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Invited Review

Nicotinamide Adenine Dinucleotide, the Sirtuins, and the Secret of a Long Health Span

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Abstract

The sirtuins constitute a family of proteins with broad enzymatic activities that have an absolute requirement for nicotinamide adenine dinucleotide (NAD^+) as a cosubstrate. Evidence has been mounting over the past 15 years or so that implicates the sirtuins in a vast array of critical cellular functions. In addition to transcription silencing and DNA repair, these functions include the regulation of ion channels, neuronal functions, cell growth, circadian rhythm, inflammatory response, mitochondrial biogenesis, insulin secretion, fat oxidation, and glucose metabolism. The sirtuins are critical for maintaining mitochondrial health, energy homeostasis, and redox balance. The sirtuins are widely believed to be behind the extension of lifespan brought about by calorie restriction and fasting. Changes in sirtuins activities have been linked to most age-related pathologies including cardiovascular disease, cancer, and Alzheimer's disease (AD). NAD^+ is familiar to students of biological chemistry as an enzyme cofactor whose presence is essential for the progress of hundreds of vital biochemical reactions. This compound is present in virtually all living organisms. Simply put, without NAD+, life as we know it would not exist. The progress of these reactions requires the cleavage of NAD^+, whose intracellular levels are known to decline steadily with age. This decline and its negative impact on the activities of these enzymes and on metabolism in general are implicated in the development of metabolic and age-linked diseases. Therefore, maintaining high intracellular NAD^+ levels throughout life may not only extend the lifespan but is likely to extend the health span as well. In this paper I review how the intracellular pool of NAD^+ is maintained and summarize the functions and regulation of sirtuins activities.

Keywords: Long health span, nicotinamide adenine dinucleotide, the sirtuins

Nicotinamide Adenine Dinucleotide

Nicotinamide adenine dinucleotide (NAD^+) is one of the most abundant substances in the human body. Its average content is estimated at about 3 g. NAD^+ is an essential participant, either as an enzyme cofactor or

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as a cosubstrate, in over 500 biochemical reactions. It plays key roles in many cellular processes that are vital for cell survival including redox balance and energy homeostasis.[1,2] An adequate intracellular level of NAD^+ is particularly critical for optimal mitochondrial function. As the cell's primary hydrogen carrier, NAD^+ functions as a coenzyme that is essential for the progress of many biochemical reactions. In the course of these and other reactions, it may serve as a signaling molecule or participate in the generation of many such molecules. In those reactions where it participates as a coenzyme, NAD^+ accepts a hydride and is reduced to NADH, which is then oxidized back to NAD^+ as it loses its electron in the course of mitochondrial oxidative phosphorylation, when ATP, CO_2, H_2O, heat, and reactive oxygen species (ROS) are generated. Thus, in this redox process, there is no net loss of NAD^+ as the sum of NAD^+ plus NADH remains unchanged. NAD^+ may also be converted to the $NADP^+$/NADPH redox couple which is important for biosynthetic reactions and for protection against ROS. However, in reactions in which it serves as a cosubstrate, NAD^+ is constantly broken down and its intracellular level in adult organisms is known to decline steadily with age resulting in mitochondrial dysfunction, altered metabolism, and increased susceptibility to disease.[2,3] Mitochondrial dysfunction and oxidative stress are believed to play a prominent role in the pathogenesis of aging-associated neurodegenerative diseases. The importance of NAD^+-dependent signaling pathways lies in their ability to protect against the damage caused by oxidative stress.[4]

In addition to its role as a coenzyme in redox reactions, NAD^+ is a necessary cosubstrate in several signaling pathways in which NAD^+ is cleaved in the course of posttranslational modifications (PTMs) of proteins and the production of second messengers. An example is provided by reactions catalyzed by the ectoenzymes CD38 and CD157 in which NAD^+ is cleaved to produce nicotinamide (NAM) plus a number of important signaling compounds such as ADP-ribose (ADPR), cyclic ADP-ribose, and nicotinic acid adenine dinucleotide phosphate. All of these compounds constitute important triggers for intracellular Ca^{2+}, the oldest and most common cellular signaling system responsible for regulating a wide range of physiological functions.[5] Reactions catalyzed by poly-ADP-ribose polymerase 1 (PARP1) consume significant amounts of NAD^+, and PARP1 deletion in laboratory mice has been shown to effectively boost cellular NAD^+ levels. PARP1 is the first member of the PARPs family of enzymes that catalyze the transfer of ADP-ribose units from NAD^+ onto a target protein substrate. They are activated by DNA breaks and play a crucial role in DNA repair.[6,7]

The intracellular level of NAD^+ is determined by the balance between the rates of NAD^+ synthesis and degradation. Unless matched by a comparable rate of NAD^+ synthesis either by recycling its breakdown products or by *de novo* synthesis, NAD^+ consuming reactions result in a net loss of NAD^+ [Figure 1]. Low NAD^+ level can have serious negative consequences for many cellular functions including mitochondrial energy balance and key enzymatic systems, such as the sirtuins, the PARPs, and ectoenzymes CD38, CD78, and CD157 since their activities are dependent on the availability of NAD^+.[8] Multiple NAD^+-dependent enzymes such as the sirtuins are critical for synaptic plasticity and neuronal resistance to oxidative stress. Interfering with these processes compromises protective mechanisms, accelerates aging, and promoting neurodegenerative diseases.[3,9] As discussed in greater detail below, increased intracellular NAD^+ level increases sirtuin activity, enhances protection against oxidative stress, and lengthens both the lifespan and health span of the organism. In young adults, intracellular NAD^+ level is maintained relatively constant through recycling (salvage) and *de novo* synthesis. It should be noted that cells are unable to acquire preformed NAD^+ from the extracellular fluid.

De novo Synthesis: From L-Tryptophan to Nicotinamide Adenine Dinucleotide

Two major pathways contribute to the maintenance of the intracellular pool of NAD^+: The first and quantitatively more important is the recycling of its immediate precursors through the salvage pathway. The second is the *de novo* synthesis pathway starting with L-tryptophan (L-TRP) which takes

place mainly in the liver and kidney [Figure 2]. However, although the contribution of the *de novo* biosynthesis of NAD^+ is relatively minor, the details of the pathway are critical to understanding the pathophysiology of age-associated disorders that may be linked to inadequate intracellular NAD^+ levels.

In mammalian cells, the essential amino acid L-TRP is metabolized mainly through the kynurenine (KRN) pathway, which accounts for the catabolism of over 90% of dietary L-TRP.[10-12] The kynurenine pathway is a cascade of 8 enzymatic steps resulting in the production of a number of biologically active metabolites such as KRN, 3-hydroxy

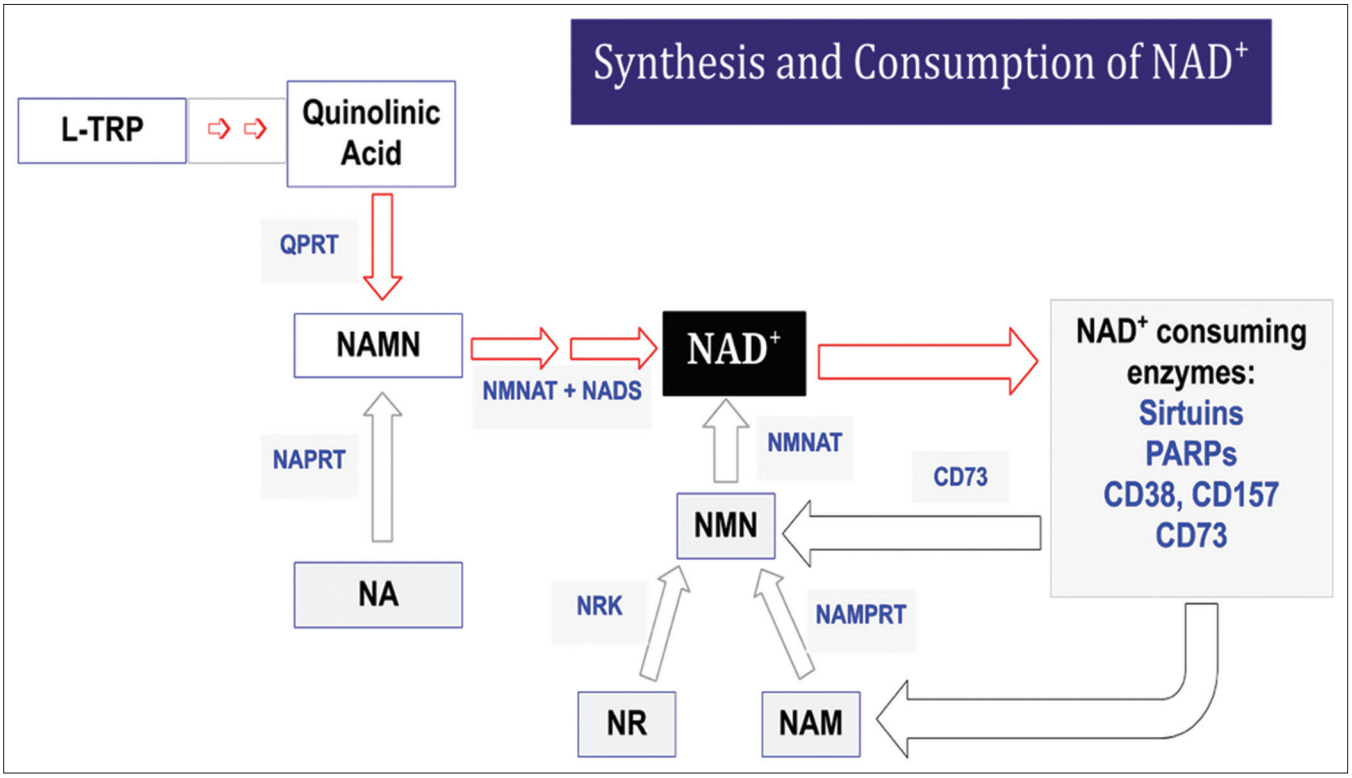

Figure 1: The balance between the rates NAD^+ synthesis and its cleavage by the nicotinamide adenine dinucleotide-consuming enzymes: Sirtuins, PARPs, and the ectozymes (CD38, CD157, and CD73)

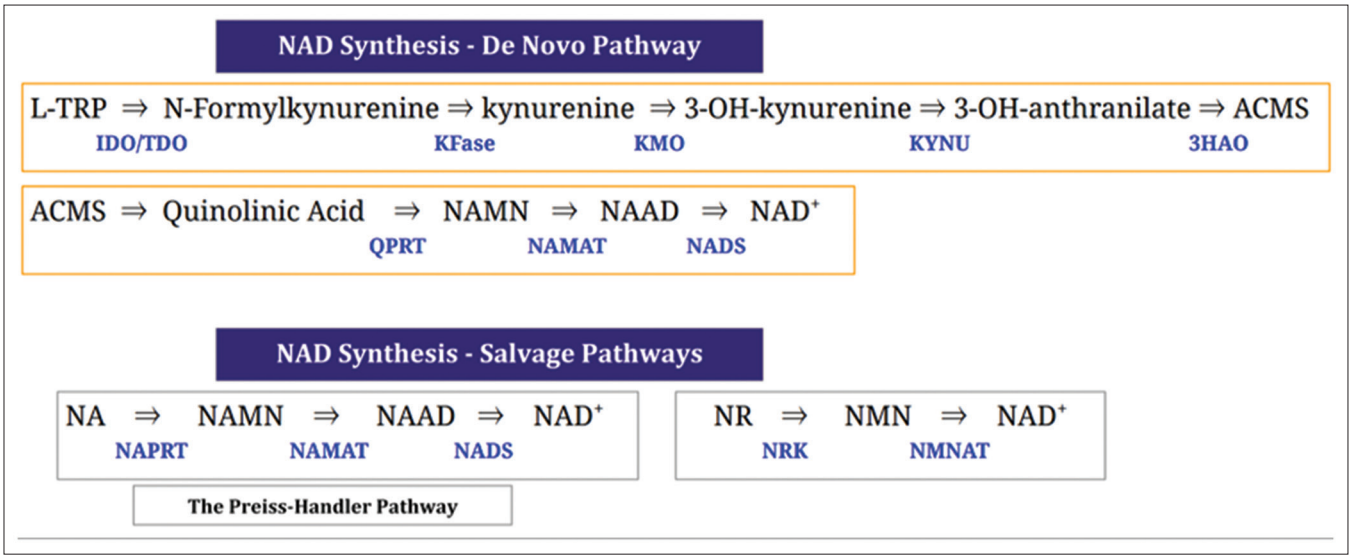

Figure 2: The two general mechanisms responsible for replenishing the intracellular pools of nicotinamide adenine dinucleotide

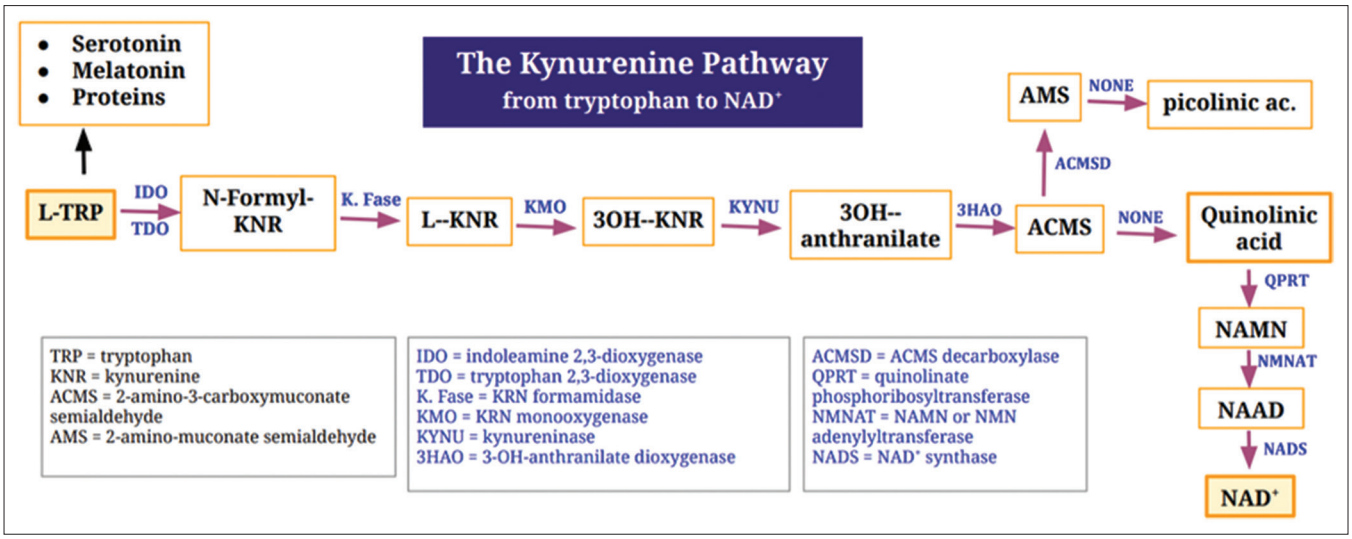

Figure 3: Details of the kynurenine pathway. Based on the subcellular location of the enzymes, these reactions take place in the cytosol

KRN (3-OH-KRN), kynurenic acid (KA), picolinic acid (PA), and quinolinic acid (QA) [Figure 3]. These metabolites play an important role in modulating the immune response, and are critical in limiting cell aging. Minor alternative channels for TRP metabolism include incorporation into proteins and the pathway leading to serotonin and melatonin. In the latter pathway, the sequence of chemical transformations consists of hydroxylation, decarboxylation, and acetylation. L-TRP > (TRPH) > 5-hydroxy-TRP > (AAAD) > serotonin > (NAT) > N-acetylserotonin > (HIOMT) > melatonin. Where: TRPH = TRP hydroxylase; AAAD = aromatic L-amino acid decarboxylase; NAT = N-acetyltransferase; HIOMT = hydroxyindole O-methyltransferase. L-TRP crosses the blood–brain barrier (BBB) through the L-amino-acid transporter. Once inside the neuron, it follows the same metabolic pathways as in peripheral tissues. In the course of L-TRP catabolism, the kynurenine pathway produces both neuroprotective and neurotoxic metabolites. The neuroprotective metabolites include KA, PA, and NAD^+, while the neurotoxic metabolites include QA and 3-OH-KRN. Elevated concentrations of these neurotoxic metabolites in the brain are believed to be at the heart of the pathophysiology of neuroinflammatory and neurodegenerative diseases such as AD, amyotrophic lateral sclerosis, Huntington's Chorea, and Parkinson's disease. When present at high (micromolar) levels in brain tissue, QA is highly toxic to neurons, astrocytes, and oligodendrocytes through multiple mechanisms of neurotoxicity.[13,14] QA is considered as an excitotoxin since it acts as an N-methyl-d-aspartate receptor agonist. It also interferes with the glutamate-glutamine cycle resulting in abnormal glutamate metabolism. In addition, it promotes lipid peroxidation which damages cell membranes and compromises the BBB. This toxic effect is attributed to the activation of nitric oxide synthase and increased rate of free radical production.[15]

The kynurenine pathway [Figure 3] begins with L-TRP, which is first converted to N-formylkynurenine in a reaction catalyzed by one of three rate-limiting enzymes. In the liver, the key enzyme is TRP 2,3-dioxygenase. In extrahepatic tissues, the reaction is catalyzed by indoleamine 2,3-dioxygenase, of which exist at least two isoforms (IDO1 and IDO2). Overexpression of these enzymes in certain cancers suggests that some kynurenine intermediates may act as signaling molecules promoting cancer progression.[16] N-formylkynurenine undergoes a series of enzymatic reactions leading to 2-amino-3-carboxymuconate semialdehyde (ACMS), a key branching point. Normally, most of the generated ACMS is decarboxylated by a specific decarboxylase (ACMSD) to form 2-amino-muconate semialdehyde (AMS). The fate of AMS is either complete oxidation through the glutarate pathway and the TCA

cycle or to undergo spontaneous (nonenzymatic) conversion to PA. A relatively small fraction of ACMS undergoes spontaneous cyclization to form QA, which will serve as a precursor of NAD^+. The rate of QA formation is increased when the capacity of ACMSD is exceeded, and more of ACMS is available for the nonenzymatic cyclization. QA is converted to nicotinic acid mononucleotide (NAMN) by a key rate-limiting enzyme, quinolinate phosphoribosyltransferase, utilizing phosphoribosyl pyrophosphate. NAMN is then channeled through the *Preiss-Handler pathway* leading to NAD^+ as shown in Figure 2. Two additional enzymes are necessary to complete the transformation of QA to NAD^+: nicotinamide mononucleotide adenylyl-transferase (NMNAT), which converts NAMN to NA adenine dinucleotide (NAAD) at the expense of ATP. There are at least three NMNAT isoforms with various organ and subcellular distributions.[16] The final step (NAAD ⇒ NAD^+) is catalyzed by NAD^+ synthetase (NADS) and requires the input of ATP as well as an amide group from glutamine.[17]

The Salvage Pathways

The salvage pathways recycle NAD^+ breakdown products and related compounds found throughout the cell and in the extracellular fluid. These precursors include NAM, NAM riboside (NR), NMN, and nicotinic acid (NA). The latter is also known as niacin or Vitamin B3. However, sometimes the term "niacin" includes both NAM and NA. These precursors are generated endogenously both inside and outside the cell, and may also be acquired from exogenous sources, in food or supplements. It should be noted that normally NMN is not present in unprocessed food, but it is available as a dietary supplement. Vitamin B3 deficiency is endemic in some parts of the world and may be severe resulting in the nutritional deficiency disease known as pellagra, which is much less common nowadays than it was in the early 20th century.[18,19] Pellagra is a disease characterized by the triad of dermatitis (dark skin rash), diarrhea, and dementia.[20] Untreated pellagra leads eventually to the patient's death. By contrast, mild Vitamin B3 deficiency is likely to result in lower metabolic rate, cold intolerance, and in the young, impaired brain development.

NAD^+-consuming ectoenzymes such as the glycohydrolases CD38 and CD157 and the nucleotide phosphatase CD73 are responsible for the cleavage of extracellular NAD^+. Cleavage by CD73 produces both NMN and NR, while NAD^+ cleavage by CD38 or CD157 yields NAM. Intracellular NAD^+ is also consumed in reactions catalyzed by such enzymes as the sirtuins (discussed below), the poly -ADP-Ribosyl Polymerases (PARPs), and SARM1 (Sterile Alpha and TIR Motif-containing protein 1). SARM1, which is highly expressed in the brain, is an essential mediator of axon degeneration after nerve injury. It initiates a local destruction program involving rapid NAD^+ cleavage.[21]

NAM is converted to NMN in a reaction catalyzed by the rate-limiting enzyme NAM phosphoribosyl transferase (NAMPT) (aka NAMPRT, NMNPRT, or NMNPT) while the conversion of NR to NMN is catalyzed by NR kinase (NRK). NMN is then converted directly to NAD^+ by NMNAT. By contrast, NA is first converted to its mononucleotide (NAMN) by NAPRT then to NAAD by the same enzyme that acts on NMN (NMNAT = NAMNAT). NAAD is then converted to NAD^+ by NADS as depicted in the Preiss-Handler pathway [Figure 2].

Thus, it is clear that intracellular pools of NAD^+ may be sustained or augmented either by inhibiting the NAD^+-consuming enzymes and the breakdown of NAD^+ or by stimulating the salvage pathways through supplementation with appropriate precursors such as NR and NAM.[22]

The Sirtuins

The sirtuins are a family of NAD^+-dependent, evolutionarily highly conserved enzymes. Like NAD^+, they are present in virtually all living organisms, from bacteria to humans.[23,24] They possess broad enzymatic activities that are critical for a myriad of cellular functions ranging from metabolic regulation and energy homeostasis to DNA repair, genome stability, stress response, and cell survival. Given their broad spectrum of vital

functions, the sirtuins are critical determinants of both the lifespan and health span of the organism.[25] Over the past 20 years, evidence has accumulated indicating that the dysregulation of sirtuins is involved in major aging-related disorders, including metabolic, cardiac, and neurodegenerative diseases, and cancer. Hence, the sirtuins are almost always associated with longevity and aging.

Seven sirtuins (sirt1-sirt7) have so far been identified in mammals, each located predominantly in a particular subcellular compartment and is associated with its distinct functions. Mammalian sirtuins are orthologous to the protein encoded by the yeast's Silent Information Regulator-2 (SIR-2) gene. The discovery of sirtuins is the result of extensive research, conducted mostly in the 1990s, on the 4 SIR genes involved in transcriptional silencing in the baker's yeast (Saccharomyces cerevisiae).[26]

Nearly 20 years ago, it was discovered that overexpression of the SIR-2 gene extended the yeast's lifespan while its inactivation accelerated aging.[27] Within a year of this discovery the sirt2 protein was identified as an NAD^+-dependent histone deacetylase (HDAC) that catalyzes the removal of acetyl groups from lysine residues #9 and #14 of histone 3 (H3), and lysine residue #16 of histone 4 (H4). The deacetylation reaction is coupled to the breakdown of NAD^+ which yields ADP-ribose and NAM. The acetyl groups removed from the target proteins are transferred to the ADP-ribose moieties to form 2-O-acetyl-ADP-ribose, which acts as a regulatory molecule.[28] The sirtuins catalytic activity is not limited to deacetylation but includes various deacylations and ADP-ribosylation as detailed below. By regulating histone as well as non-histone proteins at specific points, sirtuins regulate PTMs resulting in chromatin silencing and post-transcriptional repression of protein synthesis.

In the core of each sirtuin is the NAD^+-binding and catalytic domain. Because of their dependence on NAD^+ as a cosubstrate rather than requiring Zn as a cofactor, the sirtuins were classified at some point as Class III deacetylases (HDACs).[29] Acetylation and deacetylation of lysine residues of the nucleosome histone core will stop or allow the expression of the genes located in that area of the histone.

The early findings in the yeast were later extended to other prokaryotic and eukaryotic organisms indicating that the sirtuins are conserved throughout evolution.

Sirt1 is found mainly in the nucleus and to a lesser extent in the cytosol depending of the phase of the cell cycle. Sirt2 has the reverse distribution; it is located mainly in the cytosol and to a lesser extent in the nucleus. Sirt2 also appears to be particularly abundant in adipose tissue. Sirtuins 3, 4, and 5 are found almost exclusively in the mitochondria. Sirt6 is located in the nucleus, while sirt7 is mostly confined to the nucleolus. All seven sirtuins except sirt4 possess deacetylase activity catalyzing the removal of acetyl groups from acetylated lysine residues of target proteins while at the same time cleaving NAD^+ generating NAM and ADP-ribose. The acetyl group is transferred to the ADP-ribose to form 2'-O-acetyl-ADP-Ribose. Here is the net reaction. Acetylated Protein + NAD+ > (Sirt1) > Deacetylated Protein + 2-O-acetyl-ADP-ribose + NAM.

The salient functions of each of the seven mammalian sirtuins are outlined below and summarized in Table 1.[30-49]

Sirtuin 1

Sirt1 is the most extensively studied of all mammalian sirtuins. Through its deacetylase activity, sirt1 is responsible for the regulation of a number of transcriptional factors, including those responsible for suppressing the expression of several pro-inflammatory genes.[50,51] Sirt1 also controls the deacetylation of the nucleosome histone as well as over a dozen non-histone targets such as p53, PGC1α, FOXO1, FOXO3, FOXO4, and Notch.[36,52] Under low nutrients high NAD^+ conditions, the sirt1-catalyzed deacetylation and subsequent activation of PGC1α, the PPARγ coactivator, plays an important role in the regulation of mitochondrial biogenesis, glucose and fatty acid metabolism, and adaptive thermogenesis. The transcriptional activity of PGC1α enriches skeletal muscles with mitochondria-rich highly oxidative, fatigue-resistant slow-contracting

Table 1: The sirtuins, their location, enzymatic activity and physiological functions

Sirtuin	Location: Primary/secondary	Enzymatic activity	Physiological functions
Sirt 1	Nucleus/cytosol	Deacetylase	In the nucleus, it is the main deacetylase for acetylated histone (H1K26 and H4K16) and nonhistone target proteins (p53, FOXO1, FOXO3, FOXO4, PGC1α, Notch, etc.)
			Activation of sirt1 and deacetylation of p53, the tumor suppressor protein, leads to inhibition of p53. In the aging cell, this negative control tends to mitigate the effects of oxidative stress and reduce DNA damage and cell apoptosis, thus decelerating aging[30]
			Specific inhibition of sirt1 activates p53 inducing apoptosis of cancer cells
			It activates PGC1α, a transcriptional coactivator that regulates the genes involved in mitochondrial biogenesis and energy metabolism. PGC1α also plays an important role in antioxidant defense. Impaired PGC1α function is implicated in the pathogenesis of neurodegenerative diseases such as Alzheimer's and Parkinson's
			It activates the hypoxia-inducible transcription factors (HIF1α, HIF2α, HIF3α), and together function as oxygen sensors enabling the organism to adapt and survive in an environment where hypoxia and redox imbalance prevail[31]
			It regulates expression of HIF2α responsible for the renal hypoxia-induced erythropoietin production[32]
			Suppressing tumors and neurodegenerative diseases
			Stem cell differentiation and hepatocyte regeneration
Sirt 2	Cytosol/nucleus	Deacetylase	It is located primarily in the nucleus, and also in the cytosol
		ADP-ribosyltransferase	It deacetylases tubulin and regulate muscle differentiation
			It accumulates with age in the CNS neurons, where its deacetylase action on the microtubules is linked to age-associated neurodegenerative diseases[33,34]
			It deacetylates and activates the cell polarity regulator protein Par-3, which plays a role in maintenance of neural structures [e.g. ability of Schwann cells to myelinate nerve fibers[35]
			Through the deacetylation of FOXO1 and PGC1α, sirt2 promotes FA oxidation and plays an important role in the regulation of adipocyte metabolism[36]
			During hypoglycemia, it stimulates gluconeogenesis by deacetylation and activation of PEPCK
			It contributes to the maintenance of cellular Fe levels through the deacetylation NRF2, reduced expression of FPN1, and ultimately reduced cellular iron transport[37,38]
			Sirt 2 modulates microvascular inflammation and affects recovery from sepsis[39]
Sirt 3	Mitochondria	Deacetylase	It is the main deacetylase in the mitochondrial, where it deacetylates numerous enzymes that are essential for maintaining metabolic homeostasis.[40] These include long chain acyl CoA dehydrogenase, acetyl-CoA synthetase 2, and the TCA cycle enzymes isocitrate dehydrogenase and glutamate dehydrogenase
			During prolonged fasting, it activates key enzymes controlling lipolysis, FA oxidation, and ketone bodies production[41]
			By targeting specific components in each complex of the oxidative phosphorylation system, the sirt3 activity plays a critical role in the regulation of mitochondrial energy production[40]
			Protects against oxidative stress through the deacetylation and activation of SOD2 and IDH2 increasing reduced-to-oxidized (GSH/GSSG) ratio. These effects are stimulated by calorie restriction. In addition, sirt3 enhances the expression of mitochondrial Mn-SOD, peroxiredoxins, and thioredoxin-2[42]
Sirt 4	Mitochondria	ADP-ribosyl-transferase, Lipo-amidase	It is exclusively mitochondrial and does not have deacetylase activity. It acts as an ADP-ribosyl transferase (aka ADP-ribosylase) with GDH as its primary target inhibiting its activity. This effect on GDH is in opposition to that of sirt3[36]
			Sirt4 inhibits FA oxidation, an action that is also directly opposed to that of sirt3
			Sirt4 possesses lipoamidase activity which inhibits PDH[43]
			Sirt4 blocks insulin secretion in response to amino acid administration
Sirt 5	Mitochondria/cytosol	Deacylases (deacetylase demalonylase desuccinylase deglutarylase decrotonylase)	It is primarily a demalonylase and desuccinylase
			It acts on CPS1 during fasting, leading to the activation of the urea cycle[44]
			Laboratory mice lacking sirt5 develop severe ammonia intoxication
			It helps protect against ROS by deacetylating such enzymes as SOD1
			During starvation, sirt5 may protect mitochondria from fragmentation and degradation[45]

Contd...

Table 1: Contd...

Sirtuin	Location: Primary/secondary	Enzymatic activity	Physiological functions
Sirt 6	Nucleus/endoplasmic reticulum	ADP-ribosyl-transferase Deacetylase	Resides in the nucleus, where it plays an important role in maintaining genome stability and telomere function through the deacetylation of key histones, H3K9, and H3K56[46] Under conditions of oxidative stress, it stimulates the activity of PARP1 promoting repair of damaged DNA. The activation of PARP1 is accomplished through the transfer of ADP-ribose from nicotinamide adenine dinucleotide to PARP1 By maintaining DNA integrity and the expression of key enzymes, sirt6 plays a critical role in the regulation of metabolism. Mice lacking sirt6 develop high glycolytic rates and severe hypoglycemia, which leads to their premature death As a tumor suppressor, it regulates aerobic glycolysis (the main energy source of cancer cells)[47]
Sirt 7	Nucleolus	Deacetylase	Appears to be confined to the nucleolus with multiple targets and functions involving ribosome biogenesis and cell cycle progression, metabolic regulation, stress resistance, aging, and tumorigenesis. The mechanisms of these functions are yet to be elucidated[48] It promotes the transcription of RNA polymerase I and controls the transcription of rDNA[49]

HIF: Hypoxia inducible factor, CNS: Central nervous system, PEPCK: Phosphoenolpyruvate carboxykinase, FPN1: Ferroportin 1, FA: Fatty acid, SOD2: Superoxide dismutase 2, IDH2: Isocitrate dehydrogenase 2, GSH: Glutathione, Mn-SOD: Manganese superoxide dismutase, GDH: Glutamate dehydrogenase, PDH: Pyruvate dehydrogenase, CPS1: Carbamoyl phosphate synthetase 1, ROS: Reactive oxygen specie, PARP1: Poly-ADP-ribose polymerase 1, GSSG: Oxidized glutathion, ADP: Adenosine diphosphate, TCA: Tricarboxylic acid cycle

myofibers.[53] Thus, the sirtuin-PGC1α-PPARγ sequence is now recognized as a major regulatory pathway.[54] Through its deacetylase activity, sirt1 activates the hypoxia-inducible transcription factors (HIF1α, HIF2α, HIF3α), and together function as oxygen sensors enabling the organism to adapt and survive in an environment where hypoxia and redox imbalance prevail.[31] The HIFs are responsible for inducing the transcription of more than 100 genes involved in such physiological processes as the regulation of glucose transport and metabolism (stimulating glucose uptake and promoting anaerobic glycolysis), angiogenesis, erythropoiesis, inflammation, cell proliferation, and cancer metastasis.[55]

The sirt1 catalyzed deacetylation of nuclear factors (NF) p53 and FOXO1 limits cell apoptosis and senescence. Other critical functions of sirt1 include support of liver function and stimulation of hepatic regeneration[56] and protection against neurodegenerative diseases.[57,58] Activators and inhibitors of sirtuins are currently an active reach area.[59,60] Treatment of mice with resveratrol, a sirt1 activator, improves mitochondrial functions and protects against the obesity induced by a high-fat diet, while in obese mice, it leads to extended health span and lifespan. Other activators such stac-5 and stac-8 show promising activities against different metabolic and aging-associated diseases such as obesity, inflammatory and autoimmune disorders, cardiovascular disease, hepatic steatosis, neurodegenerative diseases, and cancer.[61]

Sirtuin 2

Sirt2, which is located primarily in the nucleus, has been shown to deacetylase tubulin and regulate muscle differentiation. It has been observed to accumulate with age in the neurons of the central nervous system, where its deacetylase action on the microtubules is linked to age-associated neurodegenerative diseases.[33] In addition, sirt2 is involved in a variety of physiological functions whose dysregulation are also implicated in a number of neurodegenerative diseases.[34] For instance, it plays an important role in the maintenance of neural structures. It deacetylates and activates the cell polarity regulator protein Par-3. The establishment of cell polarity is critical for nerve regeneration, and it is essential for the ability of Schwann cells to myelinate peripheral nerve fibers.[35] Through the deacetylation of FOXO1 and PGC1α, sirt2 promotes fatty oxidation and plays an important role in the regulation of adipocyte metabolism.[36] Under hypoglycemic conditions, sirt2 stimulates gluconeogenesis as a result of deacetylation, stabilization, and activation of phosphoenolpyruvate carboxykinase. This is the rate-limiting enzyme that catalyzes the conversion of oxaloacetate to phosphoenolpyruvate, a key step in the gluconeogenesis pathway. In addition,

sirt2 appears to play an important role in the maintenance of cellular iron levels through the deacetylation of a critical nuclear transcription factor (NF E2-related factor 2), leading ultimately to reduced expression of ferroportin 1 and reduced cellular iron transport.[37,38] In addition, observations in laboratory animals indicate that sirt2 modulates microvascular inflammation and affects recovery from sepsis.[39]

Sirtuin 3

Sirt3 is the main deacetylase in the mitochondrial, where it deacetylates numerous enzymes that are essential for maintaining metabolic homeostasis.[40] These targets include long-chain acyl CoA dehydrogenase (LC acyl-CoA DH), acetyl-CoA synthetase 2, and the TCA cycle enzymes isocitrate dehydrogenase (IDH) and glutamate dehydrogenase (GDH). During prolonged fasting, it activates key enzymes controlling lipolysis, FA oxidation, and ketone bodies production.[41] Yet another important role of sirt3 is the deacetylation of the E1-alpha component of the pyruvate dehydrogenase complex (PDHC) leading to its activation. A certain level of acetylation of E1-alpha is normally maintained by acetyl-CoA acetyltransferase 1. The acetylation/deacetylation status of the PDHC is an important determinant of its activity as reflected in the rate at which pyruvate is converted to acetyl-CoA, which is channeled into the TCA cycle. This, in turn, is an important determinant of the rate of glycolysis. Sirt3 also targets specific components in each complex of the oxidative phosphorylation system,[60] and plays a critical role in the regulation of mitochondrial energy production and the generation of reactive oxygen species.[40] Further, sirt3 protects against oxidative stress through the deacetylation and activation of superoxide dismutase (SOD2) and isocitrate dehydrogenase 2 (IDH2) raising the ratio of reduced-to-oxidized glutathione. These effects are enhanced by calorie restriction, a condition under which ROS production is reduced significantly. Under these conditions, Sirt3 has been shown to protect against age-related hearing loss.[61] In view of its critical roles in the regulation of mitochondrial substrate metabolism, protection against oxidative stress, and cell survival, sirt3 is regarded as the mitochondrial fidelity protein.

Sirtuin 4

Sirt4 is located exclusively in the mitochondria and possesses virtually no deacetylase activity. It is primarily an ADP-ribosyl transferase using NAD^+ as the donor of the ADP-ribose group to be transferred to the target protein. The primary target for sirt4 activity is GDH, which is inhibited as a result of the attachment of the ADP-ribose group. This negative effect on GDH activity is in opposition to the stimulatory effect of sirt3 on GDH.[36] Further, sirt4 inhibits fatty acid oxidation, another action that is also directly opposed to that of sirt3. In addition to its ADP-ribosylase activity, sirt4 possesses lipo-amidase activity whose primary target is the pyruvate dehydrogenase (PDH) complex which is inactivated as a result.[43] Sirt4 is induced by DNA damage as observed during exposure to gamma radiation, and it protects against the development of cancer by inhibiting mitochondrial glutamine metabolism and arresting the cell cycle and cell proliferation. In colorectal cancer, sirt4 inhibits cell proliferation, invasion, and migration.[62]

Sirtuin 5

Sirt5 is a deacylase rather than strictly a deacetylase. It selectively cleaves ε-N-carboxyacyl-lysine derivatives based on malonate, succinate, glutarate, crotonate, etc. However, it is mostly known for its demalonylase and desuccinylase activities. In the course of these reactions, it generates NAM from NAD^+ and removes the acyl group (malonyl, succinyl, etc.,) from the target protein to use in the formation of 2'-O-acyl-ADP-ribose (e.g., 2'-O-malonyl-ADP-ribose) [Figure 4].

The physiological functions of sirt5 are not fully elucidated. However, it has been shown to regulate several metabolic enzymes such as carbamoyl phosphate synthetase 1 (CPS1), succinate dehydrogenase, and 3-hydroxy-3-methylglutaryl -CoA synthase 2. CPS1 is a mitochondrial matrix enzyme that catalyzes the first step in the urea cycle ($NH_4^+ + CO_2 + 2$ ATP $\rhd$ [*CPS1*] > Carbamoyl Phosphate + 2 ADP + 2 P_i). During fasting, the

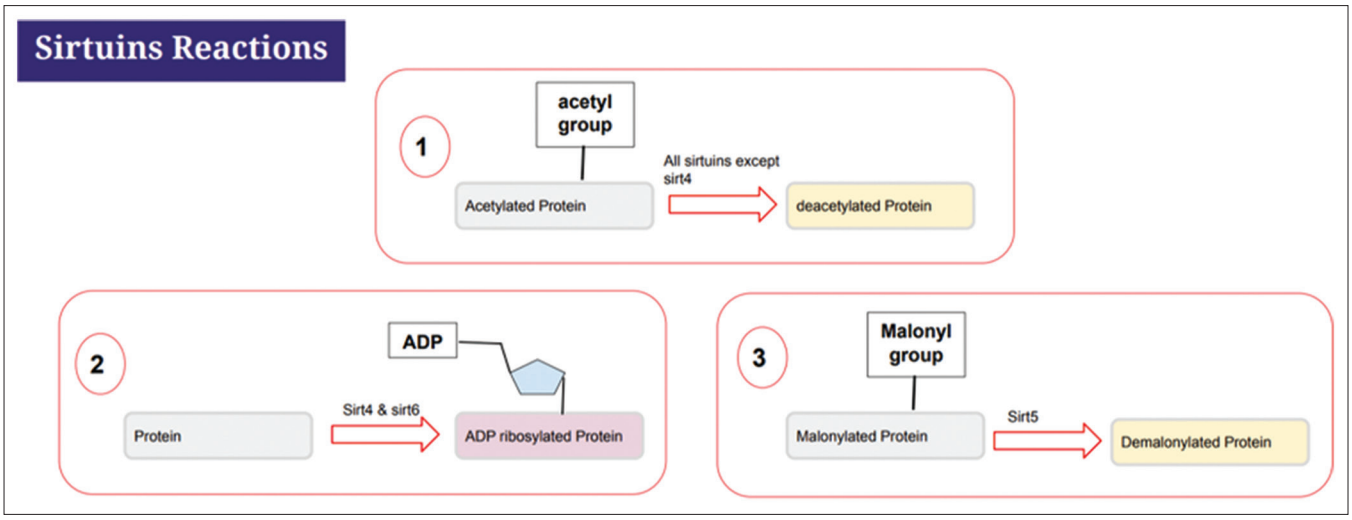

Figure 4: Three types of well-documented reactions are catalyzed by sirtuins: (1) Deacetylation; (2) ADP-ribosyl transfer; (3) deacylation (demalonylation shown as an example)

activation of CPS1 leads to the activation of the urea cycle.[44] Laboratory mice lacking sirt5 develop severe ammonia intoxication. In addition, sirt5 is known to play an important role in protecting cells against oxidative stress by regulating key enzymes involved in ROS detoxification (SOD1, IDH2, and G6PD).[63] Further, there is evidence suggesting that sirt5 may protect mitochondria from fragmentation and degradation during starvation.[45]

Sirtuin 6

Sirt6 is found exclusively in the nucleus and possesses both deacetylase and ADP-ribosylase activities. It acts as an epigenetic guardian for cellular differentiation and plays critical roles for cellular homeostasis through the regulation of chromatin signaling and genomic integrity (DNA repair), fatty acids and glucose metabolism, and telomere maintenance. Telomere length is considered an important molecular measure of cellular aging. The role of telomeres in protecting cells against DNA damage and slowing the aging process has been reviewed in detail by Aubert and Lansdorp.[64] Thus, like sirt1, sirt6 is known for its anti-aging activities. Compared to the controls, sirt6 deficient mice age faster and die earlier with the immediate cause of death being severe hypoglycemia. Genetic studies indicate that sirt6 stimulates pancreatic insulin secretion and inhibits hepatic gluconeogenesis. It also inhibits triglyceride synthesis. The negative effect on gluconeogenesis appears to be mediated through the activation of the acetyltransferase GCN5.[65]

Through its interaction with and inhibition of HIF1α, sirt6 directly suppresses the expression of a number of genes that are central to glucose metabolism, including PDK1, LDH, PFK1, and GLUT1.[66] Related to its interaction with HIF1α, sirt6 plays a vital role as a tumor suppressor by modulating cancer cell metabolism, as evidenced by the inhibition of the metabolic reprogramming of tumor cells that results in aerobic glycolysis becoming their main energy source.[47,66,67]

Sirt 7

Sirt7 is located in the nucleolus and appears to be required for the transcription of ribosomal DNA (rDNA).[49] The main targets of its deacetylase activity include H3K18ac (acetylated lysine residue #18 in H3), an epigenetic tumor biomarker, and the hypoxia-inducible factors HIF-1α and HIF-2α. Sirt7 has been shown to inhibit the HIFs through a mechanism that does not appear to involve its enzymatic activity.[54] The HIFs are critical for the development of the organism's adaptive responses to hypoxia under such conditions as obstructive sleep apnea induced intermittent hypoxia, prenatal hypoxia, or solid tumor hypoxia. HIF1α is responsible for inducing the transcription of more than 100 genes that are involved in various

physiological processes such as the regulation of glucose transport and metabolism, angiogenesis, erythropoiesis, inflammation, pH regulation, cell proliferation, and cancer metastasis.[55] Thus, the benefit of inhibiting sirt7 becomes obvious in the context of solid tumors, where oxygen deficiency and a low energy state prevail.

Regulation of sirtuins

Regulation of the sirtuins' activities is complex and occurs at multiple levels beginning with their subcellular segregation, as indicated in Table 1. In addition to the influences of the concentrations of the cosubstrates (e.g., NAD^+) and products (e.g., NAM), sirtuins are subject to multiple regulatory mechanisms: (1) control of gene expression; (2) post-transcriptional regulation (control of mRNA translation); (3) protein post-translational modifications (PTM); and (4) formation of complexes with regulatory proteins. In the following discussion, the focus will be primarily on sirt1, which has been extensively investigated in numerous species, organs, and experimental conditions more than any other sirtuin.

The sirt1 gene promoter possesses a number of binding sites for transcription factors; and therefore, it is under multifactorial control. These transcription factors include FOXO1 protein [Forkhead Box 1], CREB (cAMP response element-binding), ChREBP (carbohydrate response element-binding protein), PPARα (Peroxisome Proliferator-Activated Receptor α), PPARβ/δ, PPARγ, and PARP2 (poly-[ADP -Ribose]-Polymerase 2) among others. FOX01, CREB, PPARα, and PPARβ/δ promote the expression of sirt1 whereas ChREBP, PARP2, and PPARγ repress it. It is through these factors that nutrient availability and energy status exert their marked influence on the activities of the sirtuins in general.[68] Nutrient deficiency and low energy status (as occurs during fasting or calorie restriction) stimulate sirt1 expression while the opposite repress it.

Posttranscriptional regulation involves the binding of a micro-RNA (miRNA) to the trailer sequence or 3'UTR of the sirtuin's mRNA blocking its translation. miRNAs are short stretches of noncoding RNA that bind to the 3'UTR end of mRNA and block it. The 3'UTR is the untranslated region of the mRNA that comes immediately after the stop codon, the signal for the termination of the coding region. Some two dozen miRNAs have been studied in connection with sirt1 in various tissues and species.[69,70] miRNA-34a, the best studied of these miRNAs, constitutes together with sirt1 and p53 an effective regulatory circuit in which p53 inhibits the transcription of the SIRT1 gene and simultaneously stimulates the expression of miR-34a. The miR-34a, then, represses the translation of the sirt1 mRNA. Thus, the production of the sirt1 protein is inhibited at both the transcription and translation levels. Similar regulatory circuits involving miRNA-34a exist also for other sirtuins, namely sirt6 and sirt7. It is noteworthy that miRNA-34a may be involved in the genesis of the metabolic syndrome since it is found to be consistently elevated in obese and diabetic laboratory animals.[70]

An important level of regulation is the PTMs, which are achieved primarily through the covalent bonding of certain chemical groups at specific points of the target protein (e.g., a sirtuin). Examples include phosphorylation involving the c-Jun N-terminal kinases (JNK1 and JNK2),[71] methylation, S-glutathionylation, ubiquitination, or sumoylation. The latter refers to the conjugation through the small ubiquitin-related modifier conjugation system. Sumoylation and ubiquitination are chemically similar but can have different regulatory effects. Sumoylation modulates the properties of many proteins including the sirtuins, thereby playing an important role in the regulation of a vast number of cell functions. Sumoylation increases sirt1 activity while the removal of this modification (i.e., desumoylation) in response to oxidative stress inactivates sirt1 and promotes cell apoptosis.[72]

Yet another post-translational means of regulating sirtuin activities is through the formation of complexes of sirtuins with regulatory proteins. For instance, the complex of sirt1 with AROS

(Active Regulator of Sirt1) leads to the activation of sirt1 and the suppression of p53 with the overall effect of reducing cell apoptosis in response to DNA damage. The observation that sirt1 level is increased in a number of tumors points to its oncogenic role as indicated by the inactivation of the oncosuppressor p53. By contrast, there are several other regulatory proteins (such as SMRT, NCoR1, and DBC1) whose complexes with sirt1 suppress its activity while activating p53 and promoting cell apoptosis.[36,73,74]

As stated above, sirtuin catalyzed reactions have an absolute requirement for NAD^+ as a cosubstrate and not merely as an enzyme co-factor. Therefore, it is not surprising that sirtuin activity is very sensitive to changes in the intracellular NAD^+ pool. Calorie restriction, fasting, and exercise are all known to increase NAD^+ level and sirt1 activity.[75] By contrast, a downward shift in the NAD^+/NADH ratio as occurs with high fat diet or high rate of glycolysis depresses sirtuins activity. A relatively high intracellular NAD^+ level may be maintained either through enhanced biosynthesis or by reducing NAD^+ consuming reactions such as those catalyzed by PARP1 and CD38 (cyclic-ADP-ribose synthase 38; EC 3.2.2) [Figure 1]. Deletion or inhibition of these enzymes in laboratory animals has been shown to boost NAD^+ level and sirt1 activity, and prevent diet-induced obesity.[76]

Sirtuins and age-related diseases

It is clear from the preceding discussion that evidence has been mounting that implicates the sirtuins in a vast array of critical cellular functions. In addition to transcription silencing and DNA repair, these functions include the regulation of ion channels, neuronal function, cell growth, circadian rhythm, inflammatory response, mitochondrial biogenesis, insulin secretion, fat oxidation, and glucose metabolism.[36] Through their interactions with multiple transcription factors, regulatory proteins, and signaling molecules, the sirtuins chart the course of aging and its associated disorders. Their activities are essential for maintaining mitochondrial health, energy homeostasis and redox balance including the NAD^+/NADH ratio. Therefore, it is not surprising that the sirtuins have been linked to many age-related conditions such as cancer,[77] metabolic and cardiovascular diseases,[78,79] and neurodegenerative disorders such as AD.[56-58,80] While little is known about the sirtuins role in Parkinson's and Huntinghton diseases, significant progress has been made in understanding their role in AD. Studies of the influence of AD pathology on the expression levels of various sirtuins in the hippocampus and temporal lobe of the human brain have revealed consistent reductions in sirt1 and sirt3 together with increases in sirt2 and sirt5, but the full significance of these changes remains largely unclear. However, there is evidence indicating that sirt1 is involved in the regulation of multiple pathways that modulate the metabolism of amyloid beta (Aβ) to maintain its concentration below toxic levels. The degradation of Aβ via autophagy is dependent on sirt1 activity.[81] Sirt1 also appears to protect against Aβ-mediated synapse loss. In addition, through its deacetylase activity, sirt1 stimulates the degradation of the tau protein preventing the development of tauopathy.

The pathophysiologic processes of AD involve significant reductions in sirt1-dependent inhibition of the NF-κB pathway, deacetylation of the tau peptides, and α-cleavage of the amyloid precursor protein. The net result is increased Aβ levels, intensified pro-inflammatory signals, and cognitive decline. Thus, it is safe to conclude that sirt1 plays an important role in reducing Aβ toxicity, oxidative stress, synapse loss, and cognitive decline.

The mitochondria have long been at the center of the various theories of aging including the mitochondrial free radical theory which proposes that the generation of free radicals as byproducts of normal mitochondrial metabolic activity leads to a gradual and progressive decline in mitochondrial function resulting from damage of mitochondrial DNA and the inactivation of vital proteins including enzymes such as the sirtuins. Naturally, the most susceptible sirtuins would be the ones located within the mitochondria, namely sirt3, sirt4, and sirt5. The importance of these three sirtuins for longevity and health span is illustrated by the impact of their loss [Table 2].[82-85]

Table 2: Impact of the Loss of Mitochondrial Sirtuins in Different Organs and Systems[82-85]

Sirt3	Sirt4	Sirt5
CNS/PNS:	Skeletal Muscle:	Liver:
(-) neuron survival	(+) Fat oxidation (↑ MCD)	(-) Urea cycle (↓ CPS1)
(+) Hearing loss		
Systemic:	Liver:	Heart:
(+) Insulin resistance	(+) Fat oxidation [(+) PPARa, (+) AMPK]	(+) Hypertrophy
(+) Obesity (WAT)		(-) Contractility
		(-) Fat oxidation [(-) HADHA]
Skeletal Muscle:	White Adipose Tissue:	
(-) Glucose uptake	(+) Fat oxidation [(+) MCD]	
(-) TCA & Oxidative Phosphorylation	(-) Obesity	
Liver:	Pancreas:	
(-) Fat oxidation	(+) Insulin secretion [(+) GDH]	
(-) Ketogenesis		
(-) Urea cycle		
Heart:	Cancer:	
(+) Hypertrophy	(+) DNA damage [(+) GDH]	
(+) Fibrosis	(+) Tumorigenesis	
(-) Contractility		
Cancer:		
(+) DNA damage [(-) SOD2]		
(+) Tumorigenesis		
(+) Glycolysis [(+) HIF1a]		

AMPK=AMP kinase; CPS1=carbamoyl phosphate synthetase 1; GDH=glutamate dehydrogenase; HADHA=Hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase trifunctional protein subunit alpha; HIF1α = hypoxia-induced factor 1α; MCD=Malonyl-CoA decarboxylase; PPARα = peroxisome proliferator activated receptor α; SOD2=superoxide dismutase 2; WAT=white adipose tissue

The sirtuins are widely believed to be behind the extension of lifespan brought about by caloric restriction and fasting. Maintaining optimal sirtuin activities by boosting cellular NAD^+ availability through dietary supplements (nutriceuticals) is now the focus of extensive laboratory and clinical research efforts.[86-88] Since sirtuins activity is NAD^+-dependent, sustaining intracellular NAD^+ levels throughout life may not only extend the lifespan but also is likely to extend the health span as well. The long-term administration of NAM NMN has been shown to mitigate age-associated physiological decline in mice, an effect attributed to enhanced cellular bioavailability of NAD^+.[89] Similar observations were obtained in mice using NR.[90] However, human data are still lacking that would demonstrate unequivocally a significant rise in intracellular NAD^+ level secondary to the oral administration of either NMN or NR. Recently, Marten and co-workers explored the effects of NR supplementation in healthy lean adults (55–79 years) with respect to NAD^+ and some parameters of cardiovascular function.[91]

Metabolite measurements were limited to the peripheral blood mononuclear cells (PBMNCs). NR raised NAD^+ concentration significantly by nearly 60%. By contrast, NR raised the level of NAAD nearly fivefold compared to placebo. NAAD is a product of NR utilization and a highly sensitive biomarker of increased NAD^+ metabolism in mammals. NR may be converted to NAD^+ either via the NRK/NMNAT pathway shown in Figure 2 or via NA and NAAD: NR > NAM > NA > NAMN NAAD > NAD+.[92] Noticeable increases that did not reach statistical significance were also observed for NADP+, NAM, and NAM NMN possibly due to the limited sample size (n = 24). Collectively, these observations are consistent with increased NAD^+ level and enhanced activity of the enzymes involved in NAD^+ metabolism. In the above cited study,[91] the mean systolic blood pressure (SBP) was 9 mmHg lower after NR versus placebo in individuals with Stage I hypertension, whereas no change was observed in subjects with initial SBP in the normal range.

Conclusions

Although human data are still limited in terms of the long-term effects of supplementation on the health span, available data are sufficiently encouraging to promote expanding research activity in this field.

Authors' contribution

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Conflicts of interest

There are no conflicts of interest.

Compliance with ethical principles

No prior ethical approval is required.

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Reviewers:
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