nature metabolism

Review article

https://doi.org/10.1038/s42255-025-01387-7

NAD$^+$ precursor supplementation in human ageing: clinical evidence and challenges

Received: 7 April 2025

Accepted: 5 September 2025

Published online: 13 October 2025

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Nicotinamide adenine dinucleotide (NAD$^+$) is an essential molecule involved in cellular metabolism, and its decline has been implicated in ageing and age-related disorders. However, evidence for an age-related decline in NAD$^+$ levels in humans has been consistently observed only in a limited number of studies. Similarly, although preclinical studies support the idea that supplementation with NAD$^+$ precursors is a promising therapeutic strategy to promote healthy ageing, human clinical trials have shown limited efficacy. Therefore, an increasing understanding of how NAD$^+$ metabolism is affected in different tissues during disease and following NAD$^+$ precursor supplementation is crucial to defining the therapeutic value of NAD$^+$-targeted therapies. In this Review, we evaluate the clinical evidence supporting the notion that NAD$^+$ levels decline with age, as well as the tissue-specific effects of NAD$^+$ precursor supplementation. Viewed in perspective, the published body of data on NAD$^+$ dynamics in human tissues remains sparse, and the extrapolation of rodent-based data is not straightforward, underscoring the need for more clinical studies to gain deeper insights into systemic and tissue-specific NAD$^+$ metabolism.

Nicotinamide adenine dinucleotide (NAD$^+$) is a versatile molecule that primarily functions as a coenzyme in cellular redox reactions, being converted to its reduced form (NADH) in fundamental metabolic processes such as glycolysis, fatty acid β-oxidation and the tricarboxylic acid cycle[1,2]. Additionally, NAD$^+$ functions as a limiting substrate for NAD$^+$-consuming enzymes, including sirtuins (SIRTs), poly(ADP-ribose) polymerases, sterile alpha and Toll/interleukin-1 receptor motif-containing 1 (SARM1), and cyclic ADP-ribose (cADPR) synthases such as the ectoenzymes CD38 and CD157 (also known as bone marrow stromal cell antigen 1 (BST1))[1,2]. These NAD$^+$-dependent enzymes are crucial for maintaining metabolic homeostasis, preserving DNA integrity and regulating gene expression[3].

Lower NAD$^+$ levels have been linked to numerous inherited and age-related diseases in animal models, including neurodegenerative diseases, metabolic diseases and cancer. Notably, in preclinical trials, these pathophysiological states could be counteracted by 'boosting' NAD$^+$ levels through the administration of NAD$^+$ precursors[4]. The promising outcomes of such studies have drawn considerable attention to the efficacy of NAD$^+$ precursors in the clinical setting. Particularly, nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN) have recently become extensively researched NAD$^+$-boosting agents. However, with the increasing momentum of NAD$^+$ research, the concept of decreased NAD$^+$ levels during ageing holds important nuances, as some tissues show a clear age-related decrease, whereas others do not

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https://doi.org/10.1038/s42255-025-01387-7

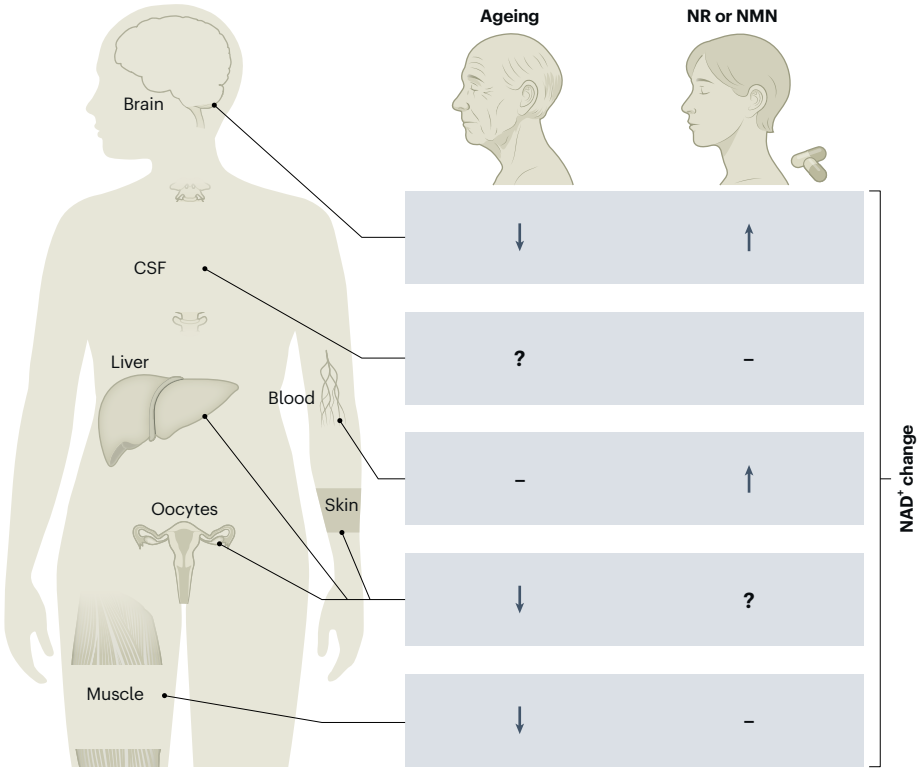

Fig. 1 | Alterations in NAD$^+$ levels across human tissues during ageing and in response to precursor supplementation. The schematic depicts the changes in NAD$^+$ levels in different tissues during ageing or following supplementation with the NAD$^+$-boosting agents NR and NMN. During ageing, NAD$^+$ levels have been reported to decline in the brain[92,94], liver[96], skin[98], oocytes[100] and muscle[88] in humans (indicated by the downward arrow). Of note, the absence of an age-related NAD$^+$ decline in the brain has also been reported[93]. No evident change in NAD$^+$ levels has been reported in the blood[75–77] (indicated by the dash). However, two studies reported an age-related decline in NAD$^+$ levels in the blood[73,74]. For the CSF, no human evidence is available regarding changes in NAD$^+$ levels during ageing (indicated by the question mark). Following supplementation with NR or NMN, NAD$^+$ levels have been reported to increase in both the brain[118] and the blood[71,72,119,121,122,135,145,164–169,171] (indicated by the upward arrow). No evident change in NAD$^+$ levels has been reported for the CSF[118] and muscle[118,119,124–126,128,163] following NAD$^+$ supplementation, and no human evidence is available for the levels in the liver, skin or oocytes.

(Fig. 1). Similarly, NAD$^+$-boosting therapies, such as the administration of NR or NMN, seem to have highly distinct effects on NAD$^+$ levels in the central and peripheral compartments[5] (Fig. 1).

In the first part of this Review, we describe general NAD$^+$ metabolism. In the second part of the article, we summarize human studies that quantify NAD$^+$ levels in various tissues and establish whether an age-related decrease is evident. Finally, we discuss published clinical trials using the NAD$^+$ precursors NR and NMN, as well as their effects on NAD$^+$ metabolism in the analysed tissues.

NAD$^+$ metabolism

NAD$^+$ metabolism is sustained through several intertwined pathways, including the Preiss–Handler pathway, the de novo synthesis pathway and the salvage pathway[3] (Fig. 2). The Preiss–Handler pathway uses nicotinic acid (NA), which, following enzymatic conversion by nicotinic acid phosphoribosyltransferase (NAPRT), yields nicotinic acid mononucleotide (NAMN). NAMN can be converted into nicotinic acid adenine dinucleotide (NAAD) by the nicotinamide mononucleotide adenylyltransferases (NMNATs) and finally to NAD$^+$ following its amidation by NAD$^+$ synthetase (NADS)[6]. The de novo synthesis pathway uses tryptophan, which, through a series of enzymatic and spontaneous reactions, produces NAMN, converging with the Preiss–Handler pathway[6]. Finally, the salvage pathway recycles nicotinamide (NAM), which is mainly generated as a product of the enzymatic consumption of NAD$^+$. NAM can be converted into NMN by nicotinamide phosphoribosyltransferase (NAMPT), which is the rate-limiting enzyme of the NAD$^+$ salvage pathway and a key regulator of the intracellular NAD$^+$ pool[7,8]. Additionally, NMN can be generated intracellularly from NR through its phosphorylation by the nicotinamide riboside kinases (NRKs)[9]. NMN is then converted into NAD$^+$ by NMNATs.

NAM can be excreted through two enzymatic systems. In the first system, NAM is oxidized to nicotinamide *N*-oxide (NAMOx) by cytochrome P450 2E1 (CYP2E1)[10] (Fig. 2). In the second system, NAM undergoes methylation catalysed by nicotinamide *N*-methyltransferase (NNMT), producing methylnicotinamide (MeNAM), which can undergo further oxidation to *N*-methyl-2-pyridone-5-carboxamide (Me2PY, sometimes referred to as 2PY) and *N*-methyl-4-pyridone-3-carboxamide (Me4PY, sometimes referred to as 4PY)[11]. In mice, aldehyde oxidase enzymes (most notably AOX2 and AOX3) predominantly generate Me2PY from MeNAM. By contrast, humans mostly express AOX1, for which MeNAM is a poor substrate, making it likely that the generation of oxidized methyl pyridines occurs through the spontaneous oxidation of MeNAM[12,13]. Excess NA is mainly excreted as nicotinuric acid following glycine conjugation by glycine *N*-acyltransferase[14]. In addition, a minor fraction of NA is excreted as trigonelline, a methylated form of NA[15].

NAD$^+$ compartmentalization

Metabolic compartmentalization, defined as separate pools of the same metabolite without being in equilibrium, adds a layer of complexity to the regulation of NAD$^+$ in addition to the pathways and enzymes described above. The different localization of NAD$^+$ synthetic and transport enzymes at the tissue, cellular and subcellular levels enables distinct NAD$^+$ pools to be independently regulated and maintained.

https://doi.org/10.1038/s42255-025-01387-7

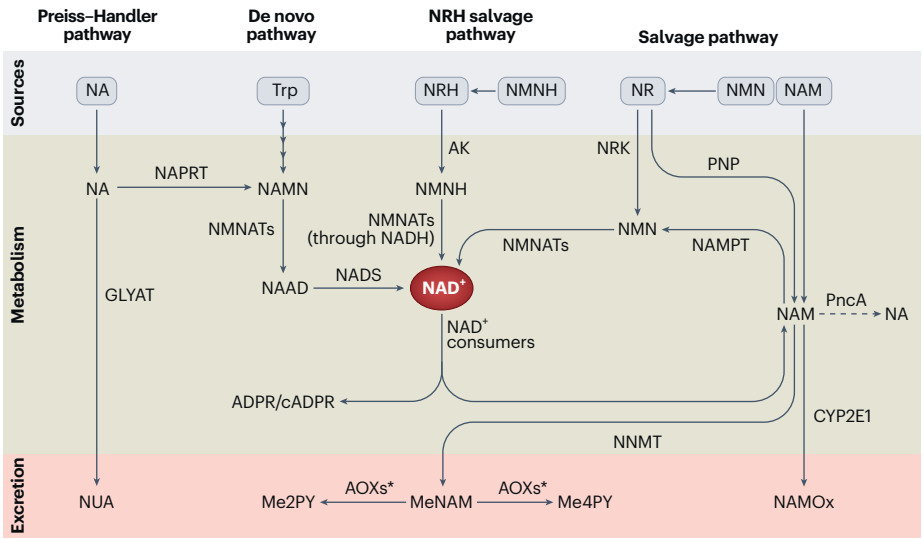

Fig. 2 | Central metabolic pathways related to NAD$^+$. The main NAD$^+$ biosynthetic pathways include the de novo pathway, the Preiss–Handler pathway, the salvage pathway and the NRH salvage pathway, which is a newly identified NAD$^+$ biosynthetic route. Three functional sections are presented in the schematic: sources, which represent NAD$^+$ precursors; metabolism, which encompasses the biosynthesis, interconversion and consumption of NAD$^+$; and excretion, which represents the excretion products of NAD$^+$. The Preiss–Handler pathway uses NA, which, following enzymatic conversion by NAPRT, yields NAMN. NAMN can be converted into NAAD by NMNATs and finally to NAD$^+$ following its amidation by NADS. The de novo synthesis pathway uses tryptophan, which, through a series of enzymatic and spontaneous reactions, produces NAMN, converging with the Preiss–Handler pathway. Finally, the salvage pathway recycles NAM, which is mainly generated as a product of the enzymatic consumption of NAD$^+$. NAM can be converted into NMN by NAMPT. Additionally, NMN can be generated intracellularly from NR through its phosphorylation by NRKs. NMN is then converted into NAD$^+$ by NMNATs. The NRH salvage pathway uses NRH, the reduced form of NR, to synthesize NAD$^+$. The pathway is initiated by the conversion of NRH to the reduced form of NMN, known as NMNH, catalysed by adenosine kinase (AK). Major NAD$^+$ consumers include SIRTs, poly(ADP-ribose) polymerases and cADPR synthases, which produce NAM that can be recycled to NMN. Alternatively, NAM can be excreted through two enzymatic systems. In the first system, NAM is oxidized to NAMOx by CYP2E1. In the second system, NAM undergoes methylation catalysed by NNMT, producing MeNAM, which can undergo further oxidation to Me2PY (sometimes referred to as 2PY) and Me4PY (sometimes referred to as 4PY). Humans express AOX isoform 1 (indicated by the asterisk), for which MeNAM is a poor substrate. Therefore, the conversion of MeNAM to Me2PY in humans most probably occurs through spontaneous oxidation. Additionally, NAM can be converted into NA in the gut by the microbial nicotinamidase PncA (indicated by the dashed arrow). Excess NA is mainly excreted as nicotinuric acid (NUA) following glycine conjugation by glycine *N*-acyltransferase (GLYAT).

Localization and regulation of subcellular NAD$^+$ pools

The subcellular localization and regulation of NAD$^+$ are exemplified by the compartment-specific expression of NMNAT enzymes, which catalyse the final enzymatic step of NAD$^+$ salvage, with NMNAT1 confined to the nucleus, NMNAT2 to the cytoplasm and NMNAT3 to the mitochondria[16]. Notably, a substantial portion of the cellular NAD$^+$ pool is localized in the mitochondrion, distinguishing this organelle as a major NAD$^+$ reservoir compared with other cellular compartments[17,18]. The different concentrations and, potentially, compartment-specific use of NAD$^+$ are enabled not only by the presence of different NMNAT isoforms but also by dedicated transporters, such as solute carrier family 25 member 51 (SLC25A51), which acts as a mitochondrial NAD$^+$ transporter[19–21]. Several studies suggest that mitochondria could function as the primary reservoirs for NAD$^+$, supplying the nucleus and cytoplasm in case of deficiency[17,22].

With the potential exception of NAM, NAD$^+$ precursors cannot freely diffuse across membranes and therefore require specific transporters to be incorporated into cells[23]. Once NAD$^+$ metabolism intermediates are inside cells, subcellular compartment membranes also become a barrier to their diffusion, and the precursors are primarily metabolized by cytosolic enzymes into NAD$^+$. However, owing to NAD$^+$-dependent enzyme activities at the organelle level, cells require compartmentalized NAD$^+$ (re)synthesis. This is exemplified by NMN, which can be generated in different compartments as a product of NAMPT activity. As NAM is generated by a number of NAD$^+$-degrading enzymes, which exist in all compartments, it can, in principle, be recycled to NAD$^+$ through local NAMPT activity. Indeed, NAMPT is found in the cytosolic and nuclear compartments of the cell[24]. It has also been reported to be located within mitochondria[24], although this finding remains controversial[25]. In the nucleus, NAMPT functions as a local biosynthetic source of NAD$^+$ even at the level of specific gene promoters[26,27]. The role of independent NAD$^+$ biosynthesis in the mitochondrion is less clear, not only because of the potential lack of NAMPT in this organelle but also in relation to the limited expression of NMNAT3 in some tissues[28]. In line with this, there are no obvious phenotypes in most tissues of mice lacking the *Nmnat3* gene[29], and the NMNAT activity observed in mitochondrial isolates may be due to nuclear NMNAT1 contamination rather than NMNAT3 itself[30]. Collectively, these observations suggest that mitochondrial NAD$^+$ levels may be largely sustained through the regulation of direct NAD$^+$ import into mitochondria, rather than through internal biosynthesis, as confirmed by SLC25A51 knockdown approaches[22].

Tissue-specific expression of NAD$^+$-related enzymes

Although NAD$^+$ metabolism is tightly regulated at the subcellular level, there are also substantial differences in the tissue expression and activity of the enzymatic machinery involved in NAD$^+$ synthesis[31]. In fact, only the liver expresses the complete set of enzymes that can metabolize all known NAD$^+$ precursors and thus has a key role in maintaining whole-body NAD$^+$ homeostasis[31]. By contrast, skeletal muscle is almost exclusively reliant on the salvage pathway, given the presence and activity of NMNATs and NAMPT[32], but with no activity of the Preiss–Handler pathway-related enzymes NAPRT and NADS[32,33]. The blood exhibits detectable activity of NADS and NMNATs, but at low levels relative to other tissues[32]. This is reflected in the NAD$^+$ turnover rate, which is likewise low compared with other tissues such as the muscle, brain, liver and kidneys[32]. Overall, the blood seems to take a passive role in NAD$^+$ homeostasis and functions as a transport medium for NAD$^+$

precursors[32]. Nonetheless, various circulating proteins influence NAD^+ precursors, thereby altering the composition of the NAD^+ metabolome.

Boosting NAD^+ levels with precursors

Owing to its size and charged nature, NAD^+ cannot passively pass through cellular membranes, including the plasma membrane. Although direct cellular uptake of NAD^+ through connexin 43 has been proposed[34], the physiological relevance of this mechanism is unclear. Therefore, the most accepted hypothesis is that, although direct NAD^+ administration increases NAD^+ levels in cultured cells[35] and even in human plasma after intravenous delivery[36], this probably occurs indirectly through the generation of NAD^+ degradation products, such as NMN, NR or NAM, which act as precursors[23]. In a clinical pilot study, intravenous infusion of NAD^+ at 3 μmol min^{-1} led to a statistically significant increase in plasma NAD^+ after 6 h of continuous infusion. Yet, this occurred in parallel with even larger increases in NAM, MeNAM or ADP-ribose (ADPR)[36], which suggests that NAD^+ was converted into other metabolites, most probably through NAD^+ glycohydrolase activities (for example, CD38, generating ADPR and NAM) or NAD^+ pyrophosphatase activities (generating NMN and AMP). Nevertheless, tracer studies in mammals will be needed to truly understand the dynamics and kinetics of NAD^+ after intravenous administration.

Classic NAD^+ precursors in health and disease

An efficient way to increase NAD^+ levels is through the administration of NAD^+ precursors (Fig. 2). NAD^+ precursors, such as tryptophan, NA and NAM, have historically been used to treat the potentially fatal disorder pellagra[37].

Tryptophan has long been recognized as a NAD^+ precursor and can indeed be used to treat pellagra. However, tryptophan is a relatively inefficient NAD^+ precursor in humans owing to its involvement in the synthesis of other biomolecules, such as kynurenic acid and serotonin[38]. Interestingly, at least in mice, tryptophan can have a notable contribution to hepatic NAD^+ biosynthesis, given the low expression of the α-amino-β-carboxy-muconate-semialdehyde decarboxylase (ACMSD) enzyme in mice compared with humans, which prevents tryptophan-derived metabolites from participating in NAD^+ synthesis[39]. Accordingly, transgenic mice with inducible expression of human ACMSD become dependent on niacin[39]. NA has demonstrated efficacy as an anti-inflammatory agent, as well as against dyslipidaemia by increasing high-density lipoprotein cholesterol and lowering both low-density lipoprotein cholesterol and total cholesterol, thus contributing to improved cardiovascular risk profiles[40,41].

An estimated 85% of physiological NAD^+ production originates from NAD^+ salvage[42]. Therefore, NAM, being the primary NAD^+ salvage substrate, represents the most direct method for boosting NAD^+. Unlike NA, NAM has not been shown to be effective against dyslipidaemia. This is in line with the fact that the benefits of NA on dyslipidaemia may not be related to its role as a NAD^+ precursor[43]. In fact, the mechanism behind the lipid-lowering effects of NA remains unclear, despite speculation about its binding to and activation of niacin receptor 1 (GPR109A). However, this claim has been refuted by studies using *Gpr109a*-deficient mice and GPR109A agonists in humans, separating the lipid-lowering effects of NA from its interaction with this receptor[44]. Although speculative, the lack of major effects of NAM on dyslipidaemia could be related to its bioavailability or ability to act as an end-product inhibitor of NAD^+-degrading enzymes such as SIRT1, indirectly contributing to the regulation of cholesterol and lipid homeostasis[6]. In addition, NAM relies on the activity of NAMPT, which is subject to feedback inhibition by NAD^+ itself, limiting its NAD^+-boosting potential[45]. Despite the limitations of NAM as a NAD^+ precursor, it is well tolerated at gram doses. This is in contrast to NA, which can cause flushing even at low doses (for example, 30 mg)[46].

More recently, attention has shifted towards NR and NMN as new frontline NAD^+-boosting therapies. A key advantage of these precursors is their excellent safety profiles and their ability to bypass NAMPT during their first conversion to NAD^+, thereby avoiding initial feedback inhibition. This offers valuable alternatives to tryptophan, NA and NAM. Given that most NAD^+-centred clinical trials rely on NR and NMN as precursors, this Review will particularly focus on these boosting agents.

Next-generation NAD^+ precursors

New alternative NAD^+ precursors are currently under investigation. Trigonelline, although often considered an excretion product of NA, can function as a NAD^+ precursor in some cases[47,48]. Recent efforts have shown that the administration of trigonelline can boost muscle NAD^+ levels comparable to NA and NAM, as well as improve muscle and mitochondrial function in preclinical models[48]. Furthermore, trigonelline demonstrated notably better stability in serum compared with NR and NMN[48]. The fact that trigonelline is a plant alkaloid abundantly found in natural products makes it a potential dietary contributor to NAD^+ homeostasis.

Other promising NAD^+ precursors include the reduced counterparts of NR and NMN, known as NRH and NMNH. These compounds are substantially more potent NAD^+ boosters than NR and NMN in cultured cells and animal models, and they are incorporated into the NAD^+ pool through an independent pathway that involves adenosine kinase[49–52]. However, preclinical studies using these compounds are scarce and rely on short-term exposure, and there is still no published data on their safety or efficacy in humans. Therefore, whether these new NAD^+ precursors can function as clinical therapeutic alternatives remains to be elucidated.

In vivo pharmacokinetics of NAD^+ precursors

NAD^+ precursors that are administered either orally or parenterally enter a complex network of metabolic conversions, microbial interactions and tissue-specific processing, which collectively shape their pharmacokinetic profiles. Unfortunately, there is a lack of studies providing side-by-side pharmacokinetic comparisons of different NAD^+ precursors in humans or preclinical models. Furthermore, most pharmacokinetic studies analysed a limited range of NAD^+ metabolites, which makes it difficult to understand the multiple metabolic conversions that may occur. Despite these limitations, this section highlights some important factors that affect the bioavailability of NAD^+ precursors.

The effect of the microbiome

Following oral administration, only a fraction of NR is absorbed by the intestinal epithelium, while the majority is degraded into NAM in the gut and further into NA by the microbial nicotinamidase PncA[53,54]. Consequently, orally administered NR or NAM boosts NAD^+ in vivo mainly through the NA that is generated by the microbiome[53] (Fig. 3). However, the microbial-mediated degradation of NAM into NA seems to be time-dependent, with a greater proportion of NR and NAM being directly absorbed through the intestinal wall within the first hour after administration[54].

Additionally, the NAM produced in host tissues can recirculate into the gut lumen and reinteract with the gut microbiome, being converted to NA to support microbial NAD^+ biosynthesis[55]. Nevertheless, the remaining NR entering the circulation is degraded to NAM within hours, most probably through purine nucleoside phosphorylase (PNP)[56,57], and only a minimal fraction of the native NR reaches peripheral tissues such as the kidneys, muscle and liver[54,55,58,59]. Despite substantial degradation of NR, treatment of NAMPT-knockout mouse models with NR protects against hepatic steatosis and sarcopenia, although it is unclear whether these effects are driven by NR itself or NR-derived NA[42,60,61].

Similar to NR, orally administered NMN is greatly hampered by the gut microbiome, which reduces its bioavailability and results in the production of nicotinic acid riboside (NAR), NAMN and NAAD, with minimal incorporation of intact NMN in peripheral tissues[62].

https://doi.org/10.1038/s42255-025-01387-7

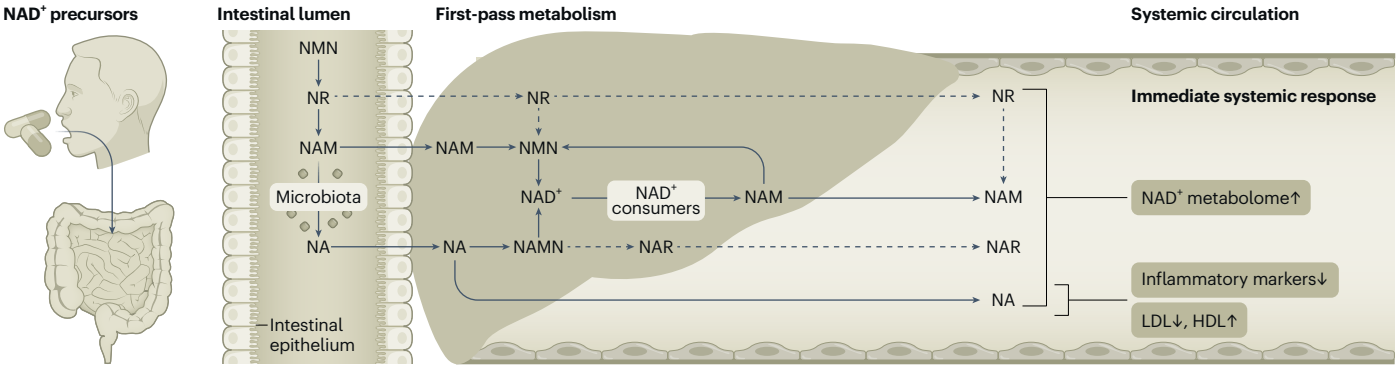

Fig. 3 | Metabolic fate of orally administered NAD$^+$ precursors. Following oral intake, NAD$^+$ precursors are metabolized by gut microorganisms in the intestinal lumen through major metabolic host routes (indicated by solid arrows). Additionally, less active metabolic pathways are also present (indicated by dashed arrows). NAM and NA, along with NR to a lesser extent, are the main compounds being transported to the liver. In the liver, extensive first-pass metabolism occurs, with predominantly NAM and NA being released into the systemic circulation. Immediate systemic responses include increased levels of NAD$^+$-related metabolites[78] and, specifically with NA supplementation, reduced inflammatory markers[122] and improved lipid profile (increasing high-density lipoprotein (HDL) cholesterol and lowering low-density lipoprotein (LDL) cholesterol)[40].

The influence of extracellular metabolism

Although the bioavailability of NAD$^+$ precursors is highly influenced by the gut microbiome, various circulating ectoenzymes can also affect their potential to reach tissues in their intact forms. These ectoenzymes include cADPR synthases (for example, CD73) that extracellularly dephosphorylate NMN to NR before intestinal absorption[5,62]. Whether NMN requires dephosphorylation to NR to be incorporated into the cell has been a contentious issue. Initial experiments using pharmacological and genetic strategies suggest a mandatory requirement for NMN dephosphorylation[23,59]. However, more recently, SLC12A8 has been described as a potential NMN transporter[63,64], although this finding remains controversial[65].

Other cADPR synthases include CD157 (BST1) and CD38. CD157 has been implicated in the extracellular metabolism of both NR and NAR. In particular, it performs base-exchange reactions between nucleosides but not nucleotides such as NMN or NAD$^+$ (ref. 54). Instead, nucleotides such as NMN are subject to degradation by CD38, whose role has recently been expanded to include the base exchange of NMN with NA, producing NAMN[66,67]. In contrast to CD157, the base exchange catalysed by CD38 was specific to NMN, with no activity towards NR[67].

Extracellular NAMPT can also influence extracellular NAD$^+$ metabolism. Extracellular NAMPT is often secreted into the circulation in the form of extracellular vesicles by adipose tissue. Extracellular vesicle-contained NAMPT can be internalized by distal tissues, enhancing NMN and NAD$^+$ synthesis intracellularly[68]. Such vesicles are especially important for organs with low intrinsic NAMPT expression, such as the brain or the pancreas[68]. For some time, it was speculated that NAMPT could enable NMN synthesis in the circulation; however, this possibility seems highly unlikely, as the absence of phosphoribosyl pyrophosphate (PRPP) and ATP in plasma would prevent NAMPT activity in the extracellular milieu[69].

Despite the similar NAD$^+$-boosting effects of NR and NMN, the circulatory stability of these two precursors seems to differ, as NR degrades in isolated mouse plasma while NMN remains stable[59,70]. The decrease in NR corresponded to a proportional increase in NAM[59]. The reasons for NR degradation in plasma are unclear but may be related to PNP1 activity[57].

Together, these observations establish that NA and NAM are the primary molecules reaching the circulation following oral supplementation with NR or NMN[53,55] (Fig. 3). Interestingly, no flushing has been reported after NR or NMN administration in humans[71,72], which suggests that if NR and NMN are converted into NA, the process may occur more gradually, preventing a spike in NA levels that would typically trigger flushing[6].

NAD$^+$ changes in human ageing tissues

Given the substantial degradation of NR and NMN in the gut and circulation, along with the tissue-specific expression and activity patterns of NAD$^+$-related enzymes, the NAD$^+$-boosting effects of these NAD$^+$ precursors are also highly tissue-specific[58]. When assessing changes in NAD$^+$ metabolism in vivo, the most commonly used readout is the abundance of NAD$^+$ in the blood or tissue. In humans, certain age- and sex-related trends in NAD$^+$ levels have been observed. Table 1 summarizes studies that quantified NAD$^+$ in various tissues and biological fluids in healthy individuals, with a particular focus on whether age-related decreases in NAD$^+$ were reliably demonstrated.

Blood

In contrast to muscle or brain tissue, the blood and its fractions offer a less invasive means of sample collection from humans. The desire to establish blood as a viable sample type for NAD$^+$ analysis in relation to human ageing is evident, as it would facilitate potential straightforward diagnostics.

Whole blood. Whole blood is the predominant type of blood sample used, with most reports indicating physiological NAD$^+$ levels ranging between 20 and 40 nmol ml^{-1} (or μM), although results can vary considerably (Table 1). There are five reports examining the relationship between whole-blood NAD$^+$ abundance and ageing, four of which are featured in Table 1 (one study is not included in Table 1 because it reports relative abundance rather than absolute concentrations). Two studies suggest a significant decrease in NAD$^+$ levels with age[73,74] (Fig. 4). Conversely, the three other studies report no significant decrease in NAD$^+$ levels with age, with one showing a normal distribution across 299 healthy individuals[75–77] (Fig. 4). The largest study, conducted on >1,500 participants, did not observe a consistent statistical difference in blood NAD$^+$ levels across five age groups, nor was there a significant change in red blood cell count, the main source of NAD$^+$ in whole blood[77] (Fig. 4). Although a declining trend in NAD$^+$ content with ageing was observed in participants under 50 years old, this trend was not observed after the age of 50 years. Notably, sex was an important factor in determining NAD$^+$ levels, with women exhibiting lower NAD$^+$ abundance in whole blood. Only after segregating by sex did a discernible trend emerge: an inverse association between ageing and blood NAD$^+$ levels was noted in men, with a significant decline after age 60 years, whereas no significant trend in NAD$^+$ levels with ageing was observed in women.

One study that observed a decrease in NAD$^+$ levels in both sexes also investigated fluctuations in NAD$^+$ levels at specific times of the day, drawing blood from six individuals at two time points:

https://doi.org/10.1038/s42255-025-01387-7

Table 1 | Human tissue-specific NAD^+ concentrations

Tissue	Fraction	Age (years)[a]	NAD^+ concentration	Reference
Brain	Cerebral cortex	21–68	0.30±0.02 nmol ml^{-1}[b] (0.27±0.02 µmol g^{-1})	92
Muscle	Not specified	44	38.763 nmol ml^{-1}[b]	136
	Vastus lateralis	17–70	0.179 nmol mg^{-1}[b]	132
		70–80	0.218 nmol mg^{-1}[b]	115
	Deltoid muscle	N/A	0.178±0.074 nmol mg^{-1}	137
	Quadriceps femoris	19–34	1.81±0.07 nmol mg^{-1}[b]	138
		23–41	1.54±0.06 nmol mg^{-1}[b]	139
Blood	Whole blood	18–70	27.7±6.0 nmol ml^{-1}[b]	76
		20–50 (F)	32.7±9.6 nmol ml^{-1}[b]	73
		20–50 (M)	44.2±18.9 nmol ml^{-1}[b]	
		50–80 (F)	24.8±9.6 nmol ml^{-1}[b]	
		50–80 (M)	25.9±9.8 nmol ml^{-1}[b]	
		18–29	33.33±5.07 nmol ml^{-1}[b]	77
		30–39	33.14±5.77 nmol ml^{-1}[b]	
		40–49	32.51±5.50 nmol ml^{-1}[b]	
		50–59	33.31±5.45 nmol ml^{-1}[b]	
		≥60	32.71±5.28 nmol ml^{-1}[b]	
		25–75	18±2 nmol ml^{-1}[b]	140
		66.4±5.3	22.0±8.1 µg ml^{-1} (33.16±12.21 nmol ml^{-1}[b])	141
		N/A	10.0±1.4–18.0±0.1 nmol ml^{-1}[b]	103
		19–68	23.4±4.05 nmol ml^{-1}[b]	75
		63±11	52.907 nmol ml^{-1}[b]	142
		N/A	16.195 nmol ml^{-1}[b]	143
		21–50	27±5 nmol ml^{-1}[b]	144
		15–49 (F)	23.0–25.6 nmol ml^{-1}[b]	145
	Red blood cells	N/A	25.40±3.88 nmol ml^{-1}[b]	102
		0–2 days	53.0 nmol ml^{-1}[b] (31.75–74.25 nmol ml^{-1}[b])	146
		30 (M)	47.31 nmol ml^{-1}[b] (33.14–61.48 nmol ml^{-1}[b])	
		N/A	50±6 nmol ml^{-1}	147
		45–70	48±6 nmol ml^{-1}	148
		N/A	79.0±8.5 nmol ml^{-1}	149
		3	112±27 nmol ml^{-1}[b]	150
		N/A	51±7 nmol ml^{-1}[b]	151
		N/A (M)	0.183±0.026 µmol g^{-1} haemoglobin	152
	Plasma	18–83	1.340 pmol ml^{-1}[b] (440–2.880 pmol ml^{-1}[b])	82
		31.20±5.84 (F, pregnant)	4.58±1.32 pmol ml^{-1}[b]	153
		N/A (M)	300.412 ng ml^{-1} (453 pmol ml^{-1}[b])	154
		20–40	54.545 pmol ml^{-1}[b]	81
		41–60	39.043 pmol ml^{-1}[b]	
		≥60	7.081 pmol ml^{-1}[b]	
		N/A	17.44–45.29 pmol ml^{-1}[b]	155
	Serum	46±10.7	17.9±3.2 µg ml^{-1} (26.98±4.82 nmol ml^{-1}[b])	156
	PBMCs	30–55	0.33–28.93 nmol ml^{-1}[b]	78
		49.3±7.1	189.4±8.9 pmol per 10^6 cells	157
		20–45	134.9–249.3 nmol ml^{-1}[b]	158
	T and B lymphocytes	25–75	358±19 pmol per 10^7 cells (0.95±0.05 nmol mg^{-1})	159

https://doi.org/10.1038/s42255-025-01387-7

Table 1 (continued) | Human tissue-specific NAD$^+$ concentrations

Tissue	Fraction	Age (years)[a]	NAD$^+$ concentration	Reference
Liver	Not specified	62.5±7.1	616 nmol ml^{-1}[b]	160
Skin	Pelvic region	0–1	8.54±1.55 ng per mg protein (12.87±2.34 pmol per mg protein[b])	98
		30–50	2.74±0.41 ng per mg protein (4.13±0.62 pmol per mg protein[b])	
		51–70	1.08±0.15 ng per mg protein (1.63±0.23 pmol per mg protein[b])	
		71–77	1.06±0.19 ng per mg protein (1.60±0.29 pmol per mg protein[b])	
Rectum	Not specified	N/A	345.5±60.7 nmol ml^{-1}[b]	99
CSF	–	N/A	0.072±0.008 nmol ml^{-1}[b]	102
Sperm	–	30 (M)	91.61±15.59 nmol per 10^6 sperm cells	101
		30–40 (M)	125.60±16.28 nmol per 10^6 sperm cells	
		>40 (M)	115.59±16.55 nmol per 10^6 sperm cells	

The table includes data partly derived or inferred from a previous meta-analysis[161]. Studies that measured total NAD(H) abundance were excluded. [a]Age is given in years unless specified otherwise. Sex is indicated for studies that included only one specified sex. M, male; F, female; N/A, not available. [b]The original unit was adjusted to ensure comparability with other studies.

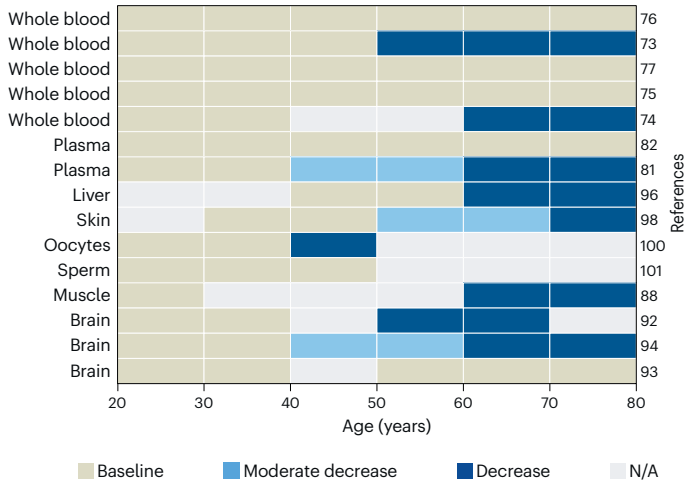

Fig. 4 | Age-driven tissue-specific changes in NAD$^+$ abundance. Ages are rounded to the nearest decade (10 years). Reported trends reflect the values cited by the authors of the referenced studies and do not represent universal values across all studies. N/A indicates that data were not available, meaning that the respective age group was not investigated in the referenced study. The baseline was defined as the level reported for individuals aged approximately 20–30 years. To guide interpretation, we compared NAD$^+$ measurements across key tissues from several studies to assess where findings were consistent and where they diverged. Notably, there are visible discrepancies between reports regarding whether NAD$^+$ levels decline in certain tissues with age.

4 a.m. and 10 a.m. There was no statistically significant difference in NAD$^+$ levels between these two time points. Additionally, the study monitored a group of six individuals, measuring NAD$^+$ levels in whole blood every 3 days over a period of more than 3 months. During this longer monitoring period, whole-blood NAD$^+$ levels were generally stable; however, notable spikes and drops were observed, raising questions about the importance of confounding factors[73].

Plasma and serum. Table 1 includes several reports on NAD$^+$ levels in plasma and one study on the levels in serum, with most studies reporting >500-fold lower NAD$^+$ levels than those in whole blood. NAD$^+$ is likely to be absent in plasma, similar to other phosphorylated compounds such as NAMN, NAAD, NADP$^+$, NMN and ADPR[78]. The limited detection of NAD$^+$ in plasma may be due to haemolysis during blood drawing or sample handling. An alternative but less substantiated explanation is that NAD$^+$ might be exported through connexin 43 (ref. 79). Although connexin 43 is known to have roles in haematopoiesis, its functionality in erythrocytes remains unconfirmed[80]. Thus, plasma NAD$^+$ may not accurately reflect intracellular conditions.

Regardless of the source of NAD$^+$ in plasma or serum, two studies have explored age-related changes in NAD$^+$ levels in plasma. One study provided evidence that NAD$^+$ decreases with age (from 20 to >60 years old)[81], whereas the other found no significant influence of increasing age on plasma NAD$^+$ levels in a healthy population (ages 18–83 years)[82] (Fig. 4).

Other cellular blood fractions. Reports on NAD$^+$ concentrations in other blood cell fractions in humans are scarce. NAD$^+$ metabolism has a crucial role in immune cell function, particularly in the context of ageing and inflammation. For instance, de novo NAD$^+$ synthesis in macrophages acts as a metabolic switch to regulate macrophage effector responses. Impaired NAD$^+$ synthesis, due to reduced expression of quinolinate phosphoribosyltransferase (QPRT), contributes to lower NAD$^+$ levels and causes innate immune dysfunction during ageing in monocyte-derived macrophages[83]. Moreover, M1-like macrophages derived from human peripheral blood mononuclear cells (PBMCs) express high levels of the NAD$^+$-consuming enzyme CD38 when compared with naive and M2 macrophages[84]; however, the direct effect of these changes on NAD$^+$ levels in humans remains unclear.

Muscle

NAD$^+$ is an essential component of healthy skeletal muscle function, having a fundamental role in numerous processes throughout a person's lifespan, including muscle development, regeneration, ageing and disease. For instance, NAD$^+$ supports muscle health by modulating SIRTs, which influence cell differentiation and regeneration—two key processes for muscle growth and repair following injury or exercise[85,86]. For a deeper explanation of NAD$^+$ functions and its mechanisms in muscle, we refer the reader to a comprehensive review by Xu and Xiao[87].

Various studies have measured NAD$^+$ concentrations in three different muscles (Table 1). These findings provide reference values for muscle NAD$^+$ levels, indicating that the concentrations are within the same range for each of these muscles. However, drawing conclusions about the potential fluctuations of NAD$^+$ levels with ageing based solely on these studies is challenging, as ageing was not their primary focus. A recent study evaluated the relationship between healthy ageing and the muscle metabolome and found that NAD$^+$ abundance in muscle decreased in older individuals compared to younger ones; however, in exercise-trained older individuals, NAD$^+$ levels were maintained at levels comparable to those of younger participants[88] (Fig. 4). Higher activity of NAD$^+$-consuming enzymes and lower activity of NAMPT, which is the main enzyme responsible for refilling the NAD$^+$ muscle pool, contribute to the age-related decrease in NAD$^+$ levels[89].

https://doi.org/10.1038/s42255-025-01387-7

BOX 1

Approaches to measuring NAD$^+$ in human studies

Quantifying NAD$^+$ levels in humans, both in vivo and ex vivo, remains methodologically challenging[181], with several approaches available[128,181]. Currently, the only method for direct in vivo NAD$^+$ quantification is MRS[92,130]. Although promising, MRS faces limited availability and patient-related challenges, such as long scan times and the need to remain still in the magnetic resonance imaging scanner, which can be uncomfortable and impractical for routine clinical use.

For ex vivo measurements, enzymatic cycling assays are the most widely used approach, mainly owing to the availability of user-friendly commercial kits. These assays offer sensitive detection of NAD(P)$^+$ and NAD(P)H, making them a low-barrier entry point for assessing NAD$^+$ status. Yet, this approach does not allow the monitoring of most NAD$^+$-related metabolites.

For a more comprehensive analysis of the NAD$^+$ metabolome, liquid chromatography–mass spectrometry (LC–MS) is the leading alternative, as it allows both targeted and untargeted profiling of a broader range of NAD$^+$-related metabolites[103]. Although it is more labour-intensive and costly, LC–MS provides greater analytical depth and throughput. However, reliable quantification depends on rigorous protocol standardization, the proper use of matched internal standards and thorough validation.

Sample matrix selection is also critical. Whole blood is one of the most accessible and informative matrices. However, it is sensitive to preanalytical variables, including freeze–thaw cycles, and has not been thoroughly validated to assess the effect of sample degradation under different storage conditions[103,129]. Dried blood spot sampling offers logistical advantages; however, reproducibility and quantitative accuracy remain under investigation, rendering measurements currently questionable. Plasma, commonly stored in hospital biobanks, is less suitable for direct NAD$^+$ quantification because NAD$^+$ itself is rarely detected in plasma owing to its intracellular localization. Nevertheless, NAD$^+$ metabolites such as NAM or MeNAM can be detected in plasma and provide indirect insights into NAD$^+$ metabolism[36]. Although valuable, tissue biopsies are invasive and less feasible for routine studies. Ultimately, the choice of method depends on balancing accessibility, depth of analysis and technical robustness, highlighting the need for standardized protocols.

However, NAMPT protein levels increase with aerobic and resistance exercise training in human skeletal muscle[90,91]. In addition, a study demonstrated a positive correlation between NAD$^+$ abundance and daily step count, thereby reinforcing the notion that even moderate exercise can help retain NAD$^+$ muscle abundance[88]. Given that NAD$^+$ levels are higher in mitochondria, differences in NAD$^+$ abundance between trained and sedentary individuals may, at least in part, reflect variations in mitochondrial content. As mitochondrial density declines with ageing, this could contribute to the decrease in NAD$^+$ levels observed in the aged muscle tissue[88].

Brain

Studying NAD$^+$ metabolism in the healthy ageing human brain poses evident challenges. However, methods based on magnetic resonance spectroscopy (MRS) can be useful (Box 1). A study using a high-field magnetic resonance in vivo NAD$^+$ assay identified a gradual but substantial decline in the NAD$^+$-to-NADH ratio in the human brain, corresponding to decreasing NAD$^+$ levels and increasing NADH levels during ageing, with the average concentration across all participants being below 1 µmol per gram of tissue[92] (Table 1 and Fig. 4). Further research on NAD$^+$ levels in the human brain has produced mixed results. One study used phosphorus-31 MRS and reported no significant differences in brain NAD$^+$ or NADH levels between younger individuals (ages 19–38 years) and middle-aged or older individuals (ages 50–72 years), which suggests that NAD$^+$ levels may remain stable across different ages[93] (Fig. 4).

By contrast, another study using proton MRS found a strong linear negative correlation between NAD$^+$ concentration and age[94] (Fig. 4). This discrepancy suggests that although some individuals may maintain stable NAD$^+$ levels into middle age, others may experience a decline as they age, possibly more closely associated with biological age rather than chronological age. The implications of NAD$^+$ metabolism extend beyond the physiological ageing process, as neurodegenerative diseases become increasingly prevalent with advancing age. Key hallmarks of brain ageing—including cellular senescence, stem cell exhaustion and mitochondrial dysfunction—are linked to these conditions and are influenced by alterations in NAD$^+$ metabolism[95].

Other tissues

NAD$^+$ concentrations in various other tissues are rarely reported and are shown in Table 1.

Liver. The function of the liver in synthesizing NAD$^+$ and releasing NAM for other tissues accounts for its high NAD$^+$ levels[3,58]. Hepatic NAD$^+$ levels in individuals over 60 years old were about 70% of those in middle-aged individuals not older than 45 years, as measured in normal-appearing liver tissue specimens obtained from patients undergoing hepatectomy[96] (Fig. 4). This was associated with a significant downregulation of NAMPT and upregulation of NADS and NMNAT in older individuals compared with middle-aged ones[96]. These findings suggest that the decline of NAMPT-controlled NAD$^+$ salvage biosynthesis is a causal factor in age-related NAD$^+$ depletion, whereas the increased NAD$^+$ de novo biosynthesis may be an adaptive response.

Adipose tissue. A recent study investigated NAD$^+$ concentrations in white adipose tissue of middle-aged individuals with obesity and insulin resistance, revealing a baseline level of 7.15 pmol mg^{-1}. This level increased following a 20% weight loss induced by bariatric surgery[97]. Additionally, research on abdominal subcutaneous adipose tissue indicated that the protein levels of NAMPT, NRK1, NMNAT1 and NMNAT2 were not influenced by age in a cohort of both young and old individuals[90].

Skin. A single study evaluated NAD$^+$ concentrations in skin biopsy specimens, aiming to identify potential age-related changes. The study found a negative correlation between NAD$^+$ levels and increasing age in both male and female individuals[98]. Interestingly, the authors found that newborns have considerably higher NAD$^+$ levels in the skin (an average of 8.54 ng per mg protein) compared with all other reported age groups. Individuals between 30 and 50 years of age had average NAD$^+$ levels of 2.74 ng per mg protein, whereas 51- to 70-year-old individuals and those in the 71–77 years age group had NAD$^+$ levels of 1.08 and 1.06 ng per mg protein, respectively (Fig. 4). This suggests that NAD$^+$ levels decline considerably during early life and then decrease more gradually in later years. However, the applied method has limitations, as NAD$^+$ was not directly measured but was instead inferred from the difference between total NAD(H) and NADH concentrations, requiring validation of these findings.

Rectum. A single report highlights that the rectal NAD$^+$ concentration is 345.5 nmol ml^{-1}, which is importantly decreased in rectal tumour tissue (83.9 nmol ml^{-1})[99].

Reproductive system. A study observed a significant age-related decline in NAD^+ levels in human oocytes, particularly at the germinal vesicle and metaphase I stages, suggesting impaired NAD^+ biosynthesis in older oocytes[100]. In the germinal vesicle oocytes, NAD^+ and its precursor NMN decreased, while other precursors such as kynurenine and NR accumulated yet failed to increase NAD^+. These findings point to a disruption in the conversion process, which may contribute to the decline in oocyte quality with advanced maternal age. One study investigated NAD^+ abundance in sperm from a group of healthy men aged <30, 30–40 or >40 years and found no correlation between NAD^+ concentration and the age of the participants[101] (Fig. 4). However, the study reported a negative correlation between intracellular sperm NAD^+ levels and sperm quality parameters, including semen concentration, motility and chromatin integrity[101].

Other bodily fluids

Cerebrospinal fluid. One study assessed the NAD^+ concentration in a pooled sample of cerebrospinal fluid (CSF). In this matrix, a concentration of 0.072 nmol ml^{-1} was found[102]. Interestingly, none of the other NAD^+-related metabolites were found in the CSF, aside from NMN, which was present at a concentration similar to that of NAD^+. This raises the question of whether these low circulating levels of metabolites in the CSF reflect their physiological abundance or are the result of contamination by a small number of cells that have undergone cytolysis[102].

Urine. Owing to its large size and low extracellular abundance, NAD^+ is not commonly analysed or detected in urine under physiological conditions. Two studies have reported the absence of NAD^+ in urine[78,103]. However, a pilot study examining the effects of a 6-h intravenous infusion of NAD^+ revealed detectable baseline levels of NAD^+ in urine, measured at 50 nmol excreted per hour. The concentration of NAD^+ significantly increased during the infusion and decreased once the infusion was stopped, suggesting the excretion of the exogenous NAD^+ (ref. 36). Although NAD^+ itself may not serve as a particularly informative biomarker in urine, several NAD^+ metabolites, such as NAM, Me2PY, Me4PY, trigonelline and NR, are excreted in urine; however, their direct relationship with ageing remains uncertain[103].

Tissue effects of NAD^+ precursors

NAD^+ depletion has been established as a common denominator during ageing (Fig. 1) and in multiple disease conditions[3,4]. Therefore, strategies aimed at restoring NAD^+ levels have become an attractive therapeutic avenue[84]. Preclinical studies have demonstrated that NR and NMN can effectively increase NAD^+ biosynthesis in the circulation as well as in tissues such as the muscle[104,105], brain[106,107], pancreas[5], lung[5], liver[5,104,105], blood vessel (aorta)[108] and brown adipose tissue[104] following oral administration. Supplementation with these NAD^+ precursors has likewise alleviated conditions such as obesity[104], neurodegenerative diseases[106,109] and vascular diseases[108,110], as well as age-related physiological decline[111]. Despite these positive findings in preclinical models, published clinical trials investigating the effect of NR and NMN have demonstrated only limited clinical improvements[112,113]. The reason for this remains debated. Tables 2 and 3 compile clinical trials that have investigated the effect of NR and NMN on the NAD^+ metabolome in human tissues. The majority of clinical trials for NR and NMN use whole blood or blood components (for example, plasma, PBMCs and erythrocytes) to assess the biochemical effects of NAD^+ precursors. In addition, muscle biopsy specimens and urine samples have been collected. Only one study assessed the NAD^+ metabolome in the cerebrum and CSF using non-invasive methods[114].

The first human trial of NR, reported in 2016, used a single 1,000 mg dose administered to one healthy volunteer, as well as increasing doses (100, 300 and 1,000 mg) in 12 other healthy volunteers[78]. Although NR itself was not reported in blood or urine samples, supplementation resulted in increased NAD^+ levels in PBMCs but not in plasma or urine. Additionally, the NAD^+ metabolome was affected, with increased levels of the NAD^+ metabolites MeNAM, Me2PY and Me4PY in PBMCs, plasma and urine samples. All three metabolites are considered biomarkers for changes in NAD^+ metabolism[78,115,116]. Strikingly, NR supplementation increased NAAD, an intermediate in the Preiss–Handler pathway (Fig. 2). This increase in NAAD in whole blood after NR administration has been replicated in several other clinical trials[114,115,117–120] (Table 2) and is likely to occur because orally administered NR is converted into NA (from NAM) by the gut microbiota. Another explanation for the increase in NAAD after NR intake could be the action of endogenous base-exchange enzymes, such as CD157 (refs. 53–55) (see the section titled 'In vivo pharmacokinetics of NAD^+ precursors').

NR and NMN consistently increase whole-blood NAD^+ levels (Fig. 1 and Table 2), firmly establishing their ability to be metabolized into NAD^+. By contrast, NAD^+ levels in muscle consistently remain unchanged[114,115,120–122] (Fig. 1). This raises the question of whether flux through NAD^+ is occurring in muscle without affecting its steady-state levels. Clearly, NR and NMN affect the NAD^+ metabolome in muscle, as evidenced by increased levels of downstream NAD^+ metabolites, such as NAMOx, MeNAM, Me2PY and Me4PY. However, these metabolites cannot be used as accurate indicators of NAD^+ flux, as NR can be directly converted to NAM and subsequently methylated or oxidized and excreted (Fig. 2). The extent to which these NAM clearance products result from the flux through NAD^+ remains unknown. The Michaelis constant (K_M) value of human NAMPT for NAM is approximately 1 µM (ref. 8), whereas NNMT has a K_M value of 430 µM for NAM[123]. This suggests that NAMPT is the primary enzyme active at low NAM concentrations. However, at increasing NAM concentrations, the enzymatic activity of NNMT would become more prominent to excrete excess NAM through MeNAM. Although NAAD in muscle may seem to be a better reflection of increased NAD^+ flux[78,114,115,120], the lack of NADS expression in muscle makes it unlikely that this is a reliable biomarker for NAD^+ flux in this tissue[32]. In either case, despite the affected muscle NAD^+ metabolism, no metabolites of the salvage pathway were increased following NR or NMN supplementation, and none of the reported studies observed improved muscle mitochondrial function[114,115,120–122,124]. This contrasts with studies performed in various rodent disease models supplemented with NR or NMN, where improved mitochondrial function was reported[42,104,111,125]. However, not all of the above rodent studies observed increased steady-state muscle NAD^+ abundance following NR or NMN supplementation[5,42].

Regarding the tissue-specific effects of NR and NMN, one study is of particular interest[114]. In this double-blinded, randomized, placebo-controlled study, 30 individuals with Parkinson's disease received 1,000 mg NR per day or placebo for 30 days. Following the study, PBMCs, skeletal muscle and CSF samples were obtained for the measurement of NAD^+ and related metabolites, whereas cerebral NAD^+ levels were analysed using MRS (Tables 2 and 3). Increased levels of cerebral NAD^+ were detected in 10 of 13 individuals, while all participants showed increased levels of NAD^+-related metabolites in PBMCs, skeletal muscle and CSF. This suggests that an increase in NAD^+ levels is possible in target organs even when peripheral tissues have unaffected NAD^+ concentrations, emphasizing the importance of the overall metabolite profile rather than a single metabolite. This study indicates that NR can increase NAD^+ levels behind the blood–brain barrier, highlighting the potential of NAD^+-boosting agents in the treatment of neurodegenerative diseases. However, further research is needed to fully understand these effects. Furthermore, other central tissues, such as the liver, kidneys and reproductive system, have not been pharmacokinetically studied or evaluated in clinical trials following NAD^+ precursor treatment. This represents a critical gap in understanding their translatability from the extensive number of preclinical studies.

https://doi.org/10.1038/s42255-025-01387-7

Table 2 | Published clinical trials using NR or NMN and their effect on the NAD^+ metabolome in skeletal muscle and whole blood

Tissue	NAD^+ precursor	Group treated	Dose regimen	Change in NAD^+	Change in NAD^+ metabolome	Reference
Skeletal muscle	NR	Individuals with peripheral artery disease	1,000 mg per day for 6 months	↔ NAD^{+a}	N/A	162
	NR	Individuals with Parkinson's disease	1,000 mg per day for 30 days	↔ NAD^{+b}	↑ NAAD, NAMOx, MeNAM, Me2PY, Me4PY ↔ NA, NAR, NR, NAM, NMN, NAMN, NADH, $NADP^+$, NADPH, NAADP, ADPR	114
	NR and exercise	Healthy	1,000 mg per day for 7 days	↔ NAD^{+a}	↑ NAMN, NAR, Me2PY, Me4PY ↔ NR, NADP, NAM, NMN, MeNAM, ADPR	122
	NR	Individuals with obesity	1,000 mg per day for 12 weeks	↔ NAD^{+a}	↔ NADH, $NADP^+$, NADPH	121
	NR	Individuals with obesity	1,000 mg per day for 6 weeks	↔ NAD^{+a}	↑ NAAD, MeNAM ↔ NADH, NADP, NADPH, NAM, NMN	120
	NR	Older adults	1,000 mg per day for 21 days	↔ NAD^{+a}	↑ NAAD, MeNAM, Me2PY ↔ NR, NAM, $NADP^+$, Me4PY	115
	NMN	Postmenopausal women with overweight or obesity	250 mg per day for 10 weeks	↔ NAD^{+a}	↑ Me2PY, Me4PY	124
Whole blood	NR	Individuals with chronic obstructive pulmonary disease	2,000 mg per day for 6 weeks	↑ NAD^{+a}	N/A	163
	NR	Older adults	250 mg per day increased by 250 mg per week up to 1,000 mg per day for 6 weeks	↑ NAD^{+a}	↑ NMN, NAAD, Me4PY ↔ ADPR	164
	NR	Individuals with ataxia telangiectasia	150–500 mg per day over 2 months; 500 mg per day for an additional 22 months	↑ NAD^{+a}	N/A	165
	NR	Individuals with Parkinson's disease	3,000 mg per day for 4 weeks	↑ NAD^{+a}	↑ NMN, NAAD, $NADP^+$, NAM, NAMOx, MeNAM, Me2PY, ADPR ↔ NAR	71
	NR	Individuals with obesity	250 mg per day increased by 250 mg per week up to 1,000 mg per day for 5 months	↑ NAD^{+a}	↑ NAAD, NMN, NAR, Me4PY, NADP, ADPR ↔ NMN, NADP, ADPR	117
	NR	Healthy	1,000 mg per day for 7 days	↑ NAD^{+a}	↑ NAAD, ADPR Me4PY	118
	NR	Individuals with heart failure	500 mg per day increased by 500 mg per week up to 2,000 mg per day for 12 weeks in total	↑ NAD^{+a}	↔ NR	166
	NR	Individuals with heart failure	500 mg per day (day 1); 1,000 mg per day (day 2); 2,000 mg per day (days 5–9)	↑ NAD^{+b}	N/A	167
	NR	Healthy	100, 300 and 1,000 mg per day for 8 weeks	↑ NAD^{+a}	N/A	168
	NR	Older adults	1,000 mg per day for 21 days	↑ NAD^{+a}	↑ NR, NMN, NAAD, NAMOx, MeNAM, Me2PY, Me4PY ↔ NAM, NADP, ADPR	115
	NR	Healthy	250 mg per day (days 1 and 2); 1,000 mg twice per day (days 7 and 8); 1,000 mg (day 9)	↑ NAD^{+a}	↑ NR	144
	NMN	Middle-aged and older adults	1,000 mg once to twice daily for 14 days	↑ NAD^{+a}	↑ Me2PY, MeNAM, NMN ↔ NAM	72
	NMN	Healthy	300 mg administered intravenously; blood collected up to 5 h after start	↑ NAD^{+a}	↔ NADH	169
	NMN	Healthy	250 mg per day for 12 weeks	↑ NAD^{+a}	↑ NAMN ↔ NMN, NAAD, NR, NAR NAM, NA, MeNAM	170
	NMN	Older adults	250 mg per day for 6 or 12 weeks	↑ NAD^{+a}	↑ NMN, NR, NAMN, NAR ↔ NA, NAM	134

'↑' and '↔' indicate a statistically significant increase or a non-significant change, respectively, in metabolite levels either between before and after treatment or between treatment and placebo. Studies that did not assess NAD^+ or NAD^+-related metabolites, as well as combination therapies such as NR and pterostilbene, were excluded. The compound was administered orally unless otherwise stated. N/A indicates that the metabolite was not reported or the level was below the detection limit. NAADP, nicotinic acid adenine dinucleotide phosphate; NADPH, nicotinamide adenine dinucleotide phosphate. [a]NAD^+ was measured as NAD^+ alone. [b]NAD^+ was measured as NAD^++NADH.

https://doi.org/10.1038/s42255-025-01387-7

Table 3 | Published clinical trials using NR or NMN and their effect on the NAD$^+$ metabolome in other tissues, cellular fractions and bodily fluids

Tissue	NAD$^+$ precursor	Group treated	Dose regimen	Change in NAD$^+$	Change in NAD$^+$ metabolome	Reference
Plasma	NR	Individuals with Werner syndrome	1,000 mg per day for 26 weeks	↑ NAD$^+$[a]	↑ NR, Me4PY, MeNAM, NAMOx	171
	NR	Individuals with ataxia telangiectasia	25 mg per kg per day (max. 900 mg) for 4 months	N/A	↑ Me2PY, Me4PY, MeNAM, NAM	172
	NR	Healthy	100, 300 and 1,000 mg per day for 8 weeks	N/A	↑ MeNAM, Me2PY	168
	NR	Healthy	100–1,000 mg single dose	N/A	↑ NAM, MeNAM, Me2PY, Me4PY	78
	NMN	Healthy	250 mg per day for 12 weeks	↑ NAD$^+$[a]	↑ NMN	173
	NMN	Healthy	100, 250 and 500 mg single dose	N/A	↑ Me2PY, Me4PY	174
Serum	NMN	Healthy	250 mg per day for 12 weeks	N/A	↑ NAM	175
	NMN	Middle-aged	300, 600 or 900 mg per day for 60 days	↑ NAD$^+$[b]	↑ NADH	176
	NMN	Postmenopausal women	300 mg per day for 8 weeks	↓ NAD$^+$[c]	↑ NAM ↔ NMN	177
PBMCs	NR	Individuals with Parkinson's disease	1,000 mg per day for 30 days	↔ NAD$^+$[b]	↑ NAAD, MeNAM ↔ NA, NR, NAM, NMN, NAMN, NADH, NADP$^+$, NADPH, NAADP, ADPR	114
	NR	Older adults	1,000 mg per day for 6 weeks	↑ NAD$^+$[a]	↑ NAAD ↔ NADP, NAM, NMN	119
	NR	Healthy	100–1,000 mg single dose	↑ NAD$^+$[a]	↑ NAAD, MeNAM, Me2PY ↔ NAM, NMN	78
	NMN	Middle-aged	125 mg per day for 8 weeks	↑ NAD$^+$[a]	N/A	178
	NMN	Individuals with hypertension	800 mg per day for 30 days	↑ NAD$^+$[a]	↑ NMN	179
	NMN	Postmenopausal women with overweight or obesity	250 mg per day for 10 weeks	↑ NAD$^+$[a]	N/A	124
Plasma-derived neuronal-origin extracellular vesicles	NR	Older adults	1,000 mg per day for 6 weeks	↑ NAD$^+$[a]	↔ NADH	180
Red blood cells	NR	Young individuals and older adults	500 mg single dose	N/A	↑ NADH, NADPH	133
Cerebrum	NR	Individuals with Parkinson's disease	1,000 mg per day for 30 days	↑ NAD$^+$[a]	N/A	114
CSF	NR	Individuals with Parkinson's disease	1,000 mg per day for 30 days	↔ NAD$^+$[a]	↑ Me2PY	114
Urine	NR	Individuals with Parkinson's disease	3,000 mg per day for 4 weeks	N/A	↑ NAR, NAM, NAMOx, MeNAM, Me2PY ↔ NR, NMN	71
	NR	Healthy	100, 300 and 1,000 mg per day for 8 weeks	N/A	↑ MeNAM, Me2PY	168
	NR	Older adults	1,000 mg per day for 21 days	N/A	↑Me4PY, NR, NAR, NAM, NAMOx, MeNAM, Me2PY	115
	NR	Individuals with obesity	2,000 mg per day for 12 weeks	N/A	↑ NR, NAM, MeNAM, NAMOx, Me2PY, Me4PY, NAR	116
	NR	Healthy	100–1,000 mg single dose	N/A	↑ NAM, MeNAM, Me2PY, Me4PY	78
	NMN	Middle-aged and older adults	1,000 mg once to twice daily for 14 days	N/A	↑ NAM, Me2PY ↔ NMN	72

'↑', '↓' and '↔' indicate a statistically significant increase, a statistically significant decrease or a non-significant change, respectively, in metabolite levels either between before and after treatment or between treatment and placebo. Studies that did not assess NAD$^+$ or NAD$^+$-related metabolites, as well as combination therapies such as NR and pterostilbene, were excluded. N/A indicates that the metabolite was not reported or the level was below the detection limit. [a]NAD$^+$ was measured as NAD$^+$ alone. [b]NAD$^+$ was measured as NAD$^+$+NADH. [c]There was uncertainty on the methodology.

Discussion and future perspectives

The mechanism behind the changes in NAD$^+$ levels with age and in age-related diseases, as well as how NAD$^+$ boosters can enhance these levels, is not yet fully elucidated. Many tissues remain insufficiently studied but hold potential interest for future research. Some tissues have not been adequately studied in humans, for obvious practical reasons, and predominantly rely on rodent models. The kidney, for example, is crucial for NAD$^+$ homeostasis, similar to the liver, and renal NAD$^+$ levels decrease with age in mice[126]. However, there are several reasons why a nuanced view on translating mouse results to humans is warranted. First, mice have a much higher metabolic rate than humans[127]. This might favour the generation of NAD$^+$ deficits

during pathophysiological challenges compared with humans, thereby magnifying the benefits of NAD^+ supplementation. Second, the use of different NAD^+ biosynthesis pathways can vary between rodents and humans. This is the case, for example, with the de novo pathway, as rodents show a higher tryptophan-to-NAD^+ conversion rate compared with humans[39]. Therefore, it cannot always be assumed that situations of NAD^+ deficiency and the corresponding therapeutic efficacy of NAD^+ precursors will be comparable across these species.

Multiple methodologies are used to quantify NAD^+ levels (Box 1). Recent advancements include the development of a luminescent-based protocol using commercially available NAD^+ and NADH detection kits, which provide improved sensitivity and are designed for straightforward and reproducible application[128]. However, differences in analytical accuracy across methods contribute to discrepancies in reported results. Additionally, NAD^+ concentration is often reported in varying units, complicating comparisons between studies, and is sometimes conflated with total NAD(H) values. Experimental studies also frequently lack detailed information on sample storage conditions and handling procedures, which can have an effect on NAD^+ stability[102]. NAD^+ levels also decrease due to the freezing and thawing of samples, which is likely to affect whole-blood samples the most[103,129]. This phenomenon is often not specifically addressed. Additionally, given that most NAD^+ is contained within red blood cells, factors such as red blood cell count and haematocrit levels are crucial for normalization purposes, as dietary intake and fluid consumption may influence the outcome. All of these factors underscore the need for thorough protocol validation before sample collection, preparation and analysis to minimize preanalytical effects. The discrepancies and limitations of the human studies mentioned in this Review highlight the need for more rigorous and specific research in these tissues to better understand the role of NAD^+ across different biological contexts.

Substantial focus has been placed on the clinical relevance of NR and NMN ever since the first clinical trial 9 years ago[78]. Despite several completed studies, the measured biochemical effects of NAD^+ precursors on human tissues remain primarily limited to blood and muscle. Non-invasive techniques, such as MRS for assessing NAD^+ metabolism in difficult-to-access tissues, should be encouraged to elucidate the full tissue-specific effects and regulation of NAD^+-boosting therapies[130].

Given the poor bioavailability of NR and NMN, their physiological roles remain largely enigmatic. As proposed in a recent review[6], it is often overlooked that several molecules are critically involved in NAD^+ metabolism. One of them is PRPP, which is a mandatory cosubstrate in the reaction catalysed by QPRT, NAPRT and NAMPT[2]. In contrast to NA or NAM, NAD^+ synthesis from nucleosides such as NR or NAR does not require PRPP. This is also true for mononucleotides such as NMN or NAMN, which enter the pathway downstream of the PRPP-dependent steps. Given the participation of PRPP in multiple cellular pathways, including the generation of purine and pyrimidine nucleotides for DNA synthesis[131], situations requiring a high rate of nucleotide synthesis might lead to limited PRPP availability, which in turn could present a bottleneck for the ability to generate NAD^+ from NA or NAM. In such scenarios, the use of NR as a NAD^+ precursor might enable cells to bypass this limitation and survive. However, whether these situations exist in human pathophysiology is still unknown.

It remains enigmatic why NR and NMN seem to have higher therapeutic success in preclinical models compared with human settings, particularly regarding their effects on muscle performance and mitochondrial function. Interestingly, a clinical study using NA at a dose of 750–1,000 mg per day for 4–10 months reported improved muscle function and mitochondrial biogenesis in patients with mitochondrial myopathy[132]. Muscle NAD^+ levels in these patients increased by 2.3-fold by month 10. Healthy individuals received the same dose of niacin (NA) for 4 months but were not included in the evaluation at 10 months. Of note, basal NAD^+ levels in the muscle of patients with mitochondrial myopathy were lower than those in healthy individuals. The lower basal NAD^+ content may represent an important parameter when attempting to increase NAD^+ levels in muscle, suggesting that a low initial NAD^+ content might be necessary to significantly boost muscle NAD^+ levels. This finding is essential in justifying the use of NAD^+ precursors, as it emphasizes the importance of the conditions under which NAD^+-boosting therapy is used. Individuals with physiological muscle homeostasis will probably not experience apparent benefits from boosting NAD^+ levels. This is supported by other clinical trials reporting slight improvements in physical performance among older adults following NR or NMN treatment, but no improvement in younger individuals[133–135]. Unfortunately, none of these studies assessed the muscle NAD^+ metabolome.

A clear trend emerges from this Review: a consistent age-related decline in NAD^+ levels is observed in muscle but not in whole blood. However, NAD^+ boosting does not seem to change muscle NAD^+ levels while easily increasing whole-blood NAD^+ concentration. Finally, focusing on NAD^+ as the only readout is insufficient to conclude a therapeutic effect, as there is little evidence of what would be improved with increased NAD^+ levels in healthy individuals of any age. As NAD^+ precursors have not been shown to mitigate any disease in humans, with the exception of pellagra—which is caused by an unbalanced diet lacking tryptophan or other NAD^+ precursors, or by secondary absorption problems—more clinical trials are needed to explore the underlying mechanisms, NAD^+ metabolism and context-dependent clinical benefits.

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Acknowledgements

The authors gratefully acknowledge the contributions of the NADIS consortium members: F.L. Bertoli (Institut Imagine Paris, France), R.P. Mato (Università degli Studi di Genova, Italy), T. Brochard (University of Oulu, Finland), J. Frank (University of Oslo, Norway), F. Matiyevskaya (University of Copenhagen, Denmark), M.E. Ermert (Khondrion BV, Nijmegen; Amsterdam UMC, Netherlands), Q. Zhang (University of Copenhagen, Denmark), L. Van Gijn (École Polytechnique Fédérale de Lausanne, Switzerland), S. Bruzzone (Università degli Studi di Genova, Italy), M. Deleidi (Institut Imagine Paris, France), M. Scheibye-Knudsen (University of Copenhagen, Denmark), E.F. Fang (University of Oslo, Norway), E. Pirinen (University of Oulu, Finland), H. Renkema (Khondrion BV, Nijmegen, Netherlands) and J. Smeitink (Khondrion BV, Nijmegen, Netherlands). K.T.V., M.M.T., C.C., G.E.J. and R.H.H. received funding from the European Union's Horizon Europe research and innovation programme through the NADIS project (#101073251). R.Z.-P. is supported by a project (PID2023-147560OA-I00) from the Ministerio de Ciencia, Innovación y Universidades-Agencia Estatal de Investigación and by the Fondo Europeo de Desarrollo Regional (FEDER, EU).

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K.T.V., M.M.T., C.C., R.Z.-P., G.E.J. and R.H.H. conceptualized the article. K.T.V. and M.M.T. wrote the manuscript, with contributions from all coauthors. K.T.V., M.M.T. and E.C. researched data and created the tables. M.v.W. provided substantial input on technical and methodological aspects. All authors reviewed, edited and approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

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Peer review information *Nature Metabolism* thanks Marie Migaud and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Primary Handling Editor: Christoph Schmitt, in collaboration with the *Nature Metabolism* team.