Received: 6 July 2021 | Revised: 13 July 2021 | Accepted: 18 July 2021

DOI: 10.1002/agm2.12170

REVIEW ARTICLE

Aging Medicine Open Access WILEY

Precursor comparisons for the upregulation of nicotinamide adenine dinucleotide. Novel approaches for better aging

Raymond D. Palmer[1] | Magdy Mahmoud Elnashar[2,3] | Mauro Vaccarezza[2]

[1]Longevity Experts, Helium-3 Biotech, Perth, WA, Australia

[2]School of Medicine, Faculty of Health Sciences, Curtin University, Perth, WA, Australia

[3]Center of Excellence, Department of Polymers, National Research Centre, Cairo, Egypt

Correspondence
Raymond D. Palmer, Longevity Experts, Helium-3 Biotech, South Perth, WA 6151, Australia.
Email: ray.palmer@longevityexperts.com

Abstract

Nicotinamide adenine dinucleotide (NAD) is a coenzyme found in every human cell and regulates a number of systems across multiple cellular compartments and tissue types via an endogenous and exogenous influence. NAD levels are demonstrated to decline with age and therefore measures to counteract the waning of NAD have been devised. A number of NAD precursor candidates such as nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), the reduced form of nicotinamide mononucleotide (NMNH), nicotinic acid (NA) nicotinamide (NAM), and dihydronicotinamide riboside (DNR) increase NAD levels in vitro and in vivo. This discussion will focus on the precursors NR, NMN, NMNH, and DNR in the upregulation of NAD. There are many publications on NAD precursors as it has become popular for human consumption in recent years due to its vital importance to the general consumer. However, there is no consensus between researchers and this was the aim of this review, to determine and discuss their areas of agreement versus disagreement, to highlight the gaps in research, and to give recommendations for future work. Bioavailability and potency of NR, NMNH, NMN, and DNR is also examined on the light of the most recent literature.

KEYWORDS

aging, nicotinamide, nicotinamide adenine dinucleotide, nicotinamide mononucleotide, nicotinamide riboside, nicotinic acid, reduced nicotinamide mononucleotide

1 | INTRODUCTION

This paper aims to perform a scoping review of existing published literature and determine inconsistencies versus consistencies, if any. The methodology here was to examine multiple published papers regarding nicotinamide adenine dinucleotide (NAD) synthesis, and from there data was extrapolated to evidence the ability of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), nicotinamide (NAM), nicotinamide mononucleotide (NMNH), and dihydronicotinamide riboside (DNR) as NAD precursors. This scoping

Abbreviations: ADP, Adenosine diphosphate; AMS, Accelerator Mass Spectrometry; CD73, Cluster of Differentiation 73; DNA, Deoxynucleicribose Acid; DNR, Dihydronicotinamide Riboside; eNAMPT, Extracellular nicotinamide phosphoribosyltransferase; ENT, Equilibrative Nucleoside Transporter; ETC, Electron Transport Train; HFD, High Fat Diet; Mg, Milligram; MNA, 1-Methylnicotinamide; NA, Nicotinic Acid; NAAD, Nicotinic Acid Adenine Dinucleotide; NAD, Nicotinamide Adenine Dinucleotide; NAM, Nicotinamide; NaMN, Nicotinic Acid Mononucleotide; NAMPT, Nicotinamide Phosphoribosyltransferase; NAPRT, NA phosphoribosyltransferase; NaR, Nicotinic Acid Riboside; NK-kb, Nuclear factor Kappa-Light-Chain-Enhancer of Activated B Cells; NMN, Nicotinamide Mononucleotide; NMNAT1, Nicotinamide mononucleotide adenylyltransferase 1; NMNH, Nicotinamide Mononucleotide H (reduced); NR, Nicotinamide Riboside; NRK, Nicotinamide Riboside Kinase; ROS, Reactive Oxygen Species; SIR2, Silent Information Regulator2; SIRT1, Sirtuin 1.

review has placed an emphasis on more recent research but also takes into account earlier research where an applicable dataset was found.

NAD^+ or NADH is the reduced form of NAD and is a coenzyme that is involved in many biochemical reactions and regulates a number of essential systems.[1,2] NAD^+ levels are demonstrated to decline with age,[3] and it is this facet that has opened inquiry to precursors that may attenuate NAD decline. Many research papers have been published on NAD due to its vital importance; however, there was no agreement reached between researchers.

In 1906 NAD was discovered by British biochemists Arthur Harden and William John Young, however Harden and Young did not manage to identify what they were seeing.[4] Then in 1936 Otto Heinrich Warburg demonstrated the nucleotide function of this coferment and identified the nicotinamide portion of it. Warburg isolated the nicotinamide adenine dinucleotide phosphate (NADP) and discovered its pivotal role for hydrogen transfer in biochemical reactions.[5] It is noted that Warburg was born in 1883 and died in 1970, making the discovery of such biochemistry in the era of 1936 impressive.[6] NAD and NADP act as redox molecules within the cell. However, NADP is more limited to biosynthetic pathways and redox protective roles.[7] Further discoveries were made by other research teams such as when Lee et al. (1989)[8] discovered a novel NAD^+ metabolite in 1989, cyclic ADP-ribose. Cyclic ADP-ribose is one of the more important moieties because it is paramount in deoxynucleic ribose acid (DNA) repair, gene regulation, and cell signalling.[9,10] Exhaustive studies were conducted and the evidence pointed toward ADP-ribose being derived directly from NAD^+.[11] Furthermore, Imai et al. (2000)[12] made the timely observation that yeast SIR2 (silent information regulator 2) and the mouse ortholog SIRT1 have NAD^+-dependent protein deacetylase activity, increasing new attention in NAD^+ biology. Indeed, human nicotinamide/nicotinic acid mononucleotide adenylyltransferase (NMNAT), an important NAD^+ biosynthetic enzyme whose activity was originally reported in 1952, was finally isolated and fully characterized in 2001.[13] Following these discoveries, nicotinamide phosphoribosyltransferase (NAMPT), the rate-limiting enzyme that triggers NAD^+ biosynthesis from nicotinamide in mammals, was also isolated and characterized.[14] In addition, nicotinamide riboside, another key NAD^+ intermediate, was shown to be incorporated into NAD^+ via nicotinamide riboside kinases (NRKs) by Bieganowski and Brenner in 2004.[15] Almost half a century after the first relevant discoveries on NAD^+ biology, humanity is now entering another exciting time of this newly rejuvenated field of biomedical science. Of note, NAD has a nonredundant role in energy metabolism in eukaryotic cells; it accepts hydride moieties to produce the reduced NADH, which provides electrons to the mitochondrial electron transport chain (ETC) to feed oxidative phosphorylation; NADH is the primary electron donor to the electron transport chain for ATP synthesis/cellular energy.[16] The roles of NAD have greatly expanded its role as a coenzyme in recent years, as NAD and its metabolites also act as degradation substrates for a wide range of enzymes and proteins such as sirtuins.[17-19] Through these activities, NAD^+ links cellular metabolism to changes in signaling and transcriptional events, highlighting its importance in human cell biology and pathophysiology.

1.1 | Nicotinamide riboside (NR) & Nicotinamide Mononucleotide (NMN) & Dihydronicotinamide Riboside (DNR) & Reduced Nicotinamide Mono-Nucleotide (NMNH)

Various experimental studies found that NMN improved cardiac function whereas NR improved mitochondrial function in muscle, liver, and brown adipose tissue.[1] Reasons for the difference in the effect between NMM and NR are not given by the authors of these studies, though it could be suspected that differences in model organisms and contrasting sample processing techniques cause the conflicting results. NR is shown to be bioavailable in mice and humans.[20,21] These studies focused on time- and dose-dependent effects on blood NAD^+ in humans. Their purpose was to demonstrate that high levels of NAD could be achieved in human subjects with relatively small doses of NR such as 100, 300, and 1000 mg daily. A different study[22] was not solely focused on NAD levels after NR administration but also focused on the biological effects of the NR supplementation as well. This study found that NR could actually decrease exercise performance in rats. Experiments gathered 18 Wistar rats, divided them into two equal groups, and gave one group a saline vehicle whilst the other was given NR at a dose of 300 mg/kg of body weight each day. After 21 days of administration, an exercise test in the form of swimming was given. Interestingly the NR group showed a significant loss in physical performance of 35%. Further research in humans is certainly required to resolve contradictory information and to establish a consistent model. This latter study in rats indicates that it is likely that NR decreases fatty acid oxidation under exercising conditions, which in turn leads to earlier fatigue. It was also indicated that NR may also disrupt redox homeostasis, which leads cells to a more reductive state.

Interestingly, Aihart and colleagues[23] demonstrated that NR levels vary considerably in the blood from person to person. Eight healthy subjects were given 250 mg of NR orally on days one and two and then again on days seven and eight. On day nine, clinical blood chemistry was measured to determine the NAD levels in each subject. Notable baseline increases in NAD at $P = .01$ and NR a $P = .03$ to day 9 were found to be $R2 = 0.72$, $P = .008$, clearly demonstrating an interrelation between NR and NAD.

Another study[24] found that NMN ameliorates loss of endothelial NAD and SIRT1 activity, augments exercise, and increases endurance in old mice. This contrasting evidence to the above-mentioned NR findings may demonstrate that NAD precursors are not equal in their beneficial pathways and the effects on their hosts. Determinations could be inferred that the metabolic process taken may have just as many effects on human physiology as the NAD levels that are pursued. Other recent research indicated that NR and NMN that are orally administered are converted to NAM by the liver.[25] This dataset indicates that that NR and NMN

may not raise NAD directly but that instead they are reduced to NAM, which then becomes the primary precursor for the formation of NAD. NR must be phosphorylated with NR kinases (NRKs) to become NMN before it can become NAD^+ once inside the cell, according to Bieganowski and Brenner's paper.[14] According to the study by Giroud-Gerbetant et al. (2019),[26] NR is not stable whilst in circulation and is rate limited by the expression of NRK's. To modify this lack of stability, Giroud-Gerbetant et al. (2019) has looked at a reduced form of NR, known as DNR. This reduced form of NR, which carries an extra hydrogen atom, is shown to be more powerful and a faster precursor in NAD^+ synthesis than NR alone. Even though the difference between NR and DNR is extremely small, Giroud-Gerbetant et al. (2019) show that it uses a new pathway for NAD^+ synthesis, which the study refers to as the NRK-1-independant pathway. This early data from Giroud-Gerbetant et al. (2019) are promising, but they are only derived from murine models; further research to determine DNR's capability in human subjects is now warranted. A reduced version of NMN is also discussed here as NMNH. Similar to DNR, NMNH also showed much more potency over its oxidized form, by increasing NAD levels faster and by a twofold rise than NMN according to Perez et al.[27] The same study showed that NMNH reduced damaged and enhanced healing in renal tubular epithelial cells upon hypoxia/reoxygenation injury. Perez et al. also found that NMNH caused a swift escalation in NAD^+ levels in kidney, liver, muscle, brain, brown adipose tissue, and heart. An additional study by Ratajczak et al. (2016)[28] shows that NMN is not taken directly into the cell, but first metabolized extracellularly into NR for cellular intake, and then converted into NAD once inside the cell. In fact NMN is dephosphorylated into NR by an extracellular receptor (CD73) by using pyrophosphatase and 5"-ectonucleotidase activity.[29] The same study[29] points out that mammalian cells have equilibrative nucleoside transporters (ENTs), which assists the entry of the recently created NR from NMN into the cellular matrix. The NR then functions as an exogenous NAD precursor that converts back into NMN via the NRK pathway, which is consistent with Bieganowski and Brenner's findings.[15] However, DNR and NMNH are both believed to enter the cell via equilibrative nucleoside transporters by Giroud-Gerbetant et al.[26] and Perez et al.[27] respectively. Whereas the DNR molecule can enter the cell and perform NAD synthesis intracellularly where it is converted to NMNH, NADH, and then NAD, the precursor NMNH must first be dephosphorylated extracellularly into DNR before it can enter the cell, and then takes the same intracellular NAD pathway as DNR.[26,27]

It has also been demonstrated that NAD levels are not raised through direct absorption of NR or NMN, but that the gut microbiome are converting NR and NMN into a different molecule known as nicotinic acid mononucleotide (NaMN) via deamidation and integration by way of the de novo pathway.[30] The breakdown of NMN into the NAD metabolome caused an increase in metabolites such as nicotinic acid riboside (NaR) and NR. This evidence suggests that NR and NMN supplementation does not directly raise NAD, but indirectly raises NAD by increasing the level of metabolites that are used in NAD synthesis.

High fat diet (HFD) was found to strongly increase fat metabolism and to improve mitochondrial function in brown fat, which mediates adaptive thermogenesis.[31] In this regard, Crisol et al. (2018)[32] reported that minimal doses of NR in male C57BL/67 mice at 400 mg/kg each day over 5 weeks demonstrated a direct correlation between NR supplementation and an increase in thermogenesis. Metabolic processes are known to produce heat and to increase body temperature in humans. The latter may lead to unwanted side effects and it may also support weight management, which was supported by Crisol's study as they found that brown adipose tissue was reduced during their study.

Moreover, NR was tested to demonstrate baseline changes across a selection of biologics (potassium, creatine kinase, glucose, uric acid, and alanine aminotransferase).[23] Even though no significant changes were found in creatine kinase, glucose, uric acid, and alanine aminotransferase (minor fluctuations of <10% were noted), serum potassium levels were lowered by the mean of −0.4 mEq/L.[23]

The data demonstrated that NR supplementation caused a statistically significant reduction in haematocrit of −2%, a reduction in hemoglobin of −0.4%, and a reduction in platelet count of −20 000 μL ($P = .005$, $P = .04$ and $P = .03$, respectively), a minor yet observable lean toward an anaemic phenotype. The loss in hemoglobin may also explain why Kourtzidis and colleagues[22] found lower physical performance in Wistar rats given the reduced oxygen carrier's presence. Other biomarkers (blood pressure, body temperature, body weight, white blood cell count, lactate dehydrogenase, or aspartate aminotransferase) were not affected in any significant way. Remarkably, Airhart et al. (2017)[23] did not find any profound differences in serum levels of sodium, chloride, urea nitrogen, creatinine, or in the differential of white blood cells. In the same human subjects, levels of NMN concentration in blood did not show any measurable difference from baseline samples collected on day nine. This is consistent with findings from Trammell et al. (2016) that have also demonstrated that after single doses of NR at 100, 300, and 1000 mg NMN exhibited low levels in peripheral blood mononuclear cells.[19] However, Trammell et al. (2016) did show that NR had superior pharmacokinetics when compared to NA and NAM. A final comment regarding NMN and NR worth noting is that the NAD salvage pathway was demonstrated to have a connection in mitochondrial clearance in wild-type hematopoietic stem cells through the initiation of a mitochondrial stress response with a higher frequency of asymmetric divisions.[33] This work demonstrated that NR and NMN were protagonists of hematopoiesis and that the protective effects of NR on mitochondrial dynamics in nonhematopoietic tissues would provide supplementary protection for ablative hematopoietic stem cell chemotherapy due to the impairment of mitochondrial function.

1.2 | Comparing the data

Differing results exist across the literature, and this has been the progenitor for many disagreements among researchers. Due to the difference in analytical methods and sample processing, different results can be garnered for the same analyte, even though the input model may have appeared similar. A novel technique that has emerged in conducting analytical research is using "mixed methods," which has gained traction in health research,[35] but the authors of this paper note that no similar mixed research methods appeared to be utilized throughout the NAD literature. This poses an interesting path forward for researchers in determining the best approach for NAD research with combined sample processing using multiple methods (mixed methods).

1.3 | NAD levels and clinical practice

There are clear differences in the use of NR, NMN, NA, and NAM to raise NAD levels, and as pointed out in the study by Williams et al. (2007),[36] low NAD levels can lead to a myriad of biological decay and disease, notably pellagra, known as the four D's; pellagra induction from low NAD levels results in dementia (delusions), dermatitis, diarrhea, and death,[54] which emphasizes the need for NAD biogenesis across all cellular compartments. Other pathologies also exist with pellagra, but this condition presents strong evidence for NAD to remain at healthy levels throughout the aging process. Pellagra captures multiple types of biological dysfunction from neurological to dermatological and demonstrates the wide-range importance of NAD levels across complex biological systems and that supplementing with precursors for NAD maintenance during age-related decline may be paramount for optimal health.[34] Other pathologies such as hypertension, heart failure, alcoholism, diabetes, and some early porphyrias may also be attributed to waning NAD levels from the aging process. In this regard, it has been recently shown in mouse models that vascular aging can also be prevented with supplementation of NMN.[24] The same study also showed that NMN supplementation can also improve blood flow and assist with angiogenesis and capillary density that in turn assist with the creation of new tissue including muscle,[24] which becomes a major reversal of age-related immobility for the aged population.

Recently it has been presented by Grozio et al. (2019)[37] that there is an amino acid and polyamine transporter known as Slc12a8 (from the solute carrier family of genes) that is an NMN transporter. Slc12a8 is found to be extremely upregulated in the small intestines of mice, which may demonstrate an organism's requirement to pull as much NMN from food as possible. It was also shown that the upregulation of Slc12a8 continued to increase into old age, creating the argument that Slc12a8 may compensate as NAD levels drop.[38,39] If Slc12a8 is upregulated to compensate for diminishing NAD levels during old age, then a conclusion could be drawn that consistent NAD levels are an essential tool to maintain youthful function, organism fitness, and furthermore, that the Slc12a8 mechanism could be regarded as a longevity mechanism to ward off age-related decline. Notably, Grozio et al. (2019)[37] appeared to show that Slc12a8 encodes for a precise NMN transporter but did not appear to assist in the transportation of NR.

However, opposing views exist to Grozio et al.'s determination for Slc12a8 being an NMN transporter. Schmidt and Brenner[40] argue that there is a lack of evidence to suggest that Slc12a8 is an NMN precursor based on the absence of background levels of NMN and NAD that were the subject of Grozio's et al.'s work. Ultimately, Schmidt and Brenner[40] point out that levels of NAD are 500 times higher than NMN in normal liver samples, and Grozio et al. (2019) had failed to examine background/control levels, which undermines any result Grozio et al. (2019) delivered. The debate remains open and no robust conclusion in this regard can be made.

Nevertheless, if confirmed this evidence adds weight to the current literature that NAD levels and novel ways to raise them are of paramount importance to fend off disease phenotype. Numerous scientific dissertations now argue that the simple way to fend off the most debilitating diseases is to simply keep an organism young. Many of the contemporary diseases seen in today's Western countries can be considered not as individual diseases but as side effects to the aging process itself. This presents opportunities for the biomedical community and biotech companies to devise novel techniques for the delivery of substances such as NMN and NR to ensure that diminishing NAD levels are kept in check. If in fact further studies show that Slc12a8 is an NMN transporter, then new similar techniques may be devised to ensure that NAD precursor molecules can be delivered to specific cellular compartments for more effective delivery. However, the argument for Slc1a8 as an NMN transporter is far from over. The story of NAD continues into many other facets of human biology and disease prevention. NAD assists with the defense mechanism known as the mitochondrial unfolded protein response that prevents unfolded proteins from proliferating and going on to cause disease such as cancer, Alzheimer's, or Parkinson's.[59-61] The sirtuins can also be activated with strong NAD levels that again affirm that NAD could be regarded as a longevity coenzyme. It becomes clear that as NAD levels taper off into adulthood, the mitochondria are unable to produce the energy for the cells they once did; this in turn leads to improper cell function that then leads to biological decay such as cardiovascular disease, cancer, neurological disease, lung disease, or other. Could this cascade of biological decay be fought off with maintaining NAD levels into old age? Consumers worldwide appear to be holding onto that thought as the market for NAD precursors grows.

1.4 | Upcoming precursor contenders

The field of NAD synthesis remains fluid with new studies and precursors and new entities entering the fray. Extracellular nicotinamide phosphoribosyltransferase (eNAMPT) is an enzyme that is showing promise in mouse studies. Scientists found that by

increasing levels of eNAMPT into the circulatory system of aged mice NAD^+ levels increased across multiple tissues.[41] The same study by Yoshida et al.[41] (2019) also demonstrated a unique delivery method whereby extracellular vesicles such as exosomes or ectosomes were the carriers of the eNAMPT, which enabled delivery directly across the wall of other cells and to the target. The same team showed that the reduction in age-associated phenotype in the mice was approximately one year, which potentially translates to a 50-year-old human having the overall physical health and ability of a 20-year-old (if such a comparison can be made at this early stage). DNR also shows a very promising profile as a future NAD enhancer as well as NMNH.[42,43] Yang et al. (2019) have also shown that DNR increased NAD^+ concentrations significantly from 2.5–10 times over the established control values. Yang's mouse study goes on to claim that DNR exceeded the levels of NAD synthesis more than NR or NMN, with no adverse reactions noted. Even though DNR has a promising outlook, further research and human trials should be conducted to establish a firm conclusion. Notably, DNR is believed to enter the cell via different transporters than NR and was found to deliver higher levels of NR into the blood plasma as opposed to NR alone.

Many other questions remain, such as which organs take up these precursors to induce NAD synthesis; can orally ingested precursors inhibit or prohibit other biological functions? Double-blind placebo trials need to be conducted in order to determine the various questions that remain about the emerging trend of NAD precursors and how to substantially manipulate their levels to influence and possibly improve human health.[44,45]

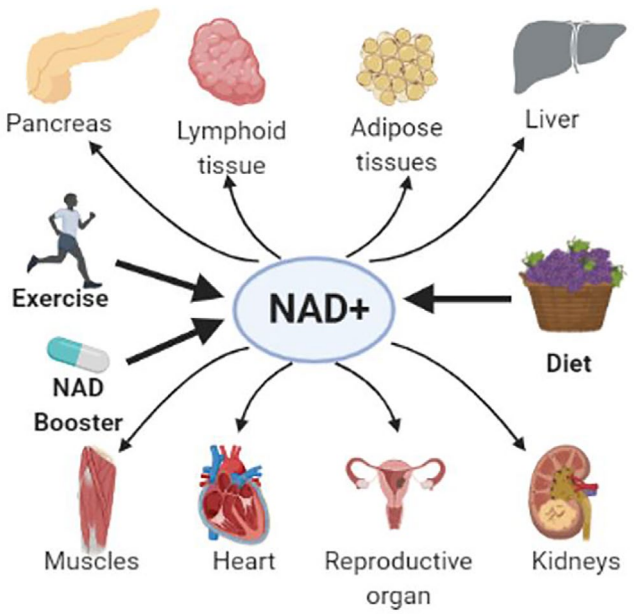

FIGURE 1 Effects of NAD-boosting molecules on human physiology. NAD^+ boosters have a substantial effect on the well-being and survival of mammals [Colour figure can be viewed at wileyonlinelibrary.com]

2 | CONCLUSIONS

The literature indicates that stable NAD levels are important for healthy aging, and NAD precursors could be considered compounds that are generally safe for human consumption; the fact that NAD precursors are easily available to the public at large and have a safe track record demonstrates that these precursors may be a very cost-effective solution in disease prevention regimes.

This paper concludes on the available data shown here that reduced forms of precursors such as DNR and NMNH may hold the most benefit for increasing NAD levels through better absorption, stability, and resistance to degradation. Despite the robust health benefits that are predicted from their experimental dosage in animal models, these NAD precursors still require further testing to determine what other effects they may deliver. Mounting data on the beneficial effects of raising and maintaining NAD levels to ward off biological decay leads to the assumption that ways to boost cellular NAD almost certainly have been shown to hold preventive and therapeutic potential in a plethora of human diseases. With documentation of NAD supplementation in humans starting to appear, the science of aging is now moving into a new era of NAD therapeutics (see Figure 1 for a synopsis on the benefit of boosting NAD levels).

Further side-by-side research of NAD precursor compounds should be conducted as a preventative measure for disease and early mortality by considering the gaps in research and the agreement/disagreement between researchers highlighted in this paper. NAD synthesis remains one of the most promising fields of research, which may be able to prevent a large percentage of the world's age-related disease.

ACKNOWLEDGEMENTS

Not Applicable

CONFLICTS OF INTEREST

RDP is Chief Scientific Officer of Helium-3 Biotech. MME and MV have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

RDP, idealization, assembly and review of the literature and writing; MME: writing, revision, and figure design; MV: draft revision, writing, and supervision.

DATA AVAILABILITY STATEMENT

Not Applicable.

ORCID

Raymond D. Palmer https://orcid.org/0000-0003-1898-0935

REFERENCES

1. Rajman L, Chwalek K, Sinclair DA. Therapeutic potential of NAD-boosting molecules: the in vivo evidence. *Cell Metab*. 2018;27(3):529-547. https://doi.org/10.1016/j.cmet.2018.02.011

2. Verdin E. NAD+ in aging, metabolism, and neurodegeneration. *Science*. 2015;350(6265):1208-1213. https://doi.org/10.1126/science.aac4854
3. Schultz MB, Sinclair DA. Why NAD+ declines during aging: it's destroyed. *Cell Metab*. 2016;23(6):965-966. https://doi.org/10.1016/j.cmet.2016.05.022
4. Harden A, John Young W. *The Alcoholic Ferment of Yeast-Juice. Part II–The Coferment of Yeast-Juice*. London: The Royal Society; 2014. https://doi.org/10.4159/harvard.9780674366701.c133
5. Otto AM. Warburg effect(s)—a biographical sketch of Otto Warburg and his impacts on tumor metabolism. *Cancer Metab*. 2016;4(1):5. https://doi.org/10.1186/s40170-016-0145-9
6. Krebs HA. Otto Heinrich Warburg, 1883–1970. *Biogr Mem Fellows R Soc*. 1972;18:629-699. https://doi.org/10.1098/rsbm.1972.0023
7. Ying W. NAD+/NADH and NADP+/NADPH in cellular functions and cell death: Regulation and biological consequences. *Antioxidants Redox Signal*. 2008;10(2):179-206. https://doi.org/10.1089/ars.2007.1672
8. Lee HC, Walseth TF, Bratt GT, Hayes RN, Clapper DL. Structural determination of a cyclic metabolite of NAD+ with intracellular Ca2+-mobilizing activity. *J Biol Chem*. 1989;264(3):1608-1615. https://doi.org/10.1016/s0021-9258(18)94230-4
9. Berger F, Ramírez-Hernández MH, Ziegler M. The new life of a centenarian: signalling functions of NAD(P). *Trends Biochem Sci*. 2004;29(3):111-118. https://doi.org/10.1016/j.tibs.2004.01.007
10. Corda D, Di Girolamo M. Functional aspects of protein mono-ADP-ribosylation. *EMBO J*. 2003;22(9):1953-1958. https://doi.org/10.1093/emboj/cdg209
11. Rodríguez-Vargas JM, Oliver-Pozo FJ, Dantzer F. PARP1 and poly(ADP-ribosyl)ation signaling during autophagy in response to nutrient deprivation. *Oxid Med Cell Longev*. 2019;2019:1-15. https://doi.org/10.1155/2019/2641712
12. Imai SI, Armstrong CM, Kaeberlein M, Guarente L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature*. 2000;403(6771):795-800. https://doi.org/10.1038/35001622
13. Schweiger M, Hennig K, Lerner F, et al. Characterization of recombinant human nicotinamide mononucleotide adenylyl transferase (NMNAT), a nuclear enzyme essential for NAD synthesis. *FEBS Lett*. 2001;492(1–2):95-100. https://doi.org/10.1016/S0014-5793(01)02180-9
14. Rongvaux A, She RJ, Mulks MH, et al. Pre-B-cell colony-enhancing factor, whose expression is up-regulated in activated lymphocytes, is a nicotinamide phosphoribosyltransferase, a cytosolic enzyme involved in NAF biosynthesis. *Eur J Immunol*. 2002;32(11):3225-3234. https://doi.org/10.1002/1521-4141(200211)32:11<3225:AID-IMMU3225>3.0.CO;2-L
15. 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*. 2004;117(4):495-502. https://doi.org/10.1016/S0092-8674(04)00416-7
16. Ahmad M & Biochemistry KCI Electron transport chain. StatPearls Publishing. 2018. http://www.ncbi.nlm.nih.gov/pubmed/30252361. Accessed March 9, 2021.
17. Blander G, Guarente L. The Sir2 family of protein deacetylases. *Annu Rev Biochem*. 2004;73:417-435. https://doi.org/10.1146/annurev.biochem.73.011303.073651
18. Haigis MC, Sinclair DA. Mammalian sirtuins: biological insights and disease relevance. *Annu Rev Pathol Mech Dis*. 2010;5:253-295. https://doi.org/10.1146/annurev.pathol.4.110807.092250
19. Kane AE, Sinclair DA. Sirtuins and NAD+ in the development and treatment of metabolic and cardiovascular diseases. *Circ Res*. 2018;123(7):868-885. https://doi.org/10.1161/CIRCRESAHA.118.312498
20. Dellinger RW, Santos SR, Morris M, et al. Repeat dose NRPT (nicotinamide riboside and pterostilbene) increases NAD+ levels in humans safely and sustainably: a randomized, double-blind, placebo-controlled study. *npj Aging Mech Dis*. 2017;3(1):17. https://doi.org/10.1038/s41514-017-0016-9
21. Trammell SAJ, Schmidt MS, Weidemann BJ, et al. Nicotinamide riboside is uniquely and orally bioavailable in mice and humans. *Nat Commun*. 2016;7:12948. https://doi.org/10.1038/ncomms12948
22. Kourtzidis IA, Stoupas AT, Gioris IS, et al. The NAD+ precursor nicotinamide riboside decreases exercise performance in rats. *J Int Soc Sports Nutr*. 2016;13(1):32. https://doi.org/10.1186/s12970-016-0143-x
23. Airhart SE, Shireman LM, Risler LJ, 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*. 2017;12(12):e0186459. https://doi.org/10.1371/journal.pone.0186459
24. Das A, Huang GX, Bonkowski MS, et al. Impairment of an endothelial NAD+-H2S signaling network is a reversible cause of vascular aging. *Cell*. 2018;173(1):74-89.e20. https://doi.org/10.1016/j.cell.2018.02.008
25. Liu L, Su X, Quinn WJ, et al. Quantitative analysis of NAD synthesis-breakdown fluxes in brief. *Cell Metab*. 2018;27:1067-1080.e5. https://doi.org/10.1016/j.cmet.2018.03.018
26. Giroud-Gerbetant J, Joffraud M, Giner MP, et al. A reduced form of nicotinamide riboside defines a new path for NAD+ biosynthesis and acts as an orally bioavailable NAD+ precursor. *Mol Metab*. 2019;30:192-202. https://doi.org/10.1016/j.molmet.2019.09.013
27. Zapata-Pérez R, Tammaro A, Schomakers BV, et al. Reduced nicotinamide mononucleotide is a new and potent nad+ precursor in mammalian cells and mice. *FASEB J*. 2021;35(4):1-17. https://doi.org/10.1096/fj.202001826R
28. Ratajczak J, Joffraud M, Trammell SAJ, et al. NRK1 controls nicotinamide mononucleotide and nicotinamide riboside metabolism in mammalian cells. *Nat Commun*. 2016;7:13103. https://doi.org/10.1038/ncomms13103
29. Poddar SK, Sifat AE, Haque S, Nahid NA, Chowdhury S, Mehedi I. Nicotinamide mononucleotide: exploration of diverse therapeutic applications of a potential molecule. *Biomolecules*. 2019;9(1):34. https://doi.org/10.3390/biom9010034
30. Kim L-J, Chalmers TJ, Smith GC, et al. Nicotinamide mononucleotide (NMN) deamidation and indirect regulation of the NAD Metabolome. 2020. https://doi.org/10.21203/RS.3.RS-87047/V1
31. Wall CE, Whyte J, Suh JM, et al. High-fat diet and FGF21 cooperatively promote aerobic thermogenesis in mtDNA mutator mice. *Proc Natl Acad Sci U S A*. 2015;112(28):8714-8719. https://doi.org/10.1073/pnas.1509930112
32. Crisol BM, Veiga CB, Lenhare L, et al. Nicotinamide riboside induces a thermogenic response in lean mice. *Life Sci*. 2018;211:1-7. https://doi.org/10.1016/j.lfs.2018.09.015
33. Vannini N, Campos V, Girotra M, et al. The NAD-booster nicotinamide riboside potently stimulates hematopoiesis through increased mitochondrial clearance. *Cell Stem Cell*. 2019;24(3):405-418.e7. https://doi.org/10.1016/j.stem.2019.02.012
34. Pieper JA. Overview of niacin formulations: differences in pharmacokinetics, efficacy, and safety. *Am J Health Syst Pharm*. 2003;60(13 Suppl 2):S9-S14. https://doi.org/10.1093/ajhp/60.suppl_2.s9
35. Tariq S, Woodman J. Using mixed methods in health research. *JRSM Short Rep*. 2013;4(6):204253331347919. https://doi.org/10.1177/2042533313479197
36. Williams AC, Ramsden DB. Pellagra: a clue as to why energy failure causes diseases? *Med Hypotheses*. 2007;69(3):618-628. https://doi.org/10.1016/j.mehy.2007.01.029
37. Grozio A, Mills KF, Yoshino J, et al. Slc12a8 is a nicotinamide mononucleotide transporter. *Nat Metab*. 2019;1(1):47-57. https://doi.org/10.1038/s42255-018-0009-4
38. Massudi H, Grant R, Braidy N, Guest J, Farnsworth B, Guillemin GJ. Age-associated changes in oxidative stress and NAD+

metabolism in human tissue. *PLoS One*. 2012;7(7):e42357. https://doi.org/10.1371/journal.pone.0042357
39. Gomes AP, Price NL, Ling AJY, et al. Declining NAD+ induces a pseudohypoxic state disrupting nuclear-mitochondrial communication during aging. *Cell*. 2013;155(7):1624-1638. https://doi.org/10.1016/j.cell.2013.11.037
40. Schmidt MS, Brenner C. Absence of evidence that Slc12a8 encodes a nicotinamide mononucleotide transporter. *Nat Metab*. 2019;1(7):660-661. https://doi.org/10.1038/s42255-019-0085-0
41. Yoshida M, Satoh A, Lin JB, et al. Extracellular vesicle-contained eNAMPT delays aging and extends lifespan in mice. *Cell Metab*. 2019;30(2):329-342.e5. https://doi.org/10.1016/j.cmet.2019.05.015
42. Liu Y, Luo C, Li T, et al. Reduced nicotinamide mononucleotide (NMNH) potently enhances NAD+ and suppresses glycolysis, the TCA cycle, and cell growth. *J Proteome Res*. 2021;20(5):2596-2606. https://doi.org/10.1021/acs.jproteome.0c01037
43. Yang Y, Mohammed FS, Zhang N, Sauve AA. Dihydronicotinamide riboside is a potent NAD concentration enhancer in vitro and in vivo. *J Biol Chem*. 2019;294(23):9295-9307. https://doi.org/10.1074/jbc.RA118.005772
44. Bonkowski MS, Sinclair DA. Slowing ageing by design: the rise of NAD+ and sirtuin-activating compounds. *Nat Rev Mol Cell Biol*. 2016;17(11):679-690. https://doi.org/10.1038/nrm.2016.93
45. Yoshino J, Baur JA, Imai S-I. NAD+ intermediates: the biology and therapeutic potential of NMN and NR. *Cell Metab*. 2018;27(3):513-528. https://doi.org/10.1016/j.cmet.2017.11.002

How to cite this article: Palmer RD, Elnashar MM, Vaccarezza M. Precursor comparisons for the upregulation of nicotinamide adenine dinucleotide. Novel approaches for better aging. *Aging Med*. 2021;00:1–7. https://doi.org/10.1002/agm2.12170