Insights in Biomedicine
ISSN: 2572-5610

Open access Mini Review

Role of Cx43 and SLC25A51 Transporters-New Perspectives in NAD$^+$ Supplementation to Improve Mitochondrial Performance

Bamboo XZ Yan[1], WeiGuo Lao[1], Chi Zhang[1], Thombas Ebjer[1], Hina Asrar[2*]

[1]*Department of Molecular Bioscience, Australian Institute of Integrative Medicine, New South Wales, Australia*
[2]*Department of Biotechnology, Dow University of Health Sciences, Karachi, Pakistan*

ABSTRACT

NAD$^+$, a linchpin for mitochondrial function, is crucial in protecting the body from aging and related diseases. The reduced biosynthesis and increased activity of NAD$^+$ consuming enzymes lower cellular contents of NAD$^+$ with aging. Since the homeostasis of the NAD$^+$ pools is vulnerable to biological aging, and thus, raises a serious concern for the healthcare staff to cure age-related disorders. Replenishing NAD$^+$ pools with the administration of relevant supplements rescues the depleting cellular NAD$^+$. Accordingly, the small-scale human clinical trials have shown ameliorative effects of exogenous NAD$^+$ in treating age-related disorders. However, validation of these studies requires considerable time. Therefore, investigations on a rapid and targeted influx of NAD$^+$ are fundamental to achieving the benefits offered by the science of anti-aging. One possible strategy is the oral administration of intact NAD$^+$ molecule followed by its forced localization to the mitochondria as an elixir of longevity. Integration of the recent findings with previous studies to dissect the trafficking of intracellular NAD$^+$ will direct to a heuristic approach to extending individual lifespan. Here we present a critical analysis of the current status of therapeutic interventions to enhance intracellular NAD$^+$ while highlighting the controversial studies and gaps in the same field. This review emphasizes using a connexion (Cx43) and mitochondrial carrier family (SLC25A51) as the chief NAD$^+$ transporters and the possible erspectives to translate this knowledge for better mitochondrial efficiency. We propose a strategy based on an improved influx of mitochondrial NAD$^+$ that will restore the redox and non-redox functions of NAD$^+$. This understanding may establish a foundation to smartly deal with situations where the inability to import the supplemented NAD$^+$ is the prime cause of mitochondria performance-related diseases..

Keywords: NAD$^+$; Transporters; Mitochondrial performance; Anti-aging science; Healthcare

INTRODUCTION

Nicotinamide adenine dinucleotide (NAD) is one of the most vital metabolites in living cells. It can act as an electron donor and acceptor by becoming oxidized (i.e., NAD$^+$) and reduced (i.e., NADH). These redox reactions are the principal source of energy (ATP) production in the mitochondria and facilitate reactions involved in glycolysis, tricarboxylic acid cycle, fatty acid beta-oxidation, etc. Although the interconversions between NAD$^+$ and NADH do not change the cellular contents of total NAD, an alteration in their content eventually leads to secondary, i.e., oxidative stress, where significant harm is done to biological macromolecules, including DNA, proteins, and lipids. Since NAD$^+$ (not NADH) also serves as a co-substrate in multiple non-redox reactions, the changes in the NAD$^+$/NADH ratio hamper the signalling and metabolic regulatory pathways [1,2]. Because of its role as a co-enzyme and co-substrate, NAD$^+$ continues to receive considerable attention in the field of aging even after decades of extensive study because of its emerging roles both as a co-enzyme and co-substrate. Aging, recognized as the hallmark of the systematic decline of NAD$^+$ in various tissues, paves its path to pathophysiologies of many

Received:	28-June-2022	**Manuscript No:**	IPIB-22-14019
Editor assigned:	30-June-2022	**PreQC No:**	IPIB-22-14019 (PQ)
Reviewed:	14-July-2022	**QC No:**	IPIB-22-14019
Revised:	19-July-2022	**Manuscript No:**	IPIB-22-14019 (R)
Published:	26-July-2022	**DOI:**	10.21767/2470-9867.7.7.88

Corresponding author Hina Asrar, Department of Molecular Biology, Dow University of Health Sciences, Pakistan, E-mail: hinaasrar@gmail.com

Citation Yan BXZ, Lao W, Zhang C, Ebjer T, Asrar S (2022) Role of Cx43 and SLC25A51 Transporters-New Perspectives in NAD$^+$ Supplementation to Improve Mitochondrial Performance. Insights Biomed.7:88.

critical diseases, including neurodegenerative, mental, and cardiovascular phenotypes. Eliminating the significant causes of these diseases may enhance life expectancy by 2-4 years [3]. The progression of these age-related disorders is strongly associated with reduced biosynthesis and increased consumption of NAD^+ [4] Figure 1 demonstrates the factors responsible for reduced NAD^+ content in the body. Recent studies have implicated the therapeutic potential of increased NAD^+ levels in treating age-associated conditions; therefore, an interest in NAD^+ boosting strategies receives particular attention. With advances in the molecular tools to measure NAD^+ content, it is becoming clear that targeted localization of intact NAD^+ into mitochondria rather than just enhancing the cellular NAD^+ is more relevant to treating and preventing age-related ailments. Understanding NAD^+ transport into mitochondria has been the most debated topic as the mitochondrial membrane was presumed impermeable to the NAD^+ molecule. Thus, by uncovering the sources of mitochondrial NAD^+ pool, better strategies to improve mitochondrial performance may be designed. The implication of upgraded information in therapeutic interventions will ensure the integration of mitochondria-targeted therapies into clinical trials. Since extensive reviews on the metabolism and function of NAD^+ have been published recently, we have summarized the fundamental information in this regard. In addition, the methods and technologies previously adopted to highlight the therapeutic potential of NAD^+ in treating age related conditions and the limitations in their implications are also reviewed. In particular, we have traced the transport of intact NAD^+ into the mitochondria to maintain the mitochondrial NAD^+ pool. Finally, we have proposed the appropriate perspectives to enhance NAD^+ contents radically and, thus, a healthy lifespan. The data source for this research included cross-sectional studies, randomized control trials, systematic reviews, and recent relevant studies. Detailed and authenticated materials by credible search engines, including Research Gate, JSTOR, PubMed, and Science, have been cited for this. (**Figure 1**)

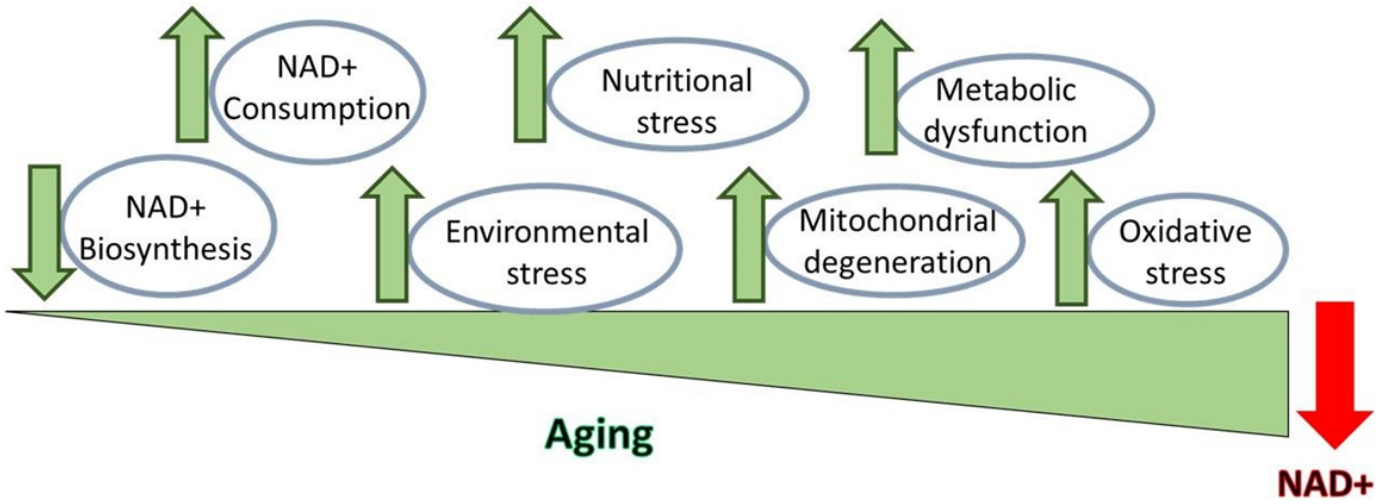

Figure 1: A presentation of factors contributing to the age-dependent decline in contents of NAD^+.

DESCRIPTION

Biosynthesis of NAD+

Various forms of vitamin B3, such as nicotinic acid (NA), nicotinamide (NAM), and nicotinamide riboside (NR), serve as a starting point for the biosynthesis of NAD^+ via two major routes: 1. The three-step Preiss-Handler pathway which generates NAD^+ from nicotinic acid (NA) and 2. The two-step Salvage pathway recycles nicotinamide (NAM) to produce NAD^+. Conversely, de novo synthesis of NAD^+ takes place by conversion of L-tryptophan into 3-hydroxyanthranilic acid through the kynurenine pathway [5]. Intermediates produced during the synthesis of NAD^+ and some alternate precursor molecules (e.g., nicotinic acid riboside (NAR)) may also serve as starting point for NAD^+ generation. However, the explanation for these alternate mechanisms is beyond the scope of this review and can be found in other works [6]. Besides, the products formed due to the consumption of NAD^+ by NAD-degrading enzymes (discussed in the next section) function to replenish NAD^+ pools. The first enzyme in the Preiss-Handler pathway, i.e., nicotinic acid phosphoribosyltransferase, produces nicotinic acid mononucleotide (NAMN) from nicotinic acid (NA). NAMN is furthered upon by nicotinamide mononucleotide adenylyltransferase (NMNAT), which adenylates NAMN to produce nicotinic acid adenine dinucleotide (NAAD). The last enzyme in the pathway, i.e., NAD^+ synthase, amidates NAAD to yield NAD^+ [7]. The completion of the Salvage pathway depends on the activity of two enzymes:

i) Nicotinamide phosphoribosyltransferase (NAMPT)

ii) Nicotinamide mononucleotide adenylyltransferase (NMNATs). NAMPT generates nicotinamide mononucleotide (NMN) from NA in a rate-limiting step, whereas NMNATs act on NMN to produce NAD^+ [8].

Consumption of NAD+

Currently, three classes of NAD^+ consuming enzymes are recognized in mammals:

i) Sirtuins

ii) Poly-ADP-ribose polymerases (PARPs)

iii) c-ADP ribose synthases [9]. In addition, NAD+ is also actively used for cellular mechanisms by many other enzymes

with activities like glycohydrolases, pyrophosphohydrolases, etc [10].

By degrading NAD^+ permanently, these enzyme families mediate a broad range of physiological processes, which range from DNA damage repair to lifespan extension. However, with a decline in the levels of NAD^+ during aging, the activities of NAD^+ dependent enzymes are affected, which eventually initiates a broad range of age-related pathophysiologies. Sirtuins are NAD^+ dependent enzymes with deacetylase/deacylase activity. By translating changes in NAD^+ levels to regulate metabolic activities, sirtuins have a central role in cellular functions such as DNA repair, circadian rhythm, and stress responses. In addition to their localization in the nucleus and cytosol, processed short forms of sirtuins (SIRT3-SIRT5) are present in the mitochondria [11]. NAD^+ is used by sirtuins as a co-substrate in the process of removal of acetyl moieties from lysines of histones proteins, liberating O-acetyl-ADP-ribose and NAM [12]. However, sirtuins also target non-histone proteins and modify their physiological functions. Different homologs of sirtuins exhibit other activities in addition to deacylation. For instance, demethylglutarylase activity by SIRT4 demalonylase and desuccinylase property of SIRT5 ADP-ribosyltransferase activity by SIRT4 and SIRT6 [13-16]. Poly-ADP-ribose polymerases (PARPs) are at the center of extreme importance because of their wide-range functions in repairing damaged DNA and mediating cellular programmed death. These enzymes have been shown to cause moderate damage to DNA as the levels of NAD^+ decline by 20%-30% compared to the normal levels. Evidence also supports the role of PARPs in influencing the functions of neurons and the endoplasmic reticulum under stress [17]. PARPs consume NAD^+ by cleaving it into NAM and ADP-ribose (ADPR). Because of a common substrate (i.e., NAD^+) to act on, the competition between PARPs and sirtuins is typically expected. However, an increase in PARPs activity while a decrease in sirtuins activity was reported as repairing damaged DNA is primarily associated with PARP-dependent NAD^+ depletion [18]. Cyclic ADP-Ribose (cADPR) synthases act as a secondary messenger and actively participate in calcium signalling, insulin regulation, and the cell cycle. The consumption of NAD^+ by the action of these enzymes produces cyclic ADP-Ribose. The most dominant CD38 and its homolog CD157 are well-recognized members of cADPR synthases. The research by China (2009) reported that the deficiency of Cd38 represented an evident increase in the NAD^+ content of the hepatic cells, cardiac cells, neurons, and muscles associated with the corresponding activation of SIRT1 [19].

Subcellular Compartmentation of NAD+

Cytoplasm, nucleus, and mitochondria are the prominent subcellular locations for NAD^+ pools. Complex redox processes interconnect these pools. The intracellular concentrations of NAD^+ are usually maintained within the range of 0.2-0.5 mm and are in the following order: mitochondria>cytosol>nucleus [20]. In addition, the NAD redox state is particular to cellular compartments. For example, the content of NAD^+ exceeds that of NADH in the cytoplasm to ensure the availability of NAD^+ for oxidative reactions. In contrast, mitochondria have a relatively higher concentration of the reduced counterpart, i.e., NADH, to ensure optimal oxidative phosphorylation [21]. Although the mechanisms by which mitochondria accumulate their NAD^+ pool are not entirely understood, 70% of total cellular NAD^+ resides within the mitochondria [22]. However, these concentrations may change up to two-fold in the presence of any physiological stimulus. Lack of NAD^+ is regarded as lethal for the metabolic reactions which demand energy. Even a change in NAD^+ redox status affects cellular behaviour irrespective of the extent to which the change occurs. The mitochondrial pool among all the NAD^+ pools is significant for vital cellular functions and cellular functions [23]. The NAD^+/NADH pair in the mitochondria generates energy (ATP) from the oxidation of diverse substrates (proteins, carbohydrates, fats), i.e., oxidative phosphorylation. While the cytoplasmic and nuclear NAD^+ pools may change in response to nutritional modifications like the cellular contents of glucose, fats, etc [24]. The mitochondrial pool has independent regulatory mechanisms even under non-optimal conditions [25]. Despite this autonomous NAD^+ pool, the homeostasis of mitochondrial NAD^+ content is considered critical for the function of multiple cellular organelles [26]. NAD^+ pools across various compartments are interconnected [27]. The concept of compartmentation is crucial to understanding the balance between co-enzymes vs. co-substrate functions of NAD^+, given the supply of this essential nucleotide becomes limited with aging. The recent findings have made this notion clear that intracellular compartmentation of NAD^+/NADH redox couple is obvious. Because of this compartmentation and the pleiotropic roles performed by NAD^+, it is assumed that pharmacological and therapeutic approaches to enhance the levels of intracellular NAD+ or to reduce the activity of NAD^+consuming enzymes may impose health risks and, therefore, should be dealt with care [28]. For instance, the overlapping effects of elevated NAD^+ in tumour prevention and promotion were demonstrated with increased content of NAD^+ precursors and the affiliated enzymes [29,30]. These discoveries highlight the importance of adapting therapeutic interventions to enhance the targeted subcellular localization of NAD^+ supplements rather than increasing the overall NAD^+ to minimize the adverse side effects.

Pleiotropic Roles of NAD+ in Physiological Processes

The redox and non-redox reactivity of NAD^+ mark NAD^+ as an essential player in cellular metabolism (**Figure 2**). The pleiotropic roles of NAD^+ range from catabolism/anabolism to cell signalling and adaptation. For the proper operation of redox functions, NAD^+ levels are maintained at a relative abundance of their reduced counterpart, i.e., NADH. The redox functions of NAD^+ are based on the deficiency of electrons in the pyridine ring and consequently on the acceptance of hydride ion. The homeostasis of the cellular redox state is crucial for the activity of metabolic pathways such as glycolysis and beta-oxidation of lipids. Alternatively, the non-redox functions of NAD^+ include the direct or indirect influence on essential cellular properties like DNA damage repair, gene expression, post-translational modifications, and stress response. These functions depend on the activity of NAD^+ consuming enzymes such as Sirtuins, PARPs, and cADPR-synthases. Thus, with declined NAD^+ levels and ceased activity of consuming enzymes, DNA damage induces genetic mutations, later translated as the pathophysiology of

chronic diseases, including tumours.

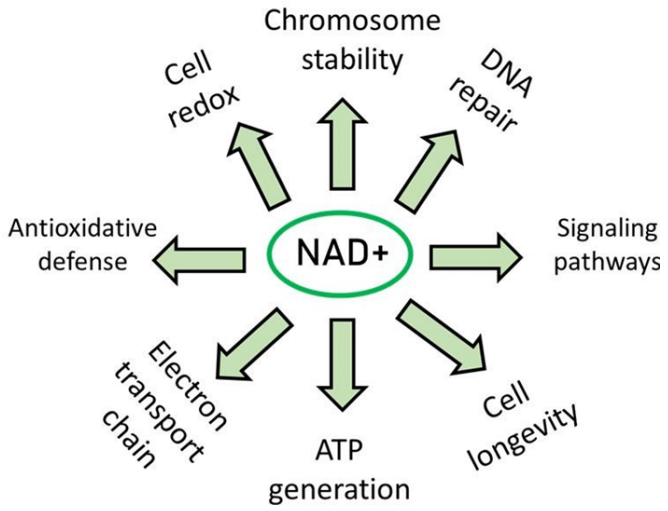

Figure 2: Representation of cellular functions performed by NAD⁺ during a cell life.

NAD+ in Redox Reaction

With advances in tools such as biosensors to measure biosynthetic enzymes, understanding NAD⁺ mediated cellular redox has improved. It has been established that NAD⁺ is responsible for significant oxidation-reduction reactions occurring in the cell. The shifting between oxidized (NAD⁺) and reduced (NADH) states permits a continuous flow of electrons across various biological processes. This NAD⁺ recycling is crucial for ATP generation via various metabolic processes like glycolysis, Krebs cycle, and electron transport in the cytosol and mitochondria. Interestingly, the activity of NAD⁺ consuming enzymes Sirtuins, i.e., SIRT3 was recently found dependent on the NAD⁺/NADH ratio rather than on the NAD⁺ content alone [21]. Such findings indicate the complexity between redox and non-redox functions of NAD⁺. Even though the redox processes provide fuel for cellular activities, the contents of NAD⁺ itself remain conserved during their operation. However, any alteration in the NAD⁺/NADH redox couple is associated with various pathological conditions such as neurogenerative and cardiovascular disorders [31]. For instance, impairments in mitochondrial oxidative phosphorylation escalated impairments in cellular redox status by enhancing the NADH/NAD⁺ ratio, which is further associated with the acylation of mitochondrial proteins [21]. Even if the production of reactive oxygen species (ROS) does not increase and ATP contents remain constant, the mitochondrial integrity may be hampered in such a case, causing epigenetic changes to increase the organ's susceptibility to chronic diseases and organ failure. On the other hand, increased ROS generation during a limited pool of reducing equivalent, i.e., NADH also paves its way to amalgamating pathogenic mechanisms of various chronic diseases. The first produced ROS, i.e., superoxide, is reactive to various biological molecules and induces oxidative stress. The increased oxidative stress then aggravates metabolic dysfunction and cellular injury. Thus, the homeostasis of the NAD⁺/NADH redox couple is vital to integrating cellular responses under metabolic and environmental shifts.

NAD+ in Non-Redox Reactions

The degradation of NAD⁺ is the basic element of multiple signalling pathways in humans [32]. By acting as a co-substrate for various enzymes, NAD⁺ regulates post-translational modifications of a diverse group of proteins with functions in DNA repair, signalling, and stress responses, thereby connecting the molecular mechanisms with physiological functions (**Figure 3**). With the advent of modern molecular tools, many more enzymes have been discovered which utilize NAD⁺ molecule to modify the structure and function of other proteins. NAD⁺ contributes to various pathways during cell signalling, from regulating intercellular calcium transients to chromatin epigenesis. In addition to acting as a co-substrate for enzymes like sirtuins, PAARPs, and c-ADPR, NAD⁺ is known to regulate the expressional response of metabolism encoding genes [27]. The extracellular NAD⁺ acts as a ligand for various cell-surface receptors, i.e., purinoreceptors, and initiates the signalling cascades [33]. Once inside the cell, the cytoplasmic NAD⁺ may act as a signalling molecule after being consumed by c-ADPR-synthases. The intermediates formed during this process, i.e., NAAD and cADPRs, bind to the endoplasmic reticulum and initiate calcium release. Thus, by enhancing cytosolic calcium levels, NAD⁺ is a precursor molecule for synthesizing spatially and temporally distinct calcium mobilizing secondary messengers [34]. The NAD⁺ localized to the nucleus regulates cellular lifespan and longevity by regulating transcription and circadian rhythm. The nucleus's NAD⁺ consuming enzymes, i.e., sirtuins, and PARPs, utilize nuclear NAD⁺ to directly control several transcription factors' activity.

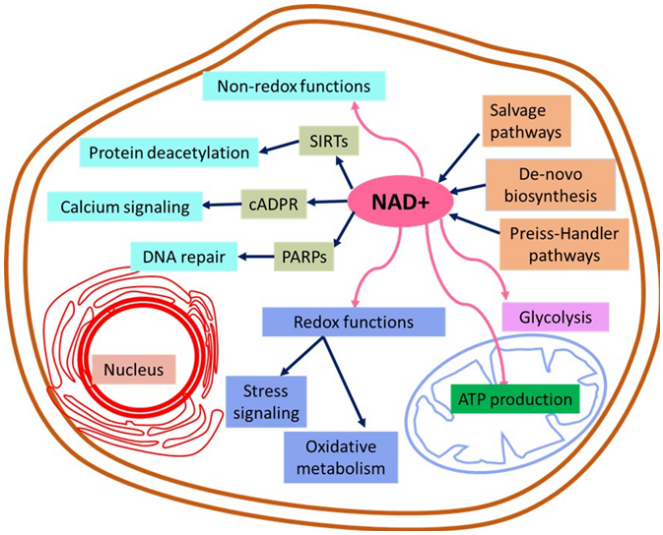

Figure 3: An illustration of the diversification of NAD⁺-related biological roles. The main 723 routes for biosynthesis and consumption of NAD⁺ along with the redox-and non-redox 7 functions of NAD⁺ are presented.

Depleting Pools of NAD+ and Related Health Complications

A diverse line of research on various organisms has confirmed that declining NAD⁺ pools are a hallmark for the development of many age-related ailments [18,35,36]. The depleted pools of NAD⁺ manifest a wide range of pathological phenotypes such as congenital deformations, neurogenerative disorders, age-related metabolic dysfunction, etc. The close relationship between NAD+ levels and human health was confirmed with the studies where impairments in NAD⁺ biosynthetic pathways at a genetic level led to devastating consequences as recorded with typical clinical signs and symptoms of age-associated disease. As mentioned earlier, many factors responsible for declined NAD⁺ contents include impaired biosynthesis, enhanced consumption,

and nutritional stress. For instance, the transcripts and protein levels of NAMPT, i.e., a key enzyme in NAD+ biosynthesis, decline over age, disturbing the NAD+ levels in various tissues and affecting the redox and non-redox pathways. Furthermore, continuous activation of PARPs lowers NAD levels by consuming it for NAD+ dependent enzymatic activity. This has been proved with a recorded increase in endogenous NAD+ when the activity of PARP was inhibited pharmacologically [37]. Other studies suggest that ectopic expression of PARPs gives rise to multiple age-related phenotypes [38] (**Figure 4**). Interestingly, with disappearing NAD+, the binding of DBC1 (deleted in breast cancer-1; a nuclear protein) with PARP increases, contributing to the accumulation of damaged DNA [39]. The deficiency of NAD+ also induces oxidative stress by altering cellular redox. The validation of the posit that reduced NAD+ is the responsible agent for causing several chronic diseases was shown with case studies where the enhanced NAD+ with the provision of NAD+ based supplements and dietary measures were sufficient to cure phenotypes of neurodegenerative diseases, metabolic disorders, and other age-related complications [40]. Therefore, replenishing NAD+ levels with either the precursors or intact NAD+ supplements appears to be a powerful therapeutic approach to boost health and prevent and treat age-related disorders.

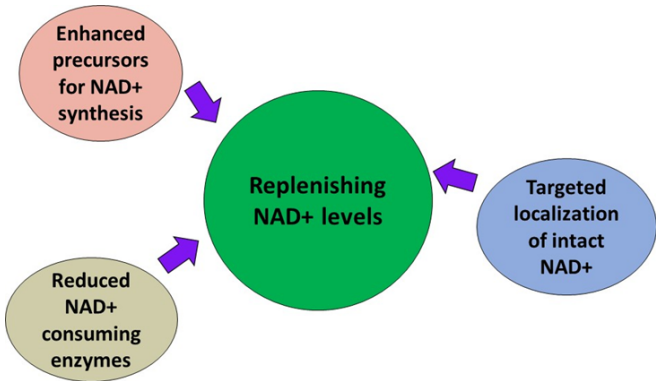

Figure 4: An overview of the possible strategies to replenish age-related declined NAD+ pools. Enhanced contents of NAD+ precursors and reduced activities of NAD+ consuming enzymes are well-known approaches to increasing cellular NAD+. Targeted localization of intact NAD+ molecules to mitochondria is another promising approach to ameliorate depleting NAD+ pools.

Supplementation of NAD+

Mounting studies have proved that an effective anti-aging intervention could be developed with a careful formulation of NAD+ supplements. Indeed, the administration of NAD+ precursors, like nicotinic acid, nicotinamide mononucleotide, nicotinamide, and nicotinamide ribose, have resulted in some beneficial effects by enhancing the cellular NAD+ levels [41]. These benefits include short-term improvement in the case of type 2 diabetes, cholesterol profile, blood sugar level, lipid profile, and a reduction in symptoms of other age-related diseases [42]. Nicotinamide mononucleotide and nicotinic acid are both used as supplements, but studies reporting their effectiveness by increasing NAD+ levels are fewer. The large-scale clinical trials such as NCT03151239, NCT03423342, NCT02835664, etc., to check the efficacy of NA in treating circulatory system ailments also showed significant side effects. Instead, a significant increase in intermediates of NAD+ metabolism after the administration of other NAD+ precursors is extensively reported [43,44]. A recent work by reported the ability of a single oral dose of nicotinamide (NAM a precursor for NAD+ synthesis) to maximize the NAD+ levels after the 12th hr [45]. of intake, an upper limit of the daily tolerable range. The increase in NAD+ was inconsistent with the increase in NAM levels suggesting the utilization of NAM into pathways other than NAD+ biosynthesis. Similarly, the metabolomics changes associated with nicotinamide supplementation were returned to baseline by 48 hrs. In contrast, the supplementation of nicotinamide ribose (NR), an intermediate of NAD+ biosynthesis, has shown more promising results in a dose-dependent manner. The exogenous application of NR has improved mitochondrial function in the liver and muscles [46]. The pharmacokinetics of NR, as conducted by (2017), indicated no associated side effects on the oral administration of NR [47]. However, the bioavailability of NR after administering a high dose, i.e., 1000 mg, was not consistent among the participants. Based on its hydrophobicity, the inter-participant variation of NR levels was assigned to the low permeability of NR across the intestinal mucosa. The instability of NR and its interconversion to other intermediates of NAD+ biosynthetic pathways are also important factors to consider. Hence, more clinical trials to understand the absorption and metabolic insights of administrated supplements are the need of the era. This lack of information addresses the concern that despite dedicating many decades of active research to enhance NAD+ levels and elucidate the possible molecular mechanisms responsible, several clinical trials can exploit NAD+ homeostasis as a therapeutic strategy. It is worth mentioning that the superiority of one NAD+ precursor over the other precursors cannot be stated precisely as the molar ranges of supplements, measurements, interventions, etc., used lack standardization previously. As extensively reviewed, the levels of most NAD+ precursors in plasma are insufficient to support increased production of NAD+ [48]. Previously, the use of intact NAD+ rather than its precursors to cure age-related degenerative disorders of the brain, heart, muscles, kidney, and skin was disapproved by researchers based on their assumption that intact NAD+ molecule is volatile. These assumptions were, however, found wrong as the positive effects related to the intravenous application of intact NADH persisted for more than 24 hrs. in Parkinson's disease patients. Thus, a stable, ingestible, and absorbable form of NAD+ as a therapeutic agent was formulated. The consumption of intact NAD+ in the form of a pill coated by the acid protective outer layer offers two advantages,

1) Patients take the oral form of NADH without any external supervision
2) The stable oral form of NADH withstands the acidic environment of the gastrointestinal tract.

As systematically reviewed by Radanovich and Verdin (2020), out of 36 human trials with published findings have implied oral administration of intact NAD+ molecule [49]. In extension, it has been shown that supplementation of intact NAD+ is more effective in a relatively low dose than the higher doses of the NAD+ precursors. The supplementation of intact NAD+ significantly increases levels of NAD+ in blood. To compare and contrast the effectiveness of different modes of NADH infusion, i.e., intravenous and oral, an open-label trial was conducted on patients with Parkinson's disease [50]. The study's findings showed that 80% of patients with Parkinson's disease in both groups experienced symptom relief. The analysis of improvement in disability (walking, pushing, and posture) indicated a more con-

vincing impact of oral in contrast to IV infusion. It was stated that the direct intake of NAD^+ increases the bioavailability of the dinucleotide and delays the decline of cognitive functions of the brain, thereby improving mental health. However, no significant improvement in the cognitive functions of dementia patients upon the administration of a 10 mg commercial NADH tablet was observed [51]. This ineffectiveness may be due to the limited number of patients tested and the absence of a control group. Meanwhile, clinical trials report improvements in certain cognitive functions and prevention of Alzheimer's disease progression upon administering 10 mg NADH [52]. The contrasting findings, therefore, indicate the need for more research on the most effective route for importing NAD^+ molecules to the mitochondrial membrane during the oral Administration of NAD^+.

Limitations in the Assimilation of Exogenous NAD+

The precursors for NAD^+ synthesis, i.e., tryptophan, NA, NAM, and NR, can be ingested through a healthy diet. However, transporting these precursors into the desired tissues and cells is fundamental to enhancing the levels of NAD^+. A layer of difficulty is added to the NAD^+ transportation into the desired cells because of the presence of lipid bilayers. Although the transporter proteins and the mechanisms responsible for importing NAD^+ precursors into the cell are mostly known, some of these findings are debated to date. For instance, the cellular uptake of NMN was considered possible only after the dephosphorylation (i.e., conversion to NR) [53]. Still, other studies proposed that cellular membranes are permeable to intact NMN molecules [54]. Moreover, despite the classical misconception that the NMN dephosphorylation event is specific to certain cell types, further research unequivocally suggests the ability of NMN to respond similarly to various cell types [55-57]. In addition to the limited availability of niacin-derived NAD^+ precursors, the declined levels of NAD^+ biosynthesis-related enzymes, such as NAMPT, restrict NAD^+ biosynthesis and, subsequently, cellular levels of NAD^+ during aging [58]. The hydrolysis of ingested NAD^+ first to NMN and then to NR with the last product as NAM, as it passes through the intestinal tract, restricts the assimilation of exogenously supplied NAD^+ [59]. In addition, cell surface proteins like CD73 catalyse the NAD^+ hydrolysis, which may cause cells not to acquire NAD^+ levels as expected with exogenous NAD [60]. Notably, the application of NAD^+ but not the precursors (nicotinamide or NMN) enhanced the levels of matrix NAD^+ in mitochondria of mammalian cells treated with FK866, i.e. an inhibitor of NAMPT. Strikingly, the activity of NAMPT was also not detected in the mitochondria isolated from other mammalian cells [25,61,62]. These findings paved the path to identifying the NAD+ transporters localized to the mitochondrial membrane.

Mitochondria-Localized Transporters of NAD+

With the development of genetically encoded biosensors to measure NAD^+ in cellular compartments it was proposed that the mitochondrial NAD^+ pool is distinct from nuclear and cytoplasmic pools [63]. The extracellular NAD^+ enters the cytoplasm after being metabolized into its precursor molecules, such as NAM and NR [44,64]. The consumption of NAD by ectoenzymes such as CD38 (which converts NAD^+ to cADPR and NAM) and CD73 (which converts NMN to NR) also produces membrane-permeable precursors for NAD+ biosynthesis. The discovery of NMNAT3 an enzyme of the Preiss-Handler pathway, in the mitochondrial matrix, supported the import of nucleocytosolic NMN as the precursor molecule to synthesize mitochondrial NAD^+ [53]. However, the exact mechanism behind the transport of NMN to the mitochondrial matrix has not been elucidated completely. The contribution of at least two fundamental mechanisms is, thus, acknowledged to maintain mitochondrial NAD+:

1) The trafficking of cytoplasmic NAD^+ into mitochondria
2) The synthesis of NAD^+ from NMN in the mitochondrial matrix. Meanwhile, indirect evidence suggesting NMN as an unlikely molecule to be transported across the mitochondria is accumulating.

For instance, the removal of NMNAT, i.e., the enzyme synthesizing NAD from NMN, did not affect the mitochondrial performance and NAD^+ levels [65]. Whereas other studies suggesting the import of intact cytosolic NAD^+ into mitochondria are emerging [61,66]. The two long-held misconceptions, i.e.,

1) The inner membrane of mitochondria is impermeable to intact NAD^+
2) The mitochondrial NAD^+ levels are in isolation with respect to cytosolic and nuclear NAD^+ pools were challenged with the increased levels of intracellular NAD^+/NADH upon the application of exogenous NAD^+ or NADH [63].

However, the mechanisms involved in the intracellular movement of NAD^+ are not completely known. It was postulated that provided NAD^+ first degrades into its intermediates which are then transported to the mitochondria to resynthesize NAD^+, but the discovery of mitochondrial NAD^+ transporters in bacteria, yeast, and plants encouraged researchers to look for its orthodox in mammals [67-69]. Surprisingly, efforts to ensure constitutive expression of plant mitochondrial NAD^+ transporters (NDT2) in human (NDT2) cells were proven inadequate for their growth despite an increased NAD^+ was recorded [70]. A detailed analysis of different transporters of mitochondrial NAD^+ in humans is therefore central to enhance its influx for various biological pathways.

Connexin43-Mediating Intercellular NAD+ Trafficking

Connexions, a large protein family of intercellular gap junction complexes, comprise about 21 members of the human genome. After their synthesis, connexions are primarily localized to the plasma membrane, performing various physiological and pathophysiological roles [71]. Different isoforms of connexion, either heterogenic or homomeric, possess membrane-spanning structures that produce gap junction channels in the adjacent cells allowing coupling and intercellular communication [72]. Among these, connexin43 (Cx43) is the most expressed and, therefore, the most extensively studied protein, which, to our surprise, also exhibits several gap-junction-independent roles. The multitude of gap-dependent and gap-independent functions of Cx43 is believed to be because of the distinctive processing of Cx43 transcripts (alternate splicing) and proteins (alternate translation). Consequently, fragments of variable length, each with a unique subcellular destination and biological activity are

produced [73]. Although the functions of connexions like enhanced apoptosis and inhibited cellular proliferation are considered toxic; the intercellular transfer of NAD^+ is their most interesting function for healthcare staff [74-79]. By regulating the trafficking of extracellular NAD^+, Cx43 facilitates mitochondrial performance and, therefore, is the centre of focus in treating critical diseases. Studies providing evidence for the function of connexion as a transporter of an intact nucleotide (e.g., NAD^+) in no coupled/isolated cells started to emerge at the end of the previous century. For instance, the NAD^+ transport property of Cx43 was also unequivocally proved in proteoliposomes reconstituted with the same Cx43 proteins. Moreover, there is evidence that localization of Cx43 to the mitochondrial membrane happens, i.e., the shuffling of cytosolic Cx43 was recorded under ischemic insult due to the expression of heat shock protein [80]. It was further revealed that upon reaching mitochondria, Cx43 is imported to the inner membrane of mitochondria and functions to improve potassium influx and respiration [81]. In addition, the discovery of channel-independent functions of Cx43, such as assistance in the intercellular transport of mitochondria, indicated the crucial role this protein performs in repairing damaged tissues in the course of physiological and environmental stress. As the size of Cx43 is not enough to work as passageway for mitochondrial movement, it still contributes to the process by binding to the docked membranes of neighbouring cells while acting as a stabilizer [82]. Since the shuffling of cytosolic Cx43 to mitochondria was recognized in the myocardial cells during ischemia-reperfusion preconditioning (IPC), its molecular function in a context other than IPC in both the myocardial and non-myocardial cells remains to be elucidated. However, the functioning of mitochondrial localized Cx43 as a mediators of potassium influx, mitochondrial uncoupling, and generation of ROS ensures the mitochondrial integrity [73]. Thus, to assure the import of intact NAD^+ supplements into the cells and improved mitochondrial performance, the use of Cx43-based medical intervention sounds promising to treat metabolic conditions associated with aging.

Slc25a51-A Regulator of NAD+ Import in the Mitochondria

Members of mitochondrial carriers (solute carrier family SLC25) facilitate the Trans membrane transport of biologically active molecules to maintain cellular homeostasis and energy conversion. The transport of these solutes is crucial for processes such as oxidative phosphorylation of carbohydrates and fats, heat production, synthesis of heme and iron clusters, etc. The presence of mitochondrial NAD^+ transporters in mammals was considered controversial, but the discovery of their counterparts in other organisms like yeast and plants provoked researchers to intensify their search [67,69]. Recently, the function of SLC25A51 was identified as a mammalian transporter for mitochondrial NAD^+. A loss in its expression caused reduced levels of mitochondrial NAD^+ and mitochondrial respiration; whereas an increase in its expression helped cells regain NAD^+ levels [80]. The authors of the study used biosensors to demonstrate decreased NAD^+ content in the tumour cells other groups demonstrated similar findings [65,84]. Mitochondria require NAD^+ to carry out the essential processes that generate fuel for respiration and facilitate cellular energy transduction. Thus, up to 70% of cellular NAD^+ is localized in the mitochondria, where ATP synthesis occurs during the mitochondrial electron transport chain (mETC). The observation that the absence of SLC25A51 has reduced the proportion of NAD^+ in mitochondria but not in other cellular components further signified the existence of SLC25A51 specifically as a transporter for mitochondrial NAD^+ [83]. The functional relevance of this gene was made clear from a data mining case study for 341 cell lines where this so-far unstudied gene (SLC25A51) was noticed to hold a position among the cluster of genes that affect processes like mETC [65]. Two other members of the SLC25 family, i.e., SLC25A52 and SLC25A53, have sequence identity with that of SLC25A51, but their expression across tissues and frequent cell lines is reported to be limited. On the other hand, the localization of SLC25A51 in the mitochondrial inner membrane was affirmed with super-resolution microscopy, even though this membrane was previously considered impermeable to NAD+. In addition to NAD^+ and NADH, the contents of TCA cycle intermediates such as cis-aconitate, α-ketoglutarate, and malate, and a loss in specific activity of complex 1 of mETC decrease in SLC25A51-deficient cells [65]. It is noteworthy; however, that depletion of redox cofactor (NAD^+) in SLC25A51-null cells enhanced their dependence on the other, i.e., flavin adenine dinucleotide (FAD).

Evidence for the Therapeutic Potential of Increased NAD+ Content

The high NAD^+ contents exert protective effects in treating ischemic strokes and cardiovascular and neurodegenerative diseases [12,85]. However, the small-scale clinical trials investigating the effects of deliberate doses of NAD^+ precursors have shown mixed results for Parkinson's and Alzheimer's diseases. Similarly, the use of NAD^+ precursors to cure metabolic conditions like muscular composition, exercise capacity, insulin resistance, diabetes type 1, and lipid profile demonstrate positive to no benefits [86-88]. The upregulated levels of NAD^+ were also shown to protect against keratosis and photoaging by facilitating the activity of NAD^+-dependent PARP enzymes to repair damaged DNA [89-90]. In another study of NR supplementation as NAD boosting molecule, the increased NAD levels ameliorated metabolic dysfunction in obese mice while enhancing their energy metabolism [46].

DISCUSSION

Restored levels of NAD^+ with NR supplements improved glucose intolerance in type II diabetic mice [56]. The elevated NAD^+ as an intervention to intervene in various medical conditions, including hypertension, schizophrenia, acute kidney damage, etc., is also reported. Still, it should be noted that most of the mentioned diseases are addressed by following a single trial study. Further trials to reproduce the positive effects of NAD^+ are still in progress. Therefore, it will be early to conclude which mode of escalating NAD^+ content, i.e., NAD^+ precursors or intact NAD^+, is more efficient in preventing NAD^+ deficiency-associated diseases. Here, we suggest increasing the number of clinical trials administrating intact oral NAD (H) and those supplementing precursors for NAD^+ biosynthesis to promptly identify the therapeutic intervention that has a solid potential to cure multiple health issues in less time. (**Figure 5**).

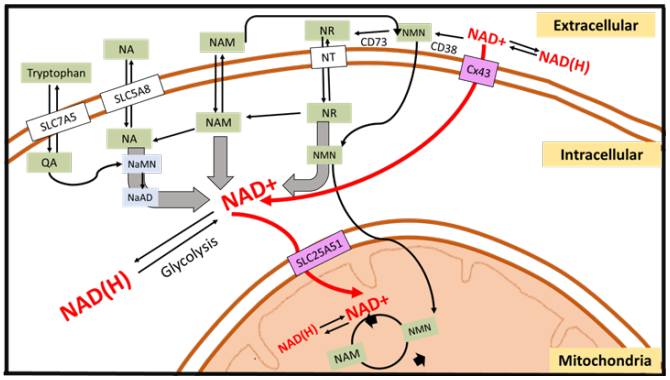

Figure 5: Integration of previous and recent discoveries to demonstrate the routes of 742 mitochondrial NAD⁺. Previously, the mitochondrial membrane was considered 743 impermeable to transport intact NAD⁺, and precursors like NMN supported synthesizing 744 NAD⁺ specifically in mitochondria. Recent findings indicate that extracellular NAD⁺ may 745 enter the cell via the connexin proteins (Cx43) and further localized to mitochondria via 746 mitochondrial carrier family proteins (SLC25A51). Hence, we propose that increased 747 expressions of these transporters by targeting their particular genes may facilitate a rapid 748 intake of intact NAD⁺ supplements. The abbreviations for the precursors, intermediates, and 749 enzymes related to NAD⁺ metabolism have been explained in the text.

CONCLUSION

Biological aging is related to mitochondrial activity at a cellular level, which ultimately depends on the available NAD⁺ content. Mitochondrial NAD⁺ influences ATP production while acting as a co-factor and co-substrate for a rising list of enzymes. Due to competition among the enzymes that require NAD⁺ as a cofactor (redox reactions) and those that require it as co-substrate (non-redox reactions), cells usually face depleted pools of this essential dinucleotide. It has become clear that NAD⁺ decline is the primary root cause to limit organismal lifespan with aging. The results for small-scale human clinical trials support using NAD⁺ boosting strategies as age effective interventions to bolster the metabolic machinery. However, the results of small-scale human clinical trials are promising. Unfortunately, replenishing NAD⁺ pools is not as simple as taking the supplements but requires the targeted localization of exogenous NAD⁺ to the desired organelle, i.e., the inner membrane of mitochondria. A better understanding of the import of the intact NAD⁺ via recently discovered transporters, i.e., members of connexions and solute carrier family, aligns well with the long-desired goal of longevity. Thus, an efficient strategy to enhance mitochondrial performance and hence, age-related diseases must ensure a rapid intracellular influx of intact NAD⁺, or NAD (H) with supplements after knowing their safety dose profile. Extended translation of such a strategy will involve designing methods to increase the expression of the NAD⁺ transporters at transcriptional and post-transcriptional levels. It will lay its groundwork to treat age-related disorders better and enhance healthy longevity. We are at an exciting time of this century where have molecular tools like crisper to effectively examine the efficiency of NAD⁺ transporters in preventing degenerative diseases related to aging by developing their gene-deficient or gene-over expressive cell lines.

ACKNOWLEDGEMENT

None.

CONFLICT OF INTEREST

The author's declared that they have no conflict of interest.

REFERENCES

1. Kim HJ, Cao W, Oh GS, Lee S, Shen A, et al. (2019) Augmentation of cellular NAD+ by NQO1 enzymatic action improves age-related hearing impairment. Aging Cell. 18:e13016.
2. Covarrubias AJ, Perrone R, Grozio A, Verdin E (2021) NAD+ metabolism and its roles in cellular processes during ageing. Nat Rev Mol Cell Biol. 22:119-141.
3. Mackenbach JP, Kunst AE, Lautenbach H, Oei YB, Bijlsma F (1999) Gains in life expectancy after elimination of major causes of death: Revised estimates taking into account the effect of competing causes. J Epidemiol Community Health. 53(1):32-37.
4. Imai SI, Guarente L (2016) It takes two to tango: NAD+ and sirtuins in aging/longevity control. NPJ Aging Mech Dis. 2(1):1-6.
5. Nikiforov A, Kulikova V, Ziegler M (2015) The human NAD metabolome: Functions, metabolism and compartmentalization. Crit Rev Biochem Mol Biol. 50(4):284-297.
6. Yang Y, Sauve AA (2016) NAD+ metabolism: Bioenergetics, signaling and manipulation for therapy. Biochim Biophys Acta. 1864(12):1787-1800.
7. Johnson S, Imai SI (2018) NAD+ biosynthesis, aging, and disease. F1000Res.7.
8. Bogan KL, Brenner C (2008) Nicotinic acid, nicotinamide, and nicotinamide riboside: A molecular evaluation of NAD+ precursor vitamins in human nutrition. Annu Rev Nutr. 28(1):115-130.
9. Cantó C, Auwerx J (2011) NAD+ as a signaling molecule modulating metabolism. Cold Spring Harb Symp Quant Biol. 76:291-298.
10. Cohen MS (2020) Interplay between compartmentalized NAD+ synthesis and consumption: A focus on the PARP family. Genes Dev. 34(5):254-262.
11. Verdin E, Hirschey MD, Finley LW, Haigis MC (2010) Sirtuin regulation of mitochondria: Energy production, apoptosis, and signaling. Trends Biochem Sci. 35(12):669-675.
12. Lautrup S, Sinclair DA, Mattson MP, Fang EF (2019) NAD+ in brain aging and neurodegenerative disorders. Cell Metab. 30(4):630-655.
13. Anderson KA, Huynh FK, Fisher-Wellman K, Stuart JD, Peterson BS, et al. (2017) SIRT4 is a lysine deacylase that controls leucine metabolism and insulin secretion. Cell Metab. 25(4):838-855.e815.
14. Du J, Zhou Y, Su X, Yu JJ, Khan S, et al. (2011) Sirt5 is a NAD-dependent protein lysine demalonylase and desuccinylase. Science. 334(6057):806-809.
15. Liszt G, Ford E, Kurtev M, Guarente L (2005) Mouse Sir2 homolog SIRT6 is a nuclear ADP-ribosyltransferase. J Biol Chem. 280(22):21313-21320.

16. Haigis MC, Mostoslavsky R, Haigis KM, Fahie K, Christodoulou DC, et al. (2006) SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic β cells. Cell. 126(5):941-954.

17. Morales J, Li L, Fattah FJ, Dong Y, Bey EA, et al. (2014) Review of poly [ADP-ribose] polymerase [PARP] mechanisms of action and rationale for targeting in cancer and other diseases. Crit Rev Eukaryot Gene Expr. 24(1):15-28.

18. Braidy N, Guillemin GJ, Mansour H, Chan-Ling T, Poljak A, et al. (2011) Age related changes in metabolism oxidative stress and Sirt1 activity in wistar rats. PloS one. 6(4):e19194.

19. Chini EN (2009) CD38 as a regulator of cellular NAD: A novel potential pharmacological target for metabolic conditions. Curr Pharm Des. 15(1):57-63.

20. Zhang T, Berrocal JG, Frizzell KM, Gamble MJ, DuMond ME, et al. (2009) Enzymes in the NAD+ salvage pathway regulate SIRT1 activity at target gene promoters. J Biol Chem. 284(30):20408-20417.

21. Karamanlidis G, Lee CF, Garcia-Menendez L, Kolwicz Jr SC, Suthammarak W, et al. (2013) Mitochondrial complex I deficiency increases protein acetylation and accelerates heart failure. Cell Metab. 18(2):239-250.

22. Stein LR, Imai SI (2012) The dynamic regulation of NAD metabolism in mitochondria. Trends Endocrinol Metab. 23(9):420-428.

23. Yang H, Yang T, Baur JA, Perez E, Matsui T, et al. (2007) Nutrient-sensitive mitochondrial NAD+ levels dictate cell survival. Cell. 130(6):1095-1107.

24. Zhao Y, Jin J, Hu Q, Zhou HM, Yi J, et al. (2011) Genetically encoded fluorescent sensors for intracellular NADH detection. Cell Metab. 14(4):555-566.

25. Sims CA, Guan Y, Mukherjee S, Singh K, Botolin P, et al. (2018) Nicotinamide mononucleotide preserves mitochondrial function and increases survival in hemorrhagic shock. JCI insight 3(17): e120182.

26. Pittelli M, Formentini L, Faraco G, Lapucci A, Rapizzi E, et al. (2010) Inhibition of nicotinamide phosphoribosyltransferase: Cellular bioenergetics reveals a mitochondrial insensitive NAD pool. JCI Insight. 285(44):34106-34114.

27. Kato M, Lin SJ (2014) Regulation of NAD+ metabolism, signaling and compartmentalization in the yeast Saccharomyces cerevisiae. DNA repair. 23:49-58.

28. Kulkarni CA, Brookes PS (2019) Cellular compartmentation and the redox/nonredox functions of NAD+. Antioxid Redox Signal. 31(9):623-642.

29. Shackelford RE, Mayhall K, Maxwell NM, Kandil E, Coppola D (2013) Nicotinamide phosphoribosyltransferase in malignancy: A review. Genes cancer. 4(11-12):447-456.

30. Nadtochiy SM, Wang YT, Nehrke K, Munger J, Brookes PS (2018) Cardioprotection by nicotinamide mononucleotide [NMN]: Involvement of glycolysis and acidic pH. J Mol Cell Cardiol. 121:155-162.

31. Ying W (2008) NAD+/NADH and NADP+/NADPH in cellular functions and cell death: Regulation and biological consequences. Antioxid Redox Signal. 10(2):179-206.

32. Houtkooper RH, Cantó C, Wanders RJ, Auwerx J (2010) The secret life of NAD+: An old metabolite controlling new metabolic signaling pathways. Endocr Rev. 31(2):194-223.

33. Durnin L, Dai Y, Aiba I, Shuttleworth CW, Yamboliev IA, et al. (2012) Release, neuronal effects and removal of extracellular β-nicotinamide adenine dinucleotide [β-NAD+] in the rat brain. Endocr Rev. 35(3):423-435.

34. Di Stefano M, Conforti L (2013) Diversification of NAD biological role: The importance of location. FEBS J. 280(19):4711-4728.

35. Gomes AP, Price NL, Ling AJ, Moslehi JJ, Montgomery MK, et al. (2013) Declining NAD+ induces a pseudohypoxic state disrupting nuclear-mitochondrial communication during aging. Cell. 155(7):1624-1638.

36. Mouchiroud L, Houtkooper RH, Moullan N, Katsyuba E, Ryu D, et al. (2013) The NAD+/sirtuin pathway modulates longevity through activation of mitochondrial UPR and FOXO signaling. Cell. 154(2):430-441.

37. Bai P, Cantó C, Oudart H, Brunyánszki A, Cen Y, et al. (2011) PARP-1 inhibition increases mitochondrial metabolism through SIRT1 activation. Cell Metab. 13(4):461-468.

38. Mangerich A, Herbach N, Hanf B, Fischbach A, Popp O, et al. (2010) Inflammatory and age-related pathologies in mice with ectopic expression of human PARP-1. Mech Ageing Dev. 131(6):389-404.

39. Li J, Bonkowski MS, Moniot S, Zhang D, Hubbard BP, et al. (2017) A conserved NAD+ binding pocket that regulates protein-protein interactions during aging. Science. 355(6331):1312-1317.

40. Zapata-Pérez R, Wanders RJ, van Karnebeek CD, Houtkooper RH (2021) NAD+ homeostasis in human health and disease. EMBO Mol Med. 13(7):e13943.

41. Yoshino J, Baur JA, Imai SI (2018) NAD+ intermediates: The biology and therapeutic potential of NMN and NR. Cell Metab. 27(3):513-528.

42. Rotllan N, Camacho M, Tondo M, Diarte-Añazco EM, Canyelles M, et al. (2021) Therapeutic potential of emerging NAD+-increasing strategies for cardiovascular diseases. Antioxidants. 10(12):1939.

43. Irie J, Inagaki E, Fujita M, Nakaya H, Mitsuishi M, et al. (2020) Effect of oral administration of nicotinamide mononucleotide on clinical parameters and nicotinamide metabolite levels in healthy Japanese men. Endocr J. 67(2):153-160.

44. Trammell SA, Schmidt MS, Weidemann BJ, Redpath P, Jaksch F, et al. (2016) Nicotinamide riboside is uniquely and orally bioavailable in mice and humans. Nat Commun. 7(1):1-14.

45. Ito TK, Sato T, Hakamata A, Onoda Y, Sato S, et al. (2020) A nonrandomized study of single oral supplementation within the daily tolerable upper level of nicotinamide affects blood nicotinamide and NAD+ levels in healthy subjects.

Trans Med Aging 4:45-54.

46. Cantó C, Houtkooper RH, Pirinen E, Youn DY, Oosterveer MH, et al. (2012) The NAD+ precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet-induced obesity. Cell Metab. 15(6):838-847.

47. Airhart SE, Laura M, Shireman Linda J, Risler Gail D, Anderson GA, et al. (2017) An open-label, non-randomized study of the pharmacokinetics of the nutritional supplement nicotinamide riboside [NR] and its effects on blood NAD+ levels in healthy volunteers. Clinical Trial. 12(12):e0186459

48. Cantó C, Menzies KJ, Auwerx J (2015) NAD+ metabolism and the control of energy homeostasis: a balancing act between mitochondria and the nucleus. Cell Metab. 22(1):31-53.

49. Radenkovic D, Verdin E (2020) Clinical evidence for targeting NAD therapeutically. Pharmaceuticals. 13(9):247.

50. Birkmayer JG, Vrecko C, Volc D, Birkmayer W (1993) Nicotinamide adenine dinucleotide [NADH]—a new therapeutic approach to Parkinson's disease: Comparison of oral and parenteral application. Acta Neurol Scand Suppl. 87:32-35.

51. Rainer M, Kraxberger E, Haushofer M, Mucke HA, Jellinger KA (2000) No evidence for cognitive improvement from oral nicotinamide adenine dinucleotide [NADH] in dementia. J Neural Transm. 107(12):1475-1481.

52. Demarin V, Podobnik SS, Storga-Tomic D, Kay G (2004) Treatment of Alzheimer's disease with stabilized oral nicotinamide adenine dinucleotide: A randomized, double-blind study. Drugs Exp Clin Res. 30(1):27-33.

53. Nikiforov A, Dölle C, Niere M, Ziegler M (2011) Pathways and subcellular compartmentation of NAD biosynthesis in human cells: From entry of extracellular precursors to mitochondrial NAD generation. J Biol Chem. 286(24):21767-21778.

54. Formentini L, Moroni F, Chiarugi A (2009) Detection and pharmacological modulation of nicotinamide mononucleotide [NMN] *in vitro* and *in vivo*. Biochem Pharmacol. 77(10):1612-1620.

55. Mills KF, Yoshida S, Stein LR, Grozio A, Kubota S, et al. (2016) Long-term administration of nicotinamide mononucleotide mitigates age-associated physiological decline in mice. Cell Metab. 24(6):795-806.

56. Yoshino J, Mills KF, Yoon MJ, Imai SI (2011) Nicotinamide mononucleotide, a key NAD+ intermediate, treats the pathophysiology of diet-and age-induced diabetes in mice. Cell Metab. 14(4):528-536.

57. Ratajczak J, Joffraud M, Trammell SA, Ras R, Canela N, et al. (2016) NRK1 controls nicotinamide mononucleotide and nicotinamide riboside metabolism in mammalian cells. Nat Commun. 7(1):1-12.

58. van der Veer E, Ho C, O'Neil C, Barbosa N, Scott R, et al. (2007) Extension of human cell lifespan by nicotinamide phosphoribosyltransferase. J Biol Chem. 282(15):10841-10845.

59. Gross CJ, Henderson LM (1983) Digestion and absorption of NAD by the small intestine of the rat. J Nutr. 113: 412-420.

60. Grozio A, Sociali G, Sturla L, Caffa I, Soncini D, et al. (2013) CD73 protein as a source of extracellular precursors for sustained NAD+ biosynthesis in FK866-treated tumor cells. J Biol Chem. 288(36):25938-25949.

61. Davila A, Liu L, Chellappa K, Redpath P, Nakamaru-Ogiso E, et al. (2018) Nicotinamide adenine dinucleotide is transported into mammalian mitochondria. Elife. 7:e33246.

62. Fletcher RS, Ratajczak J, Doig CL, Oakey LA, Callingham R, et al. (2017) Nicotinamide riboside kinases display redundancy in mediating nicotinamide mononucleotide and nicotinamide riboside metabolism in skeletal muscle cells. Mol Metab. 6(8):819-832.

63. Cambronne XA, Stewart ML, Kim D, Jones-Brunette AM, Morgan RK, et al. (2016) Biosensor reveals multiple sources for mitochondrial NAD+. Science. 352(6292):1474-1477.

64. Revollo JR, Körner A, Mills KF, Satoh A, Wang T, et al. (2007) Nampt/PBEF/visfatin regulates insulin secretion in β cells as a systemic NAD biosynthetic enzyme. Cell metab. 6(5):363-375.

65. Kory N, Uit de Bos J, van der Rijt S, Jankovic N, Güra M, et al. (2020) MCART1/SLC25A51 is required for mitochondrial NAD transport. Sci Adv. 6(43):eabe5310.

66. Felici R, Lapucci A, Ramazzotti M, Chiarugi A (2013) Insight into molecular and functional properties of NMNAT3 reveals new hints of NAD homeostasis within human mitochondria. PloS one. 8(10):e76938.

67. Todisco S, Agrimi G, Castegna A, Palmieri F (2006) Identification of the mitochondrial NAD+ transporter in Saccharomyces cerevisiae. J Biol Chem. 281(3):1524-1531.

68. Haferkamp I, Schmitz-Esser S, Linka N, Urbany C, Collingro A, et al. (2004) A candidate NAD+ transporter in an intracellular bacterial symbiont related to Chlamydiae. Nature. 432(7017):622-625.

69. Palmieri F, Rieder B, Ventrella A, Blanco E, Do PT, et al. (2009) Molecular identification and functional characterization of Arabidopsis thaliana mitochondrial and chloroplastic NAD+ carrier proteins. J Biol Chem. 284(45):31249-31259.

70. VanLinden MR, Dölle C, Pettersen IK, Kulikova VA, Niere M, et al. (2015) Subcellular distribution of NAD+ between cytosol and mitochondria determines the metabolic profile of human cells. J Biol Chem. 290(46): 27644-27659.

71. D'hondt C, Iyyathurai J, Himpens B, Leybaert L, Bultynck G (2014) Cx43-hemichannel function and regulation in physiology and pathophysiology: Insights from the bovine corneal endothelial cell system and beyond. Front Physiol. 5:348.

72. Bevans CG, Kordel M, Rhee SK, Harris AL (1998) Isoform composition of connexin channels determines selectivity among second messengers and uncharged molecules. J Biol Chem. 273(5):2808-2816.

73. Zhu, Y (2022) Gap junction-dependent and-independent functions of connexin43 in biology. Biology. 11(2):283.

74. Ma JW, Ji DD, Li QQ, Zhang T, Luo L (2020) Inhibition of connexin 43 attenuates oxidative stress and apoptosis in human umbilical vein endothelial cells. BMC Pulm Med. 20(1):1-10.

75. Johnstone SR, Best AK, Wright CS, Isakson BE, Errington RJ, et al. (2010) Enhanced connexin 43 expression delays intramitoitc duration and cell cycle traverse independently of gap junction channel function. J Cell Biochem. 110(3):772-782.

76. Islam MN, Das SR, Emin MT, Wei M, Sun L, et al. (2012). Mitochondrial transfer from bone-marrow–derived stromal cells to pulmonary alveoli protects against acute lung injury. Nat Med. 18(5):759-765.

77. Yao Y, Fan XL, Jiang D, Zhang Y, Li X, et al. (2018) Connexin 43-mediated mitochondrial transfer of iPSC-MSCs alleviates asthma inflammation. Stem cell reports. 11(5):1120-1135.

78. Rhee SK, Bevans CG, Harris AL (1996) Channel-forming activity of immunoaffinity-purified connexin32 in single phospholipid membranes. Biochemistry. 35(28):9212-9223.

79. Li H, Liu TF, Lazrak A, Peracchia C, Goldberg GS, et al. (1996) Properties and regulation of gap junctional hemichannels in the plasma membranes of cultured cells. J Cell Biol. 134(4):1019-1030.

80. Rodriguez-Sinovas A, Boengler K, Cabestrero A, Gres P, Morente M, et al. (2006) Translocation of connexin 43 to the inner mitochondrial membrane of cardiomyocytes through the heat shock protein 90–dependent TOM pathway and its importance for cardioprotection. Circ Res. 99(1):93-101.

81. Bruzzone S, Guida L, Zocchi E, Franco L, Flora AD (2001) Connexin 43 hemichannels mediate Ca2+-regulated transmembrane NAD+ fluxes in intact cells. FASEB J. 15(1):10-12.

82. Liu D, Gao Y, Liu J, Huang Y, Yin J, et al. (2021) Intercellular mitochondrial transfer as a means of tissue revitalization. Signal Transduct Target Ther. 6(1):1-18.

83. Luongo TS, Eller JM, Lu MJ, Niere M, Raith F, et al. (2020) SLC25A51 is a mammalian mitochondrial NAD+ transporter. Nature. 588(7836):174-179.

84. Goyal S, Eller JM, Cambronne L (2021) Mutational Analysis of SLC25A51 Residues Important for NAD+ Binding and Transport. Repositories.

85. Li M, Zhou ZP, Sun M, Cao L, Chen J, et al. (2016) Reduced nicotinamide adenine dinucleotide phosphate, a pentose phosphate pathway product, might be a novel drug candidate for ischemic stroke. Stroke. 47(1):187-195.

86. Van De Weijer T, Phielix E, Bilet L, Williams EG, Ropelle ER, et al. (2015) Evidence for a direct effect of the NAD+ precursor acipimox on muscle mitochondrial function in humans. Diabetes. 64(4):1193-1201.

87. Dollerup OL, Christensen B, Svart M, Schmidt MS, Sulek K, et al. (2018) A randomized placebo-controlled clinical trial of nicotinamide riboside in obese men: Safety, insulin-sensitivity, and lipid-mobilizing effects. Am J Clin Nutr. 108(2):343-353.

88. Elhassan YS, Kluckova K, Fletcher RS, Schmidt MS, Garten A, et al. (2019) Nicotinamide riboside augments the aged human skeletal muscle NAD+ metabolome and induces transcriptomic and anti-inflammatory signatures. Cell Rep. 28(7):1717-1728. e1716.

89. Jacobson EL, Kim H, Kim M, Williams JD, Coyle DL, et al. (2007) A topical lipophilic niacin derivative increases NAD, epidermal differentiation and barrier function in photodamaged skin. Exp Dermatol. 16(6): 490-499.

90. Drago F, Ciccarese G, Cogorno L, Calvi C, Marsano LA, et al. (2017) Prevention of non-melanoma skin cancers with nicotinamide in transplant recipients: A case-control study. Eur J Dermatol. 27(4):382-385.