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Cellular NAD$^+$ Level: A Key Determinant of Mitochondrial Quality and Health

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The quality of mitochondria is a key determinant of mitochondrial ATP production and reactive oxygen species generation; therefore, it plays critical roles in energy homeostasis and cellular health. Mitochondrial quality is governed by mitochondrial turnover, which involves autophagic removal of old mitochondria and biogenesis of new ones. Both activities are critically modulated by sirtuin proteins, in particular, SIRT1 and SIRT3. These proteins activate mitophagy and mitochondrial biogenesis through deacetylation-mediated activation of factors participating in key steps of autophagy and mitochondrial protein expression. They also control other factors involved in maintenance of mitochondrial integrity and in damage prevention. At the same time, the activities and protein levels of SIRT1 and SIRT3 decline during aging, contributing to mitochondrial dysfunction associated with tissue aging and many degenerative diseases. However, recent studies suggest that the activity of the sirtuin proteins can be elevated through modulation of nicotinamide adenine dinucleotide (NAD$^+$) and in physiological settings. An understanding of the underlying molecular pathways is actively sought and practical strategies are proposed based on basic research; nevertheless, their applicability at the clinical and subclinical levels could be better recognized and understood in the field of medicine. To this end, this review discusses the recent understanding of the biochemistry underlying the NAD+-redox mediated modulation of cellular sirtuin activity.

Key Words: SIRT1, SIRT3, Mitochondria, Mitophagy, Mitochondrial biogenesis

INTRODUCTION

Nicotinamide adenine dinucleotide (NAD$^+$) accepts high energy electrons from components of food to undergo reduction to NADH, which, while being oxidized back to NAD$^+$, yields electrons to produce ATP and macromolecules. Thereby, it plays critical roles in basal maintenance of metabolic rate as well as in the growth of cells while fine tuning various physiological activities.

In this energy metabolism, the ratio of NAD$^+$/NADH (or NAD$^+$ redox), determines the activity and quality of mitochondria. At least 2 characteristics of NAD$^+$ biochemistry are involved here. First, the level of NAD$^+$ redox in the mitochondria determines that of oxidative phosphorylation (OXPHOS). High NADH/NAD$^+$ levels activate the transfer of electrons to complex I; and thereby, accelerates the electron transport chain. Therefore, the NAD$^+$ redox potential directs mitochondrial ATP production. Secondly, NAD$^+$ functions as a cosubstrate of the sirtuin family of proteins and modulates their deacetylase activities[1]. The seven members of the sirtuin family (SIRT1-7) commonly break NAD$^+$ to produce ADP-ribose and nicotinamide (NAM) while detaching acetyl residues from their target proteins[2]. Because of this dependence, the activities of sirtuins are tightly regulated by the levels of NAD$^+$ (or NAD$^+$/NADH)[3]. Sirtuin proteins, especially SIRT1 and SIRT3, are critically involved in the biogenesis and degradation of mitochondria (through mitophagy)[4,5]. Thereby, their activity is critical in the maintenance of the structural and functional integrity of the mitochondria. Overall, NAD$^+$, by regulating OXPHOS and sirtuin activities, determines the levels of cellular energy metabolism and mitochondrial quality.

Mitochondria experience morphological and functional deterioration during aging and cellular senescence[6]. The loss of structural integrity and dynamics as well as of membrane potential ($\Delta\psi m$) and accumulation of high levels of reactive oxygen species (ROS) becomes prominent as cells approach senescence or become postmitotic[7]. Mitochondria also suffer severe functional deterioration in age-associated degenerative diseases, including neuro-degenerative disorders, such as Alzheimer's and Parkinson's diseases[8,9]. This mitochondrial deterioration is believed to be primarily caused by damage by ROS, which are produced mainly by the mitochondria themselves[10]. Due to the proximity to the ROS that they

produce, mitochondria are susceptible to oxidative damage[6]. Therefore, maintaining high quality mitochondria and thereby reducing ROS production might be an important factor in the prevention of degenerative diseases and delaying the functional decline of tissues during aging. Therefore, mitochondrial quality maintenance may be a prime target of therapeutic approaches in delaying aging and preventing degenerative diseases[11].

Studies so far have suggested that activation of SIRT1, a nuclear/cytoplasmic protein, and SIRT3, a sirtuin that resides primarily in mitochondria, together might be far than activation of either protein individually[12]. They commonly activate mitophagy and mitochondrial biogenesis, but also affect many different targets that positively influence mitochondrial integrity and function. Simultaneous activation of different sirtuins might be better achieved by increasing the cellular NAD^+ level rather than by administrating chemical activators that specifically target individual sirtuins. This warrants attempts to develop means to elevate cellular NAD^+ levels for the enhancement of mitochondrial quality. This review focuses on how the NAD^+ level and its redox potential changes in certain physiological states and on how these changes are modulated through pharmaceutical intervention. In particular, it introduces current understandings on the NAD^+ precursors that can be applied through diet and provides information that encourages the development of clinical, therapeutic, and dietary strategies and regimens; these strategies may allow better management of aging and degenerative diseases.

BENEFICIAL ROLES OF SIRTUIN PROTEINS IN MITOCHONDRIAL QUALITY AND HEALTH

Human sirtuin proteins, despite different localizations and substrate specificities, commonly have NAD^+-dependent deacetylase activity[13], and function in the physiology of metabolic homeostasis and stress resistance. They also play important roles in maintaining health and longevity[14]. This role that benefits health is best understood for SIRT1. It exerts positive effects on lifespan and differentiation of stem cells and thereby functions in tissue maintenance[15]. In addition, it suppresses inflammation[16]; thereby, SIRT1 attenuates the expression of inflammatory phenotypes, which become increasingly prominent in aging and are associated with diverse degenerative diseases[17]. SIRT1 appears to affect diverse aspects of cell physiology through modifying activities of specific target proteins and regulating expression of genes[18,19]. Examples include the activation of FOXO3, a transcription factor that induces the expression of anti-oxidant genes in response to oxidative stress[20] and the inactivation of RelA/p65 subunit of nuclear factor-kappa B, a pro-inflammatory transcription factor[21].

The roles of SIRT1 and SIRT3 in mitochondrial degradation and biogenesis are well understood. Mitochondrial degradation is mainly mediated by mitophagy, a type of autophagy that is responsible for the removal of mitochondria[22]. SIRT1 induces autophagy by activating Atg molecules involved in early steps of autophagy[23,24]. Deacetylation of Atg5 promotes its interaction with Atg12, which is a key step in the formation of phogophores, an initiating step in autophagy[23]. Deacetylation of Atg8, which is better known as LC3, facilitates nuclear exit of LC3 and its subsequent incorporation into the autophagosome membranes[24]. LC3 is believed to function in autophagosome membrane expansion and in cargo loading into autophagosome[25]. SIRT1 has also been shown to induce mitochondrial fission that leads to the fragmentation of mitochondria[26], which is likely required for the efficient enclosure of mitochondria in autophagosomes[27].

SIRT3 also stimulates autophagy by activating AMPK[28], which inhibits mammalian target of rapamycin C1 (mTORC1), a negative regulator of autophagy[29]. It also contributes to enhancement of mitochondrial quality by capacitating damage clearance in mitochondria. It activates 8-oxodguanie-DNA glycosylase 1 (OGG1), a DNA repair enzyme, and prevents oxidative damage to mitochondrial DNA[30]. It also increases the activities of MnSOD (SOD2)[31], a mitochondrial superoxide scavenger, and isocitrate dehydrogenase 2 (IDH2)[32], which produces NADPH and raises the level of glutathione, a key cellular antioxidant. Furthermore, as a mitochondrial sirtuin, SIRT3 deacetylates a list of mitochondrial proteins that function in the oxidation of fatty acids, amino acid degradation, TCA cycle, OXPHOS, and pyruvate dehydrogenase complex activity[33,34], thereby, SIRT3 increases the efficiency of ATP synthesis and energy homeostasis[35]. Importantly, SIRT3-mediated deacetylation of cyclophilin D (CypD)[34] causes termination of CypD attachment to mitochondrial permeability transition pores leading to the closing of these pores. This blocks mitochondrial loss of Ca^{++}, ATP, and membrane potential[36] through the open pores and protects mitochondrial integrity, functionality, and even cell viability.

Mitochondrial biogenesis is induced by activating SIRT1 and SIRT3 commonly through activation of PPAR Gamma Coactivator 1α (PGC-1α)[4,37,38], a transcription activator responsible for the expression of the transcription factors nuclear respiration factor (NRF)-1 and 2. In the nucleus, these proteins turn on the mitochondrial genes[39] and human mitochondrial transcription factor A (TFAM)[40], another transcription factor that functions on the expression of the genes in mitochondrial genome[41]. SIRT1 and SIRT3 increase the activity of PGC-1α by both directly activating the protein and by increasing its expression[42]. Overall, these activities of SIRT1 and SIRT3 function for the enhancement of mitochondrial turnover leading to an increase in mitochondrial quality. It is apparent that though SIRT1 and SIRT3 share

considerable commonality, they also affect different targets for enhancing mitochondrial quality. This suggests that a regimen which activates both sirtuins would work better than one that mobilizes either one of them alone; the enhancement of cellular NAD^+ levels would be one way to achieve this. For this reason, understanding the biochemistry of NAD^+ metabolism recently became a very active area of research.

CELLUAR LEVELS OF NAD^+

Absence of dietary supply of niacin and tryptophan, precursors of NAD^+ (through the salvage and *de novo* pathways, respectively) causes pellagra[43], a disease of diverse lesions, such as inflamed skin, severe diarrhea, and dementia. Administration of NAM successfully cures pellagra, highlighting the

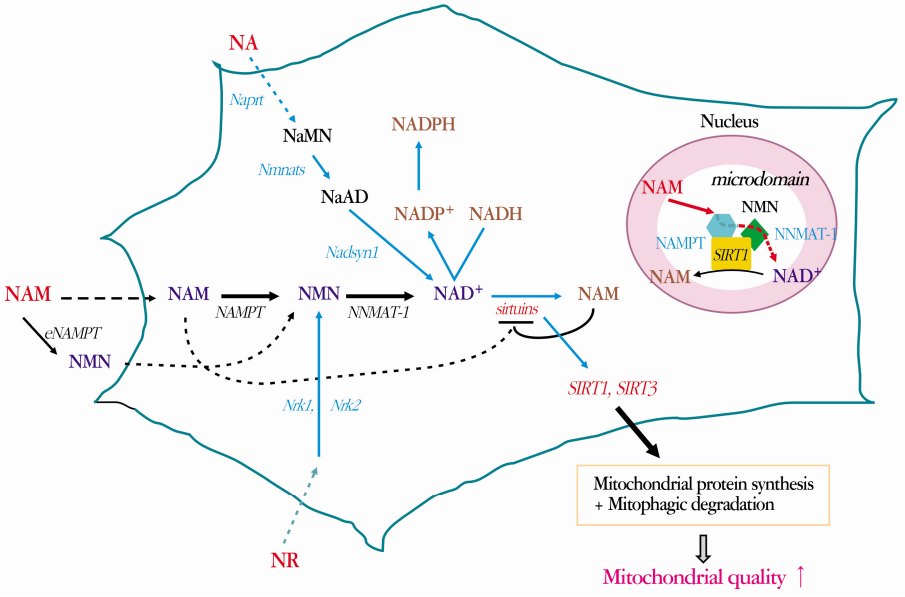

Fig. 1. Cellular metabolism of nicotinamide adenine dinucleotide (NAD^+) and its precursors. Upon administration to human fibroblasts (and mice, *in vivo*), nicotinamide (NAM) and nicotinamide mononucleotide (NMN) enter cells and are readily converted to NAD^+ through the activities of 2 enzymes, nicotinamide phosphoribosyl transferase (NAMPT) and nicotinamide nucleotide adenyl transferases (NMNATs) (salvage pathway, black arrows). Likewise, nicotinamide riboside (NR) is incorporated into the salvage pathway via NMN through the catalytic activity of nicotinamide riboside kinases (Nrks). Nicotinic acid (NA) is converted first to nicotinic acid mononucleotide (NaMN) and nicotinic acid adenine dinucleotide (NaAD) by the enzymes nicotinate phosphoribosyl transferase 1 (NAPRT1) and nicotinamide nucleotide adenyl transferases (NMNAT) 1–3. NaAD is then converted to NAD^+ by NAD synthetase 1. The elevated level of NAD^+ leads to activation of sirtuins, among which SIRT1 and SIRT3 collaborate to enhance mitochondrial quality by increasing mitochondrial turnover. NAD^+ is reduced to NADH forming NAD^+/NADH redox state, which modulates sirtuin activity as well as mitochondrial oxidative phosphorylation. NAD^+ is phosphorylated to generate cellular reducing power, NADPH, but the cellular concentrations of $NADP^+$ and NADPH are comparatively far lower and do not affect NAD^+ biochemistry. NAD^+ is also broken down to NAM and ADP-ribose by many enzymes, including PARP and sirtuins. NAM exerts feedback inhibition on sirtuins; therefore, its administration may be inhibitory to sirtuins. However, since NAM is readily converted to NAD^+, such inhibition would be transient. Studies using NAM-containing medium have found that cellular NAD^+ levels in human cells reach a peak after approximately 12–16 hours[26,89], Following the peak, the NAD^+ level decreases but is maintained at a level slightly higher than that found in untreated cells. Furthermore, it has been suggested that SIRT1 in the nucleus is located in certain microenvironments where it is present in close proximity to NAMPT and NMNAT-1 and receives a ready supply of NAD^+[100] (white circle in pink nucleus) while not being much affected by the high levels of NAM. NAMPT is present in the extracellular space (eNAMPT) and is expected to convert NAM to NMN outside cells, thereby, lowering cytosolic concentrations of NAM while elevating cellular NMN concentrations.

vital importance of NAM. NAM itself does not appear to have direct physiological activity. Once incorporated in cells, it is converted to NAD^+ through the salvage pathway. This pathway is composed of the conversion of NAM to nicotinamide mononucleotide (NMN) by nicotinamide mononucleotide phosphate transferase (NAMPT)[44] and the conversion of NMN to NAD^+ by NMN adenyl transferase (NMNAT)[45] (Fig. 1). Cultivation of cells in NAM-free medium causes a reduction in cellular NAD^+ levels by 90% in 7 days, and 99% in 14 days[46]. These observations reflect that NAD^+ (physiologically present at approximately 0.3 mM in normal animal cells[47,48]) is continuously consumed through degradative metabolism, in which classes of NAD^+-dependent ADP-ribosyl transferases are major constituents[49]. For example, poly (ADP-ribose) polymerase family proteins (PARPs), which are activated by DNA damage, degrade NAD^+ to NAM causing depletion of the cellular NAD^+ pool[50]. There are other NAD^+-consuming enzymes, such as cADP-ribose synthases and sirtuins[49]. Meanwhile, NAMPT is a rate-limiting enzyme in the salvage pathway[51], with a known K_M of approximately 5 μM for NAM[52]. Therefore, a high-dose NAM supply (commonly at a level near 5 mM) would readily push the salvage pathway and stabilize the cellular NAD^+ pool for a period of time. An intraperitoneal administration of NAM at a dose of 0.5 g/kg caused a 2- to 3-fold linear increase in NAD^+ levels in rat organs within 24 hours[53]. The complicated NAD^+ metabolome in cytosol and nucleus generates an interesting regulatory loop regarding NAD^+ and sirtuins. NAM, as a product of sirtuin reactions, exerts feedback inhibition to these enzymes but its administration eventually becomes stimulatory to the enzymes though the conversion to NAD^+. This stimulatory-inhibitory loop of NAD^+-NAM might have been developed in cells to regulate various cell physiologies including energy metabolism, circadian rhythm, and stress responses through a dietary cue. Importantly, this also provides a chance for the manipulation of sirtuins and their beneficial health effects through the modulation of NAD^+ levels.

ALTERATION OF CELLULAR LEVELS OF NAD+ AND SIRTUIN ACTIVITIES IN BIOLOGICAL CONDITIONS

In normal physiology, cellular levels of NAD^+/NADH change transiently or chronically, independently of the effect of diet. Circadian rhythm and aging are 2 important physiological conditions where NAD^+/NADH redox status fluctuates or is altered, and sirtuin activities are modified accordingly. In some of these conditions, SIRT1 levels are also altered, suggesting that a complex network that modulates sirtuin activity is active in normal physiological conditions. Meanwhile, certain pathologic conditions, such as hyperglycemia and diabetes and high levels of nutritional glucose, fatty acids, and lactate cause increased NADH/NAD^+ ratios, thereby, producing a cellular condition in which SIRT1 activity is low[54]. SIRT1 plays important roles in reducing glucose levels, increasing insulin sensitivity, and in mitochondrial functions[55], therefore, these observations constitute an important rationale for dietary intervention in metabolic disorders and provides insights for the development of such strategies.

1. Aging

During aging, both the protein level and activity of SIRT1 decline. Several factors are known to be involved in this change. In the progression of aging, the activity of PARP1, which is activated upon DNA damage and signals DNA repair enzymes, is chronically maintained high[56]. While producing poly (ADP-ribose) polymers to generate the signal, this enzyme consumes NAD^+ and causes the depletion of its cellular pool[56]. PARP1 knockout and PARP1-inhibitor treatment commonly cause elevation of NAD^+ levels, SIRT1 activity, and mitochondrial function, while its activation causes a decrease in NAD^+ levels and an increase in the extent of PGC-1α acetylation[56,57]. Meanwhile, NAMPT levels and activity also decline with aging[58] leading to low levels of NAD^+. The decrease of NAMPT levels might be associated with the decrease in circadian function during aging (See below). Circadian transcription factors regulate the expression of NAMPT and their levels decrease during aging[59]. The decline of SIRT1 expression during aging and cellular senescence has also been reported[60-62], and the involvement of the expression of a family of miRNAs has been suggested[15]. The nature of this regulation is not well understood yet.

The decline in SIRT1 function in aging and cellular senescence raises the possibility of this being responsible for mitochondrial deterioration and accumulation of ROS in aged tissues and, thereby, leads to regimens that alleviate the decline of SIRT1 activity as attractive study targets. Indeed, treatment with SIRT1 activators, such as SRT1720, resveratrol[63] or others (metformin[64] and tetramethylpyrazine[65]) was found to increase mitochondrial biogenesis and mitochondrial activities while slowing down the expression of senescence phenotypes in tissues of mice[66].

2. Circadian rhythm

Circadian rhythms function in the 24-hour oscillation of body activities, such as sleeping, feeding, core body temperature, hormone production and secretion, and cell regeneration[67]. It is governed by the suprachiasmatic nucleus (SCN), which receives neural signals from photoreceptors and ganglion cells in the retina[68]. In the SCN, SIRT1 plays an essential role in circadian rhythm modulation by enhancing the expression of the circadian transcription factors aryl hydrocarbon receptor nuclear translocator-like protein 1 (BMAL1) and CLOCK, which together are responsible for the expression

of the proteins executing the rhythm[69]. The expression of BMAL1 and CLOCK is mediated through PGC-1α[70]. Importantly, NAD^+ levels oscillate over the circadian rhythm, likely due to oscillation in the expression of NAMPT through circadian transcription factors[71]. Accordingly, the activities of SIRT1 and PGC-1α also oscillate in the body[70]. Therefore, SIRT1 and the circadian transcription factors form a mutual stimulatory loop that enforces the oscillatory pattern. Furthermore, from the role of the SIRT1-PGC-1α axis in circadian rhythm patterns, a relation between energy metabolism and circadian rhythm has been predicted. Indeed, mitochondrial activity, such as β-oxidation of fatty acids, is an important component of circadian rhythm patterns; this activity enables energy production from different sources during feeding and fasting[72]. The oscillation in SIRT3 activity and its involvement in circadian rhythm patterns has also been suggested by the accumulation of acetylated proteins in the mitochondria of BMAL1-knockout mice[72]. SIRT3 is known to modulate PGC-1α activity; therefore, its involvement in circadian rhythm patterns is expected, but this need to be examined experimentally. As predicted in other studies, the levels of SIRT1 decrease in aging along with the decline in the levels of circadian regulators[59]. This suggests that SIRT1 plays a central pacemaker role in robust circadian control, and a decay of SIRT1 activity may play an etiological role in aging. This also raises the possibility of potential therapeutic intervention in disorders associated with circadian rhythm control in aging *via* the modulation of SIRT1 activity and the level of NAD^+.

MODULATING SIRTUIN ACTIVITIES BY CALORIC RESTRICTION AND ADMINISTRATION OF NAD^+ PRECURSORS

Cellular levels of [NAD^+] or NAD^+/NADH ratio are also subject to metabolic modulations. For example, when cells are poorly fed, the flow of glycolysis decreases and thereby NAD^+ reduction to NADH decreases causing high NAD^+/NADH ratios. Therefore, glucose starvation or caloric restriction (CR) confers a cellular condition in which sirtuins become activated. Other than CR, endurance exercise is another important physiological regimen that modulates sirtuin activities through alterations in NAD^+ redox. Meanwhile, there are chemicals that elevate sirtuin activities by modulating NAD^+ levels; including NAD^+ precursors. In cells, these molecules are converted to NAD^+ and successfully increase NAD^+ concentrations in the cytosol and mitochondria[42,73]. This suggests a therapeutic feasibility of mobilization of sirtuins; not only of SIRT1 but also of those in the mitochondria.

1. Caloric Restriction and Exercise

Upon CR, mitochondrial biogenesis increases[74]. In addition, activities involving mitochondrial energy metabolism including the levels of fuel oxidation and ROS are also augmented in CR, and these seem to be attributed in part to the increase in PGC-1α activity[75]. There are evidences that PGC-1α activation in CR, which is mediated by an increase in SIRT1 activity, underlies the increased mitochondrial biogenesis. During fasting or in CR, SIRT1 expression and its activity is enhanced (through the favorable change in the NAD^+/NADH ratio) contributing to PGC-1α activation[74,76-78]. Increased SIRT1 activity would also be attributable to the enhanced autophagy observed. The expression of factors functioning upstream (Atg molecules) and downstream (lysosomal proteins) of autophagy are elevated in CR rats[79]. The age-associated decline of these molecules and autophagy were also attenuated. CR has also been proposed to activate SIRT1 indirectly through AMPK activation[80]. AMPK activates NAMPT[80] and increases OXPHOS through fatty acid oxidation[81], and thereby increases cellular levels of NAD^+/NADH. Meanwhile, AMPK activation may be attributable to potentiation of mitochondrial biogenesis during endurance exercise[82]. High ATP demand in exercise would cause AMPK activation, which would be followed by increased NAMPT expression and NAD^+ levels[83].

The enhanced β-oxidation of fatty acids is remarkable in CR[84]. Furthermore, the levels of mitochondrial enzymes are elevated in aged CR mice compared to those in aged controls[85]. SIRT3 activation has been suggested to be responsible for this change[86]. Therefore, increased biogenesis *via* SIRT1 activation and enhanced activities of mitochondrial enzymes in association with increased mitophagy together appear to function in improving the efficiency of OXPHOS in the absence of glycolysis in CR. These effects of CR are more prominent in aging where NAD^+ levels as well as SIRT1 activity decline[87] and, therefore, the effects of CR on efficiency in mitochondrial ATP production would be more apparent in old animals.

2. Nutraceutical Intervention of Cellular NAD^+ Level

Recent studies have reported the beneficial effects exerted by sirtuins when cells were administered with the precursors of NAD^+ including NAM, nicotinic acid (NA), nicotinamide riboside (NR), and NMN. Administration of high doses of NAM (e.g., 5 or 10 mM) elevates cellular NAD^+ levels as well as the ratio of NAD^+/NADH[26,88,89]. It also causes an increase in SIRT1 activity and SIRT1-mediated mitophagy[23,24,26,88,90]. Increased SIRT1 expression has also been proposed, although the underlying mechanism has not been well described. Treatment with NA, a derivative of NAM devoid of amine residues, raised the levels of NAD^+/NADH and the extent of autophagy as well[88]. NMN and NR also caused SIRT1 activation by raising NAD^+ levels[91]. In cells, these are incor-

porated in the salvage pathway either directly or through phosphorylation by NR kinases[49] (Fig. 1). NMN administration compensated for the decline of systemic NAD^+ biosynthesis in aged mice - and was able to increase autophagy in fibroblasts[90]. NR administration increased mitochondrial NAD^+ and caused deacetylation of mitochondrial proteins, and also activated SIRT1 as well as SIRT3[91]. It also induced mitochondrial biogenesis in skeletal muscle and brown fat tissue, while reducing mitochondrial abnormalities and mtDNA deletion[92]. Furthermore, NR enhanced oxidative metabolism and energy expenditure causing a physiological condition, which is similar to that induced by CR, while increasing the mitochondrial gene expression[91]. Likewise, the function of these NAD^+ precursors in mitochondrial biogenesis is well established. Although the roles of these chemicals in mitophagy have been poorly demonstrated, this possibility can be readily envisaged from the observed decrease in mitochondrial abnormalities upon NR administration[92].

A variety of natural and synthetic chemicals activate SIRT1[93] and SIRT3[94]. Resveratrol, one of the first generation activators of SIRT1, has been used to show the effect of SIRT1 in mitochondrial biogenesis and quality control[66,95]. Along with other activators, it has been shown to deacetylate PGC-1α and other substrates, even in animals under high calorie diets[95], and also induces the expression of mitochondrial protein genes in a pattern similar to that induced by CR[93]. However, these chemicals have limited specificity for the types of sirtuin proteins, SIRT1 being the primary target. And, therefore, their effects are limited to those induced by SIRT1. Besides, these agents need to penetrate mitochondrial membranes to be effective on mitochondrial sirtuins, such as SIRT3, SIRT4, and SIRT5. Furthermore, at least some of them are known to have off-target effects[96]. In this sense, NAD^+ precursors have a significant advantage over the known SIRT1 activators. They cause an increase in NAD^+/NADH ratios in cytosol as well as in mitochondria, leading to activation of most if not all of the sirtuins[73]. Accordingly, NAM treatment has been shown to effectively increase the SIRT3 activity and deacetylation of mitochondrial proteins[73].

It should be noted that NAM is a product of the deacetylation reaction of SIRT1. Thereby, NAM exerts feedback inhibition on the activity of SIRT1[97]. Since the discovery of this inhibitory effect, NAM has been extensively utilized as an inhibitor of SIRT1 to demonstrate that a given reaction is mediated by SIRT1 activation. For example, treatment with NAM was shown to decrease the expression of downstream targets of PGC-1α in muscle cells[98]. However, NAM treatment of human cells apparently elevated autophagy and removal of mitochondria in a manner dependent on SRIT1[88,90]. NAM is rapidly converted to NAD^+ once incorporated into cells; thereby, its inhibitory effect is transient and NAD^+ levels are expected to be elevated and maintained high for days. Hence, NAM treatment could remain effective in SIRT1 activation[42]. In fact, NAM treatment causes a cellular change identical to that induced by treatment with NAD^+ or NMN when measured after 24 hours[90]. Overall, these NAD^+ precursors are currently promising in subclinical trials targeting aging and aging-associated degenerative disorders.

PERSPECTIVES

Sirtuin proteins, especially SIRT1 and SIRT3, positively modulate mitochondrial quality and control the level of oxidative stress and energy as well as metabolic homeostasis; all of these factors likely contribute to longevity and health. During aging, the expression and activity of both SIRT1 and SIRT3 decrease; this decrease may likely be responsible for the decline in mitochondrial quality and consequently, for the manifestation of many metabolic as well as degenerative diseases, such as fatty liver, diabetes, and neurodegeneration. Recent studies indicate that these deteriorative changes could be intervened by enhancing the activity of SIRT1 and other sirtuin proteins through the modulation of cellular NAD^+ levels. CR provides an excellent example of this. In this review, we introduced the effectiveness of precursors of NAD^+ in sirtuin activation. At least 2 of these precursors, NAM and NR, are freely available as nutraceuticals. These easily accessible NAD^+ precursors have also been proven safe when taken at high doses for days or even years[99]. Therefore, an increased utilization of these precursors is expected in the near future. Additionally, studies are encouraged to identify wider applications of these precursors and to determine conditions for their better and safer usage. In conclusion, the physiological and dietary regimens that promote the mobilization of SIRT1 and other sirtuins require further investigation for wider application in clinical settings.

Conflicts of Interest Disclosure: The authors claim no conflicts of interest.

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