

# A Review of the Sirtuin System, its Clinical Implications, and the Potential Role of Dietary Activators like Resveratrol: Part 1

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## Abstract

The silent information regulator (SIR) genes (sirtuins) comprise a highly conserved family of proteins, with one or more sirtuins present in virtually all species from bacteria to mammals. In mammals seven sirtuin genes – SIRT1 to SIRT7 – have been identified. Emerging from research on the sirtuins is a growing appreciation that the sirtuins are a very complicated biological response system that influences many other regulator molecules and pathways in complex manners. Responses of this system to environmental factors, as well as its role in health and disease, are currently incompletely characterized and at most partially understood. This article reviews the mammalian sirtuin system, discusses the dietary, lifestyle, and environmental factors that influence sirtuin activity, and summarizes research on the importance of vitamin B3 in supporting sirtuin enzyme activity, as well as the role specifically of the amide form of this vitamin – nicotinamide – to inhibit sirtuin enzyme activity. Polyphenols, especially resveratrol, influence sirtuins. Existing evidence on these nutritional compounds, as they relate to the sirtuin system, is reviewed. In Part 2 of this review, clinical situations where sirtuins might play a significant role, including longevity, obesity, fatty liver disease, cardiovascular health, neurological disease, and cancer, are discussed. (*Altern Med Rev* 2010;15(3):245-263)

## Introduction

The silent information regulator (SIR) genes (sirtuins) comprise a highly conserved family of proteins, with one or more sirtuins present in virtually all species from bacteria to mammals. In mammals seven sirtuin genes – SIRT1 to SIRT7 – have been identified. These seven sirtuin genes code for seven distinct sirtuin enzymes that act as deacetylases or mono-ADP-ribosyltransferases. All sirtuin enzymes are dependent on oxidized nicotinamide adenine dinucleotide (NAD<sup>+</sup>).

Sirtuins are considered to be regulator genes; genes that control other genes. Sirtuins themselves can also be influenced by other genes and respond in an epigenetic manner to a variety of environmental factors. They are hypothesized to play a particularly important role in an organism's response to certain types of stress and toxicity. Sirtuins regulate reproductive and chronological lifespan in lower organisms (like yeast and bacteria) and appear to affect biological aspects of mammalian diseases of aging. This lifespan and longevity regulatory role appears to be most prominent under circumstances that represent a need for cellular adaptation, such as calorie restriction.<sup>1</sup>

Emerging from research on the sirtuins is a growing appreciation that they comprise a very complicated biological response system that influences many other regulator molecules and pathways in complex manners. Responses of this system to environmental factors, as well as its role in health and disease, are currently incompletely characterized and at most partially understood. This article reviews the current state of sirtuin research, including an overview of the mammalian sirtuin system, sirtuin biological functions, and potential clinical implications. Environmental and nutritional factors that influence the sirtuin system are also discussed.

## Overview of the Mammalian Sirtuin System

The first aspect of the sirtuin system to be identified was in the yeast *Saccharomyces cerevisiae*. This protein was named silent mating type information regulation-2 (Sir2). Sir2 was subsequently found in the fruit fly (*Drosophila melanogaster*) and

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the roundworm (*Caenorhabditis elegans*). In these organisms Sir2 is involved in the regulation of a variety of metabolic pathways including those involved in aging and longevity. In mammals, the first sirtuin gene identified was silent mating type information regulation-2 homolog (SIRT1). It is considered a homologous gene sequence (biologically equivalent gene sequence across species) to Sir2. The product of the SIRT1 gene is the SIRT1 enzyme (also known as NAD<sup>+</sup>-dependent deacetylase sirtuin-1). Six other sirtuin genes have been identified in mammals, resulting in seven genes – SIRT1 through SIRT7 – that encode for seven sirtuin enzymes in the mammalian sirtuin system. Sirtuins are found in the nucleus, cytoplasm, and mitochondria. Sirtuins are also widely expressed in a variety of tissues.

### Subcellular Location and Tissue Expression

The mammalian sirtuins occupy three different subcellular compartments. SIRT1, -2, -6, -7 are found in the nucleus; SIRT1 and SIRT2 are also found in the cytoplasm. SIRT3, -4, and -5 are found in the mitochondria (Figure 1).<sup>2,3</sup> In addition to the differences in subcellular localization, the sirtuins are also expressed in varying amounts in different tissues. Of the seven mammalian sirtuins, SIRT1 has been the most extensively studied. It is highly expressed in several brain regions including the hypothalamus, and has been found in the heart, kidney, liver, pancreas, skeletal muscle, spleen, and white adipose.<sup>2,4</sup> SIRT2 is reported to be the most abundant sirtuin in adipocytes, found in white and brown adipose tissue. It is also highly expressed in the brain and nervous system.<sup>2,5-9</sup> SIRT3 is found inside the mitochondria in skeletal muscle, brown and white adipose, heart, kidney, liver, and other metabolically active tissues.<sup>2,10-14</sup> SIRT4, another mitochondrial sirtuin, is expressed in a variety of metabolically active tissues, including the islets of Langerhans in the pancreas.<sup>2,10,15</sup> SIRT5, also a mitochondrial sirtuin, is expressed in a variety of tissues including the liver.<sup>2,10,16</sup> SIRT6 is broadly expressed, with the highest levels in adipose tissue, skeletal muscle, brain, and heart.<sup>17-19</sup> SIRT7 is found in many cells including adipocytes and cardiomyocytes.<sup>20,21</sup>

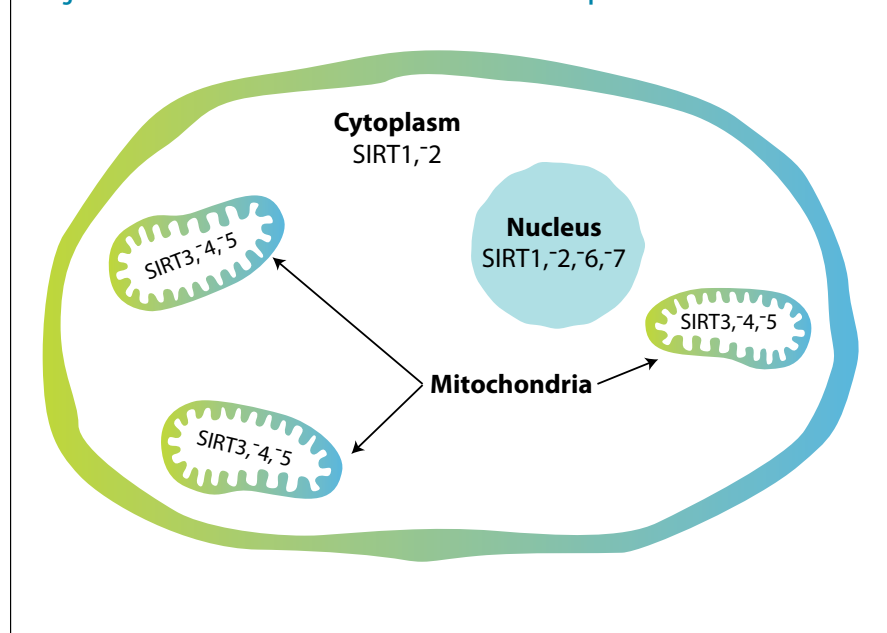
### The Sirtuin Enzymes

Sirtuin enzymes are structurally defined by the presence of two central domains that together form a highly conserved (existing in virtually all organisms) central catalytic histidine residue. The sirtuin core is flanked by variable N- and C-terminal extensions that differ among the sirtuins. The central histidine residue structure has been proposed to function as an enzymatic core.<sup>22</sup>

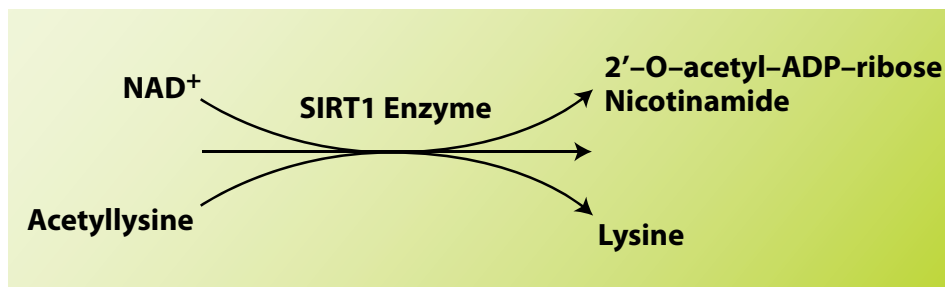
Sirtuins were originally defined as class III histone deacetylases (also called lysine deacetylases), a family of oxidized nicotinamide adenine dinucleotide (NAD<sup>+</sup>)-dependent enzymes that deacetylate lysine residues on various proteins. Sirtuin-mediated histone deacetylase reactions are specific for acetylated lysines, removing the acetyl group from the acetyllysine residue in a histone and transferring it to the ADP-ribose moiety of NAD<sup>+</sup>. This reaction cleaves the NAD<sup>+</sup> coenzyme resulting in the formation of a deacetylated protein and the release of nicotinamide and 2'-O-acetyl-ADP-ribose (Figure 2). It was later discovered that sirtuins also participate in non-histone deacetylase reactions. These non-histone deacetylations also remove acetyl groups from acetyllysine-modified proteins and transfer them to NAD<sup>+</sup>, yielding 2'-O-acetyl-ADP-ribose and nicotinamide.<sup>2,22-25</sup> Further exploration revealed that some members of this enzyme family possess mono-ribosyltransferase (mono-ADP-ribosyltransferases) activity.

**Key words:** SIRT1, sirtuin, resveratrol, SIRT, NAD, niacin, nicotinic acid, niacinamide, nicotinamide, aging, anti-aging, fasting, calorie restriction, acetylation, deacetylation

Figure 1. Location of Sirtuins in Mammalian Cellular Compartments



**Figure 2. SIRT1 Lysine Deacetylation Reaction**



In a sirtuin deacetylation reaction, the sirtuin enzyme (SIRT1 in this case) does three things: (1) hydrolyzes  $\text{NAD}^+$  into an ADP-ribose moiety and nicotinamide; (2) removes an acetyl group from a protein (acetyllysine in this case), which produces a deacetylated protein (lysine in this case); and (3) it transfers the now available acetyl group to the ADP-ribose moiety, which results in the formation of 2'-O-acetyl-ADP-ribose.

Adapted from: Yang T, Sauve AA. NAD metabolism and sirtuins: metabolic regulation of protein deacetylation in stress and toxicity. *AAPS J* 2006;8:E632-E643.

Sirtuin-mediated mono-ribosyltransferase reactions transfer the ADP-ribose group from  $\text{NAD}^+$  to acceptor proteins in a posttranslational modification called ADP-ribosylation. This reaction produces mono-ADP-ribosylated proteins and, similar to the deacetylation reactions, also yields nicotinamide.<sup>10,23,26</sup> Table 1 summarizes the mammalian sirtuins, tissue location, and sirtuin enzymes.

### Genetic Variation

Sirtuin genetic variation has been reported. Existing studies have focused primarily on genetic variation of the SIRT1 gene. SIRT1 has a variety of

single nucleotide polymorphisms (SNPs) that tag regions of the gene characterized by genetic variation. These include rs12413112, rs1467568, rs2273773, rs3758391, rs3818292, rs7069102, rs730821, and rs7895833. Homozygous, heterozygous, and noncarriers exist for the major alleles of these SNPs. As an example, the three genotypes for several of the SNPs for SIRT1, as well as their population distribution, are listed in Table 2.<sup>27</sup>

Several observational studies have attempted to elucidate whether there are any associations between SIRT1 genetic variation and health. Evidence to date suggests that there might be an

**Table 1. Sirtuin Enzyme Activities and Subcellular Locations**

| Sirtuin | Type of Enzyme                       | Subcellular Location  |
|---------|--------------------------------------|-----------------------|
| SIRT1   | Deacetylase                          | Cytoplasm and Nucleus |
| SIRT2   | Deacetylase                          | Cytoplasm and Nucleus |
| SIRT3   | Deacetylase & ADP-ribosyltransferase | Mitochondria          |
| SIRT4   | ADP-ribosyltransferase               | Mitochondria          |
| SIRT5   | Deacetylase                          | Mitochondria          |
| SIRT6   | Deacetylase & ADP-ribosyltransferase | Nucleus               |
| SIRT7   | Deacetylase                          | Nucleus               |

**Table 2. Sirtuin Genetic Variation: Distribution of Four SIRT1 Single Nucleotide Polymorphisms in a Sample Population of 917 Individuals**

| SNP            | Homozygous carrier (has both major alleles) | Heterozygous carrier (has 1 major allele and 1 minor allele) | Noncarrier (has both minor alleles) |
|----------------|---|--|-------------------------------------|
| SNP rs12413112 | 726 people were GG                          | 173 people were GA   | 18 people were AA                   |
| SNP rs2273773  | 812 people were TT                          | 103 people were TC   | 2 people were CC                    |
| SNP rs7069102  | 457 people were GG                          | 366 people were GC   | 94 people were CC                   |
| SNP rs730821   | 603 people were AA                          | 286 people were AG   | 28 people were GG                   |

Adapted from: Weyrich P, Machicao F, Reinhardt J, et al. SIRT1 genetic variants associate with the metabolic response of Caucasians to a controlled lifestyle intervention – the TULIP Study. *BMC Med Genet* 2008;9:100.

association between some aspects of SIRT1 genetic variation and risk for obesity,<sup>27-29</sup> as well as the response to lifestyle interventions for obesity.<sup>29</sup> Associations between SIRT1 genetic variation and mortality in type 2 diabetes<sup>30</sup> and cardiovascular disease<sup>31</sup> have also been reported. The association between SIRT1 genetic variation and mortality in subjects with type 2 diabetes appears to be influenced by factors such as smoking status and low niacin intake.<sup>30</sup> An association between exceptional longevity and SIRT1 genetic variation has not been observed.<sup>32</sup> Unlike with SIRT1, one observational study reported an association between a genetic variant of SIRT3 and survival in elderly subjects. The TT and GT genotypes of SNP G477T were associated with increased and decreased survival in the elderly, respectively.<sup>33</sup> One SNP in the SIRT4 gene (rs2522138) was investigated for links to type 2 diabetes, but no evidence of an association was detected.<sup>34</sup> Currently insufficient data exists to draw any definitive conclusions regarding the role of sirtuin genetic variations in health. The ability to draw inferences is also hindered since no attempt to measure actual tissue expression of SIRT1 or its enzyme activity has been made in these studies, which is a significant limitation since sirtuin expression and activity are strongly influenced by many nongenetic factors.

### Epigenetic Variation

While each sirtuin enzyme is a product of a specific sirtuin gene, expression of these genes and the activity of sirtuin enzymes in any given tissue is strongly affected by changes in the environment, diet, and lifestyle. Some of the factors that have been reported to influence the epigenetic

expression include calorie restriction (fasting), exercise, alcohol, smoking, cold exposure, oxidative stress, plant compounds (e.g., resveratrol, quercetin, and persimmon oligomeric proanthocyanidins), and melatonin. Under circumstances like calorie restriction, exercise, and resveratrol intake, the changes in sirtuin expression appear to help cells, specifically, and the organism in general, adapt to challenges.

### Calorie Restriction

The sirtuin system is strongly influenced by calorie restriction. While multiple genes respond to calorie restriction, and at least several of these are believed to be involved in the life-extending benefits attributed to long-term caloric restriction, sirtuins appear to be one of the genetic pathways involved, and a functional sirtuin system appears to be a requirement for lifespan extension to occur.<sup>35-39</sup> One piece of evidence for this inference comes from research on mice carrying two null alleles for SIRT1 (SIRT1-deficient mice). Calorie restriction does not extend lifespan of these mice.<sup>39</sup>

In mice, calorie restriction compared to high calorie diets changes the expression of over 3,000 genes, many of which can vary in expression between 10- and 50-fold. Among the genes showing the largest and most statistically significant calorie restriction-induced expression differences are those influencing the sirtuin system.<sup>40</sup> In humans SIRT1 gene expression also appears to be responsive to caloric intake. Allard et al reported that, compared with serum collected from human subjects prior to reduced calorie intake, serum collected from these same subjects after either alternate day fasting or calorie restriction resulted in increased SIRT1 expression in cells cultured in

this serum.<sup>41</sup> Civitarese et al assessed SIRT1 in overweight human volunteers. A negative energy balance was created by either restricting caloric intake by 25 percent or by restricting caloric intake by 12.5 percent and increasing exercise expenditure by 12.5 percent. In both instances, compared with controls who were fed 100 percent of energy requirements, SIRT1 expression was increased.<sup>42</sup> Crujeiras et al reported that an eight-week hypocaloric diet increased SIRT1 and SIRT2 expression in the peripheral blood mononuclear cells (PBMC) of obese subjects.<sup>43</sup> Capel et al reported that in humans, similar to findings in mice, calorie restriction influences the expression of many genes. The study involved two sets of 47 obese women who participated in 10 weeks of either a low-fat (high-carbohydrate) or a moderate-fat (low-carbohydrate) hypoenergetic diet; a control group was used for comparison. Expression of 1,000 genes in adipose tissue, including sirtuin genes, was influenced by energy restriction. SIRT3 expression was also sensitive to fat content of the hypocaloric diet, increasing in subjects consuming a moderate-fat diet compared to subjects on the low-fat diet.<sup>44</sup>

Caloric restriction (or complete fasting) modifies sirtuins in a variety of tissues (Table 3). SIRT1 increases in the liver, kidney, intestines, skeletal muscle, and white adipose, and decreases in the

stress. Presumably this response acts to promote transcription of genes that mediate adaptive metabolic and behavioral response to caloric restriction. Among the changes that appear to be related to sirtuin changes are increased lipolysis and mobilization of fatty acids from white adipose tissue,<sup>48</sup> increased hepatic gluconeogenesis, fatty acid beta oxidation, and decreased glycolysis,<sup>54-58</sup> increased fatty acid oxidation in skeletal muscle,<sup>46</sup> increased hypothalamic hunger signaling,<sup>59,60</sup> and an increase in activity and food seeking behaviors.<sup>61</sup> The sirtuin effect on specific proteins that mediate many of these changes in physiology during calorie restriction is discussed in greater detail in the section “Regulator Proteins.”

### Exercise

Exercise fails to extend maximum life span in animal experiments. Nevertheless, it does appear to influence aspects of the sirtuin system. Presumably, exercise-induced changes in sirtuins are an adaptation to the stress of exercise. Limited evidence also suggests sirtuins adapt to muscular disuse.

In rats, SIRT1 expression is increased in skeletal muscle (specifically the muscles used in exercise) after both acute endurance exercise and chronic exercise, whether the chronic exercise is low or high intensity.<sup>62</sup> Paradoxically, rat skeletal muscle SIRT1 activity was also reported to increase in an experiment intended to mimic disuse, in this case, after 21 days of denervation.<sup>63</sup> Similarly, SIRT3 increases in skeletal muscle subsequent to chronic exercise.<sup>12,13</sup> But in immobilized hind limbs, compared with the contralateral control muscle, there is reduced SIRT3.<sup>12</sup> There is also an age-related increase in SIRT1 and SIRT6 levels in rat skeletal muscle. Exercise training significantly increases the relative activity of SIRT1, but attenuates the age-associated increase in the level of SIRT6.<sup>18</sup> In rats there is an age-related decline in SIRT1 activity in the heart. Exercise training attenuates this decline.<sup>64</sup>

In humans SIRT1 is responsive to exhaustive acute exercise, increasing in skeletal muscle from pre- to post-exhaustive exercise.<sup>65</sup> SIRT1 has also been reported to significantly increase in blood polymorphonuclear cells (PMNC) after a marathon, while SIRT3 and SIRT4 decreased significantly.<sup>66</sup> SIRT3 is also responsive to chronic exercise. Lanza et al took biopsies from the vastus lateralis of 42 healthy sedentary and endurance-trained young (ages 18-30 years) and older (ages 59-76 years) subjects. SIRT3 expression was lower with age in

**Table 3. Affect of Calorie Restriction on Tissue Levels of Specific Sirtuins**

| Sirtuin | Tissue Up-regulated  | Tissue Down-regulated |
|---------|--|-----------------------|
| SIRT1   | ↑Liver, kidney, intestines, skeletal muscle, white adipose | ↓Hypothalamus         |
| SIRT2   | ↑Adipose tissue  |                       |
| SIRT3   | ↑Liver, skeletal muscle, white and brown adipose           |                       |
| SIRT5   | ↑Liver   |                       |

hypothalamus.<sup>45-48</sup> SIRT2 increases in adipose tissue.<sup>9</sup> SIRT3 is upregulated in the liver, skeletal muscle, and white and brown adipose tissue.<sup>49-52</sup> SIRT5 increases in the liver.<sup>16,53</sup> The calorie restriction-induced changes in expression of sirtuins appears to be a coordinated adaptive response to what might be described as calorie restriction



sedentary individuals, but equally elevated regardless of age in endurance-trained individuals, suggesting that exercise increases SIRT3 expression in skeletal muscle and counters age-associated declines in SIRT3 expression.<sup>67</sup> Similarly, Palacios et al reported exercise training increasing SIRT3.<sup>13</sup>

### Alcohol Consumption and Smoking

Alcohol has been variously reported as increasing SIRT1 expression in the liver of rats<sup>68</sup> and decreasing its expression.<sup>69,70</sup> More research is needed to clarify which response occurs, and whether a similar response occurs in humans. Treatment of human endothelial cells with red wine increases SIRT1.<sup>71</sup> Red and white wine have both been reported to increase SIRT1 in cardiomyocytes.<sup>72</sup> Short-term supplementation with red wine has also been reported to significantly increase vascular SIRT1 subsequent to physical training in mice.<sup>73</sup>

Several studies indicate cigarette smoke exposure alters sirtuins, specifically SIRT1. Exposure of monocyte-macrophage cells to a cigarette smoke extract causes dose- and time-dependent decreases in SIRT1 activity and levels.<sup>74,75</sup> The same response occurs in human lung epithelial cells<sup>76</sup> and human umbilical vein endothelial cells<sup>77</sup> exposed to cigarette smoke extract. Decreased SIRT1 has also been observed in the lungs of rats exposed to cigarette smoke.<sup>75,76</sup> In humans, smokers have decreased levels of SIRT1 in lung macrophages compared with nonsmokers.<sup>74</sup>

### Resveratrol and Other Plant Compounds

Resveratrol influences a variety of the sirtuin-mediated responses in many types of cells. Current evidence suggests an influence in adipocytes,<sup>4,78</sup> skeletal muscle cells,<sup>79</sup> hepatocytes,<sup>80</sup> pancreatic beta cells,<sup>81</sup> renal cells,<sup>82</sup> cardiomyocytes,<sup>83</sup> endothelial cells,<sup>84</sup> vascular smooth muscle cells,<sup>85</sup> brain and neuronal cells,<sup>86,87</sup> skin cells,<sup>88,89</sup> osteoblasts,<sup>90</sup> and lung and monocyte-macrophage cells.<sup>74</sup>

SIRT1 is not the only sirtuin influenced by resveratrol *in vitro*; exposure of cardiomyocyte cells to resveratrol caused rapid activation of SIRT1, -3, -4, and -7.<sup>91</sup>

While much research suggests a direct impact of resveratrol, other research has suggested that resveratrol might act to indirectly increase SIRT1 activity by affecting other metabolic pathways that then induce SIRT1.<sup>92</sup> Other evidence is

suggestive of a sirtuin-mimetic effect. Rather than directly or indirectly activating the sirtuin system, it might act on some of the same proteins, such as AMP-activated protein kinase (AMPK), that sirtuins target, influencing posttranscriptional activity of these proteins in ways that are similar to the sirtuin system.<sup>93-95</sup> Overall, *in vitro* and *in vivo* evidence is supportive of resveratrol impacting the sirtuin system, resulting in some of its physiological effects. Functional research on resveratrol will be reviewed in Part 2 of this review.

Several other plant compounds reportedly have effects on the sirtuin system. In mice quercetin increases SIRT1 in skeletal muscle and the brain.<sup>96</sup> In humans quercetin also increases SIRT1 in skeletal muscle.<sup>97</sup> In both of these instances, the quercetin-induced increase in SIRT1 was associated with improved exercise performance.<sup>96,97</sup>

A persimmon proanthocyanidin product, made using a mixture of freshly crushed unripened persimmon fruits and dried green tea leaves, increases SIRT1 in human fibroblast cells *in vitro*<sup>98</sup> and increases SIRT1 expression *in vivo* in the mouse brain.<sup>99</sup>

**Table 4. Protein Targets of the Less-Researched Sirtuins**

| Sirtuin | Protein Targets   |
|---------|---|
| SIRT2   | PPAR $\gamma$ , FOXO1, p53, p300, $\alpha$ -tubulin, histone H4, and homeobox transcription factor (HOXA10) <sup>6,9,114-120</sup>  |
| SIRT3   | AMPK, PGC-1 $\alpha$ , UCP1, acetyl-CoA synthetase (AceCS2), glutamate dehydrogenase, isocitrate dehydrogenase 2, forkhead box O3a (FOXO3a), mitochondrial ribosomal protein L10 (MRPL10), p53, and Ku70 <sup>14,51,121-126</sup> |
| SIRT5   | cytochrome c and carbamoyl phosphate synthetase 1 (CPS1) <sup>16,53,123</sup>   |
| SIRT6   | histone H3, tumor necrosis factor- $\alpha$ (TNF- $\alpha$ ), and possibly PPAR <sup>17,127,128</sup>   |
| SIRT7   | p53 and RNA polymerase I (Pol I) <sup>20,21</sup>   |

NOTE: SIRT4 has no deacetylase activity, although, secondary to its ADP-ribosyltransferase activity, it is involved in regulating insulin secretion by beta cells, at least in part by impacting the activity of glutamate dehydrogenase.<sup>15,129</sup>

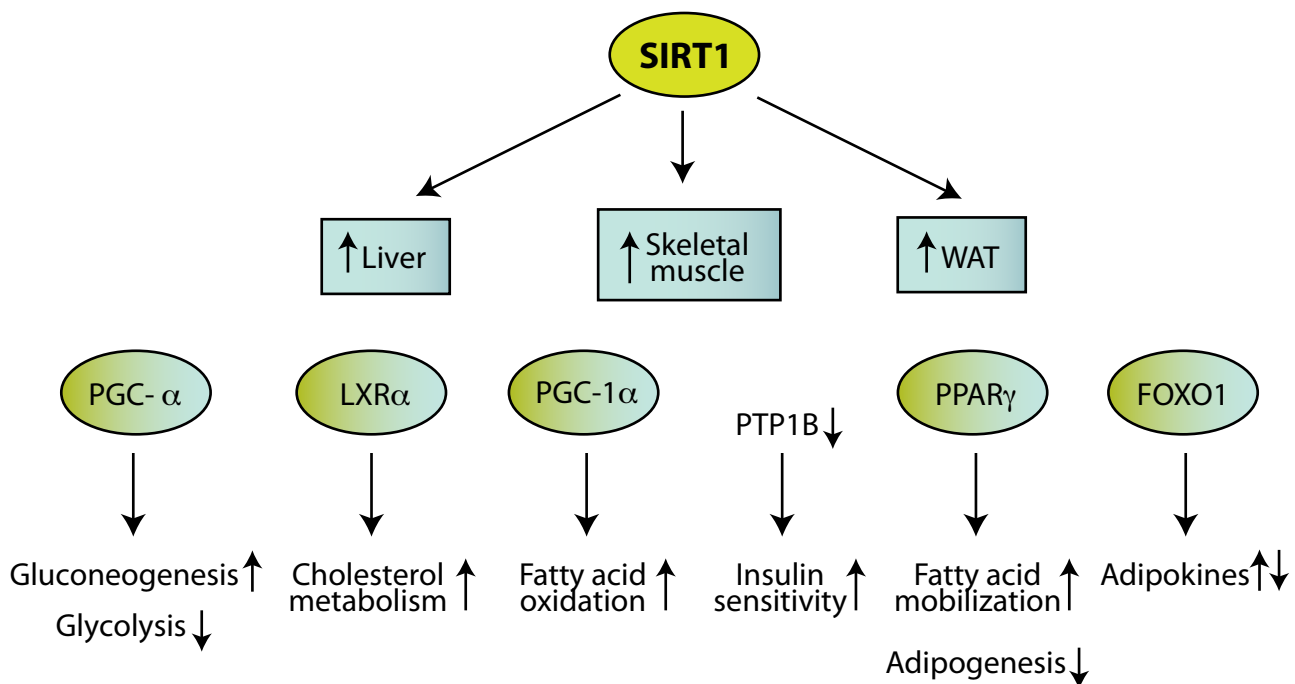
## Regulator Proteins

Acetylation and deacetylation of proteins is believed to be a fundamental regulator of the posttranslational modification of certain proteins, with the relative balance between acetylation/deacetylation adjusting biological activity of these proteins. The sirtuin system plays a significant role in this balance. Most of the sirtuin enzymes are deacetylases, enzymes which are capable of switching other proteins on and off. Because of this ability to deacetylate other proteins and hence activate and inactivate a variety of important proteins, sirtuins play an important regulatory role in many biological processes. Existing evidence suggests that, while there is some overlap in the proteins the various sirtuins deacetylate, each of the six sirtuin deacetylase enzymes (SIRT1, -2, -3, -5, -6, and -7) plays a distinct and different role in protein deacetylation by virtue of their subcellular location, tissue distributions, and protein affinities.

Of all the sirtuins, SIRT1 has received the most research attention and its impact on other proteins is best understood.

SIRT1-mediated deacetylase reactions are predominantly nuclear and target numerous proteins in a wide array of tissues, impacting the subsequent biological activity of these proteins. Proteins identified as being targets of SIRT1 include peroxisome proliferator-activated receptor-gamma (PPAR- $\gamma$ ) and its transcriptional coactivator PPAR- $\gamma$  coactivator-1alpha (PGC-1 $\alpha$ ), forkhead transcription factors (FOXO1 and FOXO3), poly(ADP-ribose) polymerase 1 (PARP1), AMPK, apurinic/aprimidinic endonuclease-1 (APE1), asymmetric dimethylarginine (ADMA), angiotensin II type 1 receptor (AT1R), estrogen receptor alpha (ER $\alpha$ ), androgen receptor, sterol regulatory element-binding protein 1 (SREBP-1), signal transducer and activator of transcription 3 (STAT3), uncoupling protein 2 and -3 (UCP2 and UCP3), p53,

**Figure 3. Calorie Restriction and SIRT1-Associated Tissue Effects**



This illustrates the SIRT1 response to fasting (calorie restriction) and the different proteins, as well as the metabolic responses they elicit.

Adapted from: Imai S. The NAD World: a new systemic regulatory network for metabolism and aging – SIRT1, systemic NAD biosynthesis, and their importance. *Cell Biochem Biophys* 2009;53:65-74.

hairly/enhancer-of-split related with YRPW motif protein 2 (HEY2), nuclear factor-kappaB (NF-κB), phosphoenolpyruvate carboxylase kinase (PEPCK), fructose-1,6-bisphosphatase (FBPase), glucose-6-phosphatase (G6Pase), histones H1, H3, and H4, and circadian clock regulator genes including brain and muscle aryl hydrocarbon receptor nuclear translocator (AARNT)-like 1 (BMAL1), cryptochrome 1 (CRY1), period 2 (PER2), and RAR-related orphan receptor gamma (RORγ).<sup>2,54-56,60,71,75,80,100-113</sup>

Because less research has been conducted on the other sirtuins, less is known about the proteins these deacetylase enzymes target. The limited existing research suggests some protein targets for these other sirtuins (Table 4).

Some of the target proteins of sirtuins are active when they are acetylated molecules and deactivated by sirtuins. PGC-1α is an example. Via SIRT1-mediated deacetylation, PGC-1α is inactivated, which has a cascading effect on other proteins that are influenced by PGC-1α. Circumstances that result in decreased SIRT1 result in decreased deacetylation of PGC-1α, making it and proteins it upregulates become more active. Conversely, increased SIRT1 activity reduces PGC-1α and downregulates PGC-1α-influenced proteins.<sup>46,56-58</sup> A similar relationship exists with other proteins that are active in an acetylated form, including PPAR-γ, SREBP-1, NF-κB, UCP2, and p53, with specific sirtuins deacetylating, and thus reducing, the activity of these proteins and downstream proteins.<sup>75,102,112,113,130-136</sup>

While some proteins are active when they are in an acetylated form, others become active after they are deacetylated. Sirtuins can play a role in activating these proteins by deacetylating them. An example of this is seen with SIRT5 and carbamoyl phosphate synthetase 1 (CPS1). CPS1 is the first and rate-limiting step of the urea cycle. Deacetylation of CPS1 activates its enzymatic activity, catalyzing the condensation of ammonia with bicarbonate to form carbamoyl phosphate.<sup>16,53</sup>

### Effects of Fasting on Protein Regulators

By modulating protein activity, sirtuins are capable of regulating a variety of metabolic pathways that augment cellular and systemic adaptive responses to stress. This is seen with fasting, a form of nutritional stress. Fasting induces SIRT1 expression in the liver, skeletal muscle, and white adipose. Locally, SIRT1 (elevated by fasting) interacts with and deacetylates a variety

of proteins in these tissues, including PPAR-γ, and PGC-1α (Figure 3). Deacetylation of these two proteins shifts biological function in adaptive ways that counter the nutritional stress.

In the liver, PGC-1α expression influences proteins involved in gluconeogenesis, glycolysis, and fatty acid beta-oxidation. The decrease in active (acetylated) PGC-1α by increased SIRT1 deacetylation activity initiates a cascading effect on these other proteins, which leads to a relative increase in gluconeogenesis and fatty acid beta-oxidation, with a corresponding decrease in glycolysis. The net effect is an increase in hepatic glucose output that helps maintain glucose homeostasis and an increased use of fat for energy, both of which are adaptive responses during fasting.<sup>56-58</sup>

In skeletal muscle, SIRT1 deacetylation of PGC-1α is required for activation of mitochondrial fatty acid oxidation genes that, as the name implies, induce fatty acid oxidation and help maintain ATP production in response to lower glucose from fasting.<sup>46</sup>

In white adipose tissue, SIRT1-induced deacetylation of PPAR-γ alters the expression of PPAR-γ-influenced genes, shifting metabolism in favor of lipolysis and promoting free fatty acid mobilization, which can then be used for energy.<sup>48</sup> Fasting also increases the expression of SIRT2 in white adipose tissue, where it deacetylates FOXO1 and PPAR-γ and is involved in promoting the shift in favor of increased lipolysis.<sup>9</sup>

SIRT3 expression is upregulated during fasting in the liver and skeletal muscle. One result of this upregulation is seen with acetyl-CoA synthetase (AceCS2). SIRT3 deacetylates (and activates) AceCS2 in the mitochondria. This catalyzes the conversion of acetate to acetyl-CoA and enables tissues to utilize acetate more efficiently during fasting conditions.<sup>52</sup> Upregulation of SIRT3 during fasting appears to ensure that higher fatty acid oxidation in the mitochondria can occur efficiently. If not, intermediate byproducts and triglycerides can accumulate. This is apparent in mice lacking both SIRT3 alleles. These mice appear phenotypically normal under basal conditions, but show marked hyperacetylation of several mitochondrial proteins, including long-chain acyl coenzyme A dehydrogenase (hyperacetylation reduces its enzyme activity), during fasting. The result is reduced ATP levels and higher levels of fatty acid oxidation intermediate products and triglycerides that accumulate in the liver.<sup>49-51</sup> SIRT3 is also



upregulated in brown adipose with fasting, where it apparently helps with adaptive thermogenesis, allowing the organism to better tolerate cold exposure during fasting periods.<sup>49,124</sup>

Fasting also increases SIRT5 in the liver, which upregulates CPS1 activity. By upregulating CPS1, the increased ammonia generated during fasting is converting into urea.<sup>16,53</sup> While this does not completely describe the entirety of the sirtuin-mediated response to fasting, it highlights how the sirtuins are epigenetically modified (by fasting in this case) and how this change in sirtuin expression and activity modulates the activity of other proteins in multiple tissues (some of which themselves have effects on other proteins). The net result of the sirtuin response to fasting is a cascading effect on the activity of a wide variety of proteins in a diversity of tissues that ultimately allows the organism to coordinate an adaptive response to fasting.

### Circadian Variations

SIRT1 deacetylation appears to vary in a circadian manner (circadian variation of other sirtuins has not been investigated), which plays a counter-regulatory role with the circadian locomotor output cycles kaput (CLOCK) protein in the mammalian circadian system. CLOCK is a gene that encodes for the CLOCK enzyme – a histone acetyltransferase. The CLOCK histone acetyltransferase is required for the circadian expression of many genes. Its expression varies rhythmically. SIRT1 activity also varies in a circadian manner, and secondary to its role as a histone deacetylase, counteracts the activity of CLOCK. This rhythmic oscillation of acetylation (by CLOCK) and deacetylation (by SIRT1) impacts the circadian acetylation of a variety of other proteins, including key circadian regulatory genes like BMAL1 and Per1, -2, and -3.<sup>137-139</sup> SIRT1 also appears to be able to directly deacetylate BMAL1 or Per2 proteins, regulating the amplitude and the duration of their circadian gene expression.<sup>101,102</sup> The net effect is that, by counterbalancing the rhythmic acetylation activity of CLOCK, SIRT1 influences the circadian oscillations of many proteins.

### Regulated Proteins

Sirtuins are regulator proteins, but they are also regulated by other proteins that influence their expression. Deleted in breast cancer-1 (DBC1) is an example of a protein that appears to regulate sirtuins. In experiments, DBC1 modulates SIRT1 activity in multiple cell lines and tissues, with decreased DBC1 expression increasing SIRT1

activity, while increased DBC1 expression decreases SIRT1. The impact of DBC1 on modulating SIRT1 appears to be a critical component dictating the biological response to changes in diet. In mice, as an example, a high-fat diet promotes DBC1 expression and fatty liver disease. This process is associated with reduced expression of SIRT1 in the liver, presumably because of the DBC1 counter-regulatory activity on SIRT1. The importance of DBC1 is observed in mice bred to have a genetic deletion of DBC1. In these mice, SIRT1 activity is increased in several tissues, including the liver. These DBC1-deficient mice, despite becoming obese on a high-fat diet, are protected from diet-induced fatty liver disease.<sup>140</sup>

p300 is another protein that appears to have some sirtuin regulatory actions. Under experimental conditions, acetylated p300 downregulates the deacetylation activity of SIRT2.<sup>141</sup> But SIRT2 can also deacetylate p300.<sup>115</sup> This suggests that SIRT2 is capable of regulating and being regulated by p300.

A complicated regulator and regulated relationship might exist for other sirtuins as well, with evidence suggesting this is the case with insulin and SIRT1. Insulin and insulin-like growth factor 1 (IGF-1) attenuate the calorie-restriction increase in SIRT1 expression in rats.<sup>36</sup> Some evidence suggests that insulin might have more complicated interactions with SIRT1 that might be influenced by glucose levels. *In vitro*, insulin has biphasic effects on SIRT1, depending on glucose levels. Insulin evoked a massive upregulation of SIRT1 in the absence of sufficient ambient glucose, but had an opposite effect when glucose levels were high.<sup>142</sup> As a generality, SIRT1 is downregulated in insulin-resistant cells and tissues. And circumstances that inhibit or reduce the expression of SIRT1 induce insulin resistance. Conversely, circumstances that result in increased expression of SIRT1 improve insulin sensitivity, especially under insulin-resistant conditions.<sup>143</sup>

Sirtuin enzyme activity is also influenced post-transcriptionally by phosphorylation/dephosphorylation enzymes. Cell cycle-dependent kinases form complexes with and phosphorylate SIRT1, increasing its enzyme activity. Dephosphorylation by phosphatases decreases SIRT1 deacetylase activity.<sup>144,145</sup>

## Interactions with NAD<sup>+</sup> and its Metabolites and Substrates

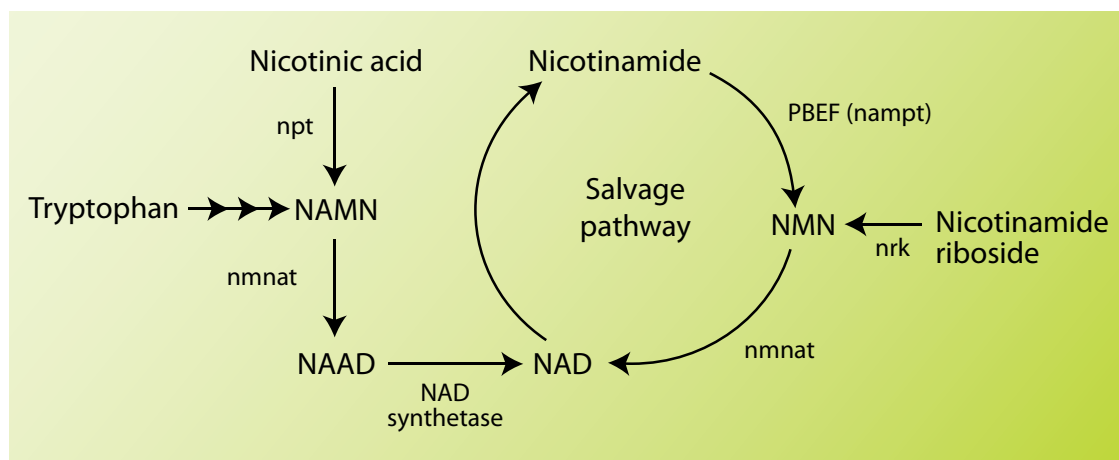
All sirtuin enzymes have an absolute dependence on the presence of the coenzyme NAD<sup>+</sup> as a cosubstrate for their activity. Sirtuin enzyme activity consumes NAD<sup>+</sup> (as does the increased activity of some of its targeted proteins) and produces nicotinamide (also known as niacinamide or nicotinic acid amide), the amide form of vitamin B<sub>3</sub>, as a byproduct.<sup>146,147</sup>

In sirtuin-NAD<sup>+</sup> reactions, the endogenously formed nicotinamide acts as an inhibitor of further sirtuin activity by promoting a base-exchange reaction – chemical reversal of a covalent reaction intermediate – at the expense of deacetylation or ribosyltransferase reactions.<sup>148,149</sup> Nicotinamide is not alone in this inhibitory activity. All metabolites of NAD<sup>+</sup> cause some inhibition of sirtuin reactions under experimental conditions, presumably by creating competition at the coenzyme binding site. However, of all NAD<sup>+</sup> metabolites tested, nicotinamide is the most potent inhibitor, inhibiting sirtuin activity *in vitro* and *in vivo*.<sup>150-153</sup> The inhibition caused by nicotinamide (and other

metabolites) affects sirtuin enzyme activity without affecting gene expression or the amount of sirtuin proteins.<sup>154</sup> These recognitions have resulted in interest in better understanding how strategies, including supplementing different forms of vitamin B<sub>3</sub> aimed at augmenting NAD<sup>+</sup> or using nicotinamide as a sirtuin inhibitor, impact sirtuins.<sup>146,147,155</sup> This section will summarize current understandings.

In mammals, NAD<sup>+</sup> is either synthesized *de novo* from the precursor amino acid tryptophan or recycled from compounds with an existing nicotinamide ring (nicotinic acid [niacin], nicotinamide, or the riboside forms of these vitamins) by one of several salvage pathways. Salvage pathways are used to produce NAD<sup>+</sup> from these nicotinamide ring compounds, whether these compounds are from the diet (dietary vitamin B<sub>3</sub>) or are formed endogenously (e.g., nicotinamide in the sirtuin-NAD<sup>+</sup> reaction). The salvage pathways for nicotinic acid, nicotinamide, and nicotinamide riboside, and the enzymes they use, are listed below. *De novo* synthesis of tryptophan and where it feeds into NAD<sup>+</sup> synthesis is also shown (Figure 4).

Figure 4. Mammalian NAD<sup>+</sup> Metabolism



The figure shows *de novo* NAD<sup>+</sup> synthesis and salvage pathways in humans. NA indicates nicotinic acid; NAMN, nicotinic acid mononucleotide; NAAD, nicotinic acid adenine dinucleotide; NR, nicotinamide riboside; NMN, nicotinamide mononucleotide; NAM, nicotinamide; npt, nicotinic acid phosphoribosyltransferase; nmnat, nicotinic acid/nicotinamide mononucleotide adenyltransferase; nrk, nicotinamide riboside kinase; nampt, nicotinamide phosphoribosyltransferase; PBEF (nampt), pre-B-cell enhancing factor; PARPs, poly(ADP-ribose) polymerases.

Adapted From: Yang T, Sauve AA. NAD metabolism and sirtuins: metabolic regulation of protein deacetylation in stress and toxicity. *AAPS Journal* 2006;8:E632-E643.

- ◆ Nicotinic acid is converted into nicotinic acid mononucleotide (NAMN) using nicotinic acid phosphoribosyltransferase. NAMN is then converted into nicotinic acid adenine dinucleotide (NAAD) via niacinamide/nicotinic acid mononucleotide adenyltransferase (NMNAT). NAAD is converted into NAD<sup>+</sup> using a glutamine-dependent NAD<sup>+</sup> synthetase.
- ◆ Nicotinamide is converted into nicotinamide mononucleotide (NMN) by nicotinamide phosphoribosyltransferase (NAMPT). NMN is converted into NAD<sup>+</sup> using the same (shared) enzyme NMNAT as in the nicotinic acid salvage pathway.
- ◆ Nicotinamide riboside is converted into NMN by nicotinamide riboside kinase. NMN is converted into NAD<sup>+</sup> using the same (shared) enzyme NMNAT as in the nicotinic acid salvage pathway.
- ◆ Tryptophan, through a series of steps, is converted into NAMN. Once NAMN is formed, it follows the same enzymatic steps as described with nicotinic acid.<sup>146,147,156-158</sup>

There is also a conserved metabolic pathway that allows for deamidation of nicotinamide to nicotinic acid in mammals. Despite the existence of this pathway, *in vivo* nicotinamide is used predominantly as a precursor for NAD<sup>+</sup> biosynthesis.<sup>159-161</sup> Note: Nicotinamide and nicotinic acid can be converted into metabolites that can be eliminated from the body.<sup>162-164</sup>

In mammals, nicotinic acid, nicotinamide, and nicotinamide riboside are incorporated into NAD<sup>+</sup> via distinct metabolic pathways that share one common enzyme (NMNAT), that can accept either NAMN (from the nicotinic acid pathway) or NMN (from the nicotinamide or nicotinamide riboside pathways) as substrates.<sup>165</sup> Existing evidence indicates that incorporation of these compounds into NAD<sup>+</sup> differs *in vivo*. Collins and Chaykin reported that nicotinamide was a better precursor for NAD<sup>+</sup> than nicotinic acid in mice.<sup>166</sup> Jackson et al reported a more complicated and tissue-specific fate of these forms of vitamin B<sub>3</sub>, with nicotinic acid appearing to be a more favorable substrate. In their rat experiments, nicotinamide and nicotinic acid administration increased tissue levels of NAD<sup>+</sup> in red blood cells, liver, heart, lung, and kidneys. Nicotinamide had a robust effect on increasing NAD<sup>+</sup> in red blood cells and the liver, and a lesser effect in heart, lung, and kidneys. Nicotinic acid increased NAD<sup>+</sup> concentrations in liver and blood to a similar degree as nicotinamide, but it had a much

more pronounced effect in the heart and kidney than in the other tissues; it had a three- to six-fold greater effect on heart and kidney NAD<sup>+</sup> than nicotinamide.<sup>167</sup> Nicotinamide riboside can also increase NAD<sup>+</sup> *in vivo*;<sup>168,169</sup> however, its effects on NAD<sup>+</sup> have not been compared to those of nicotinamide or nicotinic acid. It is currently not clear why nicotinamide appears to be a better precursor for NAD<sup>+</sup> than nicotinic acid in mice but not in rats. Nor is it clear why the changes in tissue levels of NAD<sup>+</sup> vary to such a significant degree in response to nicotinamide or nicotinic acid, or why nicotinamide and nicotinic acid appear to be utilized to increase NAD<sup>+</sup> biosynthesis to the same degree in some tissues but not others. It also has not been established whether riboside forms of these vitamins offer any distinct advantage in NAD<sup>+</sup> generation.

Although what form of vitamin B<sub>3</sub> produces the best *in vivo* NAD<sup>+</sup> generation and in which tissues is currently unclear, it does appear clear there are circumstances that can place increased demands on NAD<sup>+</sup> supplies. Greater activity of the sirtuin system is one such circumstance, with increased sirtuin enzyme activity consuming available NAD<sup>+</sup>.<sup>165</sup> Without sufficient NAD<sup>+</sup> available to meet this demand, the physiological sirtuin response is blunted and can, if taken to an extreme, result in a loss of sirtuin-dependent extended longevity that normally occurs in yeast with calorie restriction.<sup>170</sup>

Some of the same factors that influence sirtuins also influence NAD<sup>+</sup> levels and salvage pathway enzyme activity. In yeast, NAD<sup>+</sup> metabolism is dynamically regulated in response to nutritional stressors, including salt restriction, amino acid deprivation, and calorie restriction.<sup>171</sup> Fasting, a form of stress that increases SIRT1 activity in the liver, increases liver NAD<sup>+</sup> concentrations by about 50 percent in mice. The increase in liver NAD<sup>+</sup> concentration is linked to the increased SIRT1 enzyme activity and appears to be needed to support some of the adaptations to fasting previously described, including enhanced deacetylation of PGC-1 $\alpha$  and the shift to increased gluconeogenesis this protein prompts.<sup>57</sup> Conversely, NAD<sup>+</sup> concentrations decrease in the pancreas of fasted mice. This local decrease in NAD<sup>+</sup> occurs in combination with, and might be influencing, decreased pancreatic SIRT1 activity, which in turn influences the activity of other proteins in the pancreas, such as UCP2, in response to fasting.<sup>132</sup> In animals and humans, blood NAD<sup>+</sup> levels are increased with moderate activity, but decreased following intense

exercise. In mice, administration of nicotinamide improves the endurance capacity when exercise is intense, presumably because it helps maintain NAD<sup>+</sup>.<sup>172</sup>

NAMPT, also known as pre-B-cell colony-enhancing factor 1 (PBEF1) or visfatin, is encoded by the PBEF1 gene in humans. It is considered a rate-limiting enzyme for the nicotinamide to NAD<sup>+</sup> salvage pathway. As previously mentioned, it catalyzes a reaction that converts nicotinamide into its mononuclear (NMN) form. Increasing this particular salvage pathway has the theoretical dual benefit of increasing NAD<sup>+</sup> availability for sirtuin enzyme activity, while reducing nicotinamide (the byproduct that if allowed to build up would inhibit sirtuin enzymes) by using it to generate NAD<sup>+</sup>. Circumstances including fasting, glucose deprivation, and exercise training have been reported to increase the expression of the PBEF1 gene, increase NAMPT activity, and consequently support increased sirtuin activity (SIRT1, SIRT3, SIRT4, and SIRT6 have been increased in different studies). Biological responses to the activation of this salvage pathway include resistance to oxidative stress, protection against cell death, cellular senescence resistance, and lengthened replicative life-spans.<sup>18,151,173-176</sup> Salvaging endogenous nicotinamide riboside appears to be essential for calorie restriction-induced life span extension and stress resistance in yeast,<sup>177</sup> suggesting that regulation of other vitamin B<sub>3</sub> salvage pathways might also play a role in the sirtuin adaptive response to stress. When increased activity of sirtuin enzymes results in an increased cellular consumption of NAD<sup>+</sup>, evidence is suggestive of upregulation of salvage pathways playing a critical role in the sirtuin response.

The limited available evidence suggests that there is less NAMPT activity (the nicotinamide salvage pathway) in pancreatic beta-cells and brain (neurons) compared to other tissues.<sup>147,160,178</sup> Therefore, it has been suggested that these two tissues might be particularly vulnerable to enough NAD<sup>+</sup> depletion to compromise sirtuin enzyme needs.<sup>147</sup>

Although nicotinamide is a substrate for NAD<sup>+</sup>, there is also ample *in vitro* and *in vivo* evidence that it is an inhibitor of sirtuin enzyme activity.<sup>46,171,179-182</sup> *In vitro*, nicotinamide also attenuates some of resveratrol's actions, presumably because it inhibits sirtuin enzyme activity.<sup>91</sup> Which role nicotinamide plays – NAD<sup>+</sup> precursor or sirtuin deacetylase enzyme inhibitor – might be context

dependent. SIRT1 deacetylase activity has been shown to be NAD<sup>+</sup> dependent in cultured cortical neurons. If cultured neurons are treated with nicotinamide, SIRT1 deacetylase activity is attenuated. In this context, nicotinamide appears to be acting preferentially to inhibit SIRT1 activity rather than acting as a NAD<sup>+</sup> substrate. If cultured neurons are exposed to an excitotoxic insult (glutamate and N-methyl-D-aspartate [NMDA] treatment), cell viability is drastically reduced. There is also a significant and concomitant decrease of cellular NAD<sup>+</sup> and SIRT1 deacetylase activity that peaks between 4-6 hours and persists for 24 hours. In this context, treating these neurons with nicotinamide preserves NAD<sup>+</sup> levels and SIRT1 deacetylase activity. It also results in improved cell survival. While nicotinamide is both a NAD<sup>+</sup> precursor and SIRT1 enzyme inhibitor, in the context of excitotoxic stress, it appears to be used preferentially to prevent depletion of NAD<sup>+</sup>.<sup>154</sup>

Another circumstance where nicotinamide appears to be preferentially used to form NAD<sup>+</sup> rather than inhibit sirtuin activity is the time period immediately following an ischemia-induced stroke. Ischemia is an example of a stress that causes a sirtuin adaptive response in brain tissue. SIRT1 activity increases as a result, with the increased activity placing demands on and consuming available NAD<sup>+</sup>. Supplying exogenous nicotinamide in the six hours following ischemia-induced stroke in mice prevents this depletion of NAD<sup>+</sup> (presumably because the salvage pathway is upregulated as part of the adaptive response to ischemia) and appears to be neuroprotective.<sup>154</sup> In this particular situation, rather than inhibiting sirtuin enzyme activity, exogenous nicotinamide appears to be needed to maintain SIRT1 deacetylase activity. It is probable that there are other circumstances (including calorie restriction and other sources of stress reported to increase salvage pathway enzyme activity) where NAD<sup>+</sup> is being consumed at higher than normal rates, but that the nicotinamide being formed in these NAD<sup>+</sup>-sirtuin deacetylase enzyme reactions is being recycled aggressively into NAD<sup>+</sup>. This area requires further research attention and it remains to be elucidated in what circumstances, with what timing, at what doses, and in what tissues supplying nicotinamide would interact positively with sirtuins to promote the most beneficial adaptive responses.

Supplying exogenous nicotinamide is one potential strategy for ensuring that sufficient vitamin B<sub>3</sub> is available for NAD<sup>+</sup> biosynthesis, and



hence sirtuin deacetylase enzyme activity; however, this has the theoretical disadvantage associated with its role as an inhibitor of this same enzyme. Nicotinic acid can also be used for NAD<sup>+</sup> synthesis and, at least in theory, does not have the same inhibitory activity. While nicotinic acid supplementation has not been studied for its effects on sirtuin enzyme activity *in vivo* in mammals, at least some evidence suggests that it has an equal or better effect on increasing NAD<sup>+</sup> in various tissues in the body under basal (nonstressed) conditions.<sup>167</sup> This evidence was from rats and is inconsistent with another study in mice in which radio-labeled nicotinic acid and nicotinamide were used (the nicotinamide reportedly used preferentially for NAD<sup>+</sup> generation).<sup>166</sup> Human data on the relative capacity of nicotinic acid versus nicotinamide to produce NAD<sup>+</sup> are sparse. In one study using humans cells, the addition of nicotinic acid, but not nicotinamide, almost doubled cellular NAD<sup>+</sup>; it also decreased oxidative stress-induced cytotoxicity.<sup>183</sup> Nicotinic acid supplementation (50 and 100 mg/day for 14 weeks) has been reported to increase lymphocyte NAD<sup>+</sup> concentrations in smokers;<sup>184</sup> thus, there is reason to believe that it can effectively generate NAD<sup>+</sup> in humans.

The same theoretical advantage exists for nicotinamide riboside as with nicotinic acid. This form of vitamin B<sub>3</sub> does increase yeast SIR2 (the eukaryote homolog of SIRT1) activity, resulting in improved gene silencing and replicative lifespan extension.<sup>168,185</sup> In mammalian cells, nicotinamide riboside increases NAD<sup>+</sup>.<sup>186</sup> Whether or not exogenous nicotinamide riboside would have the desired effects on NAD<sup>+</sup> to sustain sirtuin deacetylase activity in humans is unknown.

NAD<sup>+</sup>-consuming reactions, including sirtuin reactions, can potentially deplete NAD<sup>+</sup>. This appears to occur rapidly in some cells exposed to stress and toxicity, while in others the salvage pathway activity is upregulated and is sufficient to maintain NAD<sup>+</sup> levels. While augmenting NAD<sup>+</sup> by supplying some form of vitamin B<sub>3</sub> has been proposed, it is thought that NAD<sup>+</sup> levels might be tightly regulated *in vivo* in tissue-specific, and possibly even subcellular-specific, manners, and that this might complicate responses to vitamin B<sub>3</sub>.<sup>147,165</sup> There is currently an incomplete understanding of how tissue levels change in response to external factors. Even less is known about subcellular metabolism of NAD<sup>+</sup>. There is also an incomplete understanding about how NAD<sup>+</sup> metabolism might be manipulated for therapeutic benefit. As

an example, it is not known how effective exogenously supplied substrates for NAD<sup>+</sup> – nicotinamide as an example – are in terms of arriving at and impacting NAD<sup>+</sup> and sirtuin activity in, for example, the mitochondria of a cardiomyocyte, as opposed to the myelin of a neuron or the cytoplasm of an adipocyte.

There is still little known about in which tissues and under what circumstances NAD<sup>+</sup> is consumed by sirtuins at rates that result in insufficient sirtuin deacetylase activity. Also, there is no research comparing different forms of vitamin B<sub>3</sub> to determine which form might be better for maintaining sirtuin activity. For these reasons, it is difficult to make definitive recommendations about supplementing vitamin B<sub>3</sub> to optimize sirtuin adaptive responses. It is possible that nicotinamide and nicotinic acid might have differing *in vivo* effects on sirtuin activity in different tissues. It is also possible that these effects could change based on external factors that activate sirtuins, such as stress and toxicity. Based on current understandings, supplying nicotinic acid might be a safer choice if the goal is to support NAD<sup>+</sup> formation and minimize risk of sirtuin enzyme inhibition.

## Conclusion

The existing evidence indicates that the mammalian sirtuin system is an incredibly complicated biological response system. At least several of the seven sirtuin genes are subject to genetic variation, and several (perhaps all) of the sirtuins respond epigenetically to a variety of environmental factors. Mechanistic and functional understanding of the mammalian sirtuin system has advanced tremendously during the past five years. This system appears to be involved in coordinating a variety of cellular responses to stress, toxicity, and other challenges, which are presumably necessary for appropriate cellular responses. Calorie restriction or fasting serves as an example. When calories are restricted – a form of nutritional stress – the expression and activity of the sirtuin system changes in a variety of tissues, including the hypothalamus, liver, skeletal muscle, and adipose tissue. These local site-specific changes in sirtuin activity deactivate some proteins and activate others. One result of this cascade is shifts in metabolism, including changes in processes such as gluconeogenesis, glycolysis, lipolysis, and thermogenesis, which allow the organism to better tolerate the period of decreased food intake. Another result appears to be behavior changes, such as increased



food seeking and appetite, related to the particular form of stress. This and other evidence suggests that the sirtuin system sits at a crossroads when it comes to initiating many of the biobehavioral changes needed for both cellular and systemic adaptation to stress and toxicity.

As research has better characterized the sirtuin system, it has become apparent that this system regulates many proteins that themselves influence a variety of cellular processes. Because of their impact on the function of a diverse array of proteins, sirtuins are involved with metabolic responses and processes that influence many aspects of human function. Existing evidence strongly supports sirtuin involvement in longevity, age-related diseases, obesity, cardiovascular and neurological function, and cancer. The sirtuin response in these clinical conditions is still being characterized. The current research in these areas will be reviewed in Part 2 of this article.

While lifestyle habits and environmental factors influence sirtuins, the most promising nutritional approaches for augmenting or inhibiting the sirtuin response appear to be resveratrol and vitamin B<sub>3</sub>. Resveratrol has generally been characterized, and supported by *in vitro* and *in vivo* evidence, as a sirtuin activator. This evidence will be discussed in Part 2 of this article.

Nicotinamide is recognized as an *in vitro* and *in vivo* inhibitor of sirtuin enzyme activity. While it can play this role, exogenous nicotinamide does not always inhibit sirtuin enzyme activity. Under certain circumstances, some of which are identical to those known to stimulate increased sirtuin activity (and hence would consume NAD<sup>+</sup>), supplying nicotinamide appears to preferentially generate NAD<sup>+</sup> needed to support the increased sirtuin activity. In these instances, rather than inhibiting sirtuin activity, exogenous nicotinamide might be needed to optimize it; in other circumstances exogenous nicotinamide might inhibit sirtuin activity. More research is required to determine which clinical situations, what timing, and at what doses nicotinamide would augment or inhibit sirtuins in ways that best help to accomplish desired therapeutic outcomes. Since nicotinic acid can be used to generate NAD<sup>+</sup> and does not have the inhibitory effects of nicotinamide, it might be a better choice when the goal is to augment sirtuin enzyme activity.

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