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REVIEW PAPER

Sirtuins: A Family of Proteins With Implications for Human Performance and Exercise Physiology

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The sirtuin family of proteins consists of seven members in mammals (SirT1-T7). Sirtuins share NAD dependency for their enzymatic activity, but some show NAD-dependent deacetylase activity, others exhibit ADP ribosyltransferase activity or both. Sirtuins have gained considerable attention due to their impact as physiological targets for treating diseases associated with aging. Sirtuins interact with metabolic pathways and may serve as entry points for drugs. This review discusses the biology of sirtuins and their potential as mediators of caloric restriction and pharmacological targets. Reduced insulin sensitivity, mitochondrial dysfunction, and others are consequences of aging or secondary to physical inactivity. Moreover, understanding human energy metabolism through sirtuins may provide a novel approach to exercise physiology. Quercetin, a natural polyphenolic flavonoid that has been widely investigated for its other health benefits, may act as an inducer of SirT1. The benefits of quercetin for exercise performance may have implications for athletes and extended to disease prevention.

KEYWORDS *sirtuins, metabolism, exercise, performance, lifespan*

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INTRODUCTION

Originally isolated from the budding yeast *Saccharomyces cerevisiae*, the silent information regulator 2 (Sir2) and ultimately four members of the protein family (Sir1-Sir4) have been identified as important regulators of mating-type locus silencing, telomeric DNA, and the lifespan (Frye 2000; Rine et al. 1979). In mammals, the sirtuins consist of seven members of Sir2 homologues (SirT1-7; Frye 1999, 2000), with SirT1 sharing the closest similarity to eukaryotic Sir2 (Frye 2000). They all contain the Rossman catalytic domain consisting of a series of conserved sequence motifs in organisms ranging from bacteria to humans (Brachmann et al. 1995; Finnin, Donigian, and Pavletich 2001). Biochemically, Sir2 functions as a deacetylase, more specifically a histone deacetylase, which can cleave the acetyl group from acetylated proteins such as histones. On the other hand, Sir2 can also cleave the acetyl groups from several transcription factors, and in this way can change their activities. Sir2 works in a unique reaction requiring the energetic intermediate NAD+ as a cofactor (Imai et al. 2000), but sirtuins can also act as mono-ADP-ribosyltransferases (Frye 1999; Revollo, Grimm, and Imai 2004). NAD-dependent deacetylase and also ribosyltransferase activities have been demonstrated for mammalian SirT1-T3 (Frye 1999).

Due to their variable N- and C- terminal ends, sirtuins display distinct subcellular localizations and probably exert different biological functions. Among the mammalian sirtuins, SirT1 is predominantly found in the nucleus while targeting a wide range of transcriptional regulators and has therefore emerged as an important regulator of aging, metabolism, stress resistance, and cell survival. For recent reviews, see Haigis and Sinclair (2010) and Liang et al. (2009). SirT1 mediates is functions through deacetylation of key transcription factors, such as the tumor suppressor protein p53, nuclear factor kappa-B (NF-kB), the Forkhead box class Os (FOXOs), and peroxisome proliferator receptor gamma coactivator-1 alpha (PGC-1α), thus improving the ability to activate downstream transcription of genes involved in oxidative metabolism (Motta et al. 2004; Nemoto, Fergusson, and Finkel 2005; Yeung et al. 2004). Another metabolic function is that SirT1 positively regulates insulin secretion in pancreatic beta cells by repressing the uncoupling protein (UCP) gene *UCP2* by binding to its promoter (Bordone et al. 2006).

SirT2 is a cytoplasmic protein that deacetylases the cytoskeletal protein α-tubulin and also FOXO1 (Jing, Gesta, and Kahn 2007), suggesting that SirT2 may play a role in cell cycle and be involved in cytoskeletal organization (Li et al. 2007). Furthermore, SirT2 expression is induced by caloric restriction in several tissues in mice (Wang et al. 2007). Mechanistically, SirT2 suppresses adipogenesis by deacetylating FOXO1 to promote its binding to peroxisome proliferator-activating receptor gamma and the subsequent repression of its transcriptional activity (Wang and Tong 2009). Furthermore,

SirT2 levels are elevated by oxidative stress, which consequently results in deacetylation of FOXO3a to activate the antioxidative stress response to reduce cellular levels of reactive oxygen species (ROS; Wang et al. 2007).

Using SirT3-deficient mice, Lombard et al. (2007) demonstrated that SirT3 is a soluble mitochondrial protein and a major mitochondrial deacetylase, which has been evolved to control reversible lysine acetylation in mammals. Indeed, SirT3 plays a key role in mitochondrial metabolism and thermogenesis in brown adipocytes following activation by proteolytic processing of its N-terminus (Schlicker et al. 2008; Shi et al. 2005). By function, SirT3 can deacetylate and thereby activate a central metabolic regulator in the mitochondrial matrix, the glutamate dehydrogenase (Schlicker et al. 2008). Furthermore, SirT3 deacetylates and activates isocitrate dehydrogenase 2, which is an enzyme that promotes regeneration of antioxidants and catalyzes a key regulation point of the citric acid cycle (Schlicker et al. 2008). Previous data also suggest that SirT3 may regulate cellular acetyl-CoA levels through the modulation of mammalian mitochondrial Acetyl-CoA synthetase-2 (AceCS2) activity, whereas AceCS1 is controlled by SirT1 (Hallows, Lee, and Denu 2006). Similar to SirT3, also SirT4 and SirT5 reside in the mitochondria, but regulate amino acid metabolism and insulin secretion (Haigis et al. 2006; Nakamura et al. 2008). In contrast to SirT3, SirT5 does not deacetylate mitochondrial matrix proteins. Instead, it can deacetylate cytochrome c, which has central function in oxidative metabolism (Schlicker et al. 2008). SirT6 and SirT7 are located in the nucleus and deacetylate some nonhistone proteins involved in the stabilization of the genome (Vakhrusheva et al. 2008).

The NAD-dependent activity makes sirtuins important in many biological functions, such as longevity (Guarente 2006; Michan and Sinclair 2007). Sirtuins have the potential to modulate biological roles of key regulatory proteins in response to alterations in the cellular redox state (Nakahata et al. 2008; Revollo et al. 2004). Moreover, due to their NAD-dependency, sirtuins are believed to constitute functional links between metabolic activity and gene silencing, apoptosis, stress resistance, senescence, genome stabilization, and aging (Bishop and Guarente 2007). Finally, because of their various functions, sirtuins have been proposed to provide novel targets for therapies to alleviate age-associated disease, such as diabetes, cancer, and cardiovascular disease, and possibly to extend the human lifespan (Michan and Sinclair 2007). This review focuses on the progress of research into sirtuins and their role in human performance.

Sirtuins, Energy Expenditure, and Lifespan

The lifespan is estimated to be determined by the accumulation of cellular damage arising from ROS, although recent evidence suggests that ROS also might be responsible for lifespan extension (Schulz et al. 2007).

Energy-restricted diets, on the other hand, have been implicated in an increase in longevity by decreasing ROS and also by reducing the severity of some metabolic disorders related to ageing (Finkel and Holbrook 2000). Notably, caloric restriction appears to improve the body's antioxidant defenses through the activity of sirtuins (Crujeiras et al. 2008). Furthermore, dietary calorie restriction has been shown to delay the onset of age-related diseases including obesity and diabetes (Hansen and Bodkin 1993), tumors and kidney diseases (Fernandes and Good 1984), as well as several types of neurodegenerative disorders (Duan et al. 2003; Mattson 2003), but the underlying mechanisms remain unclear.

Major interest in sirtuins is prompted by their possible role in lifespan extension by mediating the impact of caloric restriction. Indeed, single gene mutations can extend the lifespan of laboratory organisms, and, among these, one was shown to regulate insulin signaling (Brown-Borg et al. 1996; Clancy et al. 2001). Another, namely, Sir2, was found to independently determine the lifespan in yeast (Kaeberlein, McVey, and Guarente 1999; Tissenbaum and Guarente 2001). Thus, sirtuins seem to mediate the action of caloric restriction at least in lower organisms (Kaeberlein et al. 1999; Lamming et al. 2005; Tissenbaum and Guarente 2001; Wood et al. 2004). Because of their enormous evolutionary divergence, however, it is likely that sirtuins can determine the lifespan in a broad spectrum of organisms, including mammals. Accordingly, some studies have suggested that mammalian sirtuins, especially SirT1, are also responsive to caloric restriction (Cohen et al. 2004; Shi et al. 2005; Wang and Tong 2009) and could thereby mediate the action of caloric restriction in higher organisms. Similar to that of other sirtuins, the metabolic regulatory function of SirT2 may be the obligatory pathways underlying the effect of caloric restriction on ageing (Picard et al. 2004; Shi et al. 2005; Wang and Tong 2009).

The proposed beneficial effects of NADH oxidation on the regeneration of NAD via mitochondrial function (Piper, Harris, and Maclean 2006), where sirtuins also play a role, would help explain how aerobic exercise may delay the ageing phenotype, including the production of altered proteins, and ultimately resolve the apparent paradox that increased oxygen utilization suppresses age-related changes. Furthermore, the efficient regeneration of NAD via effective mitochondrial function is consistent with mitochondrial ageing theories, which postulate that mitochondrial dysfunction is the key element for the onset of ageing.

Adipose tissue plays a critical role in the energy homeostasis of the body and as such provides a target for the effect of sirtuins. Exposure to cold was reported to upregulate SirT3 in the brown adipose tissue, whereas elevated temperatures reduced its expression (Shi et al. 2005). Furthermore, a short-term fasting for 24 hours also induced marked expression of SirT2 in brown adipose tissue by cold exposure (Wang and Tong 2009). This indicates that also SirT2 is regulated at least to some extent by temperature, and the effect of fasting was not seen in the liver or skeletal muscle. Interestingly, a large

population-based study recently reported that genetic variants of SirT1 may influence human obesity (Zillikens et al. 2009).

Sirtuins and Exercise

Poor exercise tolerance is clearly a problem for competitive athletes and generally all individuals who engage in physically demanding activities. Increased mitochondrial biogenesis in skeletal muscle is perhaps the most important factor responsible for increased tolerance of endurance exercise in response to exercise training. Acute exercise increases the transcription of genes and upregulation of proteins responsible for mitochondrial biogenesis. AMP-activated protein kinase (AMPK) is a metabolic fuel gauge in mammals (Hardie 2007), which is transiently activated by acute exercise (Canto et al. 2009) and also may play an important role in the therapeutic effects of exercise as part of the management of type 2 diabetes (Barnes et al. 2005). A typical doubling of mitochondria in the muscle that occurs by exercise training is largely responsible for the increased oxygen utilization, shift in substrate utilization toward greater fatty acid oxidation and increased lactate threshold, the two limiting factors for endurance performance (Calvo et al. 2008, Joyner and Coyle 2008). Maximal oxygen uptake (VO_2max) is influenced by the mitochondrial oxidative capacity, but relative to the endurance capacity, it is limited by oxygen delivery through the cardiovascular system (Bassett and Howley 2000).

Endurance exercise training has been shown to elevate SirT3 expression and the mitochondrial oxidative capacity in the muscle (Lanza et al. 2008). This suggests that endurance exercise partly normalizes age-related mitochondrial dysfunction and that SirT3 expression may be restored by exercise training. Furthermore, this provides a linkage between endurance exercise and the lifespan-enhancing effects of caloric restriction (Cohen et al. 2004). Indeed, mitochondrial SirT3 has been linked to longevity, possibly by interfering with the release of an apoptosis-inducing factor (Haigis and Guarente 2006; Yang et al. 2007) in a manner similar to that by which nutrient restriction increases lifespan (Yang et al. 2007). Altogether, exercise may confer lifespan-extending effects similar to those of caloric restriction through the action of mitochondrial SirT3. Moreover, Suwa et al. (2008) were the first to demonstrate that skeletal muscle SirT1 protein expression increases with both acute endurance exercise and endurance training in rats. In the study, the authors found that SirT1 and PGC-1α protein levels were higher in the red, slow-twitch, and oxidative muscle fibers compared with white, fast-twitch, and glycolytic fibers, and also correlated positively with mitochondrial components (Suwa et al. 2008). Also Koltai et al. (2009) observed that exercise training significantly increased SirT1 activity in the skeletal muscle in young and old rats, and that SirT1-associated processes related to ageing were attenuated by exercise training. Furthermore, Canto et al. (2009) recently demonstrated that deacetylation of PCG-1α is a key mechanism by

which AMPK triggers PCG-1α activity in cultured murine myotubes and in skeletal muscle, and that SirT1 is a key mediator of AMPK action on PGC-1α transcriptional activity, the expression of genes of mitochondrial and lipid metabolism, and oxygen consumption. It has also been suggested that SirT1 and PGC-1alpha expression are independently regulated, and that although SirT1 may be involved in exercise-associated mitochondrial biogenesis, its expression may not closely correlate to changes in mitochondrial proteins (Chabi et al. 2009). Interestingly, Dumke et al. (2009) recently reported that 3 hours of exhaustive exercise in 3 consecutive days caused transient elevation in PGC-1α and SirT1 mRNA levels and cumulative increase of cytochrome c and citrate synthase mRNAs in human skeletal muscle. In addition, levels of nicotinamide phosphoribosyltransferase (NAMPT), a protein responsible for the first and rate-limiting step in the conversion of nicotinamide to NAD+, were found to be significantly increased in sedentary nonobese subjects after 3 weeks of exercise training, and also that NAMPT correlated with mitochondrial content (Costford et al. 2010). Although limited, these data suggest that SirT1 is responsive to exercise and involved in the adaptive mechanisms to endurance training. Moreover, the interdependent regulation of SirT1 and AMPK provides a finely tuned mechanism for energy homeostasis under low nutrient availability, such as in long-term physical exercise.

Sirtuins, Quercetin, and Exercise Performance

The improvement of human exercise performance by dietary supplementation has attracted increasing attention. Sirtuins are an interesting target for such a procedure mainly due to their key role in mitochondrial function. Among the most effective methods to increase sirtuins levels or their activity in tissues are caloric restriction, the use of natural flavonoids such as resveratrol, and certain drugs (Crujeiras et al. 2008; Lopez-Lluch et al. 2006; Narkar et al. 2008). An earlier work by Howitz et al. initially showed that resveratrol, a polyphenol found in red wine, lowers the Michaelis constant of SirT1 for both the acetylated substrate and NAD+, and increases cell survival by stimulating SirT1-dependent deacetylation of p53 (Howitz et al. 2003). In addition to resveratrol, a known activator of PGC-1α and SirT1 in mice (Lagouge et al. 2006), another plant-derived substance, quercetin, may be useful in the enhancement of mental and physical performance (Davis, Murphy, and Carmichael 2009a). Due to its ubiquitous presence in a wide variety of food plants including red onion, red wine, apples, and berries, quercetin plays an important role for numerous biological effects (Davis et al. 2009a; Harwood et al. 2007). Orally administered quercetin is absorbed rapidly, and up to 0.42 μM concentrations were recorded in human plasma after ingestion of a beverage containing 10 mg of quercetin (Goldberg, Yan, and Soleas 2003). Quercetin also was reported to increase the activity of recombinant SirT1 protein; however, it failed to stimulate intracellular

SirT1 activity in human HT29 cells (de Boer et al. 2006), probably because quercetin is rapidly metabolized to quercetin 3-*O*-glucuronide, the main conjugate form found in human plasma (Mullen et al. 2004). Evidently, cellular SirT1 response is strongly affected by polyphenol stability and metabolism.

Of particular interest is the recent evidence that in combination with other antioxidants and caffeine, quercetin was reported to improve human endurance performance (MacRae and Mefferd 2006). Although little is known about its effects of on mitochondrial biogenesis and exercise tolerance, Davis et al. (2009b) recently indicated that quercetin feeding increased mRNA expression of PGC-1α and SirT1, as well as mitochondrial DNA and cytochrome *c* in both skeletal muscle and the brain in mice. Furthermore, these changes were associated with an increase in maximal endurance capacity and voluntary wheel-running activity (Davis et al. 2009b). To evaluate this in humans, the authors recently reported that 1000 mg of quercetin per day for 7 days was associated with increased VO_2max by 3.9% and exercise time to fatigue by 13.2% in untrained subjects (Davis et al. 2010). In addition, Nieman et al. (2010) found that 1000 mg of quercetin per day for 2 weeks resulted in small but significant improvement in a 12-min treadmill performance trial and also modest increases in the mitochondrial DNA and mRNA levels of genes related to mitochondrial biogenesis. Quercetin also was reported to counteract with exercise-induced inflammation in trained athletes when combined with other flavonoids (Nieman et al. 2009). In contrast, some studies have not demonstrated a significant effect on physical performance (Cheuvront et al. 2009; Cureton et al. 2009; Quindry et al. 2008; Utter et al. 2009).

Most information about quercetin and exercise performance is still based upon studies *in vitro* and with animals. Therefore, more studies in humans are needed to dissect the mechanism of the quercetin-induced improvement in exercise tolerance capacity in human performance and competitive sports. Nevertheless, the available studies on the beneficial effects of quercetin upon endurance performance are promising. Studies with negative findings are of particular concern at this stage of investigation given the multitude of as-yet-unknown factors that could have affected the study outcome, including the optimal timing, dose, and delivery and metabolism of quercetin; individual differences in bioavailability; the fitness and training status of the subjects; environmental influences; and the mode and intensity of exercise, to name a few.

CONCLUSION

The dependence of cellular NAD^+ links mammalian sirtuins to the underlying metabolic state of the cell. Sirtuins have been shown to be regulated by and mediate the effects of dietary calorie restriction, and also are implicated

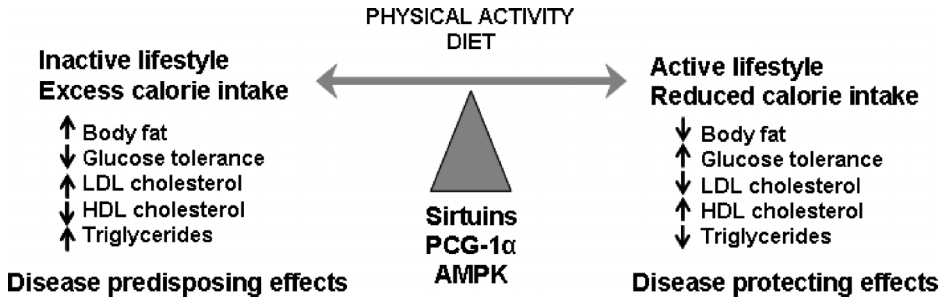

FIGURE 1 The metabolic regulators (shown middle) potentially involved in the underlying mechanisms to modify the balance. The reciprocity of the phenotype of metabolic syndrome (shown left) and calorie restriction (shown right), and their effects on disease predisposition are also indicated. Adapted by permission from Macmillan Publishers Ltd: *Nature*. Guarente, Leonard (2006). Sirtuins as potential targets for metabolic syndrome. 444(7121): 868–874, copyright 2006.

in resistance to stress and in numerous metabolic pathways. Because sirtuins are regulated by dietary factors and in turn affect multiple facets of physiology, they may act as therapeutic targets for metabolic diseases. The real excitement lies in the understanding that these disparate functions are perhaps all interconnected, the way that sirtuins as a whole serve as the bridge between what we eat and what we are (Figure 1). Furthermore, SirT1 and SirT3 may play an important role in the adaptation of cellular biogenesis by exercise. Increased physical fitness without exercise training through SirT1 and PGC-1α by nutrient supplementation with quercetin may have implications not only for performance enhancement but also for health promotion and disease prevention.

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