. During metabolic stress following fasting or calorie restriction, SIRT3 is upregulated leading to increases in fatty acid oxidation, antioxidant production, ammonia detoxification, and acetate metabolism. During HFD feeding SIRT3 is downregulated leading to decreased fatty acid oxidation, increased levels of reactive oxygen species, and decreased metabolic rate and accelerated onset of diseases of ageing.
that several subunits of the electron transport chain have increased acetylation in the absence of SIRT3, although further work is needed to confirm how hyperacetylation affects the functions of these proteins.

Finally, to address a possible role for SIRT3 in human metabolic syndrome, Hirschey and colleagues performed a genetic analysis and identified a single-nucleotide polymorphism present in human populations that correlates with increased incidence of metabolic syndrome (10). Remarkably, this polymorphism encoded a point mutation in the SIRT3 protein sequence. When they tested this mutant form of SIRT3 in vitro, they found it had reduced deacetylase activity. Thus, screening for this single-nucleotide polymorphism in human patients may eventually be a test for metabolic syndrome risk.

CONCLUSIONS

Although additional work is needed to fully understand the changes in signaling that occur due to the hyperacetylation induced in SIRT3 KO or HFD-fed mice, it is clear that these changes accelerate the development of the metabolic syndrome. These findings imply that drug therapies that increase SIRT3 activity may be useful in treating metabolic syndrome. Although SIRT3 has been identified as a PGC-1α target gene (23), little else is known about regulation of SIRT3 that should be the subject of further study. In addition, testing for SIRT3 fidelity may be a useful tool for identifying individuals predisposed to develop metabolic syndrome.

Taking into consideration the body of work published on SIRT3, it becomes clear that SIRT3 serves as an important sensor in metabolic regulation responding to changes in nutritional state and working to restore metabolic homeostasis (Figure 1). There is still much work to be done to fully understand how SIRT3 and mitochondrial acetylation influences the complex metabolic systems of living organisms. However, SIRT3 regulates an important posttranslational modification and a better understanding of how acetylation influences metabolism will lead to a better understanding of metabolism, mitochondrial function, and ultimately the diseases of aging.

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