Regulation of NAD+ metabolism in aging and disease

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Abstract

More than a century after discovering NAD+, information is still evolving on the role of this molecule in health and diseases. The biological functions of NAD^+ and NAD^+ precursors encompass pathways in cellular energetics, inflammation, metabolism, and cell survival. Several metabolic and neurological diseases exhibit reduced tissue NAD^+ levels. Significantly reduced levels of NAD^+ are also associated with aging, and enhancing NAD^+ levels improved healthspan and lifespan in animal models. Recent studies suggest a causal link between senescence, age-associated reduction in tissue NAD^+ and enzymatic degradation of NAD^+. Furthermore, the discovery of transporters and receptors involved in NAD^+ precursor (nicotinic acid, or niacin, nicotinamide, and nicotinamide riboside) metabolism allowed for a better understanding of their role in cellular homeostasis including signaling functions that are independent of their functions in redox reactions. We also review studies that demonstrate that the functional effect of niacin is partially due to the activation of its cell surface receptor, GPR109a. Based on the recent progress in understanding the mechanism and function of NAD^+ and NAD^+ precursors in cell metabolism, new strategies are evolving to exploit these molecules' pharmacological potential in the maintenance of metabolic balance.

Keywords: NAD, Niacin, Nicotinamide riboside, Nicotinamide adenine mononucleotide, niacin receptor.

1. Introduction

Nicotinamide adenine dinucleotide (NAD^+) plays a critical role in fundamental cellular processes and functions such as cellular energetics, metabolism, and survival. Several pathological conditions, including cardiovascular diseases, obesity and neurodegenerative diseases, chronological aging, and progeroid phenotypes, are associated with a dysregulation of cellular NAD^+ levels [1, 2]. NAD^+ participates in redox reactions and as a co-substrate in many others [3]. NAD^+ precursors elevate intracellular NAD^+ levels and activate sirtuins in mammalian cells (**Figures 1 and 2**) [4]. NAD^+ is also a substrate for poly(ADP-ribose) polymerase (PARP), which is a DNA damage sensor, and reduced levels of NAD^+ can impact DNA repair mechanisms [5]. The reduced form of NAD^+, NADH, is an electron donor in redox reactions. Because of the broad metabolic functions of NAD^+-dependent activities, fluctuations in cellular levels of NAD^+ impact cellular metabolism, gene expression regulation, DNA repair, mitochondrial functions, redox reactions, inflammation, intracellular trafficking, aging, and cell death. This review focuses on the transporters and receptors of NAD^+ precursors and the metabolic regulation of NAD^+ in health and disease conditions.

2. Transporters and receptors involved in the uptake and metabolism of NAD^+ precursors

The precursors of NAD^+ include niacin (nicotinic acid), nicotinamide, and nicotinamide riboside, collectively called Vitamin B3 [6] (**Figure 1**). Vitamin B3 is a water-soluble vitamin, and it is a member of the vitamin B group, which plays an essential role in the living cells. Nicotinamide mononucleotide (NMN) is an intermediate metabolite and an immediate precursor to niacin. The mechanism by which these precursors are absorbed from the intestine or transported from the extracellular fluid into the cells involves a variety of transporters. They may also activate specific cell surface receptors resulting in the induction of intracellular signaling pathways.

2.1. Transporters of NAD^+ precursors in NAD^+ metabolism*:* One of the precursors of NAD^+ is niacin, and the organism obtains niacin from endogenous or exogenous sources. The endogenous source is mainly derived from niacin produced by tryptophan metabolism, while exogenous sources can be of dietary origin or the gut microbiota [7]. Severe deficiency of niacin leads to pellagra, which is characterized by skin lesions, diarrhea, mucosal inflammation, and dementia. The molecular identity of the mediators of niacin transport across the intestinal barrier is yet to be defined [8]. Though sodium-coupled monocarboxylate transporter (SMCT) 1 and 2 have been suggested in intestinal transport of niacin, their high Km and lack of substrate

specificity were of concern [9, 10]. It is possible that the high Km transporter facilitates the absorption of pharmacological doses of niacin [10]. An H^+-coupled MCT1 mediated transport of niacin across the intestinal brush-border was also reported [11, 12]. Nevertheless, the studies establish the existence of a high-affinity carrier-mediated mechanism for niacin uptake, which is regulated by the substrate levels [8].

NMN, another NAD^+ precursor, is absorbed into blood circulation within a few minutes after oral administration, with a rapid tissue uptake within 30 min followed by a spike in NAD^+ levels [13, 14]. There was no definitive description of an NMN transporter in the intestine or other tissues until the demonstration that Slc12a8 directly transports NMN in a sodium-dependent manner [15]. Slc12a8 is strongly expressed in the small intestine, and a significant reduction in NMN absorption was observed in the gut when Slc12a8 was knocked down [15]. Furthermore, the deficiency of Slc12a8 in the small intestine significantly reduced NMN uptake and reduced NAD^+ in the jejunum and ileum. The whole body Slc12a8 knockout mice also showed reduced NMN transport and NAD biosynthesis in the jejunum and ileum [15]. As Slc12a8 expression is predominantly in the intestine and pancreas but not in skeletal muscle, liver, or white adipose tissue, the role of this transporter is likely more important in intestinal uptake of NMN rather than transport in other tissues. The NMN transport is likely not indispensable to ensure sufficient cellular NAD+ levels in many tissues as the Slc12a8 deficient mouse model shows the flexibility in ensuring sufficient cellular NAD+ supply in multiple tissues. This may suggest the existence of additional NMN transporters.

It was previously thought that NMN was converted to nicotinamide riboside prior to intracellular transport, and nicotinamide riboside transported across the plasma membrane gets converted back to NMN by NRK1/2 [15, 16]. However, it is likely that nicotinamide riboside is translocated from the extracellular compartment to the intracellular compartment by its transporter(s). PnuC has been identified as a nicotinamide riboside transporter in several prokaryotes, including *Salmonella typhimurium* and *Haemophilus influenza* [17-20]. PnuC functions in collaboration with NadR, a cytoplasmic nicotinamide riboside kinase, and converts translocated nicotinamide riboside to NMN and subsequently to NAD^+ [21]. The PnuC family is related to the eukaryotic SWEET sugar-transporter family [20, 22]. A mammalian transporter for nicotinamide riboside remains unidentified, though a recent study using HEK293 cells shows SLC29 family proteins ENT1, ENT2, and ENT4 may be involved in importing extracellular nicotinamide riboside into cultured human cells [23].

Mitochondria maintains an NAD^+ pool distinct from the cytoplasmic pool [24]. Maintaining a distinct pool of NAD^+ in these two compartments could explain why mitochondrial NAD^+ levels are not depleted for a prolonged period of time even after depleting the cytoplasmic NAD^+ pool [25, 26]. The mitochondrial pool is essential in generating electron carriers, and the cytoplasmic NAD^+ is necessary for the glycolytic process and other reactions such as sirtuin-catalyzed deacetylations. While nicotinamide riboside and NMN are efficiently converted to NAD^+ in mammalian cells, how or whether the cytoplasmic NAD^+ is translocated into mitochondria is unknown [24]. Many reports suggest that mitochondrial inner membrane is not permeable to NAD^+; nevertheless, the NAD^+ precursor NMN has been shown to translocate into mitochondria and metabolize to NAD^+ by mitochondrial NMNAT3 [27, 28]. The mechanism of NMN transport across the mitochondrial membrane remains to be determined [28]. It is interesting to note that there is no known mammalian transporter for NAD^+. However, one report demonstrated that exogenous NAD^+ is transported in the hypothalamus via a connexin 43-dependent mechanism [29]. Though the long-held view is that NAD^+ cannot cross the mitochondrial inner membrane, it was also challenged in a recent study that showed NAD^+ import into the matrix, using isotopically labeled NAD^+ [30]. The unanswered question is whether there is an inner membrane transporter for NAD^+ in the mitochondria. The transporters of NAD^+ and its precursors are critical to their entry, intracellular redistribution, and metabolism.

2.2. Receptors of NAD^+ precursors in NAD^+ metabolism: Niacin reduces cellular cyclic AMP (cAMP) levels by inhibiting adipocyte adenylyl cyclase and suppress lipolysis in adipose tissue, independent of its metabolism to NAD^+. This observation led to the discovery of the G-protein coupled receptor 109a (Gpr109a) as an endogenous receptor for niacin [31, 32] (**Figure 3**).

Gpr109a (also known as HCA2, HM74a, NIACR1, or PUMA-G) was identified as a specific and high-affinity receptor for niacin independently by three groups in 2003 [33-35]. Gpr109a belongs to a family of G-protein–coupled receptors that share significant sequence homology, and its cognate ligands are metabolites of hydroxycarboxylic acid (HCA) [36]. The most homologous protein to Gpr109a is Gpr109b, found in humans but not in rodents, shares nearly 96% homology, and is a low-affinity receptor for niacin [33]. Gpr109a is highly expressed in adipocytes, spleen, intestinal epithelium and the retinal pigment epithelium and some immune cell types including neutrophils, macrophages, keratinocytes and Langerhans cells [37-40]. The discovery of Gpr109a as a receptor for niacin proved that the mechanism of niacin-mediated effects is not only through NAD^+ generation but also due to the activation of downstream signaling following niacin binding to the cell surface receptor [41]. We recently demonstrated

this by comparing the effect of niacin and NMN (as NMN metabolizes to NAD^+ but does not bind Gpr109a) in injury resolution in wild-type and Gpr109a deficient mice [41].

In adipocytes, activation of Gpr109a by niacin results in a G(i)-mediated decrease in cAMP levels, resulting in decreased hormone-sensitive lipase (HSL) activity and reduced hydrolysis of triglycerides to free fatty acids (FFA) [35]. cAMP activates protein kinase A and regulates the HSL activity [42]. It is important to note that different nutritional situations and hormones regulate lipolysis and the production of lipoproteins. In PUMA-G knockout mice, niacin–induced decrease in FFA and triglyceride plasma levels was abrogated, indicating that Gpr109a mediates the anti-lipolytic and lipid-lowering effects of niacin [35]. Furthermore, the lack of niacin-induced flushing in these mice suggested that niacin mediates the flushing through its cell surface receptor [43]. While niacin can activate Gpr109a, other NAD^+ precursors do not bind Gpr109a.

There is very little information on the extracellular actions of NAD^+. The extracellular NAD^+ concentration is much lower than intracellular levels. However, in response to cell injury, NAD^+ is released from cells [44] and the extracellular NAD^+ binds to several subtypes of purinergic receptors to induce intracellular signaling and modulate immune responses [45] **(Figure 3**).

3. NAD^+ Metabolism

In eukaryotic cells, energy metabolism is mainly mediated by oxidative phosphorylation in the inner membrane of the mitochondrion. NAD^+ plays a vital role as electron carriers (NADH) in the oxidation/reduction (redox) reactions generating adenosine triphosphate (ATP) **(Figure 2)**. NAD^+ is reduced to NADH during catabolic processes and can also be phosphorylated to $NADP^+$ via NAD^+ kinases. NAD^+ and $NADP^+$ are two important coenzymes involved in cellular metabolism and several signaling pathways such as DNA repair, mitochondria biogenesis, gene expression, cell cycle, cellular stress response, and cellular communication [2, 46-50]. More than 400 proteins are associated with NAD^+ and NADH in various biological reactions [51].

NAD^+ is produced from niacin by the Preiss-Handler pathway named after the co-discoverers Jack Preiss and Philip Handler [48] (**Figure 1**). The first step in converting niacin to its mononucleotide form, nicotinic acid mononucleotide (NaMN), is a reaction catalyzed by nicotinic acid phosphoribosyltransferase (NaPRT). PRPP (5-phosphoribosyl-1-pyrophosphate) is a cosubstrate in this reaction. Subsequently, NaMN is transformed into its dinucleotide form, nicotinic acid-adenine dinucleotide (NaAD), by a group of ATP-dependent isoenzymes collectively called nicotinamide mononucleotide adenylyltransferases (NMNAT). In the third and

final step, glutamine-dependent NAD^+ synthase catalyzes the amidation of NaAD to generate NAD^+ [52]. However, nicotinamide and nicotinamide riboside are converted to NAD^+ through NMN. Nicotinamide is converted to NMN by the rate-limiting enzyme nicotinamide phosphoribosyltransferase (NAMPT) and subsequently to NAD^+ by NMNAT. Nicotinamide riboside is metabolized to NMN by the enzyme nicotinamide riboside kinase (NRK). While salvage pathways allow the organism to recycle the pyridine ring from NAD^+ precursors and are essential in humans, most organisms synthesize NAD^+ from tryptophan or aspartic acid-generating quinolinic acid, which is converted to NaMN by the transfer of a phosphoribose group, also called de novo pathway. The expression of the enzymes involved in each of these pathways may vary in tissues resulting in differential utilization of the NAD^+ precursors in different tissues [6]. The NAD^+ level in tissues and intracellular compartments is a major determinant in the metabolic balance.

Recent studies show that M1-like macrophages accumulate in metabolic tissues during aging, express high levels of CD38, and reduce tissue NAD^+ levels as CD38 possesses NADase activity [53]. Similar results were published simultaneously by another group demonstrating senescence induced upregulation of CD38, leading to increased NAD^+ consumption [54]. These results demonstrate a causal link between tissue macrophages in the aging tissues, senescence, and age-associated reduction of NAD^+ levels [53]. The net tissue NAD^+ level may therefore be determined by the balance between the rate of synthesis from precursors and degradation by CD38. However, the predominantly extracellular orientation of CD38 adds another layer of uncertainty here, as most of the NAD^+ is within the intracellular compartment [55]. This paradox may at least be partially addressed by the finding that CD38 can also metabolize extracellular NMN and nicotinamide riboside, thereby indirectly reducing tissue NAD^+ levels [56].

4. NAD^+ in health and diseases

Niacin has been in clinical use since the 1950s when Rudolf Altschul observed a decreased plasma cholesterol in rabbits treated with niacin [57, 58]. He also found that high doses of niacin reduced plasma cholesterol levels in normal and hypercholesterolaemic human subjects, making niacin the oldest lipid-lowering drug [57, 59, 60]. Niacin at high doses increased circulating HDL [61]. HDL transports cholesterol from peripheral tissues to the liver, acting like a scavenger, and low serum HDL is generally considered a risk factor for coronary artery diseases [62]. The increased plasma stability of newly synthesized HDL following niacin treatment was attributed to the stimulatory effect of niacin in HDL production in the liver and

niacin enhanced the lipidation of ApoA-1 by increasing the expression of ABCA1 [63]. Furthermore niacin reduced hepatic expression of HDL receptor, thereby increasing HDL in the plasma that is available to bind extra-hepatic cholesterol [64]. In addition to lowering LDL cholesterol, niacin can also lower triglycerides and very low-density lipoprotein (VLDL) [65]. Despite a significant body of clinical evidence on the beneficial effect of nicotinic acid in preventing the progression of atherosclerosis and the occurrence of cardiovascular events (3), the mechanisms by which pharmacological doses of nicotinic acid exert their effects have been elusive.

The mechanism for the lipid-lowering effect of niacin was initially described to be due to its ability to inhibit lipolysis in adipose tissue leading to a reduction in plasma-free fatty acids [66]. However, following the discovery of the receptor for nicotinic acid, the focus of mechanistic studies turned to Gpr109a, which was found to be a receptor for nicotinic acid, but not nicotinamide or other NAD^+ precursors [67]. Interestingly, nicotinamide did not affect the plasma lipid levels [59]. Niacin activates the Gi-protein-coupled Gpr109a, lowers intracellular cAMP resulting in reduced PKA-mediated activation of hormone-sensitive lipase leading to reduced triglyceride hydrolysis and FFA release [68]. Another hypothesis is that niacin can noncompetitively inhibit diacylglycerol acyltransferase 2 (DGAT2) activity [69]. DGAT1 and DGAT2 are the enzymes catalyzing the last committed step in triglyceride (TG) synthesis. This speculation was contradicted in later investigations as the effects observed in murine models could not be reproduced in primary human, rhesus, and cynomolgus hepatocytes or in a primate in vivo model [70]. In a recent study, it was observed that niacin-activated Gpr109a alters lipid metabolism by inhibition of hepatocyte lipogenesis and fatty acid absorption as well as promotion of brown adipose tissue (BAT) thermogenesis [71]. Whereas in another study, identical changes to serum lipids were observed when Gpr109a-deficient or wild-type mice were treated with niacin [72]. These investigators concluded that lipid-lowering effect of niacin is independent of both the niacin receptor Gpr109a and free fatty acid suppression. According to them, Gpr109a does not mediate lipid efficacy shown by niacin. Though the issue is unsettled, it may be pertinent to note that other NAD^+ precursors such as nicotinamide, nicotinamide riboside, and NMN do not bind Gpr109a but metabolize to increase cellular NAD^+. A recent clinical trial conducted in Japan investigated the safety of single oral administration of NMN in 10 healthy men of 40 to 60 years of age and found no significant deleterious effects, including changes in heart rate, blood pressure, oxygen saturation, and body temperature [73]. The study suggests that NAD^+ levels in the body can be safely elevated by giving NMN. As NAD^+ itself is

not given to humans directly, NAD^+ precursors become potential candidates to augment NAD^+ levels *in vivo* to maintain cellular homeostasis and improve health [74].

4.1. Diabetes and Cardiovascular Diseases

Chronic heart failure is characterized by myocardial metabolic impairment. In mouse models of heart failure, myocardial NAD^+ levels were observed to be low [75]. A decrease in the level of nicotinamide phosphoribosyltransferase enzyme which converts nicotinamide to NAD^+ and an increase in the nictotinamide riboside kinase 2 (NRK2) that utilizes nicotinamide riboside are observed in both murine and human heart failure [76, 77]. Supplementing mice with NAD^+ precursors have been found to increase NAD^+ levels and improve glucose tolerance in high fat diet (HFD)-induced diabetes [13]. NMN oral supplementation in mice significantly improved both insulin release and action in age- and diet-related type 2 diabetic or obese mouse models [13, 78]. A declined SIRT1 activity has been suggested to play a role in reduced insulin sensitivity. NAD^+ is a co-substrate for SIRT1, and therefore, supplementation of NAD^+ precursors such as NMN activates SIRT1, enhances hepatic insulin sensitivity, and restores gene expression related to oxidative stress, inflammatory response, and circadian rhythm [13]. Diguet et al. recently showed that the NAD^+ precursor nicotinamide riboside preserves cardiac function in a murine model of dilated cardiomyopathy [76]. They showed that dietary supplementation of nicotinamide riboside reduces the development of heart failure in mice by elevating myocardial NAD^+ levels [76]. While NRK2 is more restricted to muscle, NRK1 is more ubiquitous [16]. NRK1 is rate-limiting and essential for nicotinamide riboside-induced NAD^+ synthesis. NRK1 deficiency leads to decreased gluconeogenic potential and impaired mitochondrial function. Glucose intolerance, insulin resistance and hepatosteatosis were observed in NRK1 deficient mice fed with high fat diet. They were also found to be more susceptible to diet-induced liver DNA damage suggesting that endogenous nicotinamide riboside metabolism may have an important role in organ function [79]. In cardiac tissue from HFpEF patients, the expression of genes involved in NAD^+ biosynthesis was impaired. Supplementing HFpEF mice with nicotinamide riboside led to improvement in mitochondrial function and amelioration of the HFpEF phenotype [80].

A significant improvement in lipid level but minimal glycemic control was observed following niacin treatment in a clinical trial with type 2 diabetes patients [81]. Therefore, it is suggested that when niacin is used to treat dyslipidemia in patients with or at risk for diabetes, glucose should be periodically checked and risk/benefit ratio should be evaluated [82]. Nicotinamide also prevented or delayed insulin-deficient diabetes in animal models of type 1 diabetes and

protected islet cells against cytotoxic actions [83, 84]. Calorie-rich diets alter several metabolic pathways and impact mitochondrial function. NAD^+ biosynthesis mediated by NAMPT was compromised in the metabolic organs of HFD-induced type 2 diabetes in mice [13]. However, supplementation of NMN, a product of NMPT, improved glucose tolerance and insulin sensitivity by increasing NAD^+. The authors conclude that NMN supplementation might also be effective in human T2D patients [13]. Niacin supplementation has been shown to significantly reduce cardiovascular events [85], though some studies did not show a benefit in reducing cardiovascular mortality [86]. Whether the supplementation could produce additional clinical benefits in patients with dyslipidemia treated with statins also remains controversial [12–14]. Therefore, though niacin raises HDL-C and lowers TG levels, whether this effect translates to improved cardiac outcomes remains an open question [87]. Niacin showed cardiovascular benefits in one of the first clinical trials, Coronary Drug Project, with a lipid-altering drug conducted between 1966 and 1975 on 8341 men aged 30-64 years. An additional nine years of post-trial follow-up revealed 11% lower mortality in the niacin-treated group [88-90]. A decreased mortality was also observed in the open-labeled Stockholm study in which patients received a combination of clofibrate and niacin post-myocardial infarction [91]. The patients showed improved levels of serum cholesterol and triglycerides. Furthermore, chronic use of niacin was associated with cutaneous flushing, which was reduced by the use of extended release formulation. In a clinical trial evaluating the kinetics and dose-dependency of nicotinamide riboside oral availability and safety in overweight, consumption of 100, 300 and 1000 mg nicotinamide riboside significantly increased whole blood NAD^+ and other NAD^+ metabolites within 2 weeks in a dose-dependent manner (57). There were no reports of flushing or other adverse events compared with placebo-treated groups. However, The Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides: Impact on Global Health Outcomes (AIM-HIGH) trial did not see a benefit when the extended-release formulation was added to statin therapy [92]. The significant differences between the earlier clinical trials that revealed the cardiovascular benefit of niacin and the recent trials that failed to demonstrate a benefit may be related to dyslipidemia types, niacin formulation, dosing, and timing [93].

4.2. Neurological Diseases

The brain uses glucose, lactate, and ketone bodies as energy sources [94, 95]. Reduced mitochondrial function is a hallmark of neurodegenerative diseases [96, 97]. Alzheimer's disease (AD) is the most common progressive neurodegenerative disease [98]. Studies indicate

a reduced neuronal ATP level in patients with Alzheimer's disease (AD), suggesting mitochondrial insufficiency in maintaining cellular energy balance [99, 100]. In a mouse model of AD, reduced mitochondrial oxygen consumption was observed both in the brain and skeletal muscle [101]. Mitochondrial respiratory function was restored in AD mice treated with NAD^+ precursor NMN [102]. It was also found that maintaining cellular energy by preventing NAD^+ depletion could protect neurons from excitotoxicity and act as a therapeutic intervention for neurodegenerative diseases [103]. When 250 mg/kg/day of nicotinamide riboside was given for three months to Tg2576 animals, a model of AD, cognitive decline was significantly reduced with a concomitant increase in brain NAD^+ and Pgc-1α, and reduced BACE1 and A-β [104]. Furthermore, niacin insufficiency was shown to cause neurodegenerative decline. In AD mice treated with nicotinamide for eight months, the increased cognitive performance and mitochondrial function were associated with reduced A-β and tau pathologies, suggesting that nicotinamide-mediated improvement in brain bioenergetics reduces AD pathology [105]. In a clinical study conducted in a Chicago community in 1993-2002, energy-adjusted niacin intake showed protection from AD and improvement in cognitive decline [106]. Using LC-MS/MS method, it was found that NMN levels in the brain of both male and female 3xTg AD mice were reduced compared to wild-type mice [107]. NAD^+ levels are decreased in AD, and NMN was found to decrease neuroinflammation and improve learning, memory, and motor control [102, 108]. In rodent models of AD, NMN was able to restore the levels of NAD^+ and ATP. NMN prevented the Aβ1–42 oligomer-induced inhibition of LTP and neuronal death[109]. NMN also reduced β-amyloid production, amyloid plaque burden, synaptic loss, and inflammatory responses in the brain of the AD model animals.

Parkinson's disease (PD) is a progressive neurological disorder characterized by the death of dopaminergic neurons in the substantia nigra. The main pathological characteristics of PD are cell death in the basal ganglia, protein aggregation in Lewy bodies, disruption of autophagy, declined mitochondrial function, and inflammation. Vitamin B3 was reported to reduce certain early-onset PD symptoms by elevating NAD^+ levels and restoring NAD^+/NADH ratio [110, 111]. NAD+ homeostasis and its metabolism are critical in regulating autophagy [112]. High niacin levels can also sequester transition metal ions, including Fe, into stable complexes [113] . Furthermore, vitamin B3 can reduce oxidative stress and inhibit neuroinflammation. PD brain was found to have higher levels of NNMT protein and enzyme activity, suggesting that NNMT may be associated with neuronal degeneration observed in PD [114].

It is also well known that niacin deficiency is linked to several psychiatric manifestations. Vitamin B3 was shown to contribute to the recovery of acute schizophrenia whether given with or without standard treatments [115-117]. In a recent study, nicotinate phosphoribosyltransferase domain containing 1 (NAPRT1) protein was identified as a novel schizophrenia susceptibility gene in an Indian population [118]. NAPRT1 catalyzes the first step in the conversion of niacin to NAD^+ (Preiss-Handler pathway, **Figure 1**). However, the etiopathological associations between niacin deficiency and schizophrenia as well as the mechanism of action of niacin in these diseases remain unresolved (44).

4.3. Ischemia and reperfusion injury

In cardiac ischemia and ischemia/reperfusion there is dysregulation of NAD^+ metabolism as observed by reduced level of NAD^+, key enzymes involved in NAD^+ metabolism, and declined SIRT1 activity [3, 119-122]. Niacin attenuated myocyte injury and improved survival during myocardial ischemia and reperfusion [123, 124] and this therapeutic benefit may be associated with the synergistic activation of the glutathione redox cycle, a decrease of the NADH/NAD^+ ratio and increased glycolysis and lactate efflux, reduction of hydrogen peroxide level, and up-regulation of nuclear factor erythroid 2 (Nrf2) related factor [125]. Nampt overexpression in the heart, as well as exogenous NMN also had protective effects in ischemia and ischemia/reperfusion [126]. Another study found that the protection due to NMN may be attributed to increased glycolysis and downstream ATP synthesis during ischemia [127]. However, in a swine model, exogenous supplementation of NAD^+ protected myocardium against myocardial ischemic/reperfusion injury [128]. Furthermore, NAD^+ generation during clearance of dying cells has been linked to requirements for sirtuins and, indirectly, cardiac repair [129]. Inflammation and cellular energetics play critical roles in organ dysfunction following ischemia and reperfusion injury [130, 131]. The studies from our lab demonstrated that niacin improves organ function and survival following hemorrhagic shock injury (HI) [41, 132]. We found that niacin administered to rats subjected to HI resulted in a significantly prolonged duration of survival. However, the survival duration due to niacin treatment was significantly less in $Gpr109a^{-/-}$ mice. The studies suggested that the Gpr109a receptor-mediated pathway contributed significantly to niacin mediated salutary effect **(Figure 3)**. When the wild-type animals were administered NMN instead of niacin, the survival benefit was significantly reduced, further demonstrating that the salutary effect of niacin is at least partially through binding to GPR109a. This study shows that rebalancing intracellular NAD alone is insufficient in the treatment of hemorrhagic shock. In other studies, NMN has been found to increase the level of

NAD^+ in the heart and prevented NAD^+ decline during ischemia [126]. NMN protected the heart from ischemia/reperfusion injury when it was applied 30 minutes before ischemia or just before and during reperfusion, suggesting protection in both ischemic and reperfusion phases. Furthermore, NMN application has no protective effect in cardiac-specific Sirt1 KO mice, which suggests that the effect of NMN is primarily mediated through Sirt1 [133].

5. NAD^+ metabolism and aging

The process of aging is characterized by a progressive loss of functional integrity, with physiological changes such as metabolic dysfunction, DNA instability, chronic inflammation, and increased vulnerability to injury, diseases, and mortality [52, 134]. The aging phenotype is also associated with increased prevalence of senescent cells in various tissues; senescence is characterized by upregulation of p53 and/or p21, and down regulation of cyclins and cyclin dependent kinases leading to cell cycle arrest [132, 135-137]. Senescence may be a consequence of telomere shortening, cell stress, DNA damage or oncogene activation, and these cells are known to secrete inflammatory factors called SASP (senescence associated secretory phenotype) . Though SASPs were considered to have senescence-inducing and tumor suppressive potential, the autocrine and paracrine role of SASPs are controversial in wound healing, tissue repair and tumor progression [138-140]. Recently it was shown that the rate-limiting enzyme of the NAD+ salvage pathway, NAMPT, modulates SASP independent of senescence-associated cell cycle arrest and promotes tumorigenicity [139]. Cellular NAMPT and NAD^+ levels decline during chronological aging and in progeroid states [55, 139, 141]. The decline of NAD^+ levels with aging in various tissues is well documented in worms, rodents, and humans, though the degree of decline may vary from tissue to tissue [142]. The reasons for the observed age-related decline in NAD^+ include activation of NAD^+ consuming enzymes such as CD38 and SARM1, reduced mitochondrial function, and reduced NAD^+ synthesis [142]. NAD^+ is also a key molecular activator of sirtuins, which are important regulators of aging and longevity. [143]. An increased SIRT1 activity and NAD^+ levels were observed with exercise and caloric restriction interventions, and associated with age-related health benefits [144]. SIRT1 activity can also be modulated by its co-substrate NAD^+ as well as its natural and synthetic activators such as resveratrol and SRT1720 [145]. Modulation of NAD^+ to restore homeostatic levels was found to ameliorate age-associated pathophysiology and prolong both health and life spans [143].

Since the finding that increased longevity induced by calorie restriction was dependent on the activation of Sir2 by NAD^+, strategies to increase NAD^+ levels through NAD^+-precursor

supplementation to extend healthspan and lifespan were successfully tested in several animal models [146, 147]. These precursors include niacin, NMN, nicotinamide, and nicotinamide riboside. The discovery of its anti-aging, life-span prolonging property in various experimental models made NAD^+-precursors attractive as potential therapeutic candidates [74, 143, 148]. Long-term administration of NMN can boost NAD^+, increase SIRT1 activity in tissues, and mitigate age-associated physiological decline [14]. Orally administered NMN remarkably suppressed age-associated body weight gain, reversed age-associated organ dysfunction and oxidative stress, prevented age-associated gene expression, enhanced energy metabolism, promoted physical activity, improved insulin sensitivity and plasma lipid profile, and ameliorated other pathophysiological changes [14, 149]. In aging mice, NMN increased arterial SIRT1 activity and reversed age-associated arterial dysfunction and oxidative stress. NMN also restored the elasticity of capillary walls and reversed blood vessel damage caused by age [126].

Exogenous nicotinamide riboside increased net NAD^+ synthesis, improved Sir2 function, and extended life-span in yeast [4]. It is also reported that the life-span extension induced by compounds that enhance NAD^+ levels in worms was strictly dependent on daf-16 (mammalian homolog is FOXO) expression [150]. Based on the results of studies in animal models, methods to augment NAD^+ bioavailability have been proposed as a strategy for improving cardiovascular and other physiological functions with aging in humans. A randomized, double-blind, placebo-controlled, crossover clinical trial showed that chronic nicotinamide riboside supplementation was well-tolerated in healthy middle-aged and older adults, and acute supplementation with nicotinamide riboside is effective for stimulating NAD^+ metabolism in humans[151]. In a study on aged participants, oral supplementation of 1 g nicotinamide riboside per day for 21 days in a placebo-controlled, randomized, double-blind trial, an elevated muscle NAD^+ metabolome and a reduced systemic inflammation were observed suggesting potential health benefits [152]. However, how exogenous supplementation of NAD+-augmenting anti-ageing dietary supplements regulate SASP and tumorigenicity may also need to be further examined in light of a recent report [139]. A more recent study did not support the hypothesis that dietary nicotinamide riboside supplementation has a significant impact on skeletal muscle mitochondria in obese and insulin-resistant men as the supplementation did not alter mitochondrial respiration, content, or morphology [153]. Despite the many studies performed using experimental models, the beneficial effects of NAD^+ precursor supplementation for improved health span and lifespan in the humans remains to be better understood.

6. Conclusion

After more than a hundred years since discovering NAD^+, it is now established that alterations in NAD^+ levels significantly impact cellular metabolism and energetics. Studies have demonstrated that NAD^+ levels decrease with aging, and alterations in NAD^+ homeostasis are observed in age-related diseases, including cancer, cardiovascular diseases, diabetes, neurodegenerative and metabolic disorders. Methods that restore NAD^+ levels and activate NAD^+ metabolism demonstrated beneficial effects in health and disease conditions including life-span extension. Furthermore, the discovery of transporters and receptors involved in NAD^+ precursor metabolism allowed for a better understanding of the molecular mechanisms involved in NAD^+ biology. The results of the studies conducted so far in the animal and human models establish a central role for NAD^+ in cellular metabolic homeostasis and healthy living.

6.1. Future directions: Despite the critical role of NAD^+ in cell metabolism, our knowledge of the transporters and receptors of NAD^+ and NAD^+ precursors in various cell types and mitochondria are minimal and evolving. While the vast majority of studies show a beneficial effect for elevated tissue NAD^+ levels, one recent study recommended a cautious approach as they found a profound secretion of SASP in the presence of high NAD^+ levels [139]. If this finding holds, future studies may have to consider this scenario and balance the effect of NAD supplementation. It is also important to note that though several clinical trials are underway to understand the effect of NAD^+ precursor supplementation on metabolic health, translation of the results of pre-clinical studies is lagging.

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8. Conflict of Interest: The authors declare no conflict of interest

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FIGURE LEGENDS

Figure 1: NAD biosynthesis pathways: The figure illustrates three critical pathways in NAD^+ biosynthesis. Abbreviations: Quinolinate phosphoribosyltransferase (QAPRT), niacin (NA), nicotinic acid mononucleotide (NaMN), Nicotinic acid Adenine Dinucleotide (NaAD), nicotinamide (NAM), Nicotinamide mononucleotide (NMN), nicotinamide adenine dinucleotide (NAD), reduced NAD (NADH), nicotinamide rioboside (NR), Nicotinamide adenine dinucleotide phosophate (NADP), reduced NADP (NADPH), Nicotinate phosphoribosyltransferase (NAPRT), Nicotinamide phosphoribosyltransferase (NAMPT), Nicotinamide mononucleotide adenylyltransferase(NMNAT), Nicotinamide riboside kinase (NRK), NAD+ synthase (NADS), NAD+ Kinase (NADK). Two arrows indicate one or more intermediate products.

Figure 2. NAD^+ metabolism and physiology. NAD^+ precursors elevate tissue NAD^+ levels resulting in improved cellular energetics and activation of NAD^+ dependent enzymes such as sirtuins and PARPs. Cytosolic NAD^+ is essential for glycolysis, whereas NAD^+ reduced to NADH serve as electron carriers. Injured cells release NAD^+ into the extracellular matrix leading to activation of P2Y/P2X receptors and inflammatory response. CD38, CD73, and SARM1 cleave NAD^+ resulting in reduced NAD^+ levels and promoting senescence. A recent study shows that high NAD^+ levels can induce SASPs (?=limited evidence). Abbreviations: Niacin (NA), nicotinamide mononucleotide (NMN), nicotinamide riboside (NR) and nicotinamide (NAM), Tricarboxylic acid cycle (TCA), Oxidative phosphorylation (OXPHOS), acetyl group (Ac), and senescence associated secretory phenotype (SASP).

Figure 3. Transporters and receptors in NAD^+ precursor metabolism. Niacin is transported into the cells from the intestinal lumen by sodium or H^+-coupled transporters (e.g., SMCT1 or MCT1). Recently a transporter (Slc12a8) for NMN was also identified (Ref: 15). Niacin precursors potentiate cellular energetics and cell metabolism by metabolizing to NAD^+, and declining levels of NAD^+ are observed in health and disease conditions. Niacin also binds and

activates the G-protein coupled cell surface receptor, GPR109a. The activation of GPR109a is attributed to its anti-inflammatory, anti-lipolytic, and flushing effects.

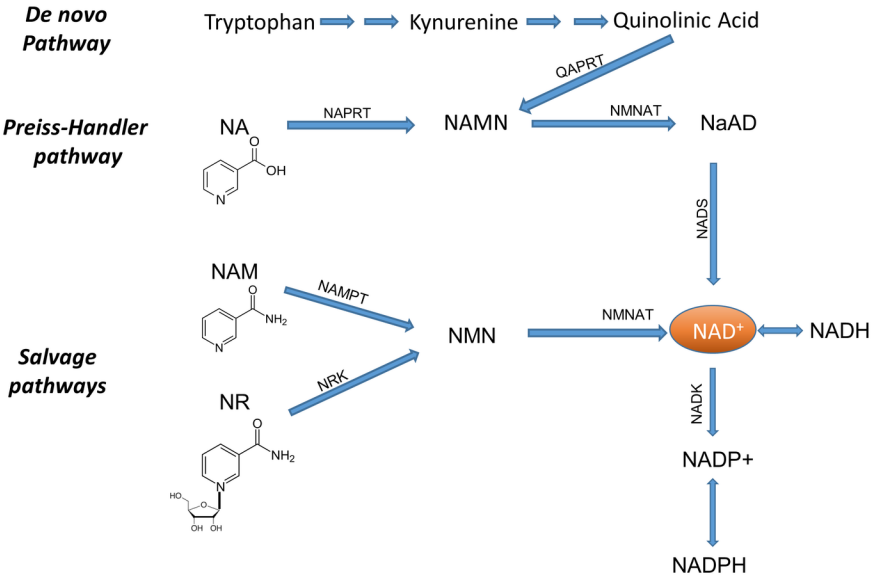
De novo Pathway
Tryptophan
Kynurenine
Quinolinic Acid
QAPRT
Preiss-Handler pathway
NA
NAPRT
NAMN
NMNAT
NaAD
NADS
NAM
NAMPT
NMN
NMNAT
NAD+
NADH
Salvage pathways
NRK
NR
NADK
NADP+
NADPH

Figure 1

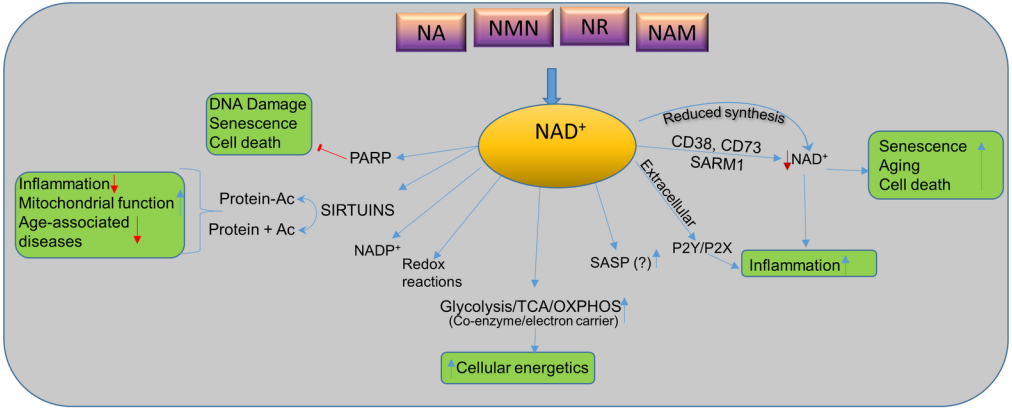
NA
NMN
NR
NAM
NAD+
DNA Damage
Senescence
Cell death
PARP
Reduced synthesis
CD38, CD73
SARM1
NAD+
Senescence
Aging
Cell death
Inflammation
Mitochondrial function
Age-associated
diseases
Protein-Ac
SIRTUINS
Protein + Ac
Extracellular
NADP+
Redox
reactions
SASP (?)
P2Y/P2X
Inflammation
Glycolysis/TCA/OXPHOS
(Co-enzyme/electron carrier)
Cellular energetics

Figure 2

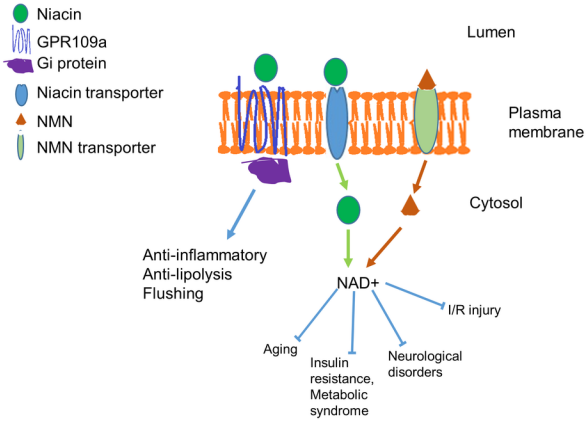
Niacin
GPR109a
Gi protein
Niacin transporter
NMN
NMN transporter
Lumen
Plasma membrane
Cytosol
Anti-inflammatory
Anti-lipolysis
Flushing
NAD+
I/R injury
Aging
Insulin resistance, Metabolic syndrome
Neurological disorders

Figure 3