HHS Public Access

Author manuscript

Nat Rev Mol Cell Biol. Author manuscript; available in PMC 2025 September 23.

Published in final edited form as:

Nat Rev Mol Cell Biol. 2024 October ; 25(10): 822–840. doi:10.1038/s41580-024-00752-w.

Regulation of and challenges in targeting NAD⁺ metabolism

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Abstract

Nicotinamide adenine dinucleotide, in its oxidized (NAD^+) and reduced (NADH) forms, is a reduction-oxidation (redox) cofactor and substrate for signaling enzymes that have essential roles in metabolism. The recognition that NAD^+ levels fall in response to stress and can be readily replenished through supplementation has fostered great interest in the potential benefits of increasing or restoring NAD^+ levels in humans to prevent or delay diseases and degenerative processes. However, much about the biology of NAD^+ and related molecules remains poorly understood. In this Review, we discuss the current knowledge of NAD^+ metabolism, including limitations of, assumptions about and unappreciated factors that might influence the success or contribute to risks of NAD^+ supplementation. We highlight several ongoing controversies in the field, and discuss the role of the microbiome in modulating availability of NAD^+ precursors such as nicotinamide riboside (NR) and mononucleotide (NMN), the presence of multiple cellular compartments that have distinct pools of NAD^+ and NADH, and non-canonical NAD^+ and NADH degradation pathways. We conclude that a substantial investment in understanding the fundamental biology of NAD^+, its detection and its metabolites in specific cells and cellular compartments is needed to support current translational efforts to safely boost NAD^+ levels in humans.

Introduction

Nicotinamide adenine dinucleotide (NAD^+) has a fundamental role in the biochemistry of life. In oxidizing reactions, it serves as a hydride (H^-) acceptor through conversion to its reduced form, NADH (Figure 1). NADH, in turn, can provide a hydride to reduce other substrates, or notably, to fuel the mitochondrial electron transport chain and thereby generate ATP. Glycolysis, β-oxidation and the tricarboxylic acid (TCA) cycle all require NAD^+. In fact, there is no sustainable path to produce ATP that does not require interconversion of NAD^+ and NADH, and the NAD^+/NADH ratio is a common point of control that links hundreds of reactions throughout the cell[1]. The phosphorylated form of NAD^+, $NADP^+$, has

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a similar role but it works with a discrete set of enzymes, allowing the $NADP^+$/NAPDH ratio to be set independently from NAD^+/NADH (Box 1).

In addition to its roles in reduction-oxidation (redox) reactions, NAD^+ serves as a substrate for several classes of non-redox enzymes; these include mono- and poly-ADP ribosyltransferases[2-4], sirtuins **(NAD^+-dependent deacylases SIRT1-7)** [5], CD38 (also known as ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 1) and its paralog CD157 (also known as BST1 or ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 2)[6,7], and NAD^+ hydrolase SARM1 (ref.[8]), all of which have important roles in cell signaling and fate decisions. The nicotinamide moiety of NAD^+ can be removed in enzymatic reactions and released as free nicotinamide **[G]** (Nam). The remaining electrophilic ADP-ribosyl moiety may be covalently attached to nucleophilic side chains (predominantly of proteins in reactions catalyzed by ADP-ribosyltransferases) or serve as an acceptor for acyl groups in reactions catalyzed by sirtuins. Moreover, it can react with water or with itself to yield ADP-ribose or cyclic-ADP-ribose (cADPR), respectively, catalyzed by CD38, CD157 or SARM1. Finally, NAD^+ is used in RNA-capping events across all kingdoms. In eukaryotes, NAD^+-capping increases degradation of RNA, whereas in prokaryotes it generally stabilizes RNA[9]. Although $NADP^+$ has been primarily considered a redox cofactor, it can also serve as a substrate in a pyridinium exchange reaction catalyzed by CD38 and SARM1, which replaces the nicotinamide moiety with a nicotinic acid moiety to generate the second messenger nicotinic acid adenine dinucleotide phosphate (NAADP)[10,11], which, like cADPR, is a potent calcium mobilizing agent[12,13]. These $NAD(P)^+$ consuming reactions, along with processes such as tissue growth or side reactions that chemically damage the structure of NAD^+, necessitate ongoing NAD^+ synthesis to maintain pools of the dinucleotides to serve as substrates and cofactors for redox reactions.

Although many of the key reactions involved in the synthesis and elimination of NAD^+ were described more than half a century ago, we are only just beginning to understand the physiological relevance of changes in NAD^+ and its metabolites in human tissues[14]. The observation that NAD^+ availability often decreases in aged tissues or disease states[15-21] has led to intense interest in the potential therapeutic value of supplemental NAD^+ precursors[22]. At the same time, evidence is mounting that our understanding of how to assess NAD^+ metabolism or the success of supplementation is woefully incomplete. Subcellular compartmentalization of discrete NAD^+ pools **[G]** along with the transport systems that establish and maintain them remain poorly characterized[23]. Discrepancies between free and total NAD^+ concentrations within each pool are thought to reflect protein binding, but the identities of such proteins and the factors controlling binding levels are unknown[24]. Differences in NAD^+ metabolism and sharing of metabolites between cell types, tissues and species remain poorly understood in most cases. Although short-term studies have consistently supported the safety of boosting NAD^+ levels through supplementation, some potential risks remain to be fully addressed with direct measurements and longer-term studies.

In this review, we discuss the complexities of NAD^+ synthesis and catabolism, focusing on how some of the less appreciated issues and unknowns such as compartmentalization and noncanonical metabolites may affect the success of NAD^+ boosting strategies. We

do not comprehensively discuss the preclinical or clinical outcomes of NAD^+ boosting that have been obtained to date, as these are exhaustively reviewed elsewhere[22,25,26]. We selectively discuss NAD^+-dependent enzymes and their downstream products only when relevant to the biochemistry of the NAD^+ metabolome. We begin with a review of the key mechanisms leading to production and degradation of NAD^+, followed by its subcellular compartmentalization, transport, and chemistry, and finally review the benefits, risks, and unknowns for strategies to increase NAD^+.

Canonical pathways of NAD^+ synthesis and degradation

The discovery of NAD^+ synthesis pathways and identification of NAD^+ itself are inextricably linked with efforts to cure pellagra, which is a chronic and often fatal disorder that we now know is the result of severe NAD^+ deficiency[27]. The demonstration that nicotinamide (Nam) or nicotinic acid (NA) are NAD^+ precursors that could restore its level led to collective designation of the two precursors as vitamin B3 **[G]** and effectively eradicated pellagra in developed countries, although it remains a challenge in some developing regions. In subsequent studies it was shown that nicotinamide and nicotinic acid are converted to mononucleotides by distinct phosphoribosyltransferases, then to dinucleotides by nicotinamide adenylyltransferases (NMNATs; Figure 2). Nicotinic acid adenine dinucleotide (NAAD) then requires the action of NAD^+ synthase 1 (NADSYN1) to convert the nicotinic acid moiety to an amide[28]. Synthesis of NAD^+ from nicotinic acid, known as the Preiss-Handler pathway was discovered first in microorganisms, but it is present also in mammals, including in humans. However, in mammals, the largest portion of NAD^+ synthesis occurs through the nicotinamide salvage pathway, which recycles nicotinamide arising from NAD^+-dependent signaling reactions (Figure 2). A third pathway, which begins from the amino acid tryptophan and is most active in the liver, has also been recognized and designated the de novo (or “kynurenine”) pathway [28]. With sufficient tryptophan availability, flux through this pathway can generate enough NAD^+ to support whole body needs (through its conversion to circulating nicotinamide) even in the absence of dietary vitamin B3 in rodents and, at least for short periods, in humans[29,30]. Conversely, biallelic mutations in *NADSYN1* (which is required for both the Preiss-Handler and de novo pathways) lead to a severe congenital disease that may be improved by supplementation with amidated NAD^+ precursors[31].

The ribosylated forms of nicotinamide and nicotinic acid — nicotinamide riboside (NR; a niacin equivalent with a complete nicotinamide moiety) and nicotinic acid riboside (NAR), respectively — can enter synthesis pathways through direct conversion to the mononucleotides through nicotinamide riboside kinases (NRKs)[32] (Figure 2). More recently, the reduced form of NR, NRH, was shown to form NADH independently of NRKs through adenosine kinase and NMNATs[33-35], although the quantitative importance of this pathway in normal metabolism remains to be established. Unlike mammals, which directly salvage nicotinamide, some evolutionary lineages including yeast, worms, flies and most microbes employ a nicotinamidase to deamidate nicotinamide into nicotinic acid for assimilation through the Preiss-Handler pathway[36] (Figure 2). Many microbes also possess an alternative de novo pathway for NAD^+ synthesis from aspartate[37].

The best studied and quantitatively most important pathway for elimination of NAD^+ equivalents is through methylation of nicotinamide by nicotinamide *N*-methyl transferase (NNMT)[38] (Figure 2). Some methylated nicotinamide (me-Nam) is eliminated directly in the urine, but most undergoes further oxidation to *N*-methyl-6-pyridone 3-carboxamide (me-6PY, often referred to as *N*-methyl-2-pyridone 5-carboxamide and abbreviated as 2PY in the literature) or *N*-methyl-4-pyridone 3-carboxamide (me-4PY, referred to as 4PY in literature)[39,40] (Figure 2). In rodents, including mice, this reaction is catalyzed by the action of multiple aldehyde oxidases (AOX)[41]. However, other mechanisms are predicted for the oxidation of me-Nam in humans, as rodent AOX1 does not recognize me-Nam as substrate and human AOX1 is the only isoform present in humans[42]. The methylated pyridones along with a small amount of unmethylated pyridones, nicotinamide *N*-oxide, and nicotinuric acid derived from excess nicotinic acid, are also cleared in the urine[43].

Subcellular NAD^+ pools, their functions and maintenance

Many cellular processes, metabolites and pathways are compartmentalized to optimize the efficiency of biochemical activities. As NAD^+ participates in as many as ~25% of all biochemical reactions[44], it needs to be available in many, if not all subcellular compartments[23]. Moreover, the multitude of NAD^+-consuming signaling reactions continuously depletes the dinucleotide. In this section, we discuss mechanisms that enable the maintenance of compartmentalized NAD^+ pools, either through local NAD^+ synthesis or by mediating import of NAD^+ or NADH.

Subcellular differences in NAD(H) concentrations

Early studies addressing subcellular distribution of NAD^+ established that mitochondria contain a large fraction of the cellular NAD^+ content, a fraction that varies between cell types and might exceed 50% in cardiomyocytes[45,46]. The need for a large mitochondrial pool has been attributed to its requirement in the TCA (Krebs) cycle and oxidative phosphorylation, the main NAD^+-dependent mechanism of ATP generation in most cells. Most of the remaining NAD^+ (typically >50% of cellular NAD^+) has been detected in the nucleus and cytosol and ascribed to a single nuclear–cytosolic pool, assuming unrestricted exchange between the two compartments based on NAD^+ being small enough to pass through the nuclear pore[45,46]. Moreover, when cells were treated with an NAD^+ synthesis inhibitor, the rate of NAD^+ depletion was similar in the nucleus and cytosol, but significantly delayed in mitochondria[47]. Given that the total volume of mitochondria is very small compared to the nucleus and cytosol, it had been clear early on that the NAD^+ concentration in mitochondria is substantially higher than its total cellular concentration. Indeed, the use of genetically encoded fluorescent NAD^+ sensors directed to the nucleus, cytosol or mitochondria confirmed the earlier observations[47]. In cultured human cells, the free NAD^+ concentration in mitochondria was estimated to be ~250 μM, whereas in both the nucleus and the cytosol, values of ~100 μM were measured[47]. Comparable but somewhat lower values for free NAD^+ were obtained using semisynthetic Förster resonance energy transfer (FRET) sensors[48]. Notably, these values are lower than the total concentrations that are extractable from cells or tissues, and this difference has been attributed to lowering the free pool of NAD^+ through protein binding.

Dehydrogenase enzymes that use NAD(H) to catalyze oxidation and reduction reactions and have K_m values for NAD(H) that are similar to its free concentration range are kinetically regulated by fluctuations in the NAD(H) concentration[44]. Many nuclear and cytosolic NAD^+-consuming enzymes, including sirtuins and ADP-ribosyltransferases[49,50], have K_m values for NAD^+ in the range of 50–200 μM. Consequently, their activity will also be modulated by physiologically relevant alterations in the NAD^+ concentration. In the nucleus, the transcription co-repressor C-terminal binding protein (CtBP) acts as NAD(H) sensor that modulates gene expression and reprogrammes metabolism in response to shifts in the redox ratio[51]. Although the capacity of redox ratio per se to regulate sirtuins is controversial, there is good evidence for regulation of sirtuin 1 (SIRT1) by circadian fluctuations in NADH levels[52,53]. In mitochondria, the K_m for NAD^+ (~250 μM) of SIRT3 suggests it is a NAD^+ sensor that adjusts metabolic regulation within these organelles according to changes in NAD^+ levels [54]. Therefore, at least some of these proteins have a potential for metabolic sensing, as their activity could be regulated by fluctuations in NAD(H) concentration.

Despite the apparent equilibration between nuclear and cytosolic NAD^+ pools, it has been reported that by changing the abundance of nuclear or cytosolic NMNATs (the enzymes that catalyze the final step of NAD^+ synthesis), temporary NAD^+ gradients between these compartments could be established, and that they might be crucial for adipocyte differentiation[55]. Thus, a subset of NAD^+-dependent enzymes can likely facilitate immediate metabolic adaptation to changes in NAD(H) abundance or in the NAD^+/NADH ratio within each cellular compartment.

The assessment of NAD^+ content in other cellular compartments has been limited by lack of adequate experimental tools (Table 1; Box 2). Given that the NAD^+-dependent oxidation of very-long-chain fatty acids takes place in peroxisomes, the presence of the dinucleotide in these organelles can be safely assumed. This assumption has been confirmed by targeted expression of the catalytic domain of poly(ADP-ribose) polymerase 1 (PARP1) in peroxisomes followed by immunocytochemical detection of ADP-ribose polymers[56], which are formed exclusively from NAD^+. Using this approach, the presence of NAD^+ was also demonstrated in the endoplasmic reticulum and the Golgi apparatus[56]. However, the roles of these subcellular pools are still unexplored.

Transport of NAD(H) across intracellular membranes

NAD^+ and its biosynthesis intermediates generally cannot passively diffuse across cellular membranes and require dedicated transporters to enter cells or membrane-bounded organelles. Nicotinamide may be an exception, especially at high concentrations, although evidence for carrier-mediated uptake also exists[57,58]. Therefore, such carriers should be key regulators of NAD^+ concentration in membrane-bounded organelles. However, knowledge about the exchange of NAD^+ or its biosynthesis intermediates across intracellular membranes has only begun to emerge.By contrast, the subcellular distribution of enzymes that can catalyze the final step of NAD^+ biosynthesis (NMNATs and NAD^+ synthase) is known and they are confined to the nucleus, cytosol and mitochondria[59]. Consequently, the presence of NAD^+ in other organelles requires dedicated NAD^+ or NADH transporters. Even

the generation of the mitochondrial NAD^+ pool is largely independent of NMNAT3, the mitochondrial NMNAT[60].

Intense research led to the identification of SLC25A51, also referred to as MCART1, as a mitochondrial NAD^+ transporter in mammalian cells and established the major pathway responsible for the generation of this important pool[61-64]. Deletion of this carrier results in mitochondria that are almost devoid of NAD^+. The liver-specific carrier protein SLC25A47 was suggested to be another mitochondrial NAD^+ transporter[65]. However, a thorough experimental verification of this function is still pending, as hepatocytes clearly express SLC25A51. Likewise, the peroxisome membrane protein SLC25A17 has been reconstituted in phospholipid vesicles and shown to transport coenzymes, including NAD^+ (ref.[66]), yet whether this carrier has a physiological function in peroxisomal NAD^+ homeostasis remains to be firmly established. How the NAD^+ pools of other organelles such as the endoplasmic reticulum and the Golgi complex are generated and maintained remains unknown.

An expanded view of organismal NAD^+ metabolism

In recent years, there has been a growing appreciation of the varied mixtures of NAD^+ precursors that we obtain from our diet, and how the gut microbiome interacts with host NAD^+ metabolism.

Dietary sources and uptake of NAD^+ precursors

A common misconception is that NAD^+ precursors arrive in the body exclusively as the conventional vitamin B3 forms — nicotinamide or nicotinic acid. In fact, all living cells contain larger amounts of the active metabolites of NAD^+ (NAD^+, NADH, $NADP^+$, NADPH), which are the major forms of “niacin equivalents” that are ingested from unprocessed plant-based or animal-based foods. “Niacin” is a somewhat ambiguous term used inconsistently in the literature to refer to both nicotinamide and nicotinic acid, or to mean nicotinic acid specifically, in which case nicotinamide may be specified as “niacinamide.” This terminology was adopted to avoid confusion with nicotine and hesitancy to consume “acid.” Here we use the chemical names except when referring to “niacin equivalents” as a collective term for molecules that contain or enable the formation of the nicotinamide moiety in NAD^+ and thus support the production of NAD^+.

As a general rule, phosphorylated nucleotides do not cross cell membranes[67], and instead NAD(P)(H) in the gut is broken down to NR (which can be taken up by equilibrative nucleoside transporters[68]) or further prior to uptake, with the majority absorbed as nicotinamide or nicotinic acid[69,70] (Figure 3). A proposed exception is that nicotinamide mononucleotides (NMN) may be imported in the phosphorylated form through SLC12A8[71], although the matter remains in debate[72,73]. Although some evidence has been presented for the association of labeled NMN with cells in an SLC12A8-dependent manner[74], its transformation into NAD^+ has required NRK activity (through nicotinamide riboside (NR) as an intermediate) or degradation to nicotinamide, in cell culture and *in vivo* studies to date[59,75]. Intact NAD^+ appears to be transported across the cell membrane through connexin 43, but the concentration gradient likely allows only export from cells through this pathway,

potentially to support extracellular signaling enzymes such as CD38 (ref.[76]). Intracellularly, connexin 43 allows movement of $NADP^+$ into endolysosomes[11].

The recommended daily intake for vitamin B3 in the United States is 16mg for men and 14mg for women[77], and various organizations have set recommendations in this range based on urinary excretion of metabolites, with the intention of providing a safe margin to prevent pellgra[78]. However, this practice leaves open the question of what the "optimal" level of B3 consumption is. In addition, tryptophan contributes substantially to NAD^+ synthesis: a study conducted in Japanese women has estimated that every ~67mg of tryptophan ingested supports NAD^+ synthesis equivalent to the amount produced from 1mg of nicotinamide[79]. In mice, tryptophan-dependent NAD^+ synthesis is sufficient to maintain the NAD^+ metabolome even in the absence of dietary vitamin B3 or microbial contributions (i.e., with the microbiome is removed by antibiotics or in germ-free mice); simultaneously inactivating the tryptophan pathway and withdrawing dietary vitamin B3 causes a true deficiency[80,81]. Humans are generally considered to depend more on vitamin B3 intake than mice, but notably they have also been shown to survive without vitamin B3 when dietary tryptophan intake is sufficient[30]. Microbial metabolism can also provide some niacin equivalents from aspartate (or dietary precursors to aspartate), but the yield from these pathways is too low to prevent pellagra and therefore of uncertain significance. Notably, whereas many countries implement a mandatory food fortification program that includes vitamin B3 (e.g., US, UK, Australia and Canada), many industrialized countries of continental Europe (e.g., France, Spain) and the Russian Federation do not. Thus, maintenance of the entire NAD^+ metabolome and the availability of its precursors, by means of diet or supplementation, depends not only on the physiological status of the host and its microbiome, but also on the approach to nutrition in the general population.

The role of the microbiome

As noted above, the gut microbiome can contribute to de novo NAD^+ synthesis. In addition, microbes themselves require NAD^+, which creates the potential for "net positive" or "net negative" influences on total niacin equivalents available to the host. However, the role of the microbiome has proven more complex and fascinating than initially suspected, with nicotinamide from the host circulation entering the gut lumen and returning largely as nicotinic acid following transformation by microbes[81] (Figure 3). This pathway accounts for ~50–80% of microbial NAD^+ synthesis and is sufficient to maintain circulating nicotinic acid levels in the host in the absence of any dietary intake. The reasons for this sharing of niacin equivalents remain speculative — it may ensure the availability of NAD^+ precursors to microbes during times of fasting or low-quality diet, and provide metabolic flexibility to host tissues, which lack the ability to generate nicotinic acid. For microbes, using deamidated NAD^+ intermediates is energetically less efficient, but more chemically stable, and avoids inhibition of bacterial DNA ligase by NMN[82,83]. There is also potential for communication through G-protein coupled receptor 109A (GPR109A; also known as HCAR2), which is present in the gut lumen[84] and responds to nicotinic acid[85]. Furthermore, microbes have a much greater metabolic role during NAD^+ precursor supplementation than was initially appreciated. The majority of the increase of NAD^+ levels observed in the liver after a bolus of nicotinamide, NR, or NMN is generated through

microbial production of nicotinic acid, rather than from direct trafficking of the administered molecule to hepatocytes[70,81,86]. With higher doses, circulating concentrations of nicotinic acid transiently reach levels sufficient to activate GPR109A throughout the body, rather than only in the gut lumen, and the potential significance of this mechanism remains unexplored[81]. Thus, the significance of microbiome composition or interventions such as antibiotics are areas that are ripe for exploration. Interestingly, providing reduced forms of NAD^+ precursors (that can be converted directly into NADH) might offer an opportunity to boost specifically host NAD(H) pools, as they appear to be much less affected by the gut microbiome[35].

The composition and structure of populations of microorganisms in the gastro-intestinal tract are affected by many factors, predominantly genetics, host physiology, diet and environmental factors. However, boosting NAD^+ has also been shown to affect the microbial flora. For instance, NAD^+ precursors have been shown to restrict the colonization of pathogenic bacteria in the intestinal tract, while improving synthesis of hepatic bile acids, which are metabolites that possess inhibitory properties against microbial proliferation[87]. The consensus is that boosting microbial NAD^+ synthesis may help maintain normal microbial metabolism, and promoting host NAD^+ levels might be beneficial to intestinal bacteria and detrimental to harmful bacteria[37,88]. However, it will be important to test whether some of these benefits extend to humans as the change in microbiome composition following supplementation may be restricted to rodents[89].

Supplementing NAD^+ metabolism

Strategies to boost NAD^+ levels have received enormous attention in recent years, with many preclinical studies suggesting it has benefits (Table 2). However, in many cases there is little mechanistic understanding, and anecdotal claims continue to outnumber proven benefits in human trials.

Choosing when to supplement

Given that the intake of niacin equivalents in developed nations is well above the threshold required to prevent pellagra, it is prudent to examine the validity of evidence that further increasing NAD^+ synthesis capacity has any benefit. In fact, except for anecdotal reports, we are unaware of any such evidence in young healthy rodents or humans, and by contrast, there are some hints of potentially detrimental effects on exercise performance (complicated by the potential influence of repeated gavages) and glucose tolerance with high doses in rodents[90-92]. However, there is abundant evidence that supplemental NAD^+ precursors are beneficial in rodents in conditions of metabolic stress, injury, or disease, and can improve some aspects of aging in mice[22]. In some, but not all, of these conditions, decreases in NAD^+ concentration have been measured in relevant tissues following supplementation, which is consistent with a model that NAD^+ levels typically found in healthy young individuals are near optimal and the goal of supplementation should be to restore NAD^+ level only when it has fallen.

One of the biggest clinical successes of NAD^+ precursors to date has been the treatment of mitochondrial myopathy with nicotinic acid[17]. In this case, the potential need for supplementation was detectable in the form of lower NAD^+ levels in muscle biopsies and whole blood of patients, with the degree of NAD^+ deficit predicting the degree of benefit. Importantly, it is not technically feasible at present to measure NAD^+ levels in rare cell types, or to assess the possibility that localized depletions occur within tissues. For this reason, even conditions in which no specific NAD^+ deficit is detected, but supplementation produces a benefit, may reflect restoration of NAD^+ levels. Moreover, the steady-state concentration of NAD^+ in a tissue is not a reliable indicator of turnover, as changes in NAD^+-consumer activity and synthesis rates that balance each other are invisible to steady-state measurements[93-95]. Excessive activation of one consumer could also limit NAD^+ availability to others, without a major change in total concentration, owing either to the kinetics or to the compartmentalization of the different enzymes. These uncertainties highlight the need for better tools and biomarkers to assess the need for and potential benefits of NAD^+ supplementation.

Choosing the most promising indications in which to invest clinical effort is a daunting task, given the broad range of rodent models that have shown benefits and the lack of readily available biomarkers or mechanistic understanding in most cases. Early trials in humans have had some successes, but disproportionately more failures in attempting to recapitulate the results from rodents. Table 2 summarizes some of the indications that appear to warrant further testing, based on evidence of efficacy in rodents, an NAD^+-driven mechanism, clinical need and availability of biomarkers for assessing success. In addition to the mitochondrial myopathy study mentioned above, conditions that might benefit from NAD^+ supplementation include inflammation, heart failure, kidney disease, several premature aging syndromes including ataxia telangiectasia (A-T) and Alzheimer disease, and aspects of healthy aging. For both heart failure[18] and kidney disease[96], decreased NAD^+ levels have been measured in affected tissues in humans, and lower circulating nicotinamide[97] and tryptophan[98] were recently noted in individuals with Alzheimer disease, although direct measurements of NAD^+ in brains of these individuals have not been reported. The longest human trial with NR to date (2 years) was performed in individuals with A-T and recently reported improvements in motor coordination and eye movements[99]. Notably, heart failure and kidney disease have clear clinical markers that can be assessed over short time frames and NR supplementation has already been shown to suppress the inflammatory phenotypes of PBMCs isolated from individuals with heart failure[100,101].

Healthy aging is perhaps the most difficult indication to assess, but also the one that is potentially the most impactful[14,22,102]. Decreases in NAD^+ levels are observed with age in multiple, but not all, tissues from rodents and in human skin[103], muscle[104], liver[105] and brain[106,107]. The decreases are mild (~30% between young and very old) and not entirely consistent between individuals, making it unclear whether they are sufficient to be functionally relevant and whether they are truly intrinsic to aging or reflect specific disease processes in a subset of individuals[108]. Supporting these concerns, depletion of the NAD^+ synthesis factor nicotinamide phosphoribosyltransferase (NAMPT) in mouse skeletal muscle decreases NAD^+ levels far more than its effect of aging, and is initially well-tolerated[109]. In humans, the age-related decrease in muscle NAD^+ levels is correlated

with muscle function, with aged trained athletes having levels that are not lower than those of young controls[104]. And although NR supplementation modestly extended lifespan in one small study in mice[110], it failed to do so in a larger multi-center test, albeit at a lower dose and altered genetic background[111]. Nevertheless, both NR and NMN improve some age-related phenotypes in mice[110,112], and age is the greatest risk factor for most of the specific conditions that NAD^+ supplementation has shown promise for. Thus, it seems quite plausible that NAD^+ supplementation could extend healthspan if not lifespan. This possibility could be tested gradually, through approval of supplements for a specific indication in aged individuals and careful assessments of their overall health as secondary outcomes. Another possibility is performing a study more akin to the tame aging with metformin trial, in which participants are enrolled prospectively and monitored for the occurrence and clinical course of multiple age-related conditions[113]. Studies testing specific mechanisms by which NAD^+ precursors have been proposed to exert antiaging effects[114] and a focus on biomarker development will greatly facilitate these translational efforts.

An overlooked concept is the need for other vitamin B-derived cofactors to support NAD^+ synthesis and functions. For instance, vitamin B1 (thiamine), which is lower in aged individuals[115], forms an obligate cofactor for the pentose pathway, which produces the 5-phosphoriboside pyrophosphate that is needed for the glycosylation step of NAD^+ biosynthesis (catalyzed by quinolinic acid phosphoribosyltransferase (QPRT), nicotinate phosphoribosyltransferase (NAPRT), or NAMPT)[116]. Similarly, thiamine-derived cofactors as well as the vitamin B2 (riboflavin)-derived cofactors flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) are necessary for the effective conversion of NADH reductive power into ATP production through the Krebs cycle and oxidative phosphorylation[116,117]. Sources and absorption of different B-vitamins are similar, and unless genetic predispositions are responsible for poor absorption, populations deficient in niacin equivalents often display other B-vitamin deficiencies[118,119]. As such, supplementation with only niacin equivalents might not be sufficient to meet the metabolic needs of an individual prone to B-vitamin deficiencies.

Current strategies for supplementing NAD^+

It is possible to simply supplement intact NAD(P)(H). In fact, intravenous NAD^+ has become fashionable although we are not aware of any scientific literature testing its effects, and a concern related to this type of administration is the potential for detrimental effects from a sustained increase in circulating adenosine[120]. In addition, there are scattered reports of supplementation with intact NAD^+ or NADH in neurological conditions that have generally not been well controlled or consistently reproduced (e.g., studies reviewed in ref.[121]). Most recent clinical efforts have focused on one of the following NAD^+ precursors:

Nicotinamide.

Nicotinamide is the body's main currency for niacin equivalents and the most straightforward way to supplement NAD^+. In fact, phase III trials have already demonstrated safety and some evidence of efficacy against skin cancer[122]. Perceived weaknesses of this approach include the fact that high concentrations of nicotinamide inhibit many NAD^+-

dependent enzymes, and that it depends on the activity of NAMPT — a key enzyme that catalyzes the phosphoribosyltransferase step in NAD^+ (re-)generation from nicotinamide (Figure 2) — which is rate-limiting, energetically costly and downregulated in some conditions including in models of heart failure[123]. NAMPT is also subject to feedback inhibition by NAD^+, thereby limiting the increase in NAD^+ level that can be achieved through routes depending on this enzyme[124]. In addition, nicotinamide is the direct substrate of the catabolic pathway initiated by NNMT, making it theoretically easy to bypass NAD^+ synthesis altogether.

Nicotinic acid.

Nicotinic acid has a long history of clinical use driven mainly by its lipid-lowering properties, which are not shared by nicotinamide and thus appear independent of NAD^+ synthesis[125]. It also comes with the disadvantage of a flushing response (temporary reddening of the skin with an unpleasant sensation of heat and/or itching) triggered by its activation of GPR109A[85]. Nevertheless, nicotinic acid supplementation is an effective strategy to raise NAD^+ levels and has been successfully applied to the treatment of mitochondrial myopathy[17]. In terms of energy demand, synthesis of NAD^+ from nicotinic acid is even more costly than from nicotinamide as the NAD^+ synthetase step adds another ATP-dependent reaction (Figure 1). Excess nicotinic acid not used in NAD^+ production is converted to nicotinuric acid (NUA; Figure 2). NUA is the only known direct catabolite of nicotinuric acid, but its putative physiological relevance has received little attention. Finally, NAD^+ synthesis relying on NAPRT rather than on NAMPT is associated with certain cancerous and precancerous lesions[126,127], which should be considered when using supplements that promote nicotinic acid availability.

Tryptophan.

Although the *de novo* pathway begins from tryptophan and total flux can be modulated by tryptophan intake (Figure 2), this supplementation strategy is less attractive because tryptophan is also the precursor to kynurenines and serotonin, which have immunomodulatory and neurological effects, respectively[128]. Excess flux into the de novo pathway may also produce the neurotoxic intermediate quinolinic acid, which is associated with delirium and mortality. However, no adverse effects were noted in a study of up to 5g/day supplemental tryptophan[129] and some studies continue to employ this strategy for NAD^+ boosting[130].

Nicotinamide riboside.

in 2004, nicotinamide riboside was recognized as an alternative entry point to the salvage pathway through NRKs[32] (Figure 2). Its main advantage is bypassing NAMPT, thereby avoiding potential feedback inhibition, dependence on expression of that enzyme, and some of the energetic cost of NAD^+ synthesis. Although these advantages clearly hold true in cell culture, much of an *in vivo* dose ends up as nicotinamide and nicotinic acid when administered orally[94,109]. Several studies have nevertheless shown advantages of NR over nicotinamide in animals[109,123], which may be related to pharmacokinetics or the small proportion of NR that reaches tissues intact.

Nicotinamide mononucleotide.

This intermediate of NAD^+ synthesis is positioned downstream of NAMPT and thus, like nicotinamide riboside, also bypasses the need for NAMPT, thereby providing similar benefits to those of nicotinamide riboside supplementation. Debate over whether there is direct uptake of NMN or whether it is first dephosphorylated to nicotinamide riboside has fuelled a discussion over which of the two molecules is the most proximal to cellular NAD^+ (ref.[71-73]). Few head-to-head comparisons testing nicotinamide riboside and NMN in the same system at the same time have been performed in rodents, and none in humans, making it unclear whether there is a substantial advantage for either molecule. Notably, both performed similarly in a model of acute kidney injury[96,131]. Because NMN was tested as a drug prior to its submission as a dietary ingredient, the FDA has ruled that NMN is an investigational drug, ineligible for distribution as a dietary ingredient or supplement in the United States under the Dietary Supplement Health and Education Act (DSHEA). By contrast, nicotinamide riboside was first granted status as a new dietary ingredient and then tested as a new investigational drug, exempting it from similar restrictions.

Other NAD^+ precursors.

The redox properties of NAD^+ are preserved in nicotinamide riboside and NMN; recently, their respective reduced forms, NRH and NMNH have also been investigated with regard to having an effect on cellular NAD^+ levels. Both compounds profoundly elevate intracellular NAD^+ levels and, despite being acid labile *in vitro*, are more effective than their oxidized counterparts in rodents, which may be partly related to improved stability in the gastro-intestinal tract and blood[34,35,132,133]. As NMNH needs to be dephosphorylated to NRH to enter cells, the intracellular conversion of the two molecules proceeds through the same route, namely through intracellular phosphorylation to NMNH by adenosine kinase, which is different from the NRK-dependent route taken by oxidized forms[33]. Whether this difference in enzymology or some other property accounts for the higher NAD^+ generation from reduced precursors remains to be fully elucidated. Ribosylated precursors entering NAD^+ synthesis through the deamidated pathway, such as NAR or nicotinic acid mononucleotide (NAMN), can maintain cellular NAD^+ levels[134]. However, their utility as NAD^+ boosters has not been well studied. Trigonelline (N-methylated nicotinic acid) occurs in plants-based foods such as coffee and it remains uncertain whether the nicotinic acid is liberated by microbial metabolism. Another consideration is whether supplementing ribose along with niacin equivalents will better support NAD^+ synthesis[135]. This moiety is effectively built in with nicotinamide riboside and NMN and could be an advantage of these molecules even if the ribose is largely separated from the nicotinamide prior to absorption. Overall, it is very clear that many supplementation strategies are available and that making rational choices about which to apply will require direct comparisons that are not yet available.

Alternative strategies to restoring or increasing NAD^+ levels

Apart from the well-studied and well-tested supplementation strategies using nicotinamide riboside or NMN, two main options can be considered to enhance or stabilize NAD^+ levels, namely, boosting NAD^+ biosynthesis through stimulation of NAD^+ synthesis enzymes and inhibition of excessive NAD^+ consumption. Given the variety of entry points into NAD^+

biosynthesis and the multitude of NAD^+ degrading enzymes, there are many potential targets, some of which have already been addressed experimentally.

Supporting NAD^+-synthesizing enzymes

NAMPT has a fundamental role in the recycling of nicotinamide, which is generated by sirtuins, ARTs, CD38 and SARM1 in all the NAD^+-degrading signaling reactions (Figure 2). As NAMPT is generally agreed to be rate-limiting in this pathway, acceleration of its activity is expected to contribute to higher NAD^+ availability. Several synthetic NAMPT activators have been identified and undergone initial characterization including demonstration of their NAD^+ boosting capacity[136-138].

Acceleration of the conversion of tryptophan to NAD^+ has also been proposed based on the inhibition of the de novo pathway enzyme ACMSD (also known as 2-amino-3-carboxymuconate-6-semialdehyde decarboxylase)[80,139]. ACMSD decarboxylates aminocarboxymuconic semialdehyde (ACMS) to picolinic acid, which is further degraded to eventually form acetyl coenzyme A. Crucially, the alternative pathway for ACMS metabolism — cyclization to quinolinic acid used to generate NAMN — is non-enzymatic[139]. Therefore, inhibition of ACMSD increases the flux towards NAD^+ and reduces the need for other niacin equivalents. However, a concern is the potential to accumulate quinolinic acid, which is a neurotoxin[140], should downstream enzymes fail to metabolize the quinolinic acid as quickly as it is produced. Upregulation of NRK2 is observed is conditions of low NAD^+ levels in the heart and skeletal muscle, suggesting that it may also provide a strategy to increase NAD^+ production[123].

Inhibition of excessive NAD^+ consumption

Inhibition of NAD^+-consuming enzymes will contribute to the stabilization of NAD^+ levels. However, it is not always clear which enzymes account for a substantial portion of NAD^+ flux and many of the beneficial effects of NAD^+-dependent processes are mediated by such enzymes, meaning their inhibition would be counterproductive. Therefore, it is important to identify those NAD^+ consumers that exhibit excessive activity and to target them specifically, achieving a dual effect: the selective downregulation of an undesired or overactivated NAD^+-dependent signaling process, along with the limitation of NAD^+ consumption. The validity of limiting consumption to maintain high NAD^+ levels was demonstrated by inhibition of PARP1 activity[141]. However, long-term PARP1 inhibition is considered undesirable owing to the important roles of this enzyme, particularly in DNA repair.

Age-related NAD^+ decline has been proposed to depend on an increase in the activity of CD38, an ectoenzyme primarily found on endothelial cells and in immune cells such as macrophages and B cells[142,143]. Moreover, it was recently shown that chronic inflammation, which is a hallmark of aging, leads to increased expression of CD38 on macrophages owing to accumulation of senescent cells. Consequently, NAD^+ cleavage is enhanced, lowering its levels in tissues[144,145]. Several inhibitors of CD38 have been developed that show promise in maintaining organismal NAD^+ levels during aging[146-148]. Another NADase of interest

is SARM1. Following mechanical or chemical insult, SARM1 mediates the rapid NAD^+ decline that ultimately drives axonal degeneration[149,150]. Although SARM1 is present in many mammalian tissues, its physiological role in non-neuronal cells has remained obscure. Inhibitors are under development[151-153] that might become of interest not only for protecting neurons, but also to generally diminishing potentially futile NAD^+ consumption. Nevertheless, it needs to be borne in mind that due to the capacity of CD38 and SARM1 to synthesize (cyclic) ADP ribose, their inhibition may have negative consequences on physiological calcium signaling. For example, oxytocin release and synaptic plasticity are impaired in mice lacking CD38 (ref.[154]).

Potential risks of NAD^+ overaccumulation

Excess intake of niacin equivalents beyond the level required to maintain NAD^+ levels is a likely consequence of a healthy diet and certain for those using supplements. While the risks of intermediate levels of precursor supplementation are being investigated, very high doses of nicotinamide or nicotinic acid can clearly be harmful[155]. For instance, according to recommendations made by the European Nicotinamide Diabetes Intervention Trial Group, nicotinamide should be considered as a drug possessing toxic potential for adult doses in excess of 3 g/day, for which unsupervised use of nicotinamide should be discouraged[156]. Although few adverse effects have been detected in preclinical studies to date, mild decreases in hemoglobin and hematocrit were noted following supplementation with nicotinamide riboside[157] and nicotinic acid[17], which may warrant monitoring in longer term studies, and some theoretical concerns remain to be fully addressed.

Inside cells, NAD^+ and NADH can be degraded to NMN and NMNH, which can be dephosphorylated to nicotinamide riboside and NRH. NAAD, an NAD^+ biosynthesis intermediate, was proposed to also be a substrate of pyrophosphatases such as NUDIX hydrolases and of phosphatases, and thereby a precursor of NAR[134]. These nucleosides can be released into the extracellular space[68]. In cell culture, the activity of equilibrative importers and exporters of nicotinamide riboside and NAR appears to depend on the phosphorylation status of nicotinamide riboside and NAR inside the cells[134], whereas the points of control of the import and export of NRH remain unknown. Circulating NAR and nicotinamide riboside are readily hydrolyzed to nicotinic acid and Nam by glycohydrolases[158] and phosphorylases[159], whereas NRH remains intact. In cells, NRH is the redox coenzyme of NQO2 (N-ribosyldihydronicotinamide:quinone dehydrogenase 2), a xenobiotic metabolizing enzyme[160,161]. Overall, it can be envisaged that excess of intracellular NAAD, NAD^+ and NADH will undergo degradation through these innocuous pathways if these molecules are not needed in redox biology or signaling, and that excess of nicotinic acid, Nam and their riboside forms enter circulation and are use in various tissues. However, overloading the system or driving chemical modification of NAD^+ or its intermediates has the potential to create unintended metabolic consequences.

Methylation of nicotinamide

Administration of NAD^+ precursors leads to a substantial increase of me-Nam levels[162,163]. Nam is methylated by NNMT (Figure 2). Although methyl-nicotinic acid (trigonelline)

is a known catabolite found in plants[164,165], nicotinic acid is not a substrate of NNMT and production of methyl-nicotinic acid in mammalian cells has not been observed. Thus, NNMT appears to be specific for the removal of excess nicotinamide[166]. Me-Nam feeds back to inhibit NNMT[167], which could be important for limiting excessive nicotinamide depletion.

Methylation of Nam consumes S-adenosylmethionine (SAM) and produces S-adenosylhomocysteine (SAH). Recycling of SAH to methionine is commonly referred to as the methyl cycle[168,169]. SAM is the second most used enzyme substrate after ATP[170-172] and its depletion is one mechanism proposed to explain the hepatotoxicity of high dose nicotinamide or nicotinic acid[173]. Although it is tempting to speculate that supplementing SAM could therefore offset effects of Nam, it was recently shown that SAM can be catabolized outside the methyl cycle into toxic catabolites, raising concerns over its safety as a freely available dietary supplement[174]. Conversely, increasing the levels of Nam might also affect the ability of SAM to contribute to the methylome, thereby reducing its effectiveness as a supplement. This effect should be of particular concern as co-supplementation of NAD^+ precursors with SAM-containing nutraceuticals is becoming more common[175]. Much remains to be elucidated in this area of NAD^+ supplement consumption in the context of co-administration.

Methylated pyridones

In mammals, me-Nam is readily oxidized to methylated pyridones: me-6PY (2PY) and me-4PY (4PY). The oxidation of me-Nam to me-PY allows the removal of excess nicotinamide by NNMT, as it removes product inhibition. These methylated pyridones exist as three possible isomers, and can be generated enzymatically or form spontaneously without the need of enzyme catalysts[40]. Often, their detection and quantification in serum, plasma and urine is limited to only one of the isomers, providing only a partial estimate of overall Nam catabolism following administration of NAD^+ precursors[176]. These oxidized forms of me-Nam have been directly associated with the presence and progression of chronic kidney diseases and have been deemed uremic toxins[177-179]. However, the toxic properties of me-PY and whether they cross cell membranes have yet to be ascertained both in eukaryotes and prokaryotes. Very recently, circulating levels of me-PY were demonstrated to correlate with major adverse cardiovascular events in humans, and suggested to act through induction of vascular cell adhesion protein 1 (ref.[178]).

Non-canonical metabolites

In addition to their catabolism through Nam, non-canonical products of NAD^+ and NADH metabolism can generate entities that are detrimental to cellular homeostasis and that counteract the benefits of increasing NAD^+ levels (Figure 4). Excess of NAD^+ and excess of NADH are likely to affect cells and organelles differently in different circumstances. For instance, under oxidative stress, mitochondrial respiration and oxidative enzymes generate reactive oxygen species (ROS) that react with electrophiles to generate oxidized derivatives[180]. In the case of $NAD(P)^+$, NMN and nicotinamide riboside, this oxidative chemistry occurs on the pyridinium ring of nicotinamide (analogous to oxidation

of me-Nam) (Figure 4); it is promiscuous and takes place intracellularly as well as extracellularly[40]. Crucially, this oxidative process leads to the formation of non-canonical degradation products with yet poorly defined properties. The three ribosylated pyridone (PYR) isomers — 2-PYR, 4-PYR and 6-PYR (Figure 4) — generated through this chemistry can be found readily in cells, tissues, blood, serum and urine, although the phosphorylated forms such as PYR triphosphate (PYR-TP) and the oxidized forms of NAD^+, ox-NAD, are restricted to biospecimens containing cells, as they are intracellular entities[181,182]. Importantly, these oxidized forms of NAD^+ are structural mimics of NAD^+ and NADH, but are not redox active and do not possess a cleavable glycosidic bond, making them potentially potent inhibitors of NAD^+-dependent processes.

NADH and NADPH, just like NRH and NMNH, can become hydrated[161,183]. This hydration leads to the formation of NADHX and NADPHX, which are NAD(P)H derivatives with properties that are poorly defined, but detrimental to their functions as redox cofactors and substrates. When studying this process on NRH, it was discovered that NRH hydration led to the formation of conjugated protein adducts, which occurred preferentially on Lys residues[161]. Importantly, NAD(P)HX formation is reversed by a two-enzyme system that is essential to cell survival[184]. Therefore, excessive amounts of NAD(P)HX that would be susceptible to this chemistry present a risk that must be tightly controlled. Enzymes such as NAD(P)H quinone dehydrogenase 1 (NQO1) and NADPH oxidases are able to convert NAD(P)H to NAD(P), although in turn they may increase the production of ROS[185,186].

Considerably-less often considered as potentially detrimental, is the ability of 1,4-NADH (the active form) to isomerize to 1,2-NADH or 1,6-NADH in the presence of riboflavin-based catalysts like FMN and FAD, either free or protein-bound (Figure 4). This isomerism is difficult to characterize in cells and tissues because it generates chemically unstable entities that can react readily with protein and generate irreversible protein conjugates, such as NAD(P)HX. Furthermore, these isomers are potent inhibitors of redox enzymes that are crucial to cellular metabolism and bioenergetics, and can potentially be detoxified through oxidation back to $NAD(P)^+$ by renalase[187,188]. As an increase in NADH can equate to an increase in the abundance of these isomers, an increase in total NAD(H) might present yet unanticipated drawbacks. In summary, an increase in NAD^+ levels through supplementation that allows for a decrease in ROS and avoids a sustained increase in NADH is most likely to offer the best physiological outcomes. It is still unknown how to best achieve this endpoint.

Conclusion and future perspective

Many open questions and controversies remain about the basic biology and metabolism of NAD^+-related molecules. Whole-tissue measurements lack the resolution to accurately determine the availability of NAD(P)(H) to enzymes in specific cells or subcellular compartments, either to diagnose deficiency or to judge the success of supplementation. We also lack tools to routinely assess NAD^+ turnover, which can vary independently from steady state concentration. Determining these parameters is crucial for understanding which NAD^+-dependent processes are relevant in a given condition. To fully exploit the therapeutic potential of modulating NAD^+ metabolism, further research efforts are needed to understand the cellular mechanisms of NAD(H) distribution and how the different pools fuel

NAD^+-dependent processes. Likewise, the interplay between different tissues in precursor generation and utilization is yet to be systematically studied. Moreover, the crucial roles of host–microbiome interactions and their interplay with other important cofactors, such as SAM, are receiving considerable interest, but will require further study to elucidate.

Rational design of translational experiments of NAD^+ boosting is going to require more sophisticated measurements and a focus on downstream mechanisms that can be tested. In humans, there is not yet any compelling reason to favor nicotinamide riboside over NMN or vice versa, and although they both have a theoretical advantage over conventional B3 vitamins, they lag behind in size and length of clinical trials. In future studies, it will be important to resolve controversies surrounding supplementation strategies to determine an optimal method for delivering niacin equivalents, whether there should be different strategies for different goals (e.g., depending on the target tissue), and reliable parameters that can be evaluated as outcomes. It is also crucial to continue assessing potential risks of NAD^+ boosting.

In summation, NAD^+ and related molecules are essential to almost every facet of metabolism and the observations that their levels decrease and can be restored suggest the potential for exciting therapeutic opportunities. Fully achieving this potential will require embracing and continuing to explore the complexities of synthesis, transport and catabolism of the NAD^+ metabolome.

Acknowledgements

M.Z. is supported by grants from the Research Council of Norway (No. 325172 — cofounded by the University of Bergen, 309567, 302314). J.A.B. is supported in part by NIH grants DK098656 and HL165792.

Competing interests

M.E.M. is supported in part by Elysium Health and holds patent on manufacturing NAD^+ precursors, their derivatives, and applications thereof. M.Z. is scientific advisor for Blue Helix Health and declares no other competing interests. J.A.B. has received research funding and materials from Pfizer, Elysium Health and Metro International Biotech and consulting fees from Pfizer, Elysium Health, Cytokinetics, and Altimmune. He holds a patent for using NAD^+ precursors in liver injury.

Glossary

ADP ribose	ADP ribose is generated by cleavage of NAD^+ and the release of Nam. ADP ribose can be attached to proteins or nucleic acids in a process called ADP-ribosylation, which is catalyzed by ADP ribosyltransferases
NAD^+ pools	Spatially or functionally separated reservoirs of NAD^+. In cells, NAD^+ pools refer to NAD^+ contents in different subcellular compartments such as mitochondria, cytosol, nucleus etc
Nicotinamide (Nam)	Also known as niacinamide; a major nutritional precursor NAD^+ and recyclable NAD^+ degradation product for NAD^+ (re-)synthesis

Sirtuins	A family of NAD^+-consuming enzymes that mediate protein modifications such as de-acylation and ADP-ribosylation, with a broad range of functions including metabolic sensing and regulation
Vitamin B3	Classically, refers to NA and Nam for their capacity to cure Pellagra; in a wider sense, natural compounds such as NR and NAR, which can be taken up in the gut and contain a pyridine moiety

References

1. Corkey BE & Deeney JT The Redox Communication Network as a Regulator of Metabolism. Front Physiol 11, 567796, doi:10.3389/fphys.2020.567796 (2020). [PubMed: 33178037]
2. Hopp AK & Hottiger MO Uncovering the Invisible: Mono-ADP-ribosylation Moved into the Spotlight. Cells 10, doi:10.3390/cells10030680 (2021).
3. Hottiger MO et al. Progress in the function and regulation of ADP-Ribosylation. Sci Signal 4, mr5, doi:10.1126/scisignal.2001645 (2011). [PubMed: 21610250]
4. Luscher B. et al. ADP-ribosyltransferases, an update on function and nomenclature. FEBS J 289, 7399–7410, doi:10.1111/febs.16142 (2022). [PubMed: 34323016]
5. Imai S, Armstrong CM, Kaeberlein M & Guarente L Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature 403, 795–800, doi:10.1038/35001622 (2000). [PubMed: 10693811]
6. Horenstein AL et al. The Circular Life of Human CD38: From Basic Science to Clinics and Back. Molecules 25, doi:10.3390/molecules25204844 (2020).
7. Gasparrini M, Sorci L & Raffaelli N Enzymology of extracellular NAD metabolism. Cellular and molecular life sciences : CMLS 78, 3317–3331, doi:10.1007/s00018-020-03742-1 (2021). [PubMed: 33755743]
8. Waller TJ & Collins CA Multifaceted roles of SARM1 in axon degeneration and signaling. Front Cell Neurosci 16, 958900, doi:10.3389/fncel.2022.958900 (2022). [PubMed: 36090788]
9. Wolfram-Schauerte M & Hofer K NAD-capped RNAs - a redox cofactor meets RNA. Trends Biochem Sci 48, 142–155, doi:10.1016/j.tibs.2022.08.004 (2023). [PubMed: 36068130]
10. Ziegler M. New functions of a long-known molecule. Emerging roles of NAD in cellular signaling. Eur J Biochem 267, 1550–1564, doi:10.1046/j.1432-1327.2000.01187.x (2000). [PubMed: 10712584]
11. Nam TS et al. Interleukin-8 drives CD38 to form NAADP from NADP(+) and NAAD in the endolysosomes to mobilize Ca(2+) and effect cell migration. FASEB J 34, 12565–12576, doi:10.1096/fj.202001249R (2020). [PubMed: 32717131]
12. Guse AH Enzymology of Ca(2+)-Mobilizing Second Messengers Derived from NAD: From NAD Glycohydrolases to (Dual) NADPH Oxidases. Cells 12, doi:10.3390/cells12040675 (2023).
13. Aarhus R, Graeff RM, Dickey DM, Walseth TF & Lee HC ADP-ribosyl cyclase and CD38 catalyze the synthesis of a calcium-mobilizing metabolite from NADP. J Biol Chem 270, 30327–30333, doi:10.1074/jbc.270.51.30327 (1995). [PubMed: 8530456]
14. Feuz MB, Meyer-Ficca ML & Meyer RG Beyond Pellagra-Research Models and Strategies Addressing the Enduring Clinical Relevance of NAD Deficiency in Aging and Disease. Cells 12, doi:10.3390/cells12030500 (2023).
15. McReynolds MR, Chellappa K & Baur JA Age-related NAD(+) decline. Exp Gerontol 134, 110888, doi:10.1016/j.exger.2020.110888 (2020). [PubMed: 32097708]
16. Breton M. et al. Blood NAD levels are reduced in very old patients hospitalized for heart failure. Exp Gerontol 139, 111051, doi:10.1016/j.exger.2020.111051 (2020). [PubMed: 32783906]

17. Pirinen E. et al. Niacin Cures Systemic NAD(+) Deficiency and Improves Muscle Performance in Adult-Onset Mitochondrial Myopathy. Cell Metab 32, 144, doi:10.1016/j.cmet.2020.05.020 (2020). [PubMed: 32640244]
18. Horton JL et al. Mitochondrial protein hyperacetylation in the failing heart. JCI insight 2, doi:10.1172/jci.insight.84897 (2016).
19. Paulionis L, Kane SL & Meckling KA Vitamin status and cognitive function in a long-term care population. BMC Geriatr 5, 16, doi:10.1186/1471-2318-5-16 (2005). [PubMed: 16351716]
20. Fang EF et al. Defective mitophagy in XPA via PARP-1 hyperactivation and NAD(+)/SIRT1 reduction. Cell 157, 882–896, doi:10.1016/j.cell.2014.03.026 (2014). [PubMed: 24813611]
21. Fang EF Mitophagy and NAD(+) inhibit Alzheimer disease. Autophagy 15, 1112–1114, doi:10.1080/15548627.2019.1596497 (2019). [PubMed: 30922179]
22. Bhasin S, Seals D, Migaud M, Musi N & Baur JA Nicotinamide Adenine Dinucleotide in Aging Biology: Potential Applications and Many Unknowns. Endocr Rev 44, 1047–1073, doi:10.1210/endrev/bnad019 (2023). [PubMed: 37364580]
23. Kulkarni CA & Brookes PS Cellular Compartmentation and the Redox/Nonredox Functions of NAD(). Antioxid Redox Signal 31, 623–642, doi:10.1089/ars.2018.7722 (2019). [PubMed: 30784294]
24. Murata MM et al. NAD+ consumption by PARP1 in response to DNA damage triggers metabolic shift critical for damaged cell survival. Mol Biol Cell 30, 2584–2597, doi:10.1091/mbc.E18-10-0650 (2019). [PubMed: 31390283]
25. Lautrup S, Sinclair DA, Mattson MP & Fang EF NAD(+) in Brain Aging and Neurodegenerative Disorders. Cell Metab 30, 630–655, doi:10.1016/j.cmet.2019.09.001 (2019). [PubMed: 31577933]
26. Yoshino J, Baur JA & Imai SI NAD(+) Intermediates: The Biology and Therapeutic Potential of NMN and NR. Cell Metab 27, 513–528, doi:10.1016/j.cmet.2017.11.002 (2018). [PubMed: 29249689]
27. Williams AC, Hill LJ & Ramsden DB Nicotinamide, NAD(P)(H), and Methyl-Group Homeostasis Evolved and Became a Determinant of Ageing Diseases: Hypotheses and Lessons from Pellagra. Curr Gerontol Geriatr Res 2012, 302875, doi:10.1155/2012/302875 (2012). [PubMed: 22536229]
28. Bogan KL & Brenner C Nicotinic acid, nicotinamide, and nicotinamide riboside: a molecular evaluation of NAD+ precursor vitamins in human nutrition. Annu Rev Nutr 28, 115–130, doi:10.1146/annurev.nutr.28.061807.155443 (2008). [PubMed: 18429699]
29. Terakata M. et al. Establishment of true niacin deficiency in quinolinic acid phosphoribosyltransferase knockout mice. J Nutr 142, 2148–2153, doi:10.3945/jn.112.167569 (2012). [PubMed: 23096007]
30. Fukuwatari T, Ohta M, Kimtjra N, Sasaki R & Shibata K Conversion ratio of tryptophan to niacin in Japanese women fed a purified diet conforming to the Japanese Dietary Reference Intakes. J Nutr Sci Vitaminol (Tokyo) 50, 385–391, doi:10.3177/jnsv.50.385 (2004). [PubMed: 15895512]
31. Szot JO et al. A metabolic signature for NADSYN1-dependent congenital NAD deficiency disorder. J Clin Invest 134, doi:10.1172/JCI174824 (2024).
32. Bieganowski P & Brenner C Discoveries of nicotinamide riboside as a nutrient and conserved NRK genes establish a preiss-handler independent route to NAD+ in fungi and humans. Cell, doi:10.1016/S0092-8674(04)00416-7 (2004).
33. Yang Y, Zhang N, Zhang G & Sauve AA NRH salvage and conversion to NAD(+) requires NRH kinase activity by adenosine kinase. Nat Metab 2, 364–379, doi:10.1038/s42255-020-0194-9 (2020). [PubMed: 32694608]
34. Yang Y, Mohammed FS, Zhang N & Sauve AA Dihydronicotinamide riboside is a potent NAD(+) concentration enhancer in vitro and in vivo. J Biol Chem 294, 9295–9307, doi:10.1074/jbc.RA118.005772 (2019). [PubMed: 30948509]
35. Giroud-Gerbetant J. et al. A reduced form of nicotinamide riboside defines a new path for NAD(+) biosynthesis and acts as an orally bioavailable NAD(+) precursor. Molecular metabolism 30, 192–202, doi:10.1016/j.molmet.2019.09.013 (2019). [PubMed: 31767171]
36. Bockwoldt M. et al. Identification of evolutionary and kinetic drivers of NAD-dependent signaling. Proc Natl Acad Sci U S A 116, 15957–15966, doi:10.1073/pnas.1902346116 (2019). [PubMed: 31341085]

37. Gazzaniga F, Stebbins R, Chang SZ, McPeek MA & Brenner C Microbial NAD metabolism: lessons from comparative genomics. Microbiol Mol Biol Rev 73, 529–541, Table of Contents, doi:10.1128/MMBR.00042-08 (2009). [PubMed: 19721089]
38. Roberti A, Fernandez AF & Fraga MF Nicotinamide N-methyltransferase: At the crossroads between cellular metabolism and epigenetic regulation. Molecular metabolism 45, 101165, doi:10.1016/j.molmet.2021.101165 (2021). [PubMed: 33453420]
39. Lenglet A. et al. N-methyl-2-pyridone-5-carboxamide (2PY)-Major Metabolite of Nicotinamide: An Update on an Old Uremic Toxin. Toxins (Basel) 8, doi:10.3390/toxins8110339 (2016).
40. Hayat F. et al. The Biochemical Pathways of Nicotinamide-Derived Pyridones. International journal of molecular sciences 22, doi:10.3390/ijms22031145 (2021).
41. Kucukgoze G & Leimkuhler S Direct comparison of the four aldehyde oxidase enzymes present in mouse gives insight into their substrate specificities. PLoS One 13, e0191819, doi:10.1371/journal.pone.0191819 (2018). [PubMed: 29370288]
42. Terao M, Garattini E, Romao MJ & Leimkuhler S Evolution, expression, and substrate specificities of aldehyde oxidase enzymes in eukaryotes. J Biol Chem 295, 5377–5389, doi:10.1074/jbc.REV119.007741 (2020). [PubMed: 32144208]
43. Stratford MR & Dennis MF High-performance liquid chromatographic determination of nicotinamide and its metabolites in human and murine plasma and urine. J Chromatogr 582, 145–151, doi:10.1016/0378-4347(92)80313-f (1992). [PubMed: 1491034]
44. Opitz CA & Heiland I Dynamics of NAD-metabolism: everything but constant. Biochem Soc Trans 43, 1127–1132, doi:10.1042/BST20150133 (2015). [PubMed: 26614649]
45. Alano CC et al. Differences among cell types in NAD(+) compartmentalization: a comparison of neurons, astrocytes, and cardiac myocytes. J Neurosci Res 85, 3378–3385, doi:10.1002/jnr.21479 (2007). [PubMed: 17853438]
46. Di Lisa F, Menabo R, Canton M, Barile M & Bernardi P Opening of the mitochondrial permeability transition pore causes depletion of mitochondrial and cytosolic NAD+ and is a causative event in the death of myocytes in postischemic reperfusion of the heart. J Biol Chem 276, 2571–2575, doi:10.1074/jbc.M006825200 (2001). [PubMed: 11073947]
47. Cambronne XA et al. Biosensor reveals multiple sources for mitochondrial NAD(+). Science 352, 1474–1477, doi:10.1126/science.aad5168 (2016). [PubMed: 27313049]
48. Sallin O. et al. Semisynthetic biosensors for mapping cellular concentrations of nicotinamide adenine dinucleotides. eLife 7, doi:10.7554/eLife.32638 (2018).
49. Anderson KA, Madsen AS, Olsen CA & Hirschey MD Metabolic control by sirtuins and other enzymes that sense NAD(+), NADH, or their ratio. Biochim Biophys Acta Bioenerg 1858, 991–998, doi:10.1016/j.bbabio.2017.09.005 (2017). [PubMed: 28947253]
50. Boehi F, Manetsch P & Hottiger MO Interplay between ADP-ribosyltransferases and essential cell signaling pathways controls cellular responses. Cell Discov 7, 104, doi:10.1038/s41421-021-00323-9 (2021). [PubMed: 34725336]
51. Fjeld CC, Birdsong WT & Goodman RH Differential binding of NAD+ and NADH allows the transcriptional corepressor carboxyl-terminal binding protein to serve as a metabolic sensor. Proc Natl Acad Sci U S A 100, 9202–9207, doi:10.1073/pnas.1633591100 (2003). [PubMed: 12872005]
52. Levine DC et al. NADH inhibition of SIRT1 links energy state to transcription during time-restricted feeding. Nat Metab 3, 1621–1632, doi:10.1038/s42255-021-00498-1 (2021). [PubMed: 34903884]
53. Chellappa K & Baur JA Reducing NAD(H) to amplify rhythms. Nat Metab 3, 1589–1590, doi:10.1038/s42255-021-00494-5 (2021). [PubMed: 34903882]
54. Canto C, Menzies KJ & Auwerx J NAD(+) Metabolism and the Control of Energy Homeostasis: A Balancing Act between Mitochondria and the Nucleus. Cell Metab 22, 31–53, doi:10.1016/j.cmet.2015.05.023 (2015). [PubMed: 26118927]
55. Ryu KW et al. Metabolic regulation of transcription through compartmentalized NAD(+) biosynthesis. Science 360, doi:10.1126/science.aan5780 (2018).

56. VanLinden MR, Niere M, Nikiforov AA, Ziegler M & Dolle C Compartment-Specific Poly-ADP-Ribose Formation as a Biosensor for Subcellular NAD Pools. Methods Mol Biol 1608, 45–56, doi:10.1007/978-1-4939-6993-7_4 (2017). [PubMed: 28695502]
57. Olsson A, Olofsson T & Pero RW Specific binding and uptake of extracellular nicotinamide in human leukemic K-562 cells. Biochem Pharmacol 45, 1191–1200, doi:10.1016/0006-2952(93)90270-7 (1993). [PubMed: 8466540]
58. Mathialagan S. et al. Nicotinic acid transport into human liver involves organic anion transporter 2 (SLC22A7). Biochem Pharmacol 174, 113829, doi:10.1016/j.bcp.2020.113829 (2020). [PubMed: 32001236]
59. Nikiforov A, Dolle C, Niere M & Ziegler M Pathways and subcellular compartmentation of NAD biosynthesis in human cells: from entry of extracellular precursors to mitochondrial NAD generation. J Biol Chem 286, 21767–21778, doi:10.1074/jbc.M110.213298 (2011). [PubMed: 21504897]
60. Yamamoto M. et al. Nmnat3 Is Dispensable in Mitochondrial NAD Level Maintenance In Vivo. PLoS One 11, e0147037, doi:10.1371/journal.pone.0147037 (2016). [PubMed: 26756334]
61. Davila A. et al. Nicotinamide adenine dinucleotide is transported into mammalian mitochondria. eLife 7, doi:10.7554/eLife.33246 (2018).
62. Luongo TS et al. SLC25A51 is a mammalian mitochondrial NAD(+) transporter. Nature, doi:10.1038/s41586-020-2741-7 (2020).
63. Kory N. et al. MCART1/SLC25A51 is required for mitochondrial NAD transport. Sci Adv 6, doi:10.1126/sciadv.abe5310 (2020).
64. Girardi E. et al. Epistasis-driven identification of SLC25A51 as a regulator of human mitochondrial NAD import. Nat Commun 11, 6145, doi:10.1038/s41467-020-19871-x (2020). [PubMed: 33262325]
65. Cheng L. et al. Hepatic mitochondrial NAD + transporter SLC25A47 activates AMPKalpha mediating lipid metabolism and tumorigenesis. Hepatology 78, 1828–1842, doi:10.1097/HEP.0000000000000314 (2023). [PubMed: 36804859]
66. Agrimi G, Russo A, Scarcia P & Palmieri F The human gene SLC25A17 encodes a peroxisomal transporter of coenzyme A, FAD and NAD+. Biochem J 443, 241–247, doi:10.1042/BJ20111420 (2012). [PubMed: 22185573]
67. Wright NJ & Lee SY Toward a Molecular Basis of Cellular Nucleoside Transport in Humans. Chem Rev 121, 5336–5358, doi:10.1021/acs.chemrev.0c00644 (2021). [PubMed: 33232132]
68. Kropotov A. et al. Equilibrative Nucleoside Transporters Mediate the Import of Nicotinamide Riboside and Nicotinic Acid Riboside into Human Cells. International journal of molecular sciences 22, doi:10.3390/ijms22031391 (2021).
69. Gross CJ & Henderson LM Digestion and absorption of NAD by the small intestine of the rat. J Nutr 113, 412–420, doi:10.1093/jn/113.2.412 (1983). [PubMed: 6218262]
70. Shats I. et al. Bacteria Boost Mammalian Host NAD Metabolism by Engaging the Deamidated Biosynthesis Pathway. Cell Metab 31, 564–579 e567, doi:10.1016/j.cmet.2020.02.001 (2020). [PubMed: 32130883]
71. Grozio A. et al. Slc12a8 is a nicotinamide mononucleotide transporter. Nat Metab 1, 47–57, doi:10.1038/s42255-018-0009-4 (2019). [PubMed: 31131364]
72. Grozio A. et al. Reply to: Absence of evidence that Slc12a8 encodes a nicotinamide mononucleotide transporter. Nat Metab 1, 662–665, doi:10.1038/s42255-019-0086-z (2019). [PubMed: 32694650]
73. Schmidt MS & Brenner C Absence of evidence that Slc12a8 encodes a nicotinamide mononucleotide transporter. Nat Metab 1, 660–661, doi:10.1038/s42255-019-0085-0 (2019). [PubMed: 32694648]
74. Unno J, Mills KF, Ogura T, Nishimura M & Imai SI Absolute quantification of nicotinamide mononucleotide in biological samples by double isotope-mediated liquid chromatography-tandem mass spectrometry (dimeLC-MS/MS). NPJ Aging 10, 2, doi:10.1038/s41514-023-00133-1 (2024). [PubMed: 38167419]

75. Ratajczak J. et al. NRK1 controls nicotinamide mononucleotide and nicotinamide riboside metabolism in mammalian cells. Nat Commun 7, 13103, doi:10.1038/ncomms13103 (2016). [PubMed: 27725675]
76. Bruzzone S. et al. A self-restricted CD38-connexin 43 cross-talk affects NAD+ and cyclic ADP-ribose metabolism and regulates intracellular calcium in 3T3 fibroblasts. J Biol Chem 276, 48300–48308, doi:10.1074/jbc.M107308200 (2001). [PubMed: 11602597]
77. in Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B(6), Folate, Vitamin B(12), Pantothenic Acid, Biotin, and Choline The National Academies Collection: Reports funded by National Institutes of Health (1998).
78. Authority, E. F. S. Scientific Opinion on Dietary Reference Values for niacin. EFSA Journal 12, 3759 (2014).
79. Fukuwatari T & Shibata K Nutritional aspect of tryptophan metabolism. Int J Tryptophan Res 6, 3–8, doi:10.4137/IJTR.S11588 (2013). [PubMed: 23922498]
80. Palzer L. et al. Alpha-Amino-Beta-Carboxy-Muconate-Semialdehyde Decarboxylase Controls Dietary Niacin Requirements for NAD(+) Synthesis. Cell reports 25, 1359–1370 e1354, doi:10.1016/j.celrep.2018.09.091 (2018). [PubMed: 30380424]
81. Chellappa K. et al. NAD precursors cycle between host tissues and the gut microbiome. Cell Metab 34, 1947–1959 e1945, doi:10.1016/j.cmet.2022.11.004 (2022). [PubMed: 36476934]
82. Sharma S. et al. Early Evolutionary Selection of NAD Biosynthesis Pathway in Bacteria. Metabolites 12, doi:10.3390/metabo12070569 (2022).
83. Chen XC et al. Development of a fluorescence resonance energy transfer assay for measuring the activity of Streptococcus pneumoniae DNA ligase, an enzyme essential for DNA replication, repair, and recombination. Anal Biochem 309, 232–240, doi:10.1016/s0003-2697(02)00302-0 (2002). [PubMed: 12413456]
84. Sivaprakasam S, Prasad PD & Singh N Benefits of short-chain fatty acids and their receptors in inflammation and carcinogenesis. Pharmacol Ther 164, 144–151, doi:10.1016/j.pharmthera.2016.04.007 (2016). [PubMed: 27113407]
85. Benyo Z, Gille A, Bennett CL, Clausen BE & Offermanns S Nicotinic acid-induced flushing is mediated by activation of epidermal langerhans cells. Molecular pharmacology 70, 1844–1849, doi:10.1124/mol.106.030833 (2006). [PubMed: 17008386]
86. Kim LJ et al. Host-microbiome interactions in nicotinamide mononucleotide (NMN) deamidation. FEBS Lett 597, 2196–2220, doi:10.1002/1873-3468.14698 (2023). [PubMed: 37463842]
87. Fang D. et al. Nicotinamide Mononucleotide Ameliorates Sleep Deprivation-Induced Gut Microbiota Dysbiosis and Restores Colonization Resistance against Intestinal Infections. Adv Sci (Weinh) 10, e2207170, doi:10.1002/advs.202207170 (2023). [PubMed: 36698264]
88. Ren Z. et al. NAD(+) and its possible role in gut microbiota: Insights on the mechanisms by which gut microbes influence host metabolism. Anim Nutr 10, 360–371, doi:10.1016/j.aninu.2022.06.009 (2022). [PubMed: 35949199]
89. Peluso AA et al. Oral supplementation of nicotinamide riboside alters intestinal microbial composition in rats and mice, but not humans. NPJ Aging 9, 7, doi:10.1038/s41514-023-00106-4 (2023). [PubMed: 37012386]
90. Kourtzidis IA et al. The NAD(+) precursor nicotinamide riboside decreases exercise performance in rats. Journal of the International Society of Sports Nutrition 13, 32, doi:10.1186/s12970-016-0143-x (2016). [PubMed: 27489522]
91. Shi W. et al. High Dose of Dietary Nicotinamide Riboside Induces Glucose Intolerance and White Adipose Tissue Dysfunction in Mice Fed a Mildly Obesogenic Diet. Nutrients 11, doi:10.3390/nu11102439 (2019).
92. Yu J. et al. Exercise-induced benefits on glucose handling in a model of diet-induced obesity are reduced by concurrent nicotinamide mononucleotide. Am J Physiol Endocrinol Metab 321, E176–E189, doi:10.1152/ajpendo.00446.2020 (2021). [PubMed: 34121447]
93. McReynolds MR et al. NAD(+) flux is maintained in aged mice despite lower tissue concentrations. Cell Syst 12, 1160–1172 e1164, doi:10.1016/j.cels.2021.09.001 (2021). [PubMed: 34559996]

94. Liu L. et al. Quantitative Analysis of NAD Synthesis-Breakdown Fluxes. Cell Metab 27, 1067–1080 e1065, doi:10.1016/j.cmet.2018.03.018 (2018). [PubMed: 29685734]
95. Hara N, Osago H, Hiyoshi M, Kobayashi-Miura M & Tsuchiya M Quantitative analysis of the effects of nicotinamide phosphoribosyltransferase induction on the rates of NAD+ synthesis and breakdown in mammalian cells using stable isotope-labeling combined with mass spectrometry. PLoS One 14, e0214000, doi:10.1371/journal.pone.0214000 (2019). [PubMed: 30875389]
96. Doke T. et al. NAD(+) precursor supplementation prevents mtRNA/RIG-I-dependent inflammation during kidney injury. Nat Metab 5, 414–430, doi:10.1038/s42255-023-00761-7 (2023). [PubMed: 36914909]
97. Dalmasso MC et al. Nicotinamide as potential biomarker for Alzheimer's disease: A translational study based on metabolomics. Front Mol Biosci 9, 1067296, doi:10.3389/fmolb.2022.1067296 (2022). [PubMed: 36685284]
98. Fathi M. et al. Dynamic changes in metabolites of the kynurenine pathway in Alzheimer's disease, Parkinson's disease, and Huntington's disease: A systematic Review and meta-analysis. Front Immunol 13, 997240, doi:10.3389/fimmu.2022.997240 (2022). [PubMed: 36263032]
99. Presterud R. et al. Long-Term Nicotinamide Riboside Use Improves Coordination and Eye Movements in Ataxia Telangiectasia. Mov Disord, doi:10.1002/mds.29645 (2023).
100. Wang DD et al. Safety and Tolerability of Nicotinamide Riboside in Heart Failure With Reduced Ejection Fraction. JACC Basic Transl Sci 7, 1183–1196, doi:10.1016/j.jacbts.2022.06.012 (2022). [PubMed: 36644285]
101. Zhou B. et al. Boosting NAD level suppresses inflammatory activation of PBMCs in heart failure. J Clin Invest 130, 6054–6063, doi:10.1172/JCI138538 (2020). [PubMed: 32790648]
102. Reiten OK, Wilvang MA, Mitchell SJ, Hu Z & Fang EF Preclinical and clinical evidence of NAD(+) precursors in health, disease, and ageing. Mech Ageing Dev 199, 111567, doi:10.1016/j.mad.2021.111567 (2021). [PubMed: 34517020]
103. Massudi H. et al. Age-associated changes in oxidative stress and NAD+ metabolism in human tissue. PLoS One 7, e42357, doi:10.1371/journal.pone.0042357 (2012). [PubMed: 22848760]
104. Janssens G. et al. Healthy aging and muscle function are positively associated with NAD+ abundance in humans. Nature Aging 2, 254–263 (2022). [PubMed: 37118369]
105. Zhou CC et al. Hepatic NAD(+) deficiency as a therapeutic target for non-alcoholic fatty liver disease in ageing. Br J Pharmacol 173, 2352–2368, doi:10.1111/bph.13513 (2016). [PubMed: 27174364]
106. Zhu XH, Lu M, Lee BY, Ugurbil K & Chen W In vivo NAD assay reveals the intracellular NAD contents and redox state in healthy human brain and their age dependences. Proc Natl Acad Sci U S A 112, 2876–2881, doi:10.1073/pnas.1417921112 (2015). [PubMed: 25730862]
107. Bagga P. et al. Single-Voxel (1) H MR spectroscopy of cerebral nicotinamide adenine dinucleotide (NAD(+)) in humans at 7T using a 32-channel volume coil. Magn Reson Med 83, 806–814, doi:10.1002/mrm.27971 (2020). [PubMed: 31502710]
108. Peluso A, Damgaard MV, Mori MAS & Treebak JT Age-Dependent Decline of NAD(+)-Universal Truth or Confounded Consensus? Nutrients 14, doi:10.3390/nu14010101 (2021).
109. Frederick DW et al. Loss of NAD Homeostasis Leads to Progressive and Reversible Degeneration of Skeletal Muscle. Cell Metab 24, 269–282, doi:10.1016/j.cmet.2016.07.005 (2016). [PubMed: 27508874]
110. Zhang H. et al. NAD(+) repletion improves mitochondrial and stem cell function and enhances life span in mice. Science 352, 1436–1443, doi:10.1126/science.aaf2693 (2016). [PubMed: 27127236]
111. Harrison DE et al. 17-a-estradiol late in life extends lifespan in aging UM-HET3 male mice; nicotinamide riboside and three other drugs do not affect lifespan in either sex. Aging Cell 20, e13328, doi:10.1111/acel.13328 (2021). [PubMed: 33788371]
112. Mills KF et al. Long-Term Administration of Nicotinamide Mononucleotide Mitigates Age-Associated Physiological Decline in Mice. Cell Metab 24, 795–806, doi:10.1016/j.cmet.2016.09.013 (2016). [PubMed: 28068222]
113. Barzilai N, Crandall JP, Kritchevsky SB & Espeland MA Metformin as a Tool to Target Aging. Cell Metab 23, 1060–1065, doi:10.1016/j.cmet.2016.05.011 (2016). [PubMed: 27304507]

114. Lautrup S, Hou Y, Fang EF & Bohr VA Roles of NAD(+) in Health and Aging. Cold Spring Harbor perspectives in medicine 14, doi:10.1101/cshperspect.a041193 (2024).
115. Wilkinson TJ, Hanger HC, George PM & Sainsbury R Is thiamine deficiency in elderly people related to age or co-morbidity? Age Ageing 29, 111–116, doi:10.1093/ageing/29.2.111 (2000). [PubMed: 10791444]
116. Tylicki A, Lotowski Z, Siemieniuk M & Ratkiewicz A Thiamine and selected thiamine antivitamins - biological activity and methods of synthesis. Biosci Rep 38, doi:10.1042/BSR20171148 (2018).
117. Saedisomeolia A & Ashoori M Riboflavin in Human Health: A Review of Current Evidences. Adv Food Nutr Res 83, 57–81, doi:10.1016/bs.afnr.2017.11.002 (2018). [PubMed: 29477226]
118. Akerele D. Household Food Expenditure Patterns, Food Nutrient Consumption and Nutritional Vulnerability in Nigeria: Implications for Policy. Ecol Food Nutr 54, 546–571, doi:10.1080/03670244.2015.1041136 (2015). [PubMed: 26083997]
119. Gila-Diaz A. et al. Multidimensional Approach to Assess Nutrition and Lifestyle in Breastfeeding Women during the First Month of Lactation. Nutrients 13, doi:10.3390/nu13061766 (2021).
120. Borea PA, Gessi S, Merighi S, Vincenzi F & Varani K Pathological overproduction: the bad side of adenosine. Br J Pharmacol 174, 1945–1960, doi:10.1111/bph.13763 (2017). [PubMed: 28252203]
121. Covarrubias AJ, Perrone R, Grozio A & Verdin E NAD(+) metabolism and its roles in cellular processes during ageing. Nat Rev Mol Cell Biol 22, 119–141, doi:10.1038/s41580-020-00313-x (2021). [PubMed: 33353981]
122. Chen AC et al. A Phase 3 Randomized Trial of Nicotinamide for Skin-Cancer Chemoprevention. N Engl J Med 373, 1618–1626, doi:10.1056/NEJMoa1506197 (2015). [PubMed: 26488693]
123. Diguet N. et al. Nicotinamide Riboside Preserves Cardiac Function in a Mouse Model of Dilated Cardiomyopathy. Circulation 137, 2256–2273, doi:10.1161/CIRCULATIONAHA.116.026099 (2018). [PubMed: 29217642]
124. Hara N. et al. Elevation of cellular NAD levels by nicotinic acid and involvement of nicotinic acid phosphoribosyltransferase in human cells. J Biol Chem 282, 24574–24582, doi:10.1074/jbc.M610357200 (2007). [PubMed: 17604275]
125. D'Andrea E, Hey SP, Ramirez CL & Kesselheim AS Assessment of the Role of Niacin in Managing Cardiovascular Disease Outcomes: A Systematic Review and Meta-analysis. JAMA Netw Open 2, e192224, doi:10.1001/jamanetworkopen.2019.2224 (2019). [PubMed: 30977858]
126. Chowdhry S. et al. NAD metabolic dependency in cancer is shaped by gene amplification and enhancer remodelling. Nature 569, 570–575, doi:10.1038/s41586-019-1150-2 (2019). [PubMed: 31019297]
127. Wang N. et al. NAPRT, but Not NAMPT, Provides Additional Support for NAD Synthesis in Esophageal Precancerous Lesions. Nutrients 14, doi:10.3390/nu14224916 (2022).
128. Klaessens S, Stroobant V, De Plaen E & Van den Eynde BJ Systemic tryptophan homeostasis. Front Mol Biosci 9, 897929, doi:10.3389/fmolb.2022.897929 (2022). [PubMed: 36188218]
129. Hiratsuka C. et al. Supplementing healthy women with up to 5.0 g/d of L-tryptophan has no adverse effects. J Nutr 143, 859–866, doi:10.3945/jn.112.173823 (2013). [PubMed: 23616514]
130. Connell NJ et al. NAD+-Precursor Supplementation With L-Tryptophan, Nicotinic Acid, and Nicotinamide Does Not Affect Mitochondrial Function or Skeletal Muscle Function in Physically Compromised Older Adults. J Nutr 151, 2917–2931, doi:10.1093/jn/nxab193 (2021). [PubMed: 34191033]
131. Fang EF et al. NAD+ Replenishment Improves Lifespan and Healthspan in Ataxia Telangiectasia Models via Mitophagy and DNA Repair. Cell Metab 24, 566–581, doi:10.1016/j.cmet.2016.09.004 (2016). [PubMed: 27732836]
132. Zapata-Perez R. et al. Reduced nicotinamide mononucleotide is a new and potent NAD(+) precursor in mammalian cells and mice. FASEB J 35, e21456, doi:10.1096/fj.202001826R (2021). [PubMed: 33724555]
133. Liu Y. et al. Reduced Nicotinamide Mononucleotide (NMNH) Potently Enhances NAD(+) and Suppresses Glycolysis, the TCA Cycle, and Cell Growth. J Proteome Res 20, 2596–2606, doi:10.1021/acs.jproteome.0c01037 (2021). [PubMed: 33793246]

134. Kulikova V. et al. Generation, Release, and Uptake of the NAD Precursor Nicotinic Acid Riboside by Human Cells. J Biol Chem 290, 27124–27137, doi:10.1074/jbc.M115.664458 (2015). [PubMed: 26385918]
135. Xue Y. et al. A Combination of Nicotinamide and D-Ribose (RiaGev) Is Safe and Effective to Increase NAD(+) Metabolome in Healthy Middle-Aged Adults: A Randomized, Triple-Blind, Placebo-Controlled, Cross-Over Pilot Clinical Trial. Nutrients 14, doi:10.3390/nu14112219 (2022).
136. Gardell SJ et al. Boosting NAD(+) with a small molecule that activates NAMPT. Nat Commun 10, 3241, doi:10.1038/s41467-019-11078-z (2019). [PubMed: 31324777]
137. Tang S. et al. Chemistry-led investigations into the mode of action of NAMPT activators, resulting in the discovery of non-pyridyl class NAMPT activators. Acta Pharm Sin B 13, 709–721, doi:10.1016/j.apsb.2022.07.016 (2023). [PubMed: 36873168]
138. Akiu M. et al. Discovery of DS68702229 as a Potent, Orally Available NAMPT (Nicotinamide Phosphoribosyltransferase) Activator. Chem Pharm Bull (Tokyo) 69, 1110–1122, doi:10.1248/cpb.c21-00700 (2021). [PubMed: 34719594]
139. Katsyuba E. et al. De novo NAD(+) synthesis enhances mitochondrial function and improves health. Nature 563, 354–359, doi:10.1038/s41586-018-0645-6 (2018). [PubMed: 30356218]
140. Lugo-Huitron R. et al. Quinolinic acid: an endogenous neurotoxin with multiple targets. Oxid Med Cell Longev 2013, 104024, doi:10.1155/2013/104024 (2013). [PubMed: 24089628]
141. Bai P. et al. PARP-1 inhibition increases mitochondrial metabolism through SIRT1 activation. Cell Metab 13, 461–468, doi:10.1016/j.cmet.2011.03.004 (2011). [PubMed: 21459330]
142. Zeidler JD et al. The CD38 glycohydrolase and the NAD sink: implications for pathological conditions. Am J Physiol Cell Physiol 322, C521–C545, doi:10.1152/ajpcell.00451.2021 (2022). [PubMed: 35138178]
143. Camacho-Pereira J. et al. CD38 Dictates Age-Related NAD Decline and Mitochondrial Dysfunction through an SIRT3-Dependent Mechanism. Cell Metab 23, 1127–1139, doi:10.1016/j.cmet.2016.05.006 (2016). [PubMed: 27304511]
144. Chini CCS et al. CD38 ecto-enzyme in immune cells is induced during aging and regulates NAD(+) and NMN levels. Nat Metab 2, 1284–1304, doi:10.1038/s42255-020-00298-z (2020). [PubMed: 33199925]
145. Covarrubias AJ et al. Senescent cells promote tissue NAD(+) decline during ageing via the activation of CD38(+) macrophages. Nat Metab 2, 1265–1283, doi:10.1038/s42255-020-00305-3 (2020). [PubMed: 33199924]
146. Tarrago MG et al. A Potent and Specific CD38 Inhibitor Ameliorates Age-Related Metabolic Dysfunction by Reversing Tissue NAD(+) Decline. Cell Metab 27, 1081–1095 e1010, doi:10.1016/j.cmet.2018.03.016 (2018). [PubMed: 29719225]
147. Li Y. et al. Discovery of a First-in-Class CD38 Inhibitor for the Treatment of Mitochondrial Myopathy. J Med Chem 66, 12762–12775, doi:10.1021/acs.jmedchem.3c00391 (2023). [PubMed: 37696000]
148. Piedra-Quintero ZL, Wilson Z, Nava P & Guerau-de-Arellano M CD38: An Immunomodulatory Molecule in Inflammation and Autoimmunity. Front Immunol 11, 597959, doi:10.3389/fimmu.2020.597959 (2020). [PubMed: 33329591]
149. Essuman K. et al. The SARM1 Toll/Interleukin-1 Receptor Domain Possesses Intrinsic NAD(+) Cleavage Activity that Promotes Pathological Axonal Degeneration. Neuron 93, 1334–1343 e1335, doi:10.1016/j.neuron.2017.02.022 (2017). [PubMed: 28334607]
150. Loreto A. et al. Neurotoxin-mediated potent activation of the axon degeneration regulator SARM1. eLife 10, doi:10.7554/eLife.72823 (2021).
151. Loring HS, Parelkar SS, Mondal S & Thompson PR Identification of the first noncompetitive SARM1 inhibitors. Bioorg Med Chem 28, 115644, doi:10.1016/j.bmc.2020.115644 (2020). [PubMed: 32828421]
152. Li WH et al. Permeant fluorescent probes visualize the activation of SARM1 and uncover an anti-neurodegenerative drug candidate. eLife 10, doi:10.7554/eLife.67381 (2021).

153. Hughes RO et al. Small Molecule SARM1 Inhibitors Recapitulate the SARM1(−/−) Phenotype and Allow Recovery of a Metastable Pool of Axons Fated to Degenerate. Cell reports 34, 108588, doi:10.1016/j.celrep.2020.108588 (2021). [PubMed: 33406435]

154. Martucci LL et al. A multiscale analysis in CD38(−/−) mice unveils major prefrontal cortex dysfunctions. FASEB J 33, 5823–5835, doi:10.1096/fj.201800489R (2019). [PubMed: 30844310]

155. Hwang ES & Song SB Possible Adverse Effects of High-Dose Nicotinamide: Mechanisms and Safety Assessment. Biomolecules 10, doi:10.3390/biom10050687 (2020).

156. Knip M. et al. Safety of high-dose nicotinamide: a review. Diabetologia 43, 1337–1345, doi:10.1007/s001250051536 (2000). [PubMed: 11126400]

157. Airhart SE et al. 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. PLoS One 12, e0186459, doi:10.1371/journal.pone.0186459 (2017). [PubMed: 29211728]

158. Yaku K. et al. BST1 regulates nicotinamide riboside metabolism via its glycohydrolase and base-exchange activities. Nat Commun 12, 6767, doi:10.1038/s41467-021-27080-3 (2021). [PubMed: 34799586]

159. Kropotov A. et al. Purine nucleoside phosphorylase controls nicotinamide riboside metabolism in mammalian cells. J Biol Chem 298, 102615, doi:10.1016/j.jbc.2022.102615 (2022). [PubMed: 36265580]

160. Megarity CF et al. The two common polymorphic forms of human NRH-quinone oxidoreductase 2 (NQO2) have different biochemical properties. FEBS Lett 588, 1666–1672, doi:10.1016/j.febslet.2014.02.063 (2014). [PubMed: 24631540]

161. Makarov MV et al. Chemical and Biochemical Reactivity of the Reduced Forms of Nicotinamide Riboside. ACS Chem Biol 16, 604–614, doi:10.1021/acschembio.0c00757 (2021). [PubMed: 33784074]

162. Trammell SA et al. Nicotinamide riboside is uniquely and orally bioavailable in mice and humans. Nat Commun 7, 12948, doi:10.1038/ncomms12948 (2016). [PubMed: 27721479]

163. Ear PH et al. Maternal Nicotinamide Riboside Enhances Postpartum Weight Loss, Juvenile Offspring Development, and Neurogenesis of Adult Offspring. Cell reports 26, 969–983 e964, doi:10.1016/j.celrep.2019.01.007 (2019). [PubMed: 30673618]

164. Zhou J, Chan L & Zhou S Trigonelline: a plant alkaloid with therapeutic potential for diabetes and central nervous system disease. Curr Med Chem 19, 3523–3531, doi:10.2174/092986712801323171 (2012). [PubMed: 22680628]

165. Konstantinidis N, Franke H, Schwarz S & Lachenmeier DW Risk Assessment of Trigonelline in Coffee and Coffee By-Products. Molecules 28, doi:10.3390/molecules28083460 (2023).

166. Loring HS & Thompson PR Kinetic Mechanism of Nicotinamide N-Methyltransferase. Biochemistry 57, 5524–5532, doi:10.1021/acs.biochem.8b00775 (2018). [PubMed: 30148963]

167. Gao Y, Martin NI & van Haren MJ Nicotinamide N-methyl transferase (NNMT): An emerging therapeutic target. Drug Discov Today 26, 2699–2706, doi:10.1016/j.drudis.2021.05.011 (2021). [PubMed: 34029690]

168. Sibani S. et al. Studies of methionine cycle intermediates (SAM, SAH), DNA methylation and the impact of folate deficiency on tumor numbers in Min mice. Carcinogenesis 23, 61–65, doi:10.1093/carcin/23.1.61 (2002). [PubMed: 11756224]

169. Ducker GS & Rabinowitz JD One-Carbon Metabolism in Health and Disease. Cell Metab 25, 27–42, doi:10.1016/j.cmet.2016.08.009 (2017). [PubMed: 27641100]

170. Cantoni GL Biological methylation: selected aspects. Annu Rev Biochem 44, 435–451, doi:10.1146/annurev.bi.44.070175.002251 (1975). [PubMed: 1094914]

171. Loenen WA S-adenosylmethionine: jack of all trades and master of everything? Biochem Soc Trans 34, 330–333, doi:10.1042/BST20060330 (2006). [PubMed: 16545107]

172. Lee YH, Ren D, Jeon B & Liu HW S-Adenosylmethionine: more than just a methyl donor. Nat Prod Rep 40, 1521–1549, doi:10.1039/d2np00086e (2023). [PubMed: 36891755]

173. McCarty MF Co-administration of equimolar doses of betaine may alleviate the hepatotoxic risk associated with niacin therapy. Med Hypotheses 55, 189–194, doi:10.1054/mehy.1999.1011 (2000). [PubMed: 10985907]
174. Fukumoto K. et al. Excess S-adenosylmethionine inhibits methylation via catabolism to adenine. Commun Biol 5, 313, doi:10.1038/s42003-022-03280-5 (2022). [PubMed: 35383287]
175. Lee A, Knox R, Reynolds M, McRoy E & Nguyen H S-adenosylmethionine and nicotinamide riboside therapy in Arts syndrome: A case report and literature review. JIMD Rep 64, 417–423, doi:10.1002/jmd2.12395 (2023). [PubMed: 37927483]
176. Dhuguru J, Dellinger RW & Migaud ME Defining NAD(P)(H) Catabolism. Nutrients 15, doi:10.3390/nu15133064 (2023).
177. Rutkowski B. et al. N-methyl-2-pyridone-5-carboxamide: a novel uremic toxin? Kidney Int Suppl, S19–21, doi:10.1046/j.1523-1755.63.s84.36.x (2003).
178. Ferrell M. et al. A terminal metabolite of niacin promotes vascular inflammation and contributes to cardiovascular disease risk. Nat Med 30, 424–434, doi:10.1038/s41591-023-02793-8 (2024). [PubMed: 38374343]
179. Kopple JD, Massry SG & Kalantar-Zadeh K Nutritional management of renal disease. Third edition. edn, (Elsevier/AP, 2013).
180. de Rosa M. et al. Enzymatic oxidation of NADP+ to its 4-oxo derivative is a side-reaction displayed only by the adrenodoxin reductase type of ferredoxin-NADP+ reductases. FEBS J 274, 3998–4007, doi:10.1111/j.1742-4658.2007.05934.x (2007). [PubMed: 17635583]
181. Pelikant-Malecka I, Sielicka A, Kaniewska E, Smolenski RT & Slominska EM 4-Pyridone-3-carboxamide-1beta-D-ribonucleoside metabolism in endothelial cells and its impact on cellular energetic balance. Nucleosides Nucleotides Nucleic Acids 33, 338–341, doi:10.1080/15257770.2014.889303 (2014). [PubMed: 24940690]
182. Slominska EM et al. A novel nucleotide found in human erythrocytes, 4-pyridone-3-carboxamide-1-beta-D-ribonucleoside triphosphate. J Biol Chem 281, 32057–32064, doi:10.1074/jbc.M607514200 (2006). [PubMed: 16920716]
183. Becker-Kettern J. et al. NAD(P)HX repair deficiency causes central metabolic perturbations in yeast and human cells. FEBS J 285, 3376–3401, doi:10.1111/febs.14631 (2018). [PubMed: 30098110]
184. Van Bergen NJ et al. NAD(P)HX dehydratase (NAXD) deficiency: a novel neurodegenerative disorder exacerbated by febrile illnesses. Brain 142, 50–58, doi:10.1093/brain/awy310 (2019). [PubMed: 30576410]
185. Vermot A, Petit-Hartlein I, Smith SME & Fieschi F NADPH Oxidases (NOX): An Overview from Discovery, Molecular Mechanisms to Physiology and Pathology. Antioxidants (Basel) 10, doi:10.3390/antiox10060890 (2021).
186. Pey AL, Megarity CF & Timson DJ NAD(P)H quinone oxidoreductase (NQO1): an enzyme which needs just enough mobility, in just the right places. Biosci Rep 39, doi:10.1042/BSR20180459 (2019).
187. Czerwinska K, Poreba R & Gac P Renalase-A new understanding of its enzymatic and non-enzymatic activity and its implications for future research. Clin Exp Pharmacol Physiol 49, 3–9, doi:10.1111/1440-1681.13594 (2022). [PubMed: 34545616]
188. Moran GR & Hoag MR The enzyme: Renalase. Arch Biochem Biophys 632, 66–76, doi:10.1016/j.abb.2017.05.015 (2017). [PubMed: 28558965]
189. Lu M, Zhu XH & Chen W In vivo (31) P MRS assessment of intracellular NAD metabolites and NAD(+) /NADH redox state in human brain at 4 T. NMR Biomed 29, 1010–1017, doi:10.1002/nbm.3559 (2016). [PubMed: 27257783]
190. Zhang Q, Piston DW & Goodman RH Regulation of corepressor function by nuclear NADH. Science 295, 1895–1897, doi:10.1126/science.1069300 (2002). [PubMed: 11847309]
191. Blacker TS et al. Separating NADH and NADPH fluorescence in live cells and tissues using FLIM. Nat Commun 5, 3936, doi:10.1038/ncomms4936 (2014). [PubMed: 24874098]
192. Parshina YP et al. Simultaneous Probing of Metabolism and Oxygenation of Tumors In Vivo Using FLIM of NAD(P)H and PLIM of a New Polymeric Ir(III) Oxygen Sensor. International journal of molecular sciences 23, doi:10.3390/ijms231810263 (2022).

193. Veskoukis AS, Margaritelis NV, Kyparos A, Paschalis V & Nikolaidis MG Spectrophotometric assays for measuring redox biomarkers in blood and tissues: the NADPH network. Redox Rep 23, 47–56, doi:10.1080/13510002.2017.1392695 (2018). [PubMed: 29088980]

194. Ummarino S. et al. Simultaneous quantitation of nicotinamide riboside, nicotinamide mononucleotide and nicotinamide adenine dinucleotide in milk by a novel enzyme-coupled assay. Food Chem 221, 161–168, doi:10.1016/j.foodchem.2016.10.032 (2017). [PubMed: 27979136]

195. Zhao Y. et al. SoNar, a Highly Responsive NAD+/NADH Sensor, Allows High-Throughput Metabolic Screening of Anti-tumor Agents. Cell Metab 21, 777–789, doi:10.1016/j.cmet.2015.04.009 (2015). [PubMed: 25955212]

196. Zou Y. et al. Illuminating NAD(+) Metabolism in Live Cells and In Vivo Using a Genetically Encoded Fluorescent Sensor. Dev Cell 53, 240–252 e247, doi:10.1016/j.devcel.2020.02.017 (2020). [PubMed: 32197067]

197. Tao R. et al. Genetically encoded fluorescent sensors reveal dynamic regulation of NADPH metabolism. Nat Methods 14, 720–728, doi:10.1038/nmeth.4306 (2017). [PubMed: 28581494]

198. Bilan DS et al. Genetically encoded fluorescent indicator for imaging NAD(+)/NADH ratio changes in different cellular compartments. Biochim Biophys Acta 1840, 951–957, doi:10.1016/j.bbagen.2013.11.018 (2014). [PubMed: 24286672]

199. Hellweg L. et al. A general method for the development of multicolor biosensors with large dynamic ranges. Nat Chem Biol 19, 1147–1157, doi:10.1038/s41589-023-01350-1 (2023). [PubMed: 37291200]

200. Molinari PE et al. NERNST: a genetically-encoded ratiometric non-destructive sensing tool to estimate NADP(H) redox status in bacterial, plant and animal systems. Nat Commun 14, 3277, doi:10.1038/s41467-023-38739-4 (2023). [PubMed: 37280202]

201. Chen L. et al. Ratiometric NAD(+) Sensors Reveal Subcellular NAD(+) Modulators. ACS Sens 8, 1518–1528, doi:10.1021/acssensors.2c02565 (2023). [PubMed: 36931900]

202. Shabalin K. et al. NAD Metabolome Analysis in Human Cells Using (1)H NMR Spectroscopy. International journal of molecular sciences 19, doi:10.3390/ijms19123906 (2018).

203. Nagana Gowda GA & Raftery D Whole Blood Metabolomics by (1)H NMR Spectroscopy Provides a New Opportunity To Evaluate Coenzymes and Antioxidants. Anal Chem 89, 4620–4627, doi:10.1021/acs.analchem.7b00171 (2017). [PubMed: 28318242]

204. Nagana Gowda GA, Abell L, Tian R & Raftery D Whole Body Distribution of Labile Coenzymes and Antioxidants in a Mouse Model as Visualized Using (1)H NMR Spectroscopy. Anal Chem 95, 6029–6037, doi:10.1021/acs.analchem.3c00054 (2023). [PubMed: 36988554]

205. Yoshino J & Imai S Accurate measurement of nicotinamide adenine dinucleotide (NAD(+)) with high-performance liquid chromatography. Methods Mol Biol 1077, 203–215, doi:10.1007/978-1-62703-637-5_14 (2013). [PubMed: 24014409]

206. Johnson S, Yoshioka K, Brace CS & Imai SI Quantification of localized NAD(+) changes reveals unique specificity of NAD(+) regulation in the hypothalamus. NPJ Aging 9, 1, doi:10.1038/s41514-023-00098-1 (2023). [PubMed: 36697402]

207. Trammell SA & Brenner C Targeted, LCMS-based Metabolomics for Quantitative Measurement of NAD(+) Metabolites. Comput Struct Biotechnol J 4, e201301012, doi:10.5936/csbj.201301012 (2013). [PubMed: 24688693]

208. Hiefner J, Rische J, Bunders MJ & Worthmann A A liquid chromatography-tandem mass spectrometry based method for the quantification of adenosine nucleotides and NAD precursors and products in various biological samples. Front Immunol 14, 1250762, doi:10.3389/fimmu.2023.1250762 (2023). [PubMed: 37799723]

209. Giner MP et al. A Method to Monitor the NAD(+) Metabolome-From Mechanistic to Clinical Applications. International journal of molecular sciences 22, doi:10.3390/ijms221910598 (2021).

210. Petucci C. et al. Measurement of Pyridine Nucleotides in Biological Samples Using LC-MS/MS. Methods Mol Biol 1996, 61–73, doi:10.1007/978-1-4939-9488-5_7 (2019). [PubMed: 31127548]

211. Pencina KM et al. Nicotinamide Adenine Dinucleotide Augmentation in Overweight or Obese Middle-Aged and Older Adults: A Physiologic Study. J Clin Endocrinol Metab 108, 1968–1980, doi:10.1210/clinem/dgad027 (2023). [PubMed: 36740954]

212. El-Kady RR, Ali AK, El Wakeel LM, Sabri NA & Shawki MA Nicotinamide supplementation in diabetic nonalcoholic fatty liver disease patients: randomized controlled trial. Ther Adv Chronic Dis 13, 20406223221077958, doi:10.1177/20406223221077958 (2022). [PubMed: 35222903]

213. Osar Z, Samanci T, Demirel GY, Damci T & Ilkova H Nicotinamide effects oxidative burst activity of neutrophils in patients with poorly controlled type 2 diabetes mellitus. Exp Diabesity Res 5, 155–162, doi:10.1080/15438600490424244 (2004). [PubMed: 15203886]

214. Remie CME et al. Nicotinamide riboside supplementation alters body composition and skeletal muscle acetylcarnitine concentrations in healthy obese humans. Am J Clin Nutr 112, 413–426, doi:10.1093/ajcn/nqaa072 (2020). [PubMed: 32320006]

215. Lapatto HAK et al. Nicotinamide riboside improves muscle mitochondrial biogenesis, satellite cell differentiation, and gut microbiota in a twin study. Sci Adv 9, eadd5163, doi:10.1126/sciadv.add5163 (2023). [PubMed: 36638183]

216. Yoshino M. et al. Nicotinamide mononucleotide increases muscle insulin sensitivity in prediabetic women. Science, doi:10.1126/science.abe9985 (2021).

217. Yi L. et al. The efficacy and safety of beta-nicotinamide mononucleotide (NMN) supplementation in healthy middle-aged adults: a randomized, multicenter, double-blind, placebo-controlled, parallel-group, dose-dependent clinical trial. Geroscience 45, 29–43, doi:10.1007/s11357-022-00705-1 (2023). [PubMed: 36482258]

218. Liao B. et al. Nicotinamide mononucleotide supplementation enhances aerobic capacity in amateur runners: a randomized, double-blind study. Journal of the International Society of Sports Nutrition 18, 54, doi:10.1186/s12970-021-00442-4 (2021). [PubMed: 34238308]

219. Okabe K. et al. Oral Administration of Nicotinamide Mononucleotide Is Safe and Efficiently Increases Blood Nicotinamide Adenine Dinucleotide Levels in Healthy Subjects. Front Nutr 9, 868640, doi:10.3389/fnut.2022.868640 (2022). [PubMed: 35479740]

220. Dollerup OL et al. A randomized placebo-controlled clinical trial of nicotinamide riboside in obese men: safety, insulin-sensitivity, and lipid-mobilizing effects. Am J Clin Nutr 108, 343–353, doi:10.1093/ajcn/nqy132 (2018). [PubMed: 29992272]

221. Igarashi M. et al. Chronic nicotinamide mononucleotide supplementation elevates blood nicotinamide adenine dinucleotide levels and alters muscle function in healthy older men. NPJ Aging 8, 5, doi:10.1038/s41514-022-00084-z (2022). [PubMed: 35927255]

222. Fukamizu Y. et al. Safety evaluation of beta-nicotinamide mononucleotide oral administration in healthy adult men and women. Scientific reports 12, 14442, doi:10.1038/s41598-022-18272-y (2022). [PubMed: 36002548]

223. Dollerup OL et al. Effects of Nicotinamide Riboside on Endocrine Pancreatic Function and Incretin Hormones in Nondiabetic Men With Obesity. J Clin Endocrinol Metab 104, 5703–5714, doi:10.1210/jc.2019-01081 (2019). [PubMed: 31390002]

224. Martens CR et al. Chronic nicotinamide riboside supplementation is well-tolerated and elevates NAD(+) in healthy middle-aged and older adults. Nat Commun 9, 1286, doi:10.1038/s41467-018-03421-7 (2018). [PubMed: 29599478]

225. Ahmadi A. et al. Randomized crossover clinical trial of coenzyme Q10 and nicotinamide riboside in chronic kidney disease. JCI insight 8, doi:10.1172/jci.insight.167274 (2023).

226. Elhassan YS et al. Nicotinamide Riboside Augments the Aged Human Skeletal Muscle NAD(+) Metabolome and Induces Transcriptomic and Anti-inflammatory Signatures. Cell reports 28, 1717–1728 e1716, doi:10.1016/j.celrep.2019.07.043 (2019). [PubMed: 31412242]

227. Wu J. et al. Boosting NAD+ blunts TLR4-induced type I IFN in control and systemic lupus erythematosus monocytes. J Clin Invest 132, doi:10.1172/JCI139828 (2022).

228. Han K. et al. Boosting NAD preferentially blunts Th17 inflammation via arginine biosynthesis and redox control in healthy and psoriasis subjects. Cell Rep Med 4, 101157, doi:10.1016/j.xcrm.2023.101157 (2023). [PubMed: 37586364]

229. Dollerup OL et al. Nicotinamide riboside does not alter mitochondrial respiration, content or morphology in skeletal muscle from obese and insulin-resistant men. J Physiol 598, 731–754, doi:10.1113/JP278752 (2020). [PubMed: 31710095]

230. Kaufman W. The use of vitamin therapy to reverse certain concomitants of aging. J Am Geriatr Soc 3, 927–936, doi:10.1111/j.1532-5415.1955.tb00947.x (1955). [PubMed: 13271047]

231. Dolopikou CF et al. Acute nicotinamide riboside supplementation improves redox homeostasis and exercise performance in old individuals: a double-blind cross-over study. Eur J Nutr 59, 505–515, doi:10.1007/s00394-019-01919-4 (2020). [PubMed: 30725213]

232. Huang H. A Multicentre, Randomised, Double Blind, Parallel Design, Placebo Controlled Study to Evaluate the Efficacy and Safety of Uthever (NMN Supplement), an Orally Administered Supplementation in Middle Aged and Older Adults. Front Aging 3, 851698, doi:10.3389/fragi.2022.851698 (2022). [PubMed: 35821806]

233. Katayoshi T. et al. Nicotinamide adenine dinucleotide metabolism and arterial stiffness after long-term nicotinamide mononucleotide supplementation: a randomized, double-blind, placebo-controlled trial. Scientific reports 13, 2786, doi:10.1038/s41598-023-29787-3 (2023). [PubMed: 36797393]

234. Jensen JB et al. A randomized placebo-controlled trial of nicotinamide riboside and pterostilbene supplementation in experimental muscle injury in elderly individuals. JCI insight 7, doi:10.1172/jci.insight.158314 (2022).

235. Nascimento EBM et al. Nicotinamide Riboside Enhances In Vitro Beta-adrenergic Brown Adipose Tissue Activity in Humans. J Clin Endocrinol Metab 106, 1437–1447, doi:10.1210/clinem/dgaa960 (2021). [PubMed: 33524145]

236. Kim M. et al. Effect of 12-Week Intake of Nicotinamide Mononucleotide on Sleep Quality, Fatigue, and Physical Performance in Older Japanese Adults: A Randomized, Double-Blind Placebo-Controlled Study. Nutrients 14, doi:10.3390/nu14040755 (2022).

237. Martin AJ et al. Neurocognitive Function and Quality of Life Outcomes in the ONTRAC Study for Skin Cancer Chemoprevention by Nicotinamide. Geriatrics (Basel) 4, doi:10.3390/geriatrics4010031 (2019).

238. Orr ME et al. A randomized placebo-controlled trial of nicotinamide riboside in older adults with mild cognitive impairment. Geroscience 46, 665–682, doi:10.1007/s11357-023-00999-9 (2024). [PubMed: 37994989]

239. Poyan Mehr A. et al. De novo NAD(+) biosynthetic impairment in acute kidney injury in humans. Nat Med 24, 1351–1359, doi:10.1038/s41591-018-0138-z (2018). [PubMed: 30127395]

240. Phelan MJ, Mulnard RA, Gillen DL & Schreiber SS Phase II Clinical Trial of Nicotinamide for the Treatment of Mild to Moderate Alzheimer's Disease. J Geriatr Med Gerontol 3 (2017).

241. Brakedal B. et al. The NADPARK study: A randomized phase I trial of nicotinamide riboside supplementation in Parkinson's disease. Cell Metab 34, 396–407 e396, doi:10.1016/j.cmet.2022.02.001 (2022). [PubMed: 35235774]

242. Berven H. et al. NR-SAFE: a randomized, double-blind safety trial of high dose nicotinamide riboside in Parkinson's disease. Nat Commun 14, 7793, doi:10.1038/s41467-023-43514-6 (2023). [PubMed: 38016950]

243. de la Rubia JE et al. Efficacy and tolerability of EH301 for amyotrophic lateral sclerosis: a randomized, double-blind, placebo-controlled human pilot study. Amyotroph Lateral Scler Frontotemporal Degener 20, 115–122, doi:10.1080/21678421.2018.1536152 (2019). [PubMed: 30668199]

244. Carrera-Julia S. et al. Effect of the Mediterranean diet supplemented with nicotinamide riboside and pterostilbene and/or coconut oil on anthropometric variables in amyotrophic lateral sclerosis. A pilot study. Front Nutr 10, 1232184, doi:10.3389/fnut.2023.1232184 (2023). [PubMed: 37810917]

245. Veenhuis SJG et al. Nicotinamide Riboside Improves Ataxia Scores and Immunoglobulin Levels in Ataxia Telangiectasia. Mov Disord 36, 2951–2957, doi:10.1002/mds.28788 (2021). [PubMed: 34515380]

246. Steinbrucker K, Tiefenthaler E, Schernthaner EM, Jungwirth J & Wortmann SB Nicotinamide Riboside for Ataxia Telangiectasia: A Report of an Early Treated Individual. Neuropediatrics 54, 78–81, doi:10.1055/a-1959-9404 (2023). [PubMed: 36223879]

247. Drago F. et al. Prevention of non-melanoma skin cancers with nicotinamide in transplant recipients: a case-control study. Eur J Dermatol 27, 382–385, doi:10.1684/ejd.2017.3025 (2017). [PubMed: 28468736]

248. Surjana D, Halliday GM, Martin AJ, Moloney FJ & Damian DL Oral nicotinamide reduces actinic keratoses in phase II double-blinded randomized controlled trials. J Invest Dermatol 132, 1497–1500, doi:10.1038/jid.2011.459 (2012). [PubMed: 22297641]

249. Allen NC et al. Nicotinamide for Skin-Cancer Chemoprevention in Transplant Recipients. N Engl J Med 388, 804–812, doi:10.1056/NEJMoa2203086 (2023). [PubMed: 36856616]

250. Jonas WB, Rapoza CP & Blair WF The effect of niacinamide on osteoarthritis: a pilot study. Inflamm Res 45, 330–334, doi:10.1007/BF02252945 (1996). [PubMed: 8841834]

251. Braidy N & Liu Y NAD+ therapy in age-related degenerative disorders: A benefit/risk analysis. Exp Gerontol 132, 110831, doi:10.1016/j.exger.2020.110831 (2020). [PubMed: 31917996]

252. Lerner F, Niere M, Ludwig A & Ziegler M Structural and functional characterization of human NAD kinase. Biochem Biophys Res Commun 288, 69–74, doi:10.1006/bbrc.2001.5735 (2001). [PubMed: 11594753]

253. Pollak N, Niere M & Ziegler M NAD kinase levels control the NADPH concentration in human cells. J Biol Chem 282, 33562–33571, doi:10.1074/jbc.M704442200 (2007). [PubMed: 17855339]

254. Ohashi K, Kawai S & Murata K Identification and characterization of a human mitochondrial NAD kinase. Nat Commun 3, 1248, doi:10.1038/ncomms2262 (2012). [PubMed: 23212377]

255. Zhang R. MNADK, a novel liver-enriched mitochondrion-localized NAD kinase. Biol Open 2, 432–438, doi:10.1242/bio.20134259 (2013). [PubMed: 23616928]

256. Kim H. et al. The mitochondrial NAD kinase functions as a major metabolic regulator upon increased energy demand. Molecular metabolism 64, 101562, doi:10.1016/j.molmet.2022.101562 (2022). [PubMed: 35944895]

257. Love NR et al. NAD kinase controls animal NADP biosynthesis and is modulated via evolutionarily divergent calmodulin-dependent mechanisms. Proc Natl Acad Sci U S A 112, 1386–1391, doi:10.1073/pnas.1417290112 (2015). [PubMed: 25605906]

258. Tai L. et al. Calmodulin Is the Fundamental Regulator of NADK-Mediated NAD Signaling in Plants. Front Plant Sci 10, 681, doi:10.3389/fpls.2019.00681 (2019). [PubMed: 31275331]

259. Hoxhaj G. et al. Direct stimulation of NADP(+) synthesis through Akt-mediated phosphorylation of NAD kinase. Science 363, 1088–1092, doi:10.1126/science.aau3903 (2019). [PubMed: 30846598]

260. Estrella MA et al. The metabolites NADP(+) and NADPH are the targets of the circadian protein Nocturnin (Curled). Nat Commun 10, 2367, doi:10.1038/s41467-019-10125-z (2019). [PubMed: 31147539]

261. Ding CC et al. MESH1 is a cytosolic NADPH phosphatase that regulates ferroptosis. Nat Metab 2, 270–277, doi:10.1038/s42255-020-0181-1 (2020). [PubMed: 32462112]

262. Li T. et al. Monitoring NAD(H) and NADP(H) dynamics during organismal development with genetically encoded fluorescent biosensors. Cell Regen 11, 5, doi:10.1186/s13619-021-00105-4 (2022). [PubMed: 35103852]

263. Skupienski R, Do KQ & Xin L In vivo (31)P magnetic resonance spectroscopy study of mouse cerebral NAD content and redox state during neurodevelopment. Scientific reports 10, 15623, doi:10.1038/s41598-020-72492-8 (2020). [PubMed: 32973277]

264. de Graaf RA & Behar KL Detection of cerebral NAD(+) by in vivo (1)H NMR spectroscopy. NMR Biomed 27, 802–809, doi:10.1002/nbm.3121 (2014). [PubMed: 24831866]

265. Azouaoui D. et al. Meta-analysis of NAD(P)(H) quantification results exhibits variability across mammalian tissues. Scientific reports 13, 2464, doi:10.1038/s41598-023-29607-8 (2023). [PubMed: 36774401]

266. Wu JT, Wu LH & Knight JA Stability of NADPH: effect of various factors on the kinetics of degradation. Clin Chem 32, 314–319 (1986). [PubMed: 3943190]

Box 1 |

NADP(H) and NAD$^+$ kinase

Even though the redox properties of nicotinamide adenine dinucleotide (NAD$^+$) and its phosphorylated form NADP$^+$ are nearly identical, all organisms depend on the presence of both pyridine nucleotides. In contrast to NAD(H), the ratio of the phosphorylated adenine dinucleotides is maintained in favor of the reduced form, NADPH. Thus, a reservoir of reducing equivalents is maintained for key cellular functions including detoxification, oxidative defense, reductive anabolic pathways and generation of reactive oxygen reactive oxygen species by NADPH oxidase.

The only known reaction to generate NADP is the phosphorylation of NAD$^+$ to NADP$^+$, which is catalyzed by NAD$^+$ kinase (NADK). Therefore, NADK is essential and ubiquitously present in all organisms. In humans, by far the predominant form of the enzyme is a cytosolic protein[252,253]. A mitochondrial counterpart has been reported[254,255], but its physiological function still needs to be established as its catalytic activity is 2–3 orders of magnitude slower compared to cytosolic NADK[254,255]. Additionally, *Nadk*-null mice are viable, and their mitochondria still contain substantial amounts of NADP(H)[256]. Genetic modulation of *Nadk*, which encodes the cytosolic enzyme, by overexpression or depletion correlates with the total cellular NADPH concentration, supporting the predominant role of this enzyme form[253].

Cytosolic NADK activity is stimulated by a direct Ca^{2+}-dependent and calmodulin-dependent mechanism[257], similar to kinases from other animals and plants[258]. The human enzyme is also activated through phosphorylation by calmodulin-dependent kinase II[257] or AKT[259].

Recent research has demonstrated that the level of NADP(H) is tightly controlled by phosphatases, both in mitochondria (by nocturnin[260]) and the cytosol (by MESH1 (ref.[261])). These enzymes dephosphorylate NADP(H) back to NAD(H), indicating that the balance between the phosphorylated and unphosphorylated forms needs to be tightly controlled. Intriguingly, nocturnin expression undergoes circadian oscillations, and was proposed to establish an important link between metabolism and the circadian clock[260].

Given its activity, NADK is in principle also a NAD$^+$-consuming enzyme. However, this reaction probably doesn't significantly affect the NAD$^+$ pool, because relative to NAD(H), the concentration of NADP(H) is substantially lower[262], and there are many fewer reactions known that degrade NADP(H). By contrast, NAD$^+$-boosting could increase also the NADP(H) pool; however, this possibility has not been well explored. In principle, increased NADP(H) could have favorable effects that might have been incorrectly ascribed to direct NAD$^+$-dependent mechanisms.

Box 2 |

Challenges in NAD$^+$ metabolome measurements

Even though NAD$^+$, NADH, NADP$^+$ and NADPH are comprehensively characterized molecules, their measurement *in vivo* and *ex vivo* remains challenging. Currently, direct NAD(P)(H) quantifications in humans can only be achieved by magnetic resonance spectrometry (MRS), and indirect measurements are achieved through invasive biopsy followed by extraction and quantitation.

Although potentially very informative, measurements of NAD$^+$ by MRS are limited to only a few research sites and remain qualitative rather than quantitative, as internal standards are difficult to identify and characterize. Furthermore, both proton and phosphorus MRS require refinements to improve signal-to-noise ratios and in the case of phosphorus MRS, other abundant biochemical entities possessing similar chemical properties can produce overlapping signals in the same analytical window (e.g., NADH, NADP(H), ADP ribose, coenzyme A and uridine diphosphate sugars, although the α-phosphate of ATP can be the most obstructive signal[107,189,263,264]).

Isolation and processing of biospecimens is currently required to achieve quantitative measurements. These measurements can be carried out on cell, blood, tissue and fluid samples. Initial processing, which requires separating water-soluble materials that include all known NAD$^+$ metabolites, from macromolecules and lipids, is often necessary and is usually followed by high performance liquid chromatography separation combined with detection by ultraviolet light (UV) or mass spectrometry[209]. This sequence, which is applicable to all biospecimens, has provided much impetus to understanding the biology of the NAD$^+$ metabolome. However, measurements have proven to be highly variable, depending on the operator, the protocol implemented, and the separation and detection technique applied[265].

Crucial to measuring NAD$^+$, NADH, NADP and NADPH is the reactivity of NAD(P)H towards oxygen, particularly in the presence of riboflavin-containing proteins, which can result in their rapid conversion to NAD(P)$^+$[161]. This reaction renders an accurate and absolute quantification of all four cofactors challenging. Freeze–thaw is particularly detrimental to the reduced forms due to chemical degradation and hydrolysis[209]. Additional consideration should be given to the time between sample harvest and processing, which should be kept consistent across experiments. Moreover, since NAD(P)(H) are chemicals sensitive to pH[266]4, attention should be given to the extraction conditions and how these affect NAD(P)(H) distribution and abundance. If using liquid chromatography coupled with mass spectrometry (n multidimensional) (LC-MS^n) as an analytical method to measure the NAD$^+$ metabolome, use of isotopically labeled NAD(P)(H) as internal standards adequately resolves this issue and is strongly recommended to improve the consistency and accuracy of measurement.

Measurements of NAD(P)(H) in whole cells using protein reporters are gaining momentum and used to better understand the role of NAD(P)(H) pools. However, improvements are needed to expand the dynamic ranges of measurements, signal-to-noise ratios, and the ability to provide quantitations in absolute terms for the individual

NAD(P)(H) pools. Notably, the cells of interest have to be genetically modified and therefore this method is not applicable to humans. Even in mice, the use of protein reporters is only feasible in the context of dedicated models, which take time to generate.

Finally, some NAD^+ catabolites detected by LC-MS^n or LC-UV are misidentified, ignored, or reported with conflicting nomenclature (e.g., 6-me-PY and 4-me-PY reported indiscriminately as PY, 2PY, or even ribosylated pyridones, although each one of these chemicals is also a NAD^+ catabolite[176]). NAD(P)(H) and its precursors are not only subject to enzymatic conversion, but also readily chemically modified, and these chemical modifications can be predicted in order to help generate chemical standards to better explore the NAD(P)(H) metabolome and its functions. Although the complete characterization of the NAD(P)(H) metabolome lacks consistency, especially when considering species-specific $NAD(P)^+$ catabolism, a more systematic approach to characterizing the NAD^+ metabolome is now emerging[207,209]

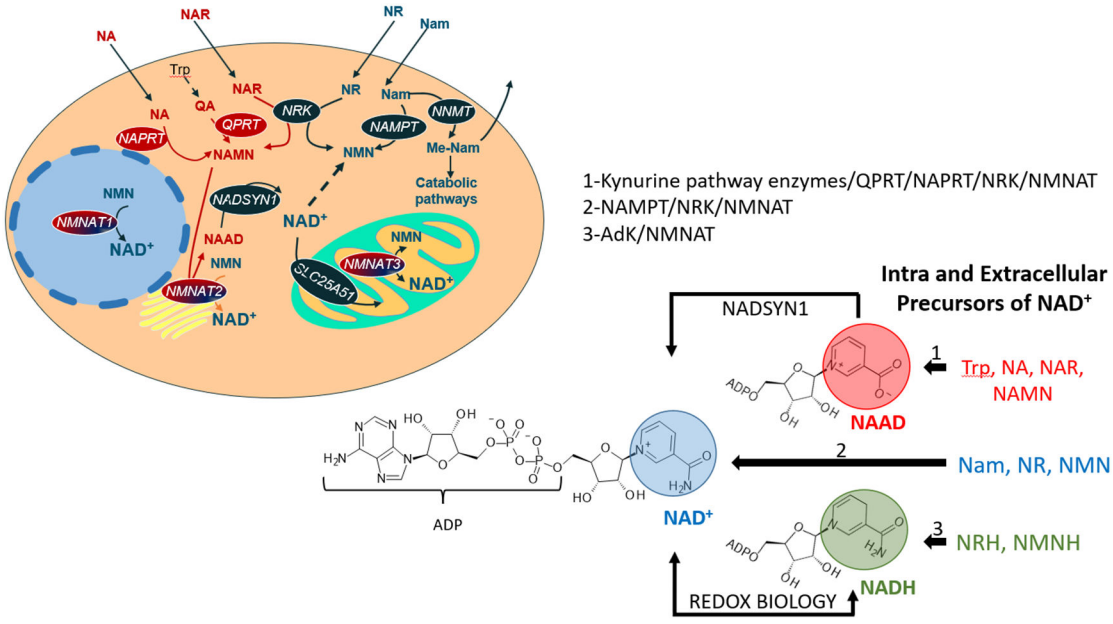

Figure 1 |. Structures and compartmentalization of pyridine adenine dinucleotides.

Oxidized nicotinamide adenine dinucleotide (NAD^+), its reduced form (NADH) and its acidic precursor nicotinic acid adenine dinucleotide phosphate (NAAD) are shown with their pyridine rings highlighted. NAD^+ and NADH are reversibly interconverted in a multitude of redox reactions. The colour coding of the different pyridine moieties is maintained in the subsequent figures (top). Key enzymes involved in the generation and maintenance of NAD^+ pools are shown within their respective cellular compartments (bottom). Dashed arrows indicate multiple steps. NA – nicotinic acid; Nam- nicotinamide; me-Nam – methylated nicotinamide; NR; nicotinamide riboside; NMN – nicotinamide mononucleotide; NAR – nicotinic acid riboside; NRH – reduced form of NR; NMNH – reduced form of NMN; NAMN – nicotinic acid mononucleotide; Trp – tryptophan; AdK – adenosine kinase; NMNAT - NMN adenylyltransferase; NAPRT – nicotinic acid phosphoribosyltransferase, QPRT - quinolinic acid phosphoribosyltransferase; NADSYN1 – NAD^+ synthase 1; NRK – NR kinase; NAMPT – nicotinamide phosphoribosyltransferase; NNMT – nicotinamide *N*-methyltransferase; SLC25A51 – solute carrier family 25 member 51.

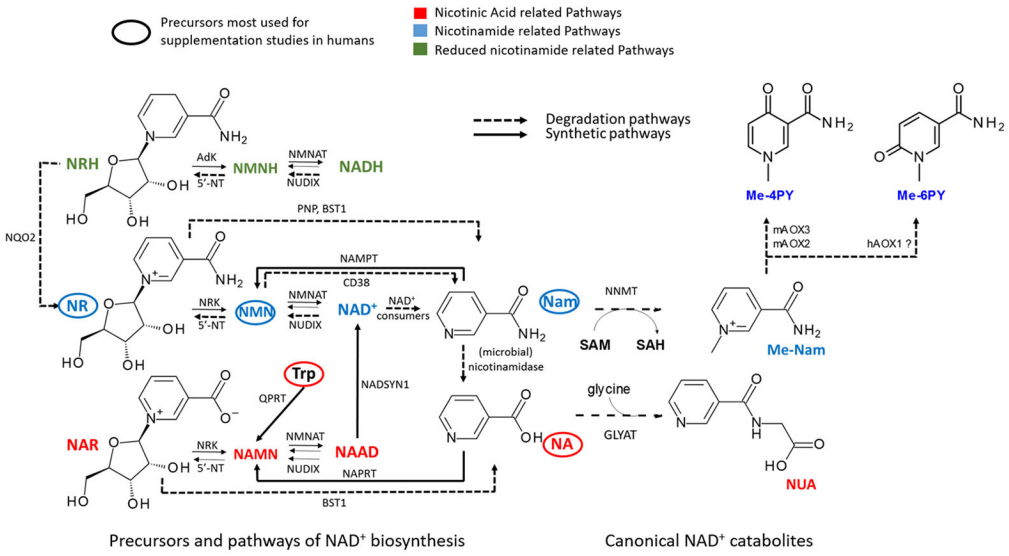

Figure 2 |. Biosynthesis and canonical degradation pathways of NAD$^+$.

Nicotinamide (Nam) and nicotinic acid (NA) are the classical vitamin B3 precursors; nicotinamide riboside (NR) and nicotinic acid riboside (NAR) are their respective derivatives; and nicotinamide mononucleotide (NMN) and nicotinic acid mononucleotide (NAMN) are their corresponding mononucleotides. Tryptophan (Trp) is degraded in the de novo pathway to quinolinic acid (not shown), which is converted to NAMN by quinolinic acid phosphoribosyltransferase (QPRT). The reactions that transform Nam and NA into their mononucleotides are catalyzed by nicotinamide phosphoribosyltransferase (NAMPT) and nicotinic acid phosphoribosyltransferase (NAPRT), respectively. NR and NAR are phosphorylated to NMN and NAMN, respectively, by NR kinases (NRKs), whereas the kinase responsible for transforming reduced NR (NRH) to reduced NMN (NMNH) is adenosine kinase (AdK). NRH can also be oxidized to NR by N-ribosyldihydronicotinamide quinone reductase 2 (NQO2). Formation of the dinucleotides in all pathways requires the activity of NMN adenylyltransferases (NMNATs). As indicated, several intermediates can be degraded to their original substrates or to other precursors by the indicated enzymes 5'-nucleotidase (5'-NT), the hydrolase NUDIX (also known as NUD22), purine nucleoside phosphorylase (PNP), CD38 or BST1. NAD$^+$ is degraded to Nam (and to ADP-ribose adducts) by "NAD$^+$ consumers", including ADP-ribosyltransferases, sirtuins or NAD$^+$ glycohydrolases/ADP-ribosylcyclases. In lower organisms, Nam is deamidated to NA by nicotinamidase, which is absent in mammals. NA can be converted to nicotinuric acid (NUA) by glycine-N-acyltransferase (GLYAT). In mammals, when not recycled into NAD$^+$, Nam is methylated by Nam-N-methyltransferase (NNMT) to form N-methylnicotinamide (me-Nam). NNMT uses the universal methyl donor S-adenosylmethionine (SAM), which is concomitantly converted to S-adenosylhomocysteine (SAH). Me-Nam is further converted to methyl pyridones (me-PY) with the oxo group added to the 4 (me-4PY) or 6 (me-6PY) position of the ring. Me-4PY generation is predominant in mice and catalyzed by aldehyde oxidase 2 (mAOX2) and mAOX3. Me-6PY is the major species produced in humans; although this reaction is often attributed to hAOX1, it is more likely non-enzymatic, as me-NAM is inherently susceptible to oxidation and mAOX1 cannot recognize me-NAM

as a substrate. Note that several enzymes, including CD38 and BST1, can catalyze base-exchange reactions that are not represented here.

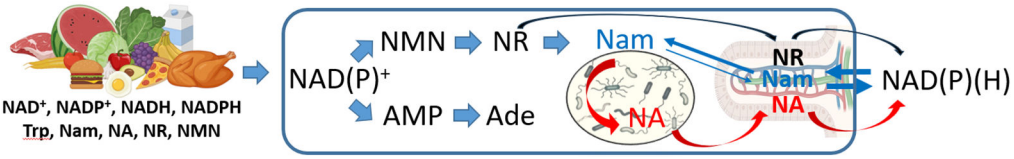

Figure 3 |. Fate of dietary precursors of NAD(P)(H).

Unprocessed foods are rich in nicotinamide (Nam)-containing dinucleotides that are degraded by host metabolism, beginning in the acid environment of the stomach (dashed rectangle). Although some nicotinamide can be absorbed directly, the net flux of this metabolite in the presence of a functional microbiome is from host circulation into the intestinal lumen. Microbial enzymes deamidate nicotinamide to nicotinic acid (NA), which can be used for microbial synthesis of oxidised nicotinamide adenine dinucleotide (NAD^+) or absorbed by the host. Within host tissues, incoming nicotinic acid, nicotinamide and nicotinamide riboside (NR) generate NAD^+, which is further converted to NADH or NADP(H), or consumed to generate nicotinamide that can then be salvaged to regenerate NAD^+ (not shown). NMN, nicotinamide mononucleotide; Trp, tryptophan.

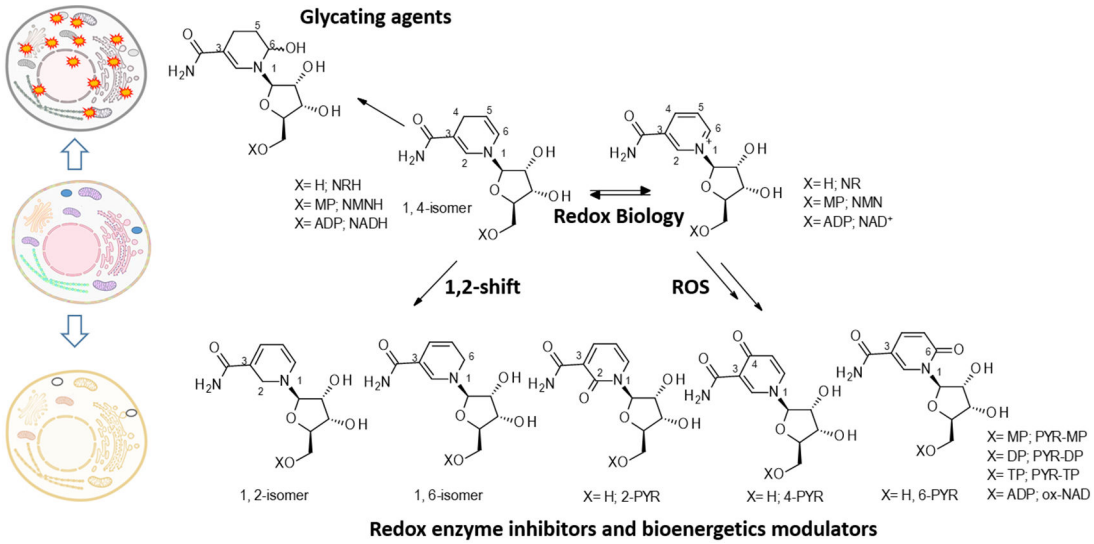

Figure 4 |. Non-canonical degradation of NAD^+ and NADH and of their ribosylated precursors. Reduced nicotinamide adenine dinucleotide (NADH) and its ribosylated precursors can undergo hydration to generate glycating species, or isomerization, thereby yielding non-redox-active derivatives that interfere with redox biochemistry. The pyridinyl riboside of NAD^+ and its ribosylated precursors also react with electron-rich reactive oxygen species (ROS) such as superoxide to generate stable ribosylated pyridones. The nucleotides, such as pyridone riboside monophosphate (PYR-MP), and dinucleotides such as oxidized NAD^+ (ox-NAD^+) are hydrolyzed to pyridone ribosides (2-PYR, 4-PYR and 6-PYR) that are released into the extracellular environment, where they circulate and are taken up by other cells. Once inside cells, PYR can be converted to their nucleotidic (monophosphate, MP, diphosphate, DP, or triphosphate, TP) and dinucleotidic (ADP-linked) forms, with unknown consequences for nucleic acid synthesis and NAD^+-dependent enzymes. Legends indicate the moiety represented by “X” in each of the listed molecules.

Table 1 |

Analytical methods to measure NAD^+ and $NAD(P)^+$ + NAD(P)H

Method		Sensitivity	Biospecimen types	Metabolites measured	Limitations (L) and advantages (A)	Ref.
Direct detection methods						
MRS	^{1}H MRS	High-μM–mM	Human tissues	NAD^+	L: expensive A: in situ measurements	107
	^{31}P MRS	High-μM–mM	Human tissues	NAD(P)(H)	L: expensive; overlapping measurements; lack of internal standard for quantification A: in situ measurements	189
Intrinsic Fluorescence	Two-photon spectrometry	nM	Whole cells	NADH	L: limited number of cells can be monitored, and only reduced forms are detectable A: spatial or redox redistribution can be monitored in real time	190
	FLIM	nM	Whole cells	NAD(P)H		191,192
Indirect detection methods						
Enzymatic assays		nM–mM	Cell, blood, and tissues extracts.	NAD(P)(H)[a]	L: requires specimen isolation and sample processing *,** A: easily implemented; numerous commercial kits are available	Reviewed in[193,194]
NAD(P)(H) genetically encoded fluorescent sensors		ratiometric	Whole cells, organelles	NAD(P)(H) specific	L: limited dynamic range A: in situ measurements; minimal interference from NAD precursors	195-198
FRET-based semisynthetic sensors		ratiometric	Whole cells, organelles	NAD(P)(H)	L: requires several components (relatively-large, overexpressed protein with two self-labelling tags and two tag-specific fluorophores) A: useable in a wide concentration range	199,48
Redox sensitive sensors		ratiometric	Whole cells, organelles.	NAD(P)(H), but not limited to it.	L: provides no information about absolute quantities A: immediate read-out of the redox status (and its changes)	200,201
^{1}H-NMR spectrometry-metabolomics		high μM and above	Extracts of Cells, blood and tissues	Nam, NR, NMN, NAD(P)(H)	L: requires specimen isolation and sample processing *,** with low sensitivity; requires high-field NMR spectrometer A: analyses of ATP and of other key energetics metabolites can be performed concurrently.	202-204
LC-UV		mid μM to high mM	Extracts of Cells, blood and tissues	All NAD metabolites possessing UV-absorbance properties	L: requires specimen isolation and sample processing *,** with relatively low sensitivity for which small sample sizes are not suitable; detection efficiency is dependent on the spectral properties of the metabolite being detected; quantification only of metabolites with known UV properties is possible *** LC with detection by MS, rather than by UV is preferable. A: low cost and easy to implement.	205,206
LC-MS^n metabolomics		low nM to high mM	Extracts of Cells, blood and tissues	All NAD metabolites	L: requires specimen isolation and sample processing *,** A: ability to measure the	207-210

Method	Sensitivity	Biospecimen types	Metabolites measured	Limitations (L) and advantages (A)	Ref.
				full NAD^+ metabolome and discover new NAD^+-related entities; ability to trace how NAD^+ precursors are used and distributed using stable isotopologs.	
Poly-ADP-ribose generation	Detection of relative changes	Organelles	NAD^+	L: no absolute quantification; suitable only for membrane-bounded organelles. A: sensitive, robust, easy to implement	[56]

*Low reproducibility due to variability in extraction and experimental protocols, detection of free vs. protein bound NAD(P)(H), and/or effects of extraction pH on NAD(P)(H) abundance. Results can be affected by sample handling time.

**Normalization to protein concentration, volume or tissues mass is required.

***LC-UV has been supplanted by LC-MS^n, which provides increased sensitivity and ability to detect NAD metabolites regardless of their individual spectral properties.

FLIM, fluorescence-lifetime imaging microscopy; FRET, Förster resonance energy transfer; LC, liquid chromatography; LC-UV, liquid chromatography coupled with ultraviolet spectrometry; LC-MS^n, liquid chromatography coupled with mass spectrometry (n multidimensional); MRS, magnetic resonance spectroscopy; NMR, nuclear magnetic resonance.

Table 2 |

Evidence of benefits of supplemental NAD^+ precursors in humans

Parameter/disease	Improved by	Unchanged by	Comments
Physiological parameters			
Body weight	NMN[211]	NA[17] Nam[212,213] NR[214-215] NMN[216-217] NMN[218])[a]	No clear trend in most studies
Adiposity	NA[17] NR[214] (NMN[219])	NR[220,215] NMN[216,221] NMN[211]* (NMN[218,222])	May be dose and/or duration dependent *Only abdominal fat is measured
Insulin sensitivity, glucose homeostasis	NMN[216]* Nam[212]	NMN[210,218,217] NR[214,220,223, 224]	* Attributed specifically to muscles
Blood lipids (cholesterol, triglycerides)	NR[225] NMN[211] Nam[212]*	NR[220] NMN[216,219] (NMN[222])	* Beneficial for improving cholesterol, but not triglyceride, levels
Inflammation	NR[226] NR[100,227]* (NR[101]*) (NR[228])	NR[214]	* Assessments performed in PBMCs, monocytes or T cells isolated after treatment
Mitochondrial function	NA[17] NR[100]* NR[215]**	Trp/NA/Nam[130] NR[214,226,229]	* Assessments performed in PBMCs isolated after treatment ** Biogenesis, but not function, is measured in adipose tissue
Physical function	NA[17] NAM[230] NMN[217] (NMN[210,221,220,230]) (NR[231]*)	Trp/NA/Nam[130] NR[223,226] NMN[232] NR[225])	* A single dose improved function in old individuals, but not in young individuals
Hypertension and/or vascular dysfunction	NR[224]* NMN[211] (NMN[233])	NR[214] NMN[219,232]	* Nominally significant (before multiple comparisons correction)
Muscle regeneration	none	NR/PT[234]	In individuals aged 55–80
Brown adipose thermogenesis	none	NR[235]	Positive results achieved in *ex vivo* treated adipocytes, but not *in vivo*
Sleep quality	(NMN[236])	none	PM administration more promising than AM
Cognitive function	none	Nam[237]* NR[238]	* In individuals with previous skin cancer
Diseases/injuries			
Acute kidney injury	Nam[239]	none	Injury associated with cardiac surgery
Chronic kidney disease	none	NR[225]	Primary disease measures unchanged, but some evidence obtained of metabolic improvement
Mitochondrial myopathy	NA[17]	none	Contributions from NAD^+-independent effects on GPR109A or lipids cannot be excluded
Heart failure	none	NR[100]	Functional parameters unchanged; PMBCs had reduced inflammatory potential
Alzheimer disease	none	Nam[240]	No safety concerns noted
Parkinson disease	(NR[241]) (NR[242]*)	none	* Clinical improvement detected, but confounded by shorter interval since levodopa treatment in patients receiving NR
ALS	NR/PT[243,244]	none	NR-independent effects of PT have not been ruled out
Ataxia telangiectasia	NR[245] NR[99] (NR[246])	none	Improvements not associated with any change in neurofilament light chain (NfL)

Parameter/disease	Improved by	Unchanged by	Comments
Skin cancer, UV protection	Nam[122,247-248]	Nam[249]*	* 12-month follow up in transplant recipients
Osteoarthritis/joint immobility	Nam[230,250]	none	Multiple prior studies summarized in ref.[230]

[a] Parentheses indicate studies of duration < 4 weeks and n < 10, that have mixed outcomes on related measures, or that highlight nonsignificant trends.

This table is not a complete listing of NAD^+-related studies and specifically excludes extensive literature on using NA to lower circulating lipids (through a mechanism not shared by nicotinamide and therefore likely NAD^+-independent), numerous promising but inconsistently controlled studies on intact NAD^+ and NADH supplementation[121], and early studies on Nam supplementation that often are based on case reports (e.g., [230]). The table also excludes topical formulations, which have shown benefits in skin[251]. With the exception of a phase III trial for skin cancer[122], all studies in this table are early stage investigational studies and in some cases may have failed to detect effects due to limited statistical power. We are not aware of human clinical studies that demonstrated detrimental outcomes for any of the listed parameters. NA, nicotinic acid; Nam, nicotinamide; NMN, nicotinamide mononucleotide; NR, nicotinamide riboside; NR/PT, nicotinamide riboside with pterostilbene.