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NAD+ homeostasis in health and disease

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The conceptual evolution of nicotinamide adenine dinucleotide (NAD+) from being seen as a simple metabolic cofactor to a pivotal cosubstrate for proteins regulating metabolism and longevity, including the sirtuin family of protein deacylases, has led to a new wave of scientific interest in NAD+. NAD+ levels decline during ageing, and alterations in NAD+ homeostasis can be found in virtually all age-related diseases, including neurodegeneration, diabetes and cancer. In preclinical settings, various strategies to increase NAD+ levels have shown beneficial effects, thus starting a competitive race to discover marketable NAD+ boosters to improve healthspan and lifespan. Here, we review the basics of NAD+ biochemistry and metabolism, and its roles in health and disease, and we discuss current challenges and the future translational potential of NAD+ research.

NAD, the first cofactor described, was discovered in 1906 (ref. [1]), when it was observed that yeast juice separated into low- and high-molecular-weight fractions lost the capacity to perform alcoholic fermentation. However, addition of the low-molecular-weight fraction to yeast accelerated the fermentation reaction, even if it was boiled beforehand. This observation suggested that a yet-unknown heat-stable factor present in the low-molecular-weight fraction was required for fermentation. At that time, this factor was named 'cozymase'. Almost three decades later, the chemical composition of cozymase, comprising an adenine, a phosphate and a reducing-sugar group, was reported[2]. The actual function of NAD+, however, remained unclear until 1936, when cozymase was shown to be capable of transferring hydride between molecules with the nicotinamide base as its redox site[3]. This research on NAD+ was rewarded by three Nobel prizes. In the early 2000s, interest in NAD+ experienced a resurrection, after a novel role of NAD+ as a cosubstrate for the sirtuin enzyme family, important regulators of longevity and metabolism, was discovered[4–6].

A short introduction to NAD+

NAD+ and NADH and related redox reactions. The NAD+ and NADH (NAD(H)) redox couple is essential for a variety of electron-exchange-dependent biochemical reactions, particularly redox reactions involving oxidoreductase-mediated hydride transfer. NAD+ acts as an electron acceptor in these reactions, whereas NADH acts as an electron donor. Many reactions that require NAD(H) as a coenzyme are linked to catabolism and harvesting of metabolic energy (Fig. 1): the oxidized form, NAD+, serves a cofactor for enzymes involved in (1) alcohol metabolism (alcohol and aldehyde dehydrogenases), (2) glycolysis (glyceraldehyde phosphate dehydrogenase), (3) oxidative decarboxylation of pyruvate to acetyl-CoA (pyruvate dehydrogenase), (4) fatty acid β-oxidation (3-hydroxyacyl-CoA dehydrogenase) and (5) tricarboxylic acid cycle (α-ketoglutarate, isocitrate and malate dehydrogenases). NAD+ also acts as a cofactor for lactate dehydrogenase (LDH) in the liver during the Cori cycle. However, in most cases, LDH catalyses the reverse reaction, the reduction of pyruvate to lactate, thereby harvesting electrons from NADH and hence regenerating cellular NAD+ to assist in glycolysis (Fig. 1).

Similarly to LDH, glyceraldehyde phosphate dehydrogenase, which normally catalyses the sixth step of glycolysis, can catalyse the reverse reaction in the liver by using NADH as coenzyme during gluconeogenesis (Fig. 1). NADH also acts as a cofactor for desaturases, enzymes involved in the synthesis of highly unsaturated fatty acids. Intriguingly, this process has recently been reported as an alternative mechanism to restore the cellular NAD+ pool and fuel glycolysis[7] (Fig. 1). Finally, NADH delivers electrons to the electron-transport chain and is thereby instrumental in oxidative phosphorylation (Fig. 1).

NAD(H) and NADP(H). Approximately 10% of the total NAD(H) is phosphorylated at the adenosine riboside site by NAD+ kinases, thus leading to the de novo synthesis of nicotinamide adenine dinucleotide phosphate (NADP(H))[8–10]. Despite being structurally related, NAD(H) and NADP(H) are typically recognized by different enzymes and thus exhibit distinct functions. Whereas NAD(H) primarily participates in catabolic reactions, NADP+ and its reduced counterpart, NADPH, are mainly required for anabolic reactions and cellular oxidative-stress defence.

NADP+ plays an essential role as a cofactor for the rate-limiting step of the pentose-phosphate pathway. This pathway not only produces precursors for the synthesis of nucleotides and aromatic amino acids, but also generates substantial amounts of cytosolic NADPH. Alternatively, NADPH can be generated by other NADP+-linked enzymes, including the mitochondrial and cytosolic isoforms of isocitrate dehydrogenase and malic enzyme, glutamate dehydrogenase, members of the aldehyde dehydrogenase family, as well as the nicotinamide nucleotide transhydrogenase (NNT)[11,12].

NADPH in turn is required for (1) oxidative-stress defence by glutathione reductase and (2) immune responses mediated by NADPH oxidase, which produces free radicals for the defence against pathogens ('respiratory burst')[13], as well as for the (3) reductive biosynthesis of fatty acids, cholesterol and steroids. Moreover, NADP+ serves as a precursor for the synthesis of nicotinic acid adenine dinucleotide phosphate, a critical second messenger for intracellular Ca^{2+} mobilization[14].

Interestingly, C57BL/6J mice, a widely used laboratory strain in metabolic research, have a mutation in the gene encoding NNT, thus leading to a five-exon deletion. The production of a non-functional NNT protein has been associated with several mitochondrial redox abnormalities, including changes in the ratio of oxidized to reduced glutathione, enhanced H_2O_2 release and a

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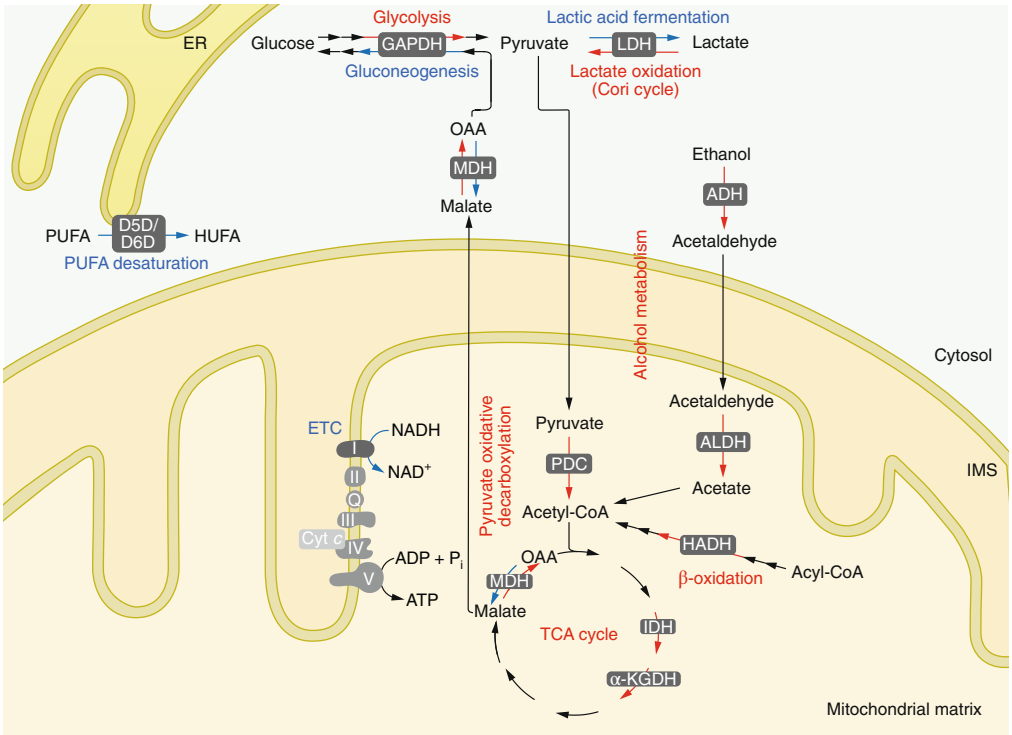

Fig. 1 | Main biochemical reactions requiring NAD⁺/NADH as a coenzyme. Many crucial metabolic pathways rely on the NAD⁺/NADH redox couple, which act as coenzymes in reactions involving electron exchange. Metabolic reactions in which NAD⁺ accepts electrons are highlighted in red, and the names of the corresponding enzymes are annotated. Metabolic processes involving NAD⁺ include glycolysis, the tricarboxylic acid (TCA) cycle, β-oxidation, alcohol metabolism, lactate oxidation as part of the Cori cycle and the oxidative decarboxylation of pyruvate. Reactions in which NADH is oxidized are highlighted in blue. These reactions include the reduction of pyruvate to lactate, desaturation required for the synthesis of highly unsaturated fatty acids, and the conversion of 1,2-bisphosphoglycerate into glyceraldehyde 3-phosphate, and of oxaloacetate (OAA) into malate required to transport OAA out of mitochondria during gluconeogenesis. Finally, NADH is recycled back into NAD⁺ by donating electrons to complex I of the respiratory chain. α-KGDH, α-ketoglutarate dehydrogenase; ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; cyt *c*, cytochrome *c*; D5D/D6D, delta-5/delta-6 desaturase; ETC, electron transport chain; GAPDH, glyceraldehyde 3 phosphate dehydrogenase; HADH, 3-hydroxyacyl-CoA dehydrogenase; HUFA, highly unsaturated fatty acids; IDH, isocitrate dehydrogenase; ER, endoplasmic reticulum; IMS, intermembrane space; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; PDC, pyruvate dehydrogenase complex; PUFA, polyunsaturated fatty acids; Q, coenzyme Q; P_i, inorganic phosphate.

diminished capacity to metabolize peroxide[15–17]. These findings not only highlight the importance of NADP(H) in cellular biology and mitochondrial function, but also should be considered in interpreting metabolic data obtained from these mice. More details on the different functions of NAD(H) and NADP(H) can be found in other reviews[18,19].

NAD⁺ as a cosubstrate in non-redox reactions. The discovery that NAD⁺ acts as cosubstrate for the sirtuin family of deacylases and thereby effectively coordinates mitochondrial function, metabolism and ageing, heralded a new era of NAD⁺ research at the very beginning of the twenty-first century. In 1979, the 'sirtuin family founder', silent information regulator 2 (SIR2), was identified in the yeast *Saccharomyces cerevisiae* as a regulator of yeast mating type[20,21]. Two decades later, SIR2 was shown to increase yeast lifespan[22] and was crucially found to be an NAD⁺-dependent histone deacetylase in the year 2000 (refs. [4,23]). Mammalian sirtuins are now well established not to act exclusively as deacetylases but to also catalyse the removal of different acyl groups, such as malonyl, succinyl and propionyl as well as several others.

Sirtuins remove an acyl group from their substrate by using NAD⁺ as a cosubstrate, thereby generating the deacylated substrate 2-*O*-acyl-ADP-ribose and nicotinamide (NAM) as end products (Fig. 2). In mammals, seven members of the sirtuin family have been identified. All the sirtuins share the highly conserved NAD⁺-binding domain[24] but differ in their Michaelis constant (K_m) values for NAD⁺ (Table 1).

On the basis of their K_m values, sirtuins can be divided into two groups: (1) those with K_m values below the physiological range (~300–700 μM)[25] of NAD⁺, including SIRT2, SIRT4, SIRT5 and SIRT6, for which NAD⁺ might not necessarily be rate limiting, and (2) those whose activity is highly dependent on NAD⁺ availability, such as SIRT1 and SIRT3 (Table 1).

Because NAD⁺ levels provide a readout for the energetic state of a cell, some sirtuins are thought to act as metabolic sensors that adjust cellular metabolism according to metabolic needs. However, not all sirtuins are tightly regulated by cellular energy status. For example, SIRT3 acts as a non-specific eraser of acyl groups within mitochondria, where its activity follows the laws of mass action and is principally governed by acyl-CoA levels and perturbed pK_a values of lysine residues[26].

Beyond sirtuins, other important NAD⁺-consuming enzymes have been identified, such as the cyclic ADP-ribose (cADPR) synthases (including CD38 and CD157) and the poly(ADP-ribose) polymerase (PARP) protein family. Comprising 17 members in humans and 16 members in mice, the PARP protein family has a central role in DNA repair and preservation of genomic integrity[27]. More specifically, PARPs are responsible for PARylation, a reversible post-transcriptional protein modification, in which a large polymer of ADP-ribose moieties is added to the target protein,

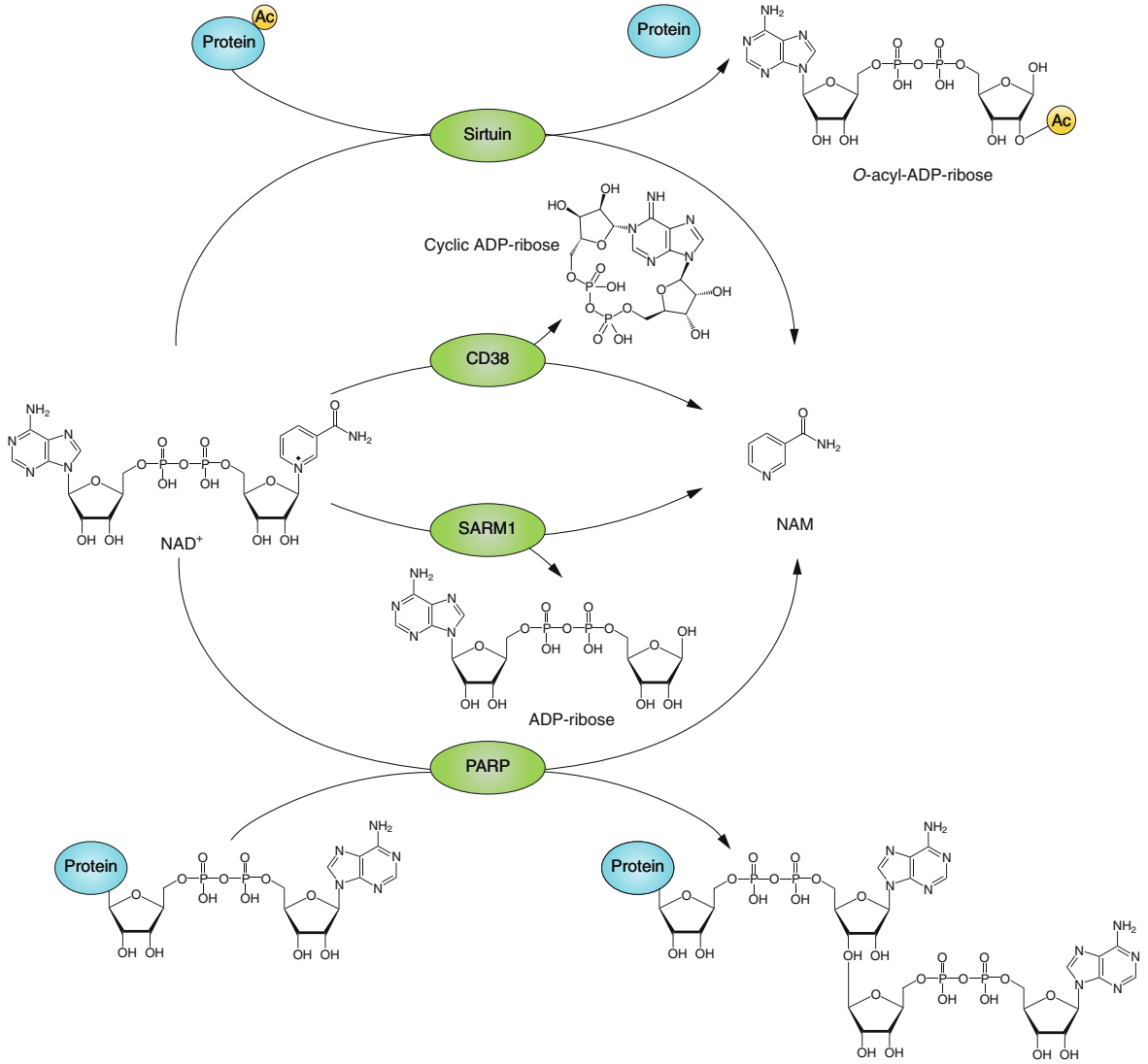

Fig. 2 | The main NAD⁺-consuming enzymes. NAD⁺ serves as a cosubstrate for a variety of enzymes with crucial roles in metabolism, ageing and cell survival. Arrows indicate reactions mediated by NAD⁺-consuming proteins (green). Molecular structures are represented for the initial and end products, along with by-products of each reaction. Comprising seven proteins in humans, the sirtuin family of enzymes mediates deacylation of several important proteins. The reactions catalysed by sirtuins involve removal of an acyl group (such as acetyl, malonyl, succinyl, crotonyl or propionyl) from the substrate, thus resulting in the generation of *O*-acyl-ADP-ribose with concomitant consumption of NAD⁺. NAD⁺ in turn is hydrolysed into nicotinamide (NAM). PARPs are another important group of NAD⁺ consumers. During the PARylation reaction catalysed by PARPs, multiple ADP-ribosyl groups are attached to protein substrates, and NAD⁺ is hydrolysed into NAM. The third group of NAD⁺-consuming enzymes is represented by cyclic ADP-ribose synthases, which regulate calcium homeostasis. CD38 catalyses the generation of cyclic ADP-ribose by using NAD⁺, which is converted into NAM. The TIR domain of SARM1 drives the cleavage of NAD⁺ into NAM and ADP-ribose (and cyclic ADP-ribose to a lesser extent), thus qualifying the TIR-domain-containing proteins as a family of NAD⁺ consumers. Ac, Acyl.

which is accompanied by NAD⁺ hydrolysis into NAM (Fig. 2). The main NAD⁺ consumers among the PARPs are PARP-1 and PARP-2. PARP-1 alone accounts for 90% of all NAD⁺ used by the PARP family[27].

In contrast to sirtuin family proteins, PARP activity appears not to be affected by fluctuations in NAD⁺ levels, because the K_m values for NAD⁺ are far below the physiological range of NAD⁺ (Table 1). Thus, PARPs have an advantage over sirtuins in competing for limiting NAD⁺ resources[27]: NAD⁺ availability would become rate limiting for sirtuin activity in situations in which PARPs are overactivated, as observed during ageing or after DNA damage[10,28–30]. In addition to PARPs, the less studied enzymes responsible for mono-ADP-ribosylation use NAD⁺ as a cosubstrate, but to a markedly lower extent than the PARPs[31].

Another family of NAD⁺ consumers is the cADPR synthases, which use NAD⁺ to produce cADPR (Fig. 2), an important cellular regulator of Ca^{2+} homeostasis[32]. cADPR synthases indirectly affect multiple Ca^{2+}-dependent activities, including cell proliferation, muscle contraction, immune responses and glucose-stimulated insulin secretion from pancreatic β-cells[33]. The most prominent cADPR synthases are CD38 and its homolog CD157. CD157 exhibits a cADPR-synthesis efficiency several hundred fold lower than that of CD38 (ref. [34]). Historically, CD38 was described as a cell-surface molecule specific to the immune system[35] but was later found to be ubiquitously expressed[33,36] and to have both extra- and intracellular enzymatic activity[37] and a low K_m value for NAD⁺ (refs. [38,39]) (Table 1). Consequently, CD38-deficient mice have up to 30-fold-higher NAD⁺ levels in tissues than wild-type mice[36].

Table 1 | NAD⁺-consuming enzymes and the corresponding K_m values of NAD⁺

Enzyme	K_m v (μM)	References
SIRT1	94–888	285–287
SIRT2	~100	285
SIRT3	280–880	288,289
SIRT4	35	290
SIRT5	26–200	291,292
SIRT6	13	293
SIRT7	-	
PARP1	20–97	294,295
PARP2	130	295
CD38	15–25	38,39
SARM1	24	41

Sterile alpha and TIR motif–containing 1 (SARM1) is an important NAD⁺ consumer in neurons. The dimerization of its Toll/interleukin receptor (TIR) domain consumes NAD⁺, generating mostly NAM and ADPR, but also small amounts of cADPR[40,41] (Fig. 2). NAD⁺ depletion caused by SARM1 is tightly associated with axonal destruction[42].

NAD⁺ RNA caps. RNA caps are nucleotide modifications, most commonly 7-methylguanylate added at the 5′ ends of primary transcripts, which regulate mRNA stability and translation. NAD⁺ linked to the 5′ ends of RNA was first observed in bacteria[43,44], in which it not only protects RNA against degradation[45] but also serves as a non-canonical initiation factor for de novo transcription[46]. The presence of NAD⁺ RNA caps was subsequently confirmed in yeast[47] and human[48] cells. In both prokaryotes and eukaryotes, the incorporation of the NAD⁺ into RNA (referred to as 'NADding') is performed by the respective RNA polymerases during transcription initiation[46,49,50] or occurs post-transcriptionally in the case of exonucleolytically generated non-coding RNAs in eukaryotes[48]. In contrast to NAD⁺ caps in bacteria, which promote RNA stability and translation, mammalian NAD⁺ caps instead accelerate RNA decay through an NAD⁺-decapping mechanism (known as 'deNADding')[48]. Recently, mitochondrial RNA polymerases have also been shown to efficiently cap mitochondrial RNA with NAD⁺ (refs. [51,52]), a finding that seems logical given the bacterial origin of mitochondria.

Despite major advances in the field, the physiological relevance of NAD⁺ capping remains to be elucidated. However, NAD⁺ capping might possibly represent a direct mechanism to modulate transcriptional output according to the cellular energy state.

NAD⁺ homeostasis and its maintenance

NAD⁺ synthesis. NAD⁺ can be produced from different forms of vitamin B_3, which include NAM, nicotinic acid (NA) and nicotinamide riboside (NR)[53]. All these molecules are known as NAD⁺ precursors. Alternatively, NAD⁺ can be synthetized via the de novo synthesis (DNS) pathway, from the essential amino acid tryptophan.

Although some authors use the term 'salvage pathway' to refer exclusively to the production of NAD⁺ from NAM[54], NAD⁺ salvage must be considered as the pathway responsible for recycling metabolites produced by cellular catabolism[55,56]. This view thus positions NAD⁺ salvage opposite of DNS, during which a relatively complex final molecule is built from low-molecular-weight compounds. With this definition, NAD⁺ salvage includes NAD⁺ production from all three forms of vitamin B_3 (that is, NAM, NA and NR) and consists of fewer biosynthetic steps than DNS, which requires formation of a pyridine ring.

The synthesis of NAD⁺ from NAM and NR is a two-step process, and these two pathways share a common intermediate, nicotinamide mononucleotide (NMN) (Fig. 3). To be transformed into NMN, NR must be phosphorylated by NR kinase (NRK), whereas a phosphoribosyl moiety must be added to NAM by NAM phosphoribosyltransferase (NAMPT). The common and the final step of these two salvage pathways is the conversion of NMN into NAD⁺ catalysed by NMN adenylyltransferase (NMNAT).

The pathway leading to NAD⁺ production from NA is known as the Preiss–Handler pathway and consists of three steps[57]. In the first reaction, NA is converted into NA mononucleotide (NAMN) by NA phosphoribosyltransferase (NAPRT). NAMN is then converted to NA adenine dinucleotide (NAAD) by NMNAT, which can use both NA mononucleotide and NAM mononucleotide as substrates and is therefore common to all three salvage pathways. The final step of the Preiss–Handler pathway consists of the conversion of NAAD into NAD⁺, catalysed by NAD synthetase (NADS) (Fig. 3).

The DNS pathway consists of seven steps, all but one of which are catalysed by an individual enzyme (Fig. 3). The single non-enzymatic reaction is the spontaneous cyclization of α-amino-β-carboxymuconate-ε-semialdehyde (ACMS) into quinolinic acid (QA). Interestingly, besides being transformed into QA, ACMS can also be decarboxylated by ACMS decarboxylase (ACMSD) (Fig. 3). Because the conversion of ACMS into QA occurs spontaneously, the proportion of ACMS leading to NAD⁺ is determined by the activity of ACMSD[58,59]. If QA is formed, it is converted into NAMN by quinolinate phosphoribosyltransferase (QPRT), which then feeds into the Preiss–Handler pathway.

Subcellular distribution of NAD⁺ and its biosynthetic enzymes. Many studies exploring subcellular NAD⁺ distribution and metabolism have experienced technical limitations in absolute NAD⁺ quantification, thus explaining divergent reports on subcellular NAD⁺ concentrations in the literature. Despite the lack of experimental evidence, however, NAD⁺ is generally recognized to be exchangeable between the cytosolic and nuclear NAD⁺ pools, most probably because the two pools exhibit comparable NAD⁺ concentrations[25,60,61]. However, the mitochondrial NAD⁺ pool was long believed to be separate from the nucleo-cytosolic pool because the regulation of mitochondrial NAD⁺ levels appears to differ from those in the rest of the cell[61]. This concept was based on several observations: (1) mitochondria have been shown to sustain NAD⁺ levels even after exhaustion of the nucleo-cytosolic NAD⁺ pool[62–64]; (2) mitochondria contain specialized enzymes for NAD⁺ synthesis[63,65,66]; and (3) the mitochondrial inner membrane is impermeable to NAD⁺ (refs. [67,68]). However, this long-held view has recently been challenged by the use of isotope-tracer methods, which have shown that mammalian mitochondria are not only capable of taking up NAD⁺ precursors, such as NMN and NR (as previously reported[69–71]), but also intact NAD(H)[72]. These results suggest the existence of a yet-unidentified mammalian mitochondrial NAD⁺ transporter. The existence of such a transporter is further supported by earlier reports showing that cytosolically produced NAD⁺ also increases the mitochondrial NAD⁺ pool[61] and that exogenously supplied NAD⁺ boosts mitochondrial NAD⁺ stores more efficiently than cytoplasmic stores[73]. Notably, mitochondrial NAD⁺ transporters are known to exist in yeast (Ndt1 and Ndt2)[74], in which loss of either of the two transporters leads to mitochondrial NAD⁺ depletion, whereas their overexpression increases the mitochondrial NAD⁺ content[74,75]. In mammalian cells, however, no comparable transporter has been identified to date[76].

Another key determinant of the compartment-specific NAD⁺ homeostasis is the subcellular distribution of the different NAD⁺ biosynthetic enzymes. The major site of de novo NAD⁺ synthesis, for example, appears to be the cytosol, because all enzymes of this pathway are localized there[25] (Table 2).

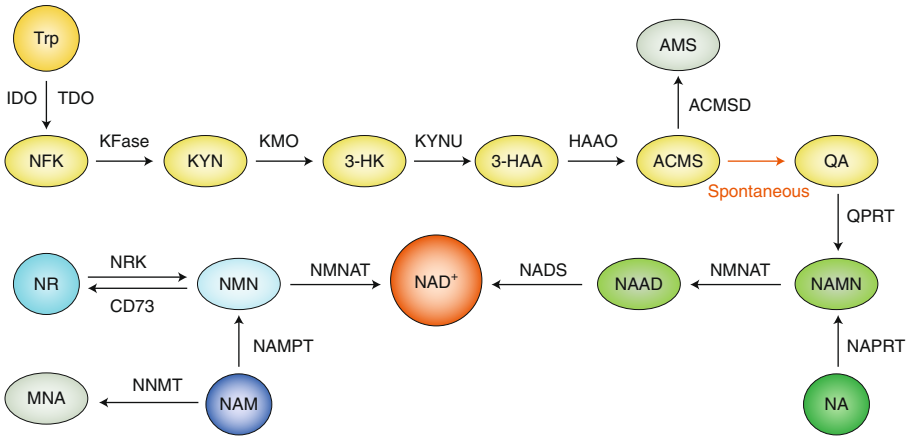

Fig. 3 | NAD⁺-biosynthesis pathways. NAD⁺ biosynthesis can be accomplished through different routes: it can be produced either de novo from the essential amino acid tryptophan (Trp) (yellow) or via salvaging, starting from different naturally occurring forms of vitamin B_3—nicotinic acid (NA) (green), nicotinamide riboside (NR) (light blue) and nicotinamide (NAM) (dark blue). The first reaction of de novo NAD⁺ biosynthesis opens the indole ring of tryptophan and is catalysed by either indoleamine-2,3-dioxygenase (IDO) or tryptophan-2,3-dioxygenase (TDO). Four consecutive enzymatic steps lead to production of 2-amino-3-carboxymuconate-6-semialdehyde (ACMS), which is then transformed into quinolinic acid (QA) in the unique non-enzymatic reaction of the de novo pathway. ACMS can otherwise be decarboxylated by ACMS decarboxylase (ACMSD), thus limiting the proportion of ACMS transformed into QA. QPRT catalyses the conversion of QA into NA mononucleotide (NAMN), which is also an intermediate in the Preiss–Handler pathway (green). In the Preiss–Handler pathway, NA is converted into NAD⁺ via three consecutive enzymatic reactions catalysed by NA phosphoribosyltransferase (NAPRT), NMN/NAMN adenylyltransferase (NMNAT) and NAD synthetase (NADS), respectively. The two remaining salvaging routes, starting either from NR or NAM, share the common intermediate NAM mononucleotide (NMN), which is generated by NR kinase (NRK) or NAM phosphoribosyltransferase (NAMPT), respectively. NAM can otherwise be methylated by NAM *N*-methyltransferase (NNMT). AMS, 2-aminomuconic-6-semialdehyde; CD73, ecto-5′-nucleotidase; 3-HAA, 3-hydroxyanthranilic acid; HAAO, 3-hydroxyanthranilic acid dioxygenase; 3-HK, 3-hydroxykynurenine; KFase, kynurenine formamidase; KMO, kynurenine 3-monooxygenase; KYN, kynurenine; KYNU, kynureninase; NAAD, NA adenine dinucleotide; NFK, *N*-formylkynurenine; MNA, 1-methyl-nicotinamide.

Similarly, many other enzymes important for NAD⁺ synthesis—including NADS, NRK1, NRK2 and NAPRT—have been solely detected in the cytosol[71,77,78]. In contrast, the three established isoforms of NMNAT, the enzyme central to all known NAD⁺-synthesis pathways (Table 2), are found in distinct subcellular compartments: NMNAT1 localizes to the nucleus[79,80], NMNAT2 is found in the cytosol and Golgi apparatus[80,81], and NMNAT3 is present in mitochondria[63,81,82].

These different subcellular distributions of the NMNAT isoforms and concomitant compartmentalization of NAD⁺ synthesis have been shown to control adipocyte differentiation: the depletion of the cellular NMN pool by increased cytosolic NMNAT2 activity limits the activity of NMNAT1 within the nucleus, thus leading to nuclear NAD⁺ depletion, which in turn results in decreased PARP-1 activity and enhanced proadipogenic transcriptional program[83].

Unlike NMNAT, NAMPT is ubiquitous in all NAD⁺-relevant cellular compartments: it is found in the nucleus[84], cytosol[84,85] and mitochondria[63]. Therefore, NAM is considered the common NAD⁺ precursor in all compartments.

The mitochondrial localization of NMNAT3 (refs. [86–88]) and NAMPT[64,71,89], however, remains controversial. For example, a recent study has found that isolated mammalian mitochondria are not able to synthesize NAD⁺ from NAM[72].

Interestingly, the different sirtuins also localize to different subcellular compartments: whereas SIRT1, SIRT6 and SIRT7 predominantly reside in the nucleus and, in the case of SIRT7, also in the nucleolus, SIRT2 typically localizes to the cytosol, and SIRT3, SIRT4 and SIRT5 localize to the mitochondria[6]. The compartmentalized regulation of NAD⁺ pools might be required to locally activate specific members of the sirtuin family and thereby adjust metabolism according to the metabolic requirements of the cell[90]. In line with this view, the subcellular NAD⁺ distribution has been found to be highly variable between tissues with different metabolic functions

Table 2 | Subcellular distribution of NAD⁺-synthesizing enzymes

Enzyme	Cytosol	Mitochondria	Nucleus	Other
NADS	x[78]			
NMNAT1			x[79,80,296]	
NMNAT2	x[80,81,297]			Golgi[81]
NMNAT3	x[82,87,88]	x[63,76,81,82] Not in mitochondria[86–88]		
NAMPT	x[84,85]	x[63] Not in mitochondria[64,71,89]	x[84]	
NAPRT	x[77]			
NRK1	x[71]			
NRK2	x[71]			
TDO	x[25]			
IDO	x[25]			
KFase	x[298–300]		x[298]	
KMO		x[301–303] (outer membrane)		
KYNU	x[304]			
HAAO	x[305]			
QPRT	x[25]			
ACMSD	x[298,306]			

The subcellular compartments where the enzymes were detected are marked with 'x'. TDO, tryptophan-2,3-dioxygenase; IDO, indoleamine-2,3-dioxygenase; KFase, kynurenine formamidase; KMO, kynurenine 3-monooxygenase; KYNU, kynureninase; HAAO, 3-hydroxyanthranilic acid dioxygenase.

Table 3 | Distribution of NAD$^+$-synthesizing enzymes in different organs, on the basis of abundance

Enzyme	Not detected	Present	Highly abundant
NMNAT1	–	Intestine, spleen, heart, brain, testis, SM, lung, pancreas, blood, adrenal gland[98,307]	Liver, kidney[98]
NMNAT2	Pancreas, adrenal gland[307]	Liver, kidney, intestine, spleen, heart, testis, SM, lung, blood[98,307]	Brain[98]
NMNAT3	Testis[307]	Intestine, spleen, brain, SM, lung, pancreas, blood, WAT[98,307]	Liver, kidney, heart, adrenal gland[98,307]
NADS	SM, lung, WAT[98,307]	Spleen, heart, testis, brain, pancreas, blood, adrenal gland[98,307]	Liver, kidney, intestine[98]
NAMPT	–	Intestine, spleen, heart, brain, testis, skeletal muscle, lung, pancreas, WAT, adrenal gland[102,103,307]	Liver, kidney, BAT[102,103]
NAPRT	Testis, SM, WAT[103,307]	Intestine, spleen, heart, brain, lung, pancreas[103]	Liver, kidney, adrenal gland[103,307]
NRK1	Spleen, WAT[107,307]	Intestine, heart, brain, testis, SM, lung, pancreas, BAT, adrenal gland[107,108,307]	Liver, kidney[107,108]
NRK2	Liver, kidney, intestine, spleen, brain, testis, lung, pancreas, BAT, adrenal gland[107–109,307]	Heart, WAT[107,108]	Skeletal muscle[107–109]
CD73	Brain, SM, BAT[107]	Heart, WAT[107]	Liver, kidney, intestine, spleen, testis, lung, pancreas, adrenal gland[107,307]
NNMT	Spleen, heart, testis, SM[307]	Kidney, intestine, brain, lung, pancreas, adrenal gland[307,308]	Liver, BAT, WAT[308,309]
TDO	Kidney, spleen, heart, brain, testis, SM, lung, pancreas, WAT, adrenal gland[307,310]	Intestine[307]	Liver[310]
IDO	Heart, brain, testis, pancreas, WAT, adrenal gland[307]	Liver, kidney, SM[310,311]	Intestine, spleen, lung[310]
KFase	Spleen, heart, SM, lung, pancreas, WAT[307]	Intestine, brain, testis, adrenal gland[307]	Liver, kidney[312]
KMO	Heart, brain, testis, pancreas, WAT, adrenal gland[307]	Intestine, spleen, SM, lung[310]	Liver, kidney, placenta[302,310]
KYNU	–	Intestine, heart, brain, testis, SM, lung, pancreas, WAT, adrenal gland[307,310,313]	Liver, kidney, spleen[310,313], placenta[304]
HAAO	Heart, brain, testis, pancreas, WAT, adrenal gland[307]	SM[310]	Liver, kidney, intestine, spleen, lung[310]
QPRT	Small intestine, spleen, heart, brain, testis, SM, lung, pancreas, WAT[103,307]	–	Liver, kidney, adrenal gland[103,307]
ACMSD	Intestine, spleen, heart, testis, SM, lung, pancreas, blood, BAT, WAT, adrenal gland[307,314]	Brain[314]	Liver, kidney[314–316]

BAT, brown adipose tissue; SM, skeletal muscle; WAT, white adipose tissue.

and needs: for example, heart cells and myocytes contain approximately 75% of the total NAD$^+$ in the mitochondria[68], whereas in hepatocytes, the bulk of NAD$^+$ is cytosolic[91].

Systemic regulation of NAD$^+$ homeostasis. In addition to the subcellular differences in NAD$^+$ homeostasis, organ-specific differences exist in both the expression of the different NAD$^+$ biosynthetic enzymes (Table 3) and the preference for specific NAD$^+$ precursors[10].

The liver and, to a lesser extent, the kidney appear to act as a hub for whole-body NAD$^+$ homeostasis, because all enzymes involved in NAD$^+$ biosynthesis, with only few exceptions, are highly expressed in these two organs (Table 3). Moreover, enzymes responsible for the de novo NAD$^+$ synthesis are expressed almost exclusively in the liver and kidney (Table 3). Accordingly, circulating tryptophan is efficiently converted into NAD$^+$ in the liver, whereas other organs appear to rely mainly on NAM or NA for NAD$^+$ synthesis[10,92].

Tryptophan, NAM and NA have the highest levels in plasma, reaching concentrations above 0.1 μM (ref. [10]). Whereas NA has been suggested to be the preferable NAD$^+$-precursor molecule[77,93–97], some studies have found NAM to be more important than NA[10,98–100]. A preference for NAM is supported by the observation that NAM is as much as ten times more abundant in plasma than NA[10,101]. Moreover, a recent study applying isotope-tracer-based technology has found that only the liver, kidney, pancreas, small intestine and spleen use NA, and do so to a much lesser extent than NAM[10]. Notably, these tissues also exhibit robust expression of the NAM- and NA-using enzymes, NAMPT and NAPRT (Table 3), respectively[102,103]. Other tissues, such as the heart, lung, brain, skeletal muscle and white adipose tissues (WAT), in which NAPRT expression is either low or completely absent[102,103] (Table 3), appear to rely mainly on NAM for NAD$^+$ synthesis[10].

The major provider of circulating NAM is the liver[10,92,104], which accounts for more than 95% of the NAM production from

tryptophan[10]. Beyond the liver, the kidney also uses tryptophan for NAD^+ production; NAD^+ is then converted into NAM by NAD^+-consuming enzymes, and NAM is subsequently excreted into the circulation, albeit at much lower rates than those in the liver[10]. Interestingly, both the liver and kidney can also produce NAD^+ from NA but generally show a preference for tryptophan over NA[10,92,104], thus positioning the long-underestimated de novo NAD^+-synthesis pathway at the centre stage of whole-body NAD^+ homeostasis.

Circulating NAM serves as an NAD^+ precursor for other organs. The rates at with organs take up NAM and produce or consume NAD^+, also called NAD fluxes, however, vary substantially among organs: whereas high NAD turnover rates have been reported in the small intestine (581 μM h^{-1}) and spleen (481 μM h^{-1}), the turnover in skeletal muscle (10 μM h^{-1}) and WAT (10 μM h^{-1}) appears to be more than 40-fold lower[10]. These pronounced differences exceed expectations, because the differences in the expression of NAD^+-synthesizing or NAD^+-consuming enzymes in the respective tissues are far less pronounced[10,102,103] (Table 3).

Despite being scarcely present in plasma under basal conditions (<10 nM)[10], NR and NMN have been shown to efficiently increase tissue NAD^+ levels[10,105,106]. Accordingly, the enzymes using NR and NMN—NRK1 and NRK2 (refs. [107–109]) and NMNAT1–3 (ref. [98]), respectively—are robustly expressed in multiple tissues (Table 3). Whereas NRK1 is ubiquitously expressed, NRK2 expression is usually specific to the skeletal muscle[107–109] but can be induced in, for example, neurons damaged by axotomy[110].

Expression of the different NMNAT isoforms also shows tissue specificity (Table 3): whereas NMNAT1 is ubiquitously expressed, NMNAT2 expression is restricted to the brain, and NMNAT3 is found mainly in liver, kidney, heart and adrenal glands[98]. Interestingly, to enter the cell, NMN must be first dephosphorylated to NR on the extracellular surface. Once inside the cell, it is rephosphorylated back to NMN by NRK[107]. However, this process might not be the only mechanism for NMN uptake, because a recent study has identified Slc12a8 as a selective NMN transporter mainly expressed in the pancreas and gut[111]. However, this finding has been questioned[112]. Whether both mechanisms coexist or complement each other remains a matter of debate.

Interestingly, large differences in NAD tissue fluxes have also been observed when NAD^+ precursors are administered orally or intravenously[10]. Orally administered tryptophan, NA, NR and NMN are largely directed to the liver, where they are metabolized either to NAD^+ locally or to NAM, which is then released into the circulation[10,92,104]. This liver-encompassing conversion step appears to be required to subsequently raise tissue NAD^+ levels[10,94]. However, some reports have demonstrated that NR can successfully increase NAD^+ content even in the absence of NAMPT activity[113,114]. In contrast, NR and NMN reach their target tissues intact when administered intravenously[10]. In the case of the brain, however, the conversion of NR and NMN to NAM is required at all times[10,115], because NR and NMN appear to be incapable of crossing the blood–brain barrier[10].

Despite major advances in the understanding of organismal and organ-specific NAD^+ homeostasis, future studies will need to shed light on unresolved questions regarding the subcellular, organ-specific and systemic regulation of NAD^+ homeostasis in health and especially in disease. The vast differences in NAD^+ homeostasis across cell types and tissues, however, hold promise in identifying targeted treatment strategies for a multitude of metabolic- and age-related diseases in the near future.

Factors affecting NAD^+ homeostasis. NAD^+ is a critical regulator of cell bioenergetics, and its abundance reflects the energy status of the cell. Energy-limiting conditions, such as caloric restriction[116–119], fasting[116,117,120], low-glucose feeding[121] or exercise[116,122–124], are characterized by an increase in NAD^+ levels. Conversely, excessive energy intake, for example, high-fat or high-fat/high-sucrose diets, results in NAD^+ depletion in various metabolic organs, including skeletal muscle[70], brown adipose tissue (BAT)[70], WAT[106] and liver[106,125–127].

Beyond energy and nutritional status, circadian rhythms also control NAD^+ levels. Liver NAD^+ levels fluctuate in a diurnal manner[128,129] and decline after loss of CLOCK or BMAL, the two master regulators of the circadian clock[129–131]. Expression of *Nampt*[128,129,131] and *Nmrk1* (ref. [130]) is controlled by the circadian machinery and is likely to be responsible for oscillations in hepatic NAD^+ content. Interestingly, the circadian-clock machinery appears to be reciprocally regulated by NAD^+ consumers: PARP-1 (ref. [132]), SIRT1 (refs. [133–136]), SIRT6 (ref. [137]) and, with less direct evidence, SIRT3 (ref. [138]), as well as by NAD^+ itself[139,140].

NAD^+-boosting strategies. Strategies to promote an increase in NAD^+ levels broadly fall into two categories: stimulation of its synthesis or inhibition of excessive NAD^+ consumption[25,141]. Several strategies can be used to promote NAD^+ synthesis:

1. Supplementation with NAD^+ precursors is efficient in vitro and in vivo, including in nematodes, flies, rodents[142] and, most importantly, humans. Dihydronicotinamide riboside has been recently reported as another biochemical precursor for NAD^+, which induces a strong NAD^+ increase in various mammalian cell lines and mouse tissues[143].
2. NAD^+ production can also be enhanced by stimulation of enzymes involved in NAD^+ synthesis (Fig. 3): both overexpression of NAMPT[144,145] and its activation with pharmacological enhancers, such as P7C3 (ref. [146]) or SBI-797812 (ref. [147]), translate into an increase in NAD^+ content. Similarly, overexpression of another NAD^+-synthetic enzyme, NMNAT, has comparable effects to those after administration of different NAD^+ precursors[110,148,149].
3. NAD(P)H-quinone oxidoreductase 1 (NQO1) is an antioxidant protein that catalyses the transformation of quinones into hydroquinones by using NADH as an electron donor, hence raising cellular NAD^+ levels. Therefore, in contrast to the examples mentioned earlier, the loss of NQO1 has been reported to decrease NAD^+ content in the liver and kidneys[150]. Conversely, activation of NQO1 by β-lapachone or dunnione increases intracellular NAD^+ levels and protects against various diseases and conditions, including chemotherapy-induced nephrotoxicity, cardiac dysfunction, intestinal damage, hearing loss[151–155], acute pancreatitis[156] and lung fibrosis[157]. Moreover, overexpression of NQO1 together with cytochrome *b*5 reductase 3 (CYB5R3) has been reported to mimic different aspects of caloric restriction, including modest lifespan extension[158].
4. The most recent wave of approaches to stimulate NAD^+ production is based on the principle of preventing the escape of intermediates from the NAD^+-biosynthetic pathway. This method can be illustrated by two enzymes: ACMSD and NAM *N*-methyltransferase (NNMT). As mentioned earlier, ACMSD catalyses the reaction at the branch point of the de novo NAD^+ synthesis and therefore acts as a gatekeeper controlling the dissipation of a pathway intermediate ACMS into the side branch (Fig. 3). Accordingly, mice overexpressing human ACMSD and maintained on a vitamin B_3-free diet have diminished NAD^+ levels and display neurological symptoms similar to those of humans with NAD^+ deficiency[59]. Conversely, downregulation and pharmacological inhibition of ACMSD efficiently increases NAD^+ levels in *Caenorhabditis elegans*, as well as in the mouse liver and kidneys[58,159].
   Similarly to ACMSD, NNMT catalyses a reaction removing a precursor molecule from the pathway normally leading to the production of NAD^+. However, in the case of NNMT, it acts on the salvage pathway; that is, NNMT N-methylates NAM, thus producing 1-methylnicotinamide (Fig. 3), which can no

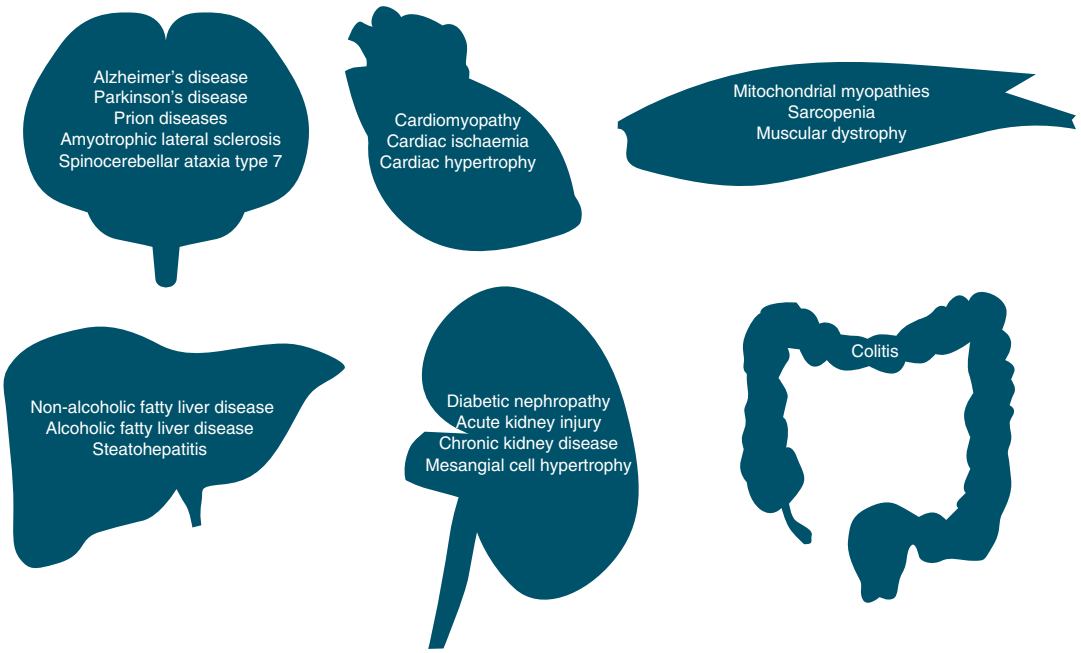

Fig. 4 | Disease conditions associated with altered NAD⁺ homeostasis. Altered NAD homeostasis and diminished NAD⁺ content have been linked to multiple diseases affecting different organs in either humans or rodent models. The first disease associated with NAD⁺ deficiency was pellagra, whose symptoms are also known as the 'three Ds' (dermatitis, diarrhoea and dementia). In the brain and nervous system, NAD⁺ depletion is associated with several proteinopathies, in which misfolded proteins accumulate in toxic protein aggregates, including AD, PD, prion disease and SCA7. In the heart, cardiomyopathies, cardiac ischaemia and hypertrophies display a decrease in tissue NAD⁺ content. In skeletal muscle, NAD⁺ depletion is detected in disorders including sarcopenia, muscular dystrophies and mitochondrial myopathies. In the intestine, approaches aiming to restore NAD⁺ homeostasis improve the regenerative capacity of ISCs and are beneficial in the context of several intestinal pathologies, including mesenteric ischaemia and colitis. In the liver, decreased NAD⁺ content is associated with alcoholic and non-alcoholic fatty liver disease, whereas preservation of hepatic NAD⁺ levels alleviates the damage even at more advanced stages of liver disease, that is, in steatohepatitis and cirrhosis. Finally, altered NAD⁺ levels are detected in AKI, diabetic nephropathy and mesangial-cell hypertrophy. Whereas NA and NAM protect against several pathological features of CKD, the renal NAD⁺ pool has not been assessed in the context of this disorder.

longer be used for NAD⁺ synthesis. Knockdown[160] and pharmacological inhibition of NNMT[161,162] both lead to an increase in NAD⁺ content.

5. Owing to their low K_m for NAD⁺ (Table 1), PARP-1 and CD38 can strongly deplete cellular NAD⁺ stores in situations in which they become overactivated (Fig. 2). Both pharmacological and genetic PARP inhibition raise NAD⁺ levels and have consequent beneficial effects on metabolic health and longevity[28,126,163–167]. Interestingly, mammalian cells appear to also be equipped with endogenous PARP inhibitors. For example, NADP⁺ has recently been shown to act as a negative regulator of PARylation and DNA-damage repair in cancer cells[168]. Similarly, NAD⁺ levels are substantially increased by genetic or pharmacological CD38 inhibition[166,169–175]. Another NAD⁺ consumer, SARM1, has been extensively studied in the context of Wallerian degeneration (Fig. 2). NAD⁺ depletion caused by SARM1 activation leads to axonal destruction[42]. SARM1 loss of function has hence been reported to restore the NAD⁺ content depleted by nerve transection and to protect axons[176].

NAD⁺ in disease and the therapeutic potential of NAD⁺ boosting

NAD⁺ depletion is a hallmark of ageing and numerous age-related disorders as diverse as neuro-muscular, cardiometabolic, liver and kidney diseases (Fig. 4). For this reason, the therapeutic and preventive potential of NAD⁺ boosting has been scrutinized in a variety of preclinical models and disease settings. The promising outcomes of these studies have triggered a series of clinical trials, which are currently testing the efficacy of NAD⁺ therapeutics in human diseases. Below, we summarize the current knowledge about the implications of NAD⁺ in diseases, placing a particular focus on disorders that have not been extensively covered before. We also provide two comprehensive tables listing most of the preclinical and clinical studies involving NAD⁺-boosting strategies. We will not be discussing ageing, as well as systemic diseases, such as diabetes, metabolic syndrome or cancer, but we instead refer readers to recent reviews covering these subjects[54,177–182].

Nervous system. Axonal degeneration, also known as Wallerian degeneration, is considered an early hallmark preceding neuronal death in many disorders of the nervous system, including Alzheimer's disease (AD) and Parkinson's disease (PD), ischaemic brain and spinal cord injuries, diabetic neuropathy, traumatic brain injury and amyotrophic lateral sclerosis (ALS)[183,184]. The link between NAD⁺ and axonal degeneration was originally established after the discovery of a spontaneous dominant mutation in mice, Wallerian degeneration slow (*wld*^{s}), which substantially delays axonal degeneration after injury[185]. In wild-type mice, a decline in NAD⁺, initiated by SARM1, has been detected in damaged axons undergoing Wallerian degeneration but has been found to be absent in *wld*^{s} mice[42,186]. The *wld*^{s} mutation results in the overexpression of a chimeric protein consisting of a fragment of the polyubiquitination factor UFD2a/UBE4b and the full-length NMNAT1 (ref. [187]).

Two different mechanisms underlying the beneficial effects of reconstituted NMNAT activity on Wallerian degeneration have been proposed. According to the first model, the accumulation of the NMNAT substrate, NMN, within the axon promotes

its degeneration[188]. The neurotoxicity of NMN is explained by its function as an endogenous SARM1 activator triggering cADPR production and consequent axonal destruction[189]. An alternative mechanism suggests that NMNAT1 prevents NAD^+ depletion by SARM1, thereby ensuring maintenance of the NAD^+ pool at the axon end[190]. Although the two models differ regarding the main cause of axonal death and the way in which NMNAT accomplishes its neuroprotective function, NAD^+ depletion associated with Wallerian degeneration remains the unifying factor in both theories.

Unexpectedly, preserving the NAD^+ pool has been found to be sufficient to protect axons from degeneration and alleviate conditions associated with axonal degeneration, including various peripheral neuropathies, ischaemic brain or spinal cord injury (Table 4). Interestingly, a neurogenesis-enhancing compound, P7C3 (ref. [191]), was found to be neuroprotective in a mouse model of PD[192] and was later shown to be an NAMPT activator[146]. P7C3 also protects against the onset of ALS[193], traumatic brain injury[194] and AD[195,196]—conditions tightly associated with Wallerian degeneration.

Neurodegenerative disorders characterized by accumulation of misfolded and aggregated proteins and associated with severe proteotoxic stress—such as AD, PD, ALS and prion disease—have also been linked to NAD^+ depletion[197,198] and altered NAD^+ homeostasis[199,200]. Mitochondrial function, which is impaired in many of these disorders, has been successfully restored with different approaches that elevate cellular NAD^+ levels (Table 4).

Recently, a decrease in NAD^+ levels has also been detected in cellular models of spinocerebellar ataxia type 7 (SCA7), a retinal–cerebellar neurodegenerative disorder characterized by mitochondrial impairment. Alterations in the NAD^+ DNS pathway, detected by metabolomics analysis, have been reported to be a signature of people with SCA7 and to lead to decreased mitochondrial NAD^+ levels—a likely cause of the mitochondrial dysfunction observed in these patients[201].

Alterations in NAD^+ homeostasis, caused by impaired NAD^+ production, also affect neural stem-cell function[202]. Accordingly, reconstituting NAD^+ levels improves both the proliferative and regenerative capacities of neural stem cells[203].

Liver. Fatty liver disease (FLD) is characterized by abnormal lipid accumulation within the liver, exceeding 5–10% of the organ's weight. FLD can occur in the setting of alcohol abuse (alcoholic FLD) or metabolic disease (non-alcoholic FLD (NAFLD)). Importantly, NAFLD is now the most common hepatic disorder in developed countries. Hepatic lipid accumulation is associated with a decrease in NAD^+ levels independently from the factor causing steatosis, such as high-fat or high-fat/high-sucrose feeding[106,125,127], a methionine/choline-deficient diet[58,126], orotic acid administration[204], a high-fat/high-cholesterol diet in *Apoe*$^{-/-}$ mice[125] or alcohol[205].

Apart from the decreased hepatic NAD^+ content, these different models of hepatic steatosis also share underlying pathogenic factors, including altered mitochondrial function, increased oxidative stress and inflammation. Importantly, preservation of NAD^+ content in these conditions appears to be an effective approach to prevent hepatic lipid accumulation (Table 4). Preserving hepatic NAD^+ levels via NMN administration has also been shown to attenuate telomere-associated liver fibrosis in a mouse model of telomere dysfunction, which is associated with downregulated hepatic expression of sirtuin proteins[206]. In an animal model of cirrhosis, a more advanced stage of FLD has been found to be associated with severe liver damage, inflammation and fibrosis, whereas PARP inhibition not only prevents but also reverses the progression of the pathology[207].

In addition, in a mouse model of hepatocellular carcinoma caused by liver-specific overexpression of unconventional prefoldin RPB5 interactor (URI), de novo NAD^+ biosynthesis has been shown to be inhibited; moreover, recovering hepatic NAD^+ content via NR administration prevents tumour formation and slows tumour progression[208].

Even though the postpartum period is not considered a disease, it is marked by strong metabolic stress in female organisms. A recent study has shown that hepatic NAD^+ content is decreased in postpartum lactating female mice, whereas circulating NAD^+ levels are increased to supply NAD metabolites to the mammary glands, thus ultimately resulting in a >20-fold increase in not only mammary NAD^+ levels but also in $NADP^+$ levels[209].

Kidney. Many kidney disorders are associated with impaired mitochondrial function and diminished sirtuin signalling, especially that of SIRT1, SIRT3 and SIRT6. These conditions include acute kidney injury (AKI)[210,211], chronic kidney disease (CKD)[212,213], renal lipotoxicity mediated by free fatty acids[214] and diabetic nephropathy[215–218].

Diminished kidney NAD^+ content has been reported in AKI[58,219,220]. Recovery of NAD^+ levels via supplementation with NMN or NAM[219,221,222], or administration of PARP inhibitors[220], ACMSD inhibitors[58] or NQO1 activators[153,154] protects against kidney damage in models of cisplatin- and ischaemia/reperfusion-induced AKI (Table 4). The de novo NAD^+-synthesis pathway appears to play a substantial role in the pathophysiology of AKI[222], and QPRT activity is particularly important for AKI resistance[219]. Accordingly, a decrease in NAMPT expression has been detected in AKI kidneys[211,223] and potentially may also contribute to AKI-associated NAD^+ depletion.

Diabetic nephropathy is associated with extracellular-matrix accumulation and is also characterized by a drop in NAD^+ levels, accompanied by a decrease in SIRT1 expression[218,224]. Upregulation of PARP1 in multiple organs of diabetic mice might be one cause of NAD^+ depletion[218,224,225], especially given that PARP inhibitors have nephroprotective effects through decreasing inflammation and oxidative stress[225]. Importantly, NMN treatment of kidney cells restores SIRT1 signalling and attenuates the expression of fibrotic factors[224]. Treatment with theobromine also successfully decreases extracellular-matrix deposition within the kidney, recovers NAD^+ levels and SIRT1 expression, and alleviates inflammation[218].

Glomerular mesangial-cell hypertrophy is an early manifestation of diabetic nephropathy and is also associated with decreased NAD^+ levels[226]. Preventing NAD^+ depletion in mesangial cells via exogenous NAD^+ administration protects them against hypertrophy via activation of the AMPK–mTOR signalling pathway[226].

Few data are available on NAD^+ metabolism in the context of CKD. NA alleviates several complications of CKD, including hypertension and proteinuria, by attenuating oxidative stress and inflammation[227]. Similarly to NA, NAM has also been reported to suppress inflammation and protect kidneys against another pathological feature of CKD, renal tubulointerstitial fibrosis[228]. Unfortunately, none of the previous studies have provided details on the mechanism through which NAM/NA exerts protection. Whether these treatments affect the renal NAD^+ pool remains to be examined.

Intestine. NAD^+ metabolism has not been extensively studied in the context of intestinal disorders, and existing reports have been contradictory. Manipulations of the intestinal NAD^+ pool should be performed with caution, because NAMPT levels have been reported to gradually increase in the intestine with colitis progression[229]. NAMPT inhibition with FK866 alleviates dextran sulfate sodium (DSS)-induced colitis and protects mice against colitis-induced colorectal cancer[229]. Decreased NAD^+ levels, followed by attenuation of PARP1 and SIRT signalling, thereby leading to suppression of inflammation, have been claimed to underpin the beneficial effects of NAMPT inhibition. Furthermore, intestine-specific loss of SIRT1 has been reported to protect against colitis-induced colorectal cancer in mice[230]. However, a study using a different

Table 4 | Therapeutic potential of NAD$^+$ boosters in rodent disease models

Supplement	Pathological condition	Health benefits/effects	References
Brain/nervous system			
NAD$^+$ (50–100 mg kg^{-1}) NAM (125–500 mg kg^{-1}) NU1025 (1–3 mg kg^{-1}) 4-ANI (1–3 mg kg^{-1})	Ischaemic injury Spinal cord injury	↓ Neuronal death	317–322
NAD$^+$ (30 mg kg^{-1} d^{-1})	Prion disease	↓ Prion-protein neurotoxicity	198
NR (250–400 mg kg^{-1} d^{-1}, 10 weeks) NAM (40–500 mg kg^{-1} d^{-1}, 9 months) NMN (500 mg kg^{-1}, 10 d) P7C3 (10–20 mg kg^{-1}, 9 or 15 months) Caloric restriction	AD	↓ Amyloid-β aggregates, ↑ synaptic plasticity, ↑ neuronal survival, ↑ BDNF levels, ↑ SIRT1 activity, ↓ oxidative stress, ↓ apoptosis, ↓ PARP-1 activity	118,196,197, 323–327
NR (200 mg kg^{-1} d^{-1}, 31 d)	Peripheral neuropathy	↓ Tactile hypersensitivity, ↓ escape-avoidance behaviours	328
NR (300 mg kg^{-1} d^{-1}, 8 weeks)	Diabetic peripheral neuropathy	↑ Motor- and sensory-neuron conduction velocity, ↑ intraepidermal nerve fibres	127
NR (1,000 mg kg^{-1} 2× per day, 7 d)	Noise-induced neurite degeneration	↓ Spiral ganglia neurite degeneration, ↓ noise-induced hearing loss	329
NAM (550 mg kg^{-1} d^{-1}, 3 or 6 months)	Glaucoma	↓ Optic nerve degeneration, ↓ axonal loss in retinal ganglion cells, ↓ thinning of retinal nerve fibre layer; protection of visual function	149
P7C3 and P7C3A20 (5–40 mg kg^{-1} d^{-1})	PD	Protection of dopaminergic neurons from MPTP-mediated cell death	192
P7C3 (20 mg kg^{-1})	ALS	Protection of ventral horn spinal cord motor neurons from cell death, ↑ motor function	193
P7C3-S243 (3–30 mg kg^{-1} d^{-1})	Traumatic brain injury	↓ Axonal degeneration, ↑ synaptic activity, ↑ learning and memory, ↑ motor coordination	194
Kidney			
NMN (500 mg kg^{-1} d^{-1}, 2 d) 3-AB (15 ml kg^{-1}) TES-1025 (15 mg kg^{-1} d^{-1}, 12 d) Dunnione (10–20 mg kg^{-1})	AKI	↑ GFR; normalization of blood creatinine and BUN; ↓ urinary excretion of collagen type IV, podocin, cystatin C and retinol-binding protein; ↓ KIM1 levels; ↓ inflammation; ↑ SIRT1 activity	58,153,220,221
NA (50 mg kg^{-1} d^{-1}, 12 weeks) NAM (200, 400 or 800 mg kg^{-1}, 13 d)	CKD	↓ Renal interstitial fibrosis, ↓ hypertension, ↓ proteinuria, ↓ histological injury, ↓ oxidative stress, ↓ inflammation	227,228
Muscle			
NR (400 mg kg^{-1} d^{-1}, 4 weeks and 4 months)	Mitochondrial myopathy	↓ Respiratory-chain defects, ↑ exercise tolerance, ↓ mitochondrial-ultrastructure abnormalities, ↓ mitochondrial-DNA deletions	251,252
NR (400 mg kg^{-1} d^{-1}, 8 weeks)	DMD	↑ Structural-protein expression, ↓ poly(ADP)-ribosylation, ↓ inflammation, ↓ fibrosis	254
NNMT inhibitor (5–10 mg kg^{-1}, 1 or 3 weeks post-injury) 78c (10 mg kg^{-1}, 4–14 weeks)	Sarcopenia	↑ Muscle stem-cell proliferation, ↑ contractile function	162,175
Cardiovascular system			
NMN (500 mg kg^{-1}) Luteolinidin (5–50 μM)	Ischaemia-induced injury	↓ Ischaemic injury, ↑ SIRT1 activation, ↓ Foxo1 acetylation, ↑ endothelium-dependent vasodilatory function, ↑ recovery of left-ventricular contractile function	171,264
NMN (500 mg kg^{-1}, 4 weeks)	Pressure-overload-induced cardiac hypertrophy	↓ Cardiac hypertrophy, ↓ mitochondrial-protein hyperacetylation	268
NMN (400 mg kg^{-1} d^{-1}, 5 d)	Haemorrhagic shock	↑ Survival, ↑ time before resuscitation is required, ↓ lactic acidosis, ↓ serum IL-6 level, ↑ mitochondrial function	245
NR (750 mg kg^{-1} d^{-1}, 5 d)	Lethal cardiomyopathy caused by severe iron depletion	↑ Survival, ↑ SIRT1 activation	267
NR (450 mg kg^{-1} d^{-1})	Dilated cardiomyopathy	↓ Risk of heart failure	113

Continued

Table 4 | Therapeutic potential of NAD$^+$ boosters in rodent disease models (continued)

Supplement	Pathological condition	Health benefits/effects	References
NR (400 mg kg^{-1} d^{-1}, 8 weeks)	DMD	↑ Cardiac function	254
Intestine			
NR (50 mg kg^{-1})	Mesenteric ischaemia	↓ Mesenteric ischaemia–reperfusion injury	235
NR (500 mg kg^{-1} d^{-1}, 6 weeks)	DSS-induced colitis	↑ ISC number and function	234
Liver			
NR (400 mg kg^{-1} d^{-1}, 2, 9 or 18 weeks) Olaparib (50 mg kg^{-1} d^{-1}, 18 weeks) TES-991 (15 mg kg^{-1} d^{-1}, 2.5 weeks)	NAFLD	↑ Hepatic β-oxidation, ↓ hepatic triglyceride levels, ↓ plasma ALAT	58,125,126
Adipose tissue			
NMN (500 mg kg^{-1} d^{-1}, 2 months) NR (400 mg kg^{-1} d^{-1}, 9 weeks)	Metabolic syndrome	Restoration of adiponectin and adipsin levels, ↑ insulin sensitivity	70,330
Pancreas			
NA (10–500 mg kg^{-1} d^{-1}, 30 d) NMN (500 mg kg^{-1} d^{-1}, 7–10 d and 2 months) 78c (10 mg kg^{-1}, 4–14 weeks) Apigenin (100 mg kg^{-1} d^{-1}, 7 d)	Metabolic syndrome	↑ Glucose tolerance, ↓ lipid levels, ↓ inflammation, ↓ oxidative stress	106,175,330–332
β-Lapachone (10 mg kg^{-1})	Acute pancreatitis	↓ Inflammation	156

ALAT, alanine transaminase; BUN, blood urea nitrogen; GFR, glomerular filtration rate; KIM1, kidney injury molecule-1; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

intestine-specific SIRT1-knockout model has found the opposite result: intestinal deletion of SIRT1 leads to abnormal activation of Paneth cells and intestinal inflammation, as well as to increased susceptibility to colitis[231]. In line with these observations, different energy-deficient conditions normally associated with sirtuin activation, including short fasting (24 hours) and caloric restriction, have been shown to improve the regenerative capacity of intestinal stem cells (ISCs)[232,233]. In the case of caloric restriction, the improvements in ISC function are dependent on SIRT1. According to the proposed model, the release of cADPR by Paneth cells activates AMPK within ISCs, thus increasing transcription of NAMPT and the subsequent SIRT1-mediated deacetylation of S6 kinase 1 (S6K1) and its consequent phosphorylation by mTORC1, and ultimately resulting in ISC expansion[232]. Moreover, NR administration to old mice rejuvenates their ISCs and restores the ISC regenerative capacity for repair of gut damage caused by DSS[234].

Another condition in which NR supplementation has demonstrated protective effects is mesenteric ischaemia–reperfusion[235]. Intestinal damage caused by cisplatin is associated with a decreased NAD$^+$/NADH ratio, owing to PARP1 overactivation. Restoring this ratio to normal via activation of NQO1 is protective against cisplatin toxicity in intestinal cells[155]. In summary, more research must be performed to understand the role of NAD$^+$ metabolism in the intestine and to establish whether sirtuin activation is desirable in this organ.

Another interesting twist on intestinal NAD$^+$ metabolism has been revealed by a study of the gut microbiome reporting an intriguing connection between the gut microbiome and neurodegeneration in a mouse model of ALS (*Sod1*-Tg mice), by showing that gut-colonizing bacteria affect the severity of the ALS pathology[200]. Intriguingly, *Akkermansia muciniphila* bacteria, which alleviate ALS symptoms, have been reported to stimulate NAM accumulation in the central nervous system in *Sod1*-Tg mice. Furthermore, systemic NAM supplementation has been found to attenuate ALS progression and improve motor phenotypes in these mice[200].

Haematopoietic system. Haematopoietic stem cells (HSCs) maintain the blood system by generating more than ten different types of mature blood cells in the process of haematopoiesis[236]. In response to injury, HSCs are capable of rapid proliferation and commitment to multipotent progenitors, which, through differentiation, replenish the depleted stores of diverse cell populations. However, to maintain the HSC pool, a subpopulation of these cells must preserve the capacity for self-renewal. Owing to their residence within hypoxic stem-cell niches, HSCs have a low mitochondrial potential and rely mainly on glycolysis for energy production. Low mitochondrial activity constitutes a functional signature of self-renewing HSCs, because differentiating cells that have lost self-renewal ability rapidly increase the capacity for oxidative phosphorylation[237]. Both NR and NMN successfully stimulate haematopoiesis in vivo and improve mouse survival after HSC transplantation by decreasing mitochondrial activity and stimulating mitochondrial clearance via mitophagy[238]. Interestingly, the effects of NR and NMN in enhancing mitophagy in HSCs are opposite from their effects in hepatocytes or muscle cells, in which both compounds stimulate mitochondrial biogenesis (Table 4).

Although NAM has been shown to increase the homing of haematopoietic progenitors and to enhance platelet production in vitro[239,240], NAM supplementation does not enhance haematopoiesis in vivo[238,241]. These differences between the effects of NAM and NR may be due to the tissue-specific preferences for NAD$^+$ precursors. Intriguingly, the positive effects of NR appear to be observed only in blood cells with nuclei. Whereas NR accelerates platelet recovery after HSC transplantation[238], it does not affect the quality or cellular lifespan of isolated platelets, although it is still able to efficiently increase NAD$^+$ content in these cells[242]. Hence, the effects of NR on platelets in vivo are probably due to its action in megakaryocytes, platelet precursors that still have nuclei.

Haemorrhagic shock is caused by rapid loss of a large volume of blood, thus leading to vasoconstriction and tissue hypoperfusion, which ultimately deprive tissues of oxygen. Without proper clinical management, haemorrhagic shock leads to organ failure, particularly of the liver and kidney. Although a dramatic decrease in the volume of circulating fluid has long been considered a major contributor to the pathophysiology of haemorrhagic shock, haematopoietic failure observed in animals and humans after haemorrhagic

Table 5 | Therapeutic potential of NAD^+ boosters in humans

Supplement	Dose	Disease or experimental model	Health benefits/effects	↑ NAD^+	References
Brain/nervous system					
Stabilized NADH	12 mg d^{-1}, 6 months	Probable AD	↑ Cognitive function, ↑ Mattis Dementia Rating Scale, ↑ verbal fluency, ↑ visual-constructional ability, ↑ abstract verbal reasoning	ND	333
NA	250 mg d^{-1}, 45 d	PD	↑ Motor and cognitive function, ↑ sleep, normalization of GPR109A, ↑ NAD/NADP ratio	ND	334
	1 g d^{-1}, 3 months	PD	↑ Routine activities, ↑ motor function, ↓ rigidity	ND	335
NAM	3 g d^{-1}, 6 months	Mild/moderate AD	No adverse effects, no effect on cognitive function	ND	336
Acipimox	250 mg, 4× per day	Hypopituitary	↓ Parasympathetic tone	ND	337
EH301	1.2 g d^{-1}, 4 months–1 y	ALS	↓ ALS progression, ↑ ALS Functional Rating Scale score	ND	338
Blood/serum biochemistry					
NA	1 g d^{-1}, 3 months	PD	↓ TG	ND	335
	3 g d^{-1}, ~6 y	CAD	↓ TG, ↓ TC	ND	339
	375–1,500 mg d^{-1}, 10 weeks	Low HDL-C	↓ TG, ↓ TC, ↓ LDL-C, ↑ HDL-C	ND	340
NA+ colestipol	0.5–6 g d^{-1}, 2.5 y	CAD	↓ TG, ↓ LDL-C, ↑ HDL-C	ND	341
	3–12 g d^{-1}, 2 y	CAD or coronary venous bypass	↓ TC, ↓ LDL-C, ↑ HDL-C	ND	342,343
NA + statins	1–4 g d^{-1}, 3 y	CAD with low HDL-C	↓ LDL-C, ↑ HDL-C	ND	344
	1 g d^{-1}, 1 y	CAD with low HDL-C	↑ HDL-C	ND	345
	2 g d^{-1}, 24 weeks	Type IIa or type IIb hyperlipidaemia	↓ TG, ↓ LDL-C, ↓ non-HDL-C, ↓ Apo B, ↓ lipid/lipoprotein ratio, ↑ HDL-C, ↑ Apo A-I	ND	346
	0.5–1 g d^{-1}, 1 y	CAD	↓ TC, ↓ LDL-C, ↑ HDL-C, ↑ Apo A	ND	347
	2 g d^{-1}, ~1 y	CAD	↓ TG, ↑ TC, ↑ HDL-C, ↑ LDL-C	ND	348
	1.5–2 g d^{-1}, 3 y	CAD	↓ TG, ↓ LDL-C, ↑ HDL-C	ND	349
	2 g d^{-1}, ~4 y	Vascular disease	↓ LDL-C, ↑ HDL-C	ND	350
NAM	~1 g d^{-1}, 12 weeks	Patients on haemodialysis	↓ Phosphate and intact parathyroid hormone levels, ↑ HDL-C, ↓ LDL-C	✓	351
NR	0.1–1 g d^{-1}	Healthy individuals	No adverse effects	✓	105
	0.25–1 g d^{-1}, 1 week	Healthy individuals	No adverse effects	✓	352
	1 g d^{-1}, 6 weeks	Healthy individuals	No effects on haematology, plasma glucose, insulin, TC and TG	✓	270
	2 g d^{-1}, 12 weeks	Obese, IR men	No effects on glucose and lipid homeostasis	ND	279
	0.1, 0.3 or 1 g d^{-1}, 2 weeks	Overweight individuals	No elevation in LDL-C, reduction in WBCs (for 0.3 g dose)	✓	353
NMN	100, 250 or 500 mg	Healthy men	↑ Bilirubin, ↓ creatinine, ↓ chloride, ↓ glucose; no adverse effects	✓	354
NRPT = NR + PT	NR (250–500 mg d^{-1}) + PT (50–100 mg d^{-1}), 60 d	Healthy individuals	↑ TG, ↑ TC, ↑ LDL-C; no effects on haematology and electrolytes	✓	355

Continued

Table 5 | Therapeutic potential of NAD^+ boosters in humans (continued)

Supplement	Dose	Disease or experimental model	Health benefits/effects	↑ NAD^+	References
Acipimox	0.75 g d^{-1}, 4–9 weeks	Hyperlipoproteinaemia	↓ TG, ↓ LDL-C, ↑ HDL-C	ND	356
	0.75–1.2 g d^{-1}, 60 d–9 months	Hyperlipoproteinaemia/ hypertriglyceridaemia	↓ TG, ↓ plasma TC, ↑ HDL-C	ND	357
	1.5 g d^{-1}, 3 d	T2DM	↓ NEFA with rebound, ↓ TG, ↓ glucose, ↓ insulin	ND	358
	1 g d^{-1}, 7 d	T2DM	↓ NEFA, ↓ insulin, ↓ glucose, ↑ glucose tolerance	ND	359
	1 g d^{-1}	Hypopituitary	↓ Glucose, ↓ NEFA	ND	337
	0.75 g d^{-1}, 2 weeks	T2DM	↑ NEFA, ↓ TC, ↓ TG	ND	360
	1.5 g d^{-1}	Healthy individuals	↓ NEFA, ↑ disposition index during 24-h fasting, ↑ insulin response, ↑ insulin sensitivity	ND	361
	0.75 g d^{-1}	Lean/obese nondiabetic individuals or those with T2DM	↓ NEFA and insulin, ↑ insulin-stimulated glucose uptake, ↑ glucose tolerance	ND	362
	1 g d^{-1}, 1 week	Normal and overweight individuals	↓ NEFA, ↓ insulin, ↓ C-peptide, ↓ HOMA index	ND	363
	0.75 g d^{-1}	Overweight individuals with hypopituitary	↓ NEFA, ↑ GLP-1, ↑ glucose-infusion rate	ND	364
	0.25 g d^{-1}	T2DM	↓ NEFA during exercise, ↓ glucose and insulin during recovery from exercise, ↑ glycaemic control	ND	365
Immune system					
NAM + calcipotriol	4% NAM + 0.005% calcipotriol 2× per day, 12 weeks	Mild to moderate psoriasis	↓ Psoriasis Area Severity Index score, ↑ patient satisfaction with lesion improvement	ND	366
Muscle					
NR	1 g d^{-1}, 6 weeks	Healthy individuals	No effect on activity, exercise performance, motor function	ND	270
	2 g d^{-1}, 12 weeks	Obese, IR men	No effect on resting energy expenditure, respiratory capacity of muscle mitochondria, mitochondrial morphology and distribution	NC	279,367
	1 g d^{-1}, 21 d	Marginally obese, aged men	No effect on muscle mitochondrial bioenergetics, muscle blood flow, systemic cardiometabolic parameters; ↓ circulating inflammatory cytokines	✓	281
NRPT	NR (250–500 mg d^{-1}) + PT (50–100 mg d^{-1}), 60 d	Healthy individuals	↑ Mobility, ↑ lower-body strength	✓	355
Acipimox	0.75 g d^{-1}, 2 weeks	T2DM	↑ Mitochondrial unfolded-protein response, ↑ ATP, ↑ muscle lipid content, ↑ mitochondrial respiration, ↓ insulin sensitivity	✓	360
	1 g d^{-1}, 7 d	T2DM	↓ Muscle fatty acyl-CoA content, ↑ glucose tolerance, ↑ insulin sensitivity	ND	359
	0.5 g d^{-1}	T2DM	↓ NEFA oxidation, ↑ intramyocellular TG and carbohydrate use, ↓ lipid content in type I muscle fibres after exercise	ND	368
EH301	1.2 g d^{-1}, 4 months	ALS	↑ Muscle strength, ↑ muscle weight, ↑ muscle function	ND	338

Continued

Table 5 | Therapeutic potential of NAD⁺ boosters in humans (continued)

Supplement	Dose	Disease or experimental model	Health benefits/effects	↑ NAD⁺	References
Liver					
NR	1 g d^{-1}, 6 weeks	Healthy individuals	No effects on liver parameters (for example, ALAT, ASAT, albumin)	ND	270
	2 g d^{-1}, 12 weeks	Obese, IR men	↓ Hepatic lipid content (not statistically significant)	✓	279
NRPT	NR (250–500 mg d^{-1}) + PT (50–100 mg d^{-1}), 60 d	Healthy individuals	↓ ALAT and ASAT, indicating improved liver function	✓	355
Kidney					
NA	100–1,000 mg d^{-1}, 8 months	CKD	↓ Phosphate, ↓ dyslipidaemia	ND	369
	500 mg d^{-1}, 6 months	CKD	↓ Phosphate, ↑ GFR, ↓ dyslipidaemia	ND	370
	1–2 g d^{-1}, 24 weeks	CKD	↓ Phosphate	ND	371
Niceritrol	750 mg d^{-1}, 8 weeks	Haemodialysis	↓ Phosphate, unchanged BUN, creatinine and calcium levels	ND	372
NAM	~1 g d^{-1}, 12 weeks	Haemodialysis	↓ Serum phosphate, ↓ parathyroid hormone	✓	351
NR	1 g d^{-1}, 6 weeks	Healthy individuals	No effect on kidney parameters (for example, estimated GFR and BUN)	ND	270
NRPT	NR (250–500 mg d^{-1}) + PT (50–100 mg d^{-1}), 60 d	Overweight individuals	No effect on kidney parameters (creatinine, sodium, potassium and chloride)	✓	355
Adipose tissue					
NA	375 –1,500 mg d^{-1}, 10 weeks	Low HDL-C	↑ Adiponectin, ↓ resistin	ND	340
NR	1 g d^{-1}, 6 weeks	Healthy individuals	No effect on body fat mass	ND	270
	2 g d^{-1}, 12 weeks	Obese, IR men	No effects on lipid oxidation, lipolysis and body composition	✓	279
Acipimox	0.75 g d^{-1}, 4–9 weeks	Hyperlipoproteinaemia	↓ AT lipolysis, ↓ plasma TG	ND	356
	1 g d^{-1}, 1 week	Normal and overweight individuals	↓ NEFA, ↓ adiponectin; no effect on fat content, body weight or body mass index	ND	363
	0.5 g d^{-1}	T2DM	↓ NEFA release from AT	ND	368
	0.25 g d^{-1}	Hyperinsulinaemia	↓ Glycerol release from subcutaneous AT	ND	373
EH301	1.2 g d^{-1}, 4 months	ALS	↓ Fat mass	ND	338
Pancreas					
NR	1 g d^{-1}, 6 weeks	Healthy individuals	No effects on plasma glucose, insulin and HOMA-IR	ND	270
	2 g d^{-1}, 12 weeks	Obese, IR men	No effects on fasting/post-glucose plasma glucose, insulin, C-peptide, glucagon and β-cell function	✓	280
Acipimox	1.5 g d^{-1}	Patients with T2DM predisposition	↑ Glucose-stimulated insulin secretion, ↑ β-cell function	ND	361
	0.25 g d^{-1}	T2DM	↓ Postprandial glucose and insulin during exercise recovery	ND	365
Cardiovascular system					
NA	3 g d^{-1}, 6 y	CAD	↓ Incidence of definite, nonfatal myocardial infarction, ↓ all-cause mortality after 5 y	ND	339

Continued

Table 5 | Therapeutic potential of NAD⁺ boosters in humans (continued)

Supplement	Dose	Disease or experimental model	Health benefits/effects	↑ NAD⁺	References
NA + colestipol	0.5–6 g d^{-1}, 2.5 y	CAD	↓ Atherosclerotic progression	ND	341
	3–12 g d^{-1}, 2 y	CAD or coronary venous bypass	↓ Atherosclerotic progression, ↑ coronary status	ND	342,343
NA + statins	1–4 g d^{-1}, 3 y	CAD with low HDL-C	↓ Coronary stenosis, ↓ occurrence of a first cardiovascular event	ND	344
	1 g d^{-1}, 1 y	CAD with low HDL-C	↓ Atherosclerotic progression	ND	345
	2 g d^{-1}, ~1 y	CAD		ND	348
	2 g d^{-1}, 24 weeks	Hyperlipidaemia	↑ Lipid profile	ND	346
	0.5–1 g d^{-1}, 1 y	CAD		ND	347
NR	1 g d^{-1}, 6 weeks	Hypertension	↓ Systolic and diastolic BP, ↓ aortic stiffness	✓	270
NMN	0.1, 0.25 and 0.5 g d^{-1}	Healthy men	No adverse effects	ND	354
NRPT	NR (250 mg d^{-1}) + PT (50 mg d^{-1}), 60 d	Healthy individuals	↓ Diastolic BP	✓	355
Acipimox	1 g d^{-1}, 1 week	Normal and overweight individuals	↓ NEFA, ↓ systolic BP, ↓ cardiac function; unchanged cardiac TG	ND	363
Lung					
EH301	1.2 g d^{-1}, 4 months	ALS	↑ Pulmonary function	ND	338
Intestine					
Acipimox	0.25 g d^{-1} 1.5 g d^{-1}	Obese individuals with and hypopituitary	↑ Systemic levels of GLP-1	ND	364
NR	2 g d^{-1}, 12 weeks	Obese, IR men	No effect on GLP-1 and gastric inhibitory polypeptide post glucose	✓	280

Studies that detected an increase in NAD⁺ levels are indicated with ✓. ND, studies in which NAD⁺ levels were not determined; NC, studies in which NAD⁺ content was quantified, but no change in NAD⁺ was detected. Apo, apolipoprotein; ASAT, aspartate transaminase; AT, adipose tissue; BP, blood pressure; CAD, coronary artery disease; GLP-1, glucagon-like peptide-1; HDL-C, high-density-lipoprotein cholesterol; HOMA, homeostasis model assessment; HOMA-IR, HOMA for insulin resistance index; IR, insulin resistant; LDL-C, low-density-lipoprotein cholesterol; NEFA, non-esterified fatty acids; PT, pterostilbene; TC, total cholesterol; TG, triglycerides; WBCs, white blood cells.

shock constitutes another important component[243,244]. In view of the stimulatory effects of NR and NMN on haematopoiesis[238], the finding that NMN administration prevents metabolic derangement in organs caused by haemorrhagic shock is unsurprising[245]. Another benefit of NMN administration comes from the decrease in circulating levels of the pro-inflammatory cytokine IL-6 (ref. [245]), a predictor of severity of haemorrhagic shock[246,247]. Anti-inflammatory effects associated with maintenance of the NAD⁺ pool have also been observed in macrophages[248]. Intriguingly, de novo NAD⁺ synthesis is essential for the preservation of NAD⁺ levels in macrophages at rest or after immune challenge. Another study in macrophages has reported NAMPT upregulation in response to inflammatory activation, such as after lipopolysaccharide (LPS) stimulation[249]. Interestingly, both studies have detected PARP overactivation in macrophages after LPS stimulation, an effect that might be the cause of the NAD⁺ depletion[248,249]. CD38 expression in macrophages is also increased by LPS[250].

Muscle. Myopathies encompass different types of disorders associated with muscle weakening and degeneration. One subgroup of myopathies is caused by mitochondrial dysfunction. Treatment with NR or PARP inhibitors leads to an increase in muscular NAD⁺ levels with a concomitant restoration of muscle function in models of mitochondrial myopathy[251,252] (Table 4).

Muscular dystrophies constitute another subgroup that is characterized by cycles of muscle degeneration and regeneration, and ultimately results in an exhaustion of the muscle stem-cell pool and loss of muscle regenerative capacity[253]. A severe depletion in muscular NAD⁺ content has been detected in *mdx* mice, a model of Duchenne's muscular dystrophy (DMD), the most common muscular dystrophy[254,255]. This decrease in NAD⁺ levels is probably due to overactivation of PARPs and/or decreased expression of NAMPT in dystrophic muscles[254]. NR can preserve muscle function in *mdx* mice by restoring the expression of structural muscle proteins and enhancing mitochondrial function[254]. It also prevents a typical feature of DMD, senescence of muscle stem cells[203].

Aged muscles also have diminished regenerative capacity[203,256,257] and NAD⁺ content[203,258], thus indicating that similar mechanisms might be implicated in ageing-related declines in muscle mass and function. Dysfunctional mitochondria are a hallmark of ageing-induced muscle stem cell (MuSC) senescence, and NR has been reported to improve mitochondrial function via activation of the mitochondrial unfolded-protein response and prohibitin pathway in the MuSC[203]. Beyond decreased mitochondrial activity, increased DNA damage has been observed in MuSCs in old mice[203], thus leading to an overactivation of PARPs in aged muscles[259]; this overactivation may be one of the causes of age-dependent NAD⁺ depletion. Finally, given that administration of NNMT inhibitors significantly improves muscle regenerative capacity after acute injury[162], enhanced NNMT activity might also contribute to age-dependent NAD⁺ depletion in muscle.

Notably, owing to efficient stimulation of mitochondrial function by increasing NAD⁺, NAD⁺-boosting techniques positively affect physical performance even in animals without compromised muscular function[70,164], although some species differences may exist[260].

Cardiovascular system. A decrease in cardiac NAD^+ levels accompanies different pathologies of the heart[113,261–264]. Cardiac ischaemia, such as that in the context of coronary artery disease and myocardial infarction, constitutes one of these conditions, and different strategies for restoring cardiac NAD^+ content after ischaemia have yielded promising results (Table 4). One reason for NAD^+ depletion in post-ischaemic hearts is the overactivation of CD38, which has been detected in endothelial cells[265]. Both genetic deletion and pharmacological inhibition of CD38 are protective in post-ischaemic hearts[171–173]. Similarly, loss of function of CD38 protects the heart against high-fat-diet-induced oxidative stress[266] and improves heart function in aged mice[175].

Disrupted NAD^+ homeostasis is common to cardiomyopathies of different types. NR administration decreases the risk of heart failure in both hypertrophic[267] and dilated[113] cardiomyopathies. Similarly to humans with DMD, *mdx* mice are prone to developing cardiomyopathy with age, and maintaining cardiac NAD^+ levels as a result of NR administration improves their cardiac function[254].

Cardiac hypertrophy is another condition accompanied by a decline in NAD^+ levels[263], and restoration of NAD^+ content via NMN administration protects against cardiac hypertrophy induced by pressure overload in a mouse model of mitochondrial complex I deficiency[268]. The increase in the heart's sensitivity to pressure overload after pharmacological inhibition of NAMPT further highlights the importance of maintaining NAD^+ homeostasis to protect cardiac function[269].

The trend towards a decrease in both systolic and diastolic blood pressure, as well as aorta stiffness in humans after short-term therapy with NR, is in line with these observations in rodents but requires confirmation in a larger study[270].

Atherosclerosis is associated with endothelial dysfunction, recruitment of macrophages and degeneration of smooth muscle cells in the vessel wall. Activation of SIRT1 exerts beneficial effects on all these cell types and is atheroprotective[271]. In agreement with a protective role of SIRT1 in atherosclerosis, boosting NAD^+ by NMN administration reverses age-related endothelial dysfunction by restoring SIRT1 activity and decreasing oxidative stress[272]. During early stages of atherosclerosis, macrophages become foam cells by ingesting modified plasma LDL cholesterol, which in turn promotes atherosclerotic-plaque formation. NAMPT loss of function has been reported to stimulate lipid accumulation in macrophages, thus further emphasizing the importance of maintaining cellular NAD^+ levels to slow atherosclerosis[273].

Finally, it is important to keep in mind that the anti-dyslipidaemic effects of nicotinic acid (niacin) were reported long before the discovery of the link between NAD^+ and sirtuins[56,274].

NAD^+ boosting in endothelial cells also restores their angiogenic capacity. Indeed, NMN administration or enhanced NAMPT activity in vitro improves the angiogenic function of endothelial cells in situations of lipid or glucose overload[275,276]. Moreover, in aged mice, NMN administration restores capillary density in the muscle, thus improving treadmill performance[277] and rescuing impaired neurovascular coupling by increasing endothelial vasodilation[278].

Future of NAD^+ research: a land of opportunities and risks

Emerging clinical evidence. Within the past decade, the NAD^+ field has experienced a genuine scientific renaissance resulting in general excitement around NAD^+ in the media. New data on the beneficial effects of different strategies to restore cellular NAD^+ levels are accumulating rapidly. Although the therapeutic potential of boosting NAD^+ levels via several approaches is undeniable, it is time to pause and ponder the potential repercussions due to the excitement surrounding NAD^+. Several issues must be resolved to determine the true translational value of all the research on NAD^+ that has been published to date.

In 2016, the first trial of NR in humans reported that oral administration of NR to healthy volunteers led to a dose-dependent increase in blood NAD^+ levels[105] (Table 5). Several other studies have been completed since then (Table 5), and more than 30 human clinical trials are currently ongoing or recruiting participants. The already available data indicate that the translation from the rodent models to humans might not be as straightforward as expected. Although the studies show that different doses and durations of NR administration all lead to an increase in NAD^+ levels in humans, and do not appear to be associated with serious adverse effects (Table 5), NR has not resulted in striking improvements in any disease setting to date[279,280].

Dietary supplementation with NR for 12 weeks has not been found to improve insulin sensitivity or glucose homeostasis in obese insulin-resistant men[279] or to have effects on β-cell function or on the levels of circulating bile acids and adipsin[280]. Despite the disappointing outcomes of the first clinical trials, several points require consideration. First, the doses of NAD^+ precursors used (mainly 1,000 mg d^{-1}) were far lower than those used in preclinical animal models. Second, 12 weeks in the context of a human study might be too short a time for NR to show full therapeutic benefit. Moreover, diminished levels of circulating inflammatory cytokines observed in older individuals after 21 days of NR supplementation[281], as well as the trend towards decreased blood pressure in healthy older participants after a 6-week NR treatment[270], appear rather positive, because hypertension and meta-inflammation are important risk factors for cardiometabolic diseases.

For NAD^+ boosters to accomplish the leap from the bench to bedside, carefully planned clinical studies of longer duration with higher doses, involving large numbers of patients should be performed. However, at this point, the natural origin of NAD^+ precursors must be considered. NAD^+ precursors as vitamins present a double-edged sword. On the one hand, their use does not require approval by the US Food and Drug Administration (FDA), thus substantially simplifying studies and sales of these compounds as food supplements. Many people are already taking such supplements outside clinical trials, and the side effects associated with their consumption often go unnoticed and are not routinely reported. A single person experiencing a serious adverse event would jeopardize the entire field of NAD^+ research and halt translation of promising preclinical results. On the other hand, because NR, NA, NMN and NAM are all natural products, the biopharmaceutical industry is hesitant to invest in large clinical trials. Unfortunately, convincing evidence that NAD^+ boosters are beneficial in the context of human diseases can be provided only by well-designed clinical trials. Consequently, the scientific and clinical community must not bend under the hype and pressure surrounding the beneficial effects of NAD^+ boosting and must by all means prevent the field from moving in the wrong direction on the basis of insufficient clinical evidence.

Remaining gaps and future directions. Even the knowledge of basic NAD^+ biology remains unexpectedly incomplete. Most published studies aiming to modulate NAD^+ metabolism have focused exclusively on measuring NAD^+ concentrations, unfortunately often with non-quantitative methods. Assessment of the entire NAD^+ metabolome constitutes a much more accurate way to obtain a comprehensive footprint of the effects of NAD^+ boosting, given that NAD^+ and its related metabolites undergo constant interconversion[282]. An additional level of complexity comes from every tissue possessing its own NAD^+ metabolome, which can still be affected by cross-talk with the NAD^+ systems of other organs. Measuring fluxes through pathways of NAD^+ synthesis and degradation is hence another complementary strategy to better characterize the dynamics of NAD^+ homeostasis[10].

Methods for accurate and reproducible NAD^+ quantification should be applied more carefully. Of all the currently available techniques, mass spectrometry has the greatest analytical power and delivers the

most robust results[282]. Fortunately, the field is constantly evolving: for example, the development of NAD^+ biosensors might shed light on different aspects of NAD^+ homeostasis by allowing for monitoring within different subcellular compartments in intact cells[61,283]. New techniques enabling the monitoring of NAD^+ and its metabolites at the 'point of care'[284] will also become essential for fast and simple, yet accurate and reproducible NAD^+ measurements in healthy individuals as well as in people with diseases to establish whether their NAD^+ dynamics are similar to those of animal disease models.

Only with systematic progress in both basic and clinical understanding of NAD^+ biology can the exciting observations be translated from the bench to the bedside, and the preventive and therapeutic potential associated with maintaining healthful NAD^+ homeostasis be exploited.

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E.K., M.R., D.H. and J.A. wrote the manuscript.

Competing interests

J.A. is a consultant to Mitobridge-Astellas, MetroBiotech and TES pharma, companies that develop NAD^+-boosting therapies. E.K., M.R. and D.H. declare no conflicts of interest.

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