Preclinical and clinical evidence of NAD\(^+\) precursors in health, disease, and ageing

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A B S T R A C T

NAD\(^+\) is a fundamental molecule in human life and health as it participates in energy metabolism, cell signalling, mitochondrial homeostasis, and in dictating cell survival or death. Emerging evidence from preclinical and human studies indicates an age-dependent reduction of cellular NAD\(^+\), possibly due to reduced synthesis and increased consumption. In preclinical models, NAD\(^+\) replenishment extends healthspan and lifespan and mitigates several conditions, such as premature ageing diseases and neurodegenerative diseases. These findings suggest that NAD\(^+\) replenishment through NAD\(^+\) precursors has great potential as a therapeutic target for ageing and age-predisposed diseases, such as Alzheimer’s disease. Here, we provide an updated review on the biological activity, safety, and possible side effects of NAD\(^+\) precursors in preclinical and clinical studies. Major NAD\(^+\) precursors focused on by this review are nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and the new discovered dihydronicotinamide riboside (NRH). In summary, NAD\(^+\) precursors have an exciting therapeutic potential for ageing, metabolic and neurodegenerative diseases.

1. Introduction

Nicotinamide adenine dinucleotide (oxidised form, NAD\(^+\)), first discovered as a coenzyme in fermentation in 1906, has been extensively studied throughout the last century with a total of four Nobel Prizes given to NAD/NADP-related discoveries (Fang et al., 2017; Harden and Young, 1906). Our understandings of the functions of NAD\(^+\) extend from a role in redox homeostasis, to its action as a fundamental metabolite participating in glycolysis, the tricarboxylic acid cycle (TCA) and mitochondrial oxidative phosphorylation (OXPHOS), to its participation in cell signalling pathways (Chalkiadaki and Guarente, 2015; Fang et al., 2017; Mouchiroud et al., 2013a; Verdin, 2015a). From a biosynthetic
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point of view, major known NAD\(^+\) precursors are nicotinic acid (NA), nicotinamide (NAM), nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and dihydronicotinamide riboside (NRH) (Bogan and Brenner, 2008; Katsyuba et al., 2020; Verdin, 2015a). Nicotinic acid (also named Vitamin B3 or niacin) has 2 other forms, (nicotinamide/NAM, also named niacinamide) and inositol hexanicotinate, which have different effects from nicotinic acid.

For many years, NAD\(^+\) precursors, such as NA and NAM, were researched and utilised in treatment of pellagra, a disease caused by a vitamin B3 deficiency that raged across Europe and the USA (Bogan and Brenner, 2008; Sydenstricker, 1958). As the knowledge of NAD and its role in metabolism and redox homeostasis expanded, NAD\(^+\) and its precursors gradually became more intensively studied in modern medicine, especially in the ageing field. Throughout the past two decades, NAD\(^+\) boosting molecules such as NA, NAM, NR, and NMN have displayed therapeutic potential in preventing ageing phenotypes and promoting healthy longevity (Canto et al., 2015; Lautrup et al., 2019). Rigorous trials of these NAD\(^+\) boosters are required in order to determine the safety and efficacy of these molecules for ageing and different diseases. This review will discuss the current state of the literature surrounding cellular metabolism, functions, and possible therapeutic applications of NAD\(^+\) precursors.

2. NAD\(^+\) biosynthesis

In this section, we provide a summary of different known NAD\(^+\) synthetic pathways. Within the cell, NAD\(^+\) is reversibly converted to NADH. It also converts to NADPH, which can be reversibly converted to NADPH. Furthermore, intracellular NAD\(^+\) is constantly consumed/ degraded by enzymes such as sirtuins (SIRTs), ADP ribosyl transfersases (ARTs) and poly (ADP-ribose) polymerases (PARPs), with NAM generated as its by-product (Fang et al., 2017). Thus, it is necessary to have efficient NAD\(^+\) synthesis in order to maintain a cellular NAD\(^+\) pool. This is achieved by a several synthesis pathways: the kynurenine pathway (de novo), the Preiss-Handler pathway, the salvage pathway, and the emerging NRH-salvage pathway. While the salvage pathway and the NRH-salvage pathway are intertwined, for clarity, we separate the two here (Fig. 1). The complexity of NAD\(^+\) biosynthesis provides multiple entry points for NAD\(^+\) augmentation, which lends itself to the potential for implementation of multiple pharmacological approaches through the administration of different precursors.

The kynurenine pathway (de novo) utilises the amino acid tryptophan (Trp) in the synthesis of NAD\(^+\) and other neuroactive substances (Vecsei et al., 2013) (Fig. 1A). Trp enters the cell through the transmembrane solute carrier (SLC) transporters SLC7A5 and SLC36A4. The internalised Trp is then catalysed to formylkynurenine (FK) via the rate limiting enzymes, indoleamine 2,3-dioxygenase (IDO) or tryptophan 2,3-dioxygenase (TDO) (Canto et al., 2015; Diani-Moore et al., 2017; Yamazaki et al., 1985). FK is then catalysed into kynurenine, which can be further converted into kynurenic acid, 3-hydroxykynurenine (3-HK) or anthranilic acid (Savitz, 2020). 3-HK is further converted to 3-hydroxynanthranilic acid (3-HAA), a-aminoo-β-carboxymuconate-ε-semialdehyde (ACMS), and quinolinic acid, sequentially. Quinolinic acid is then converted to nicotinic acid mononucleotide (NMN), which converges with the Preiss-Handler pathway (detailed below), to synthesise NAD\(^+\) (Fig. 1A). Some of these intermediates, and their associated downstream metabolites, display opposing attributes, which could explain the dichotomy in their neurotoxic or neuroprotective traits (Schwarz and Pollicicciari, 2002). Kynurenic acid prevents hyperactivation of N-methyl-D-aspartate (NMDA) receptors and inhibits neuronal excitotoxicity, whereas quinolinic acid, one of the downstream metabolites of 3-HK, has the opposite effect (Vecsei et al., 2013). Maintaining the balance of these counteracting metabolites is of great importance, as a disruption of this balance is linked to neurodegenerative and psychiatric disorders like Alzheimer’s disease (AD) and schizophrenia, respectively (Maddison and Giorgini, 2015). However, the kynurenic pathway is not efficient in generating NAD\(^+\); one reason may that the intermediate ACMS could be converted to picolinic acid by ACMS decarboxylase (ACMSD) (Bender, 1983; Houtkooper et al., 2010). While picolinic acid is considered neuroprotective (Guillemin et al., 2007), it is reported that reduction of picolinic acid via ACMSD inhibition protects against damage to the liver and kidney (Katsyuba et al., 2018). These paradoxical reports highlight the importance of tissue concentration and specificities in determining the health effects by the metabolites in the kynurenine pathway.

The Preiss-Handler pathway synthesises NAD\(^+\) by metabolising the precursor NA in a three-step manner, converging with the kynurenic pathway at the second step (Fig. 1B). NA-internalisation is mediated through the solute carrier/SLC transporters SLC5A8 and SLC22A13 in the plasma membrane and is converted to NAMN by Nicotinic acid phosphoribosyltransferase (NaPRT). As described above, quinolinic acid from the kynurenic pathway is metabolised into NAMN via QRPT, a critical step for NAD\(^+\) synthesis via the kynurenic pathway (Houtkooper et al., 2010). NAMN is then converted to nicotinic acid adenine dinucleotide (NAAD) via MNM NTA – 3 enzymes. Finally, NAAD is converted to NAD\(^+\) by the ATP-dependent NAD\(^+\) synthase (NADS) (Hara et al., 2003). To note, while NA has been used in the clinic for many years, there are some adverse effects, such as unpleasant skin flushing, mostly due to activation of the GPR109A receptor by NA (Haslam et al., 1984; Trammell et al., 2016; Winter and Boyer, 1973; Wise et al., 2003).

Compared with the kynurenic and Preiss-Handler pathways, the salvage pathway is considered to be a powerful approach to boost intracellular NAD\(^+\) in a fast and efficient manner (Fig. 1C). Within the cell, NAM is metabolised into NMN via nicotinamide phosphoribosyltransferase (NAMPT) and then by MNM NTA – 3 into NAD\(^+\) (Rongvaux et al., 2002; Samal et al., 1994; Verdin, 2015b). There is intracellular NAMPT (INAMP) and extracellular NAMPT (eNAMP). As NAMPT is the rate-limiting enzyme of the salvage pathway, the importance of iNAMP-induced initiation of NAM-dependent NAD\(^+\) recycling is highlighted (Bogan and Brenner, 2008; Verdin, 2015b). Of the three NMNAs, NMNAT1 is a nuclear enzyme with expression level tissue-specific (Emanueli et al., 2001; Yalowitz et al., 2004); there are higher levels in skeletal muscle, heart, kidney, liver, and pancreas, whereas the levels measured in the brain are sparse (Emanueli et al., 2001; Yalowitz et al., 2004). NMNAT2 is predominantly found in cytosol and Golgi apparatus, while NMNAT3 is located in the mitochondria/cytoplasm (Berger et al., 2005; Felici et al., 2013; Hikosaka et al., 2014; Yalowitz et al., 2004; Zhang et al., 2003). In addition to the endogenous NAM and NMN for the salvage pathway, exogenous molecules like NAD\(^+\), NMN, NR, and NAM, also participate in this pathway. Extracellular NAD\(^+\) and NMN are normally catabolised to NR or NAM by the membrane-bound NAADases ADP-ribosyl cyclases (CD38/CD157), and CD73 (Fig. 1C) (Bogan and Brenner, 2008; Verdin, 2015b). While the relatively small NAM molecule can diffuse into cells, the larger NR molecule is transported into the cell via the equilibrative nucleoside transporter (ENT) family ENT1, ENT2, and ENT4 (Kropotov et al., 2021). There are inconsistent reports as to whether extracellular NNM could be taken up by cells. For example, some studies showed that extracellular NNM is dephosphorylated to NR for cellular uptake (Ratajczak et al., 2016), while another report argued SLC12A8 was a cellular transporter of NNM whereby the data got challenges (Grozo et al., 2020; Schmidt and Brenner, 2015). As NAM enters the cell, it participates in the salvage pathway directly. For internalised NR, it is phosphorylated to NMN by nicotinamide riboside kinase 1 or 2 (NRK1/NRK2), then further converts to NAD\(^+\) (Bieganowski and Brenner, 2004; Ratajczak et al., 2016).

The recently described “NRH salvage pathway” is a potential new NAD\(^+\) synthesis pathway. It may converge with the original salvage pathway, elucidating yet another potential pharmacological approach to enhance NAD\(^+\) levels (Yang et al., 2020) (Fig. 1D). Initially,
Fig. 1. The NAD⁺-based synthetic pathways and its subcellular homeostasis. The biosynthesis of NAD⁺ comprises four pathways: the kynurenine pathway (the de novo pathway in mammals), the Preiss-Handler pathway, the salvage pathway and the new proposed NRH-salvage pathway. (A) The kynurenine pathway utilises tryptophan (Trp) to synthesise NAD⁺, permeating the plasma membrane and entering the cell through the SLC7A5 and SLC36A4 transporters. When Trp is incorporated intracellularly, it is converted to formylkynurenine (FK), which subsequently is catalysed into kynurenine. Kynurenine functions as a branching point in the pathway, either being converted to kynurenic acid through kynurenine aminotransferases (KATs), ultimately providing quinolinic acid, or to 3-hydroxykynurenine (3-HK) by the catalysis of kynurenine 3-monooxygenase (KMO). 3-HK is then, through tryptophan 2,3-dioxygenase (KYN2O), converted to 3-hydroxynaphthoic acid (3-HAA), which is further oxidised into α-amino-β-carboxymuconate-ε-semialdehyde (ACMS) by the governing of 3-hydroxyanthranilic acid oxidase (3HAO). ACMS then constitutes another branching point, as it is either spontaneously converted to quinolinic acid or enzymatically converted to picolinic acid. Quinolinic acid may then converge with the Preiss-Handler pathway, forming nicotinic acid mononucleotide (NMN) by administration of quinolinol or phosphoribosyltransferase (QPRT) and following the remaining steps as detailed below. Furthermore, some of the metabolites of the kynurenine pathway are neuroactive (the intermediates with one mark of "*" in the illustration possess neurotoxic traits, whereas the metabolites with two marks display neuroprotective attributes). (B) The Preiss-Handler pathway utilises nicotinic acid (NA) as a NAD⁺ precursor, providing NAD⁺ in three steps. NA enters the cell through the transmembrane SLC5A8 and SLC22A3 transporters, being converted to NAMN by nicotinic acid phosphoribosyl transferase (NaPRT). NA adenine dinucleotide (NAD⁺) is then synthesised from NAMN, formed from both NA and Trp, by Nicotinamide mononucleotide transferases (NMNATs). Finally, NAD⁺ synthase (NAD⁺S) converts NAD⁺ to NAD⁺. (C) The regular salvage pathway uses intracellular nicotinamide (NAM) as well as extracellular NAD⁺ and related metabolites. In the extracellular milieu, NAD⁺ may originate directly from NMM, through CD73, or indirectly from NAMN or NAM, as these are converted to NMMN by CD73 and the extracellular isotype of NAM phosphoribosyltransferase (eNAMPT), respectively. As NMM enters the cell through equilibrative nucleoside transporter (ENT) family ENT1, ENT2, and ENT4 (Kropotov et al., 2021). NMM is generated by the phosphorylation of nicotinamide riboside kinases (NRKs). Also, an intracellular isotype of NAMPT (iNAMPT) initiates NAD⁺ recycling by forming NMM from NAM. As these two sources of NMM converge, NAD⁺ is generated from NMM through NMNATs. (D) The NRH salvage pathway utilises dihydronicotinamide riboside (NRH), the reduced form of NR, as a biosynthetic precursor of NAD⁺. The initial step is seemingly catalysed by adeninonucleoside kinase (AK), providing NMMN from NRH. NMMN is subsequently converted to NADH through NMNATs, finally being oxidised to NAD⁺. (E) Mitochondrial homeostasis of NAD⁺. SLC25A51 has recently been discovered to be a mammalian transporter for direct NAD⁺ permeation from cytoplasm to mitochondrial matrix. The cytosolic and mitochondrial NAD⁺ pools communicate indirectly through the transportation of reducing equivalents across mitochondrial membranes in the glycerolaldehyde 3-phosphate or malate-aspartate shuttle. Although NMNAT3 has been suggested to be an essential participant of the salvage pathway in mitochondria, recent studies do not support the existence of an active mitochondrial NMNAT3 to synthesise NMN to NAD⁺ (Kory et al., 2020; Luongo et al., 2020). NAD⁺ is consumed by SIRT3-5, providing NAM, which is potentially further converted to NMMN through NAMPT. (F) Nuclear transport of NAD⁺ is also incomplete, but nuclear pore-mediated NAD⁺ diffusion has been suggested as a plausible mechanism. Furthermore, the nuclear NAMNAT-i isotype is seemingly involved in nuclear NAD⁺ salvage pathway. Finally, NAM is methylated to MeNAM to be excreted through the urine. See text for details including references.

extracellular NRH is transported in its intact form through unidentified mechanisms, across the cell membrane in order to be phosphorylated by a NRH kinase, thought to be adeninonucleoside kinase (AK) (Yang et al., 2020). Consistent with this hypothesis, AK knockout (KO) hepatocytes treated with NRH did not display the same levels of elevated NAD⁺ as WT cells (Giroud-Gerbetant et al., 2019). Thus, AK is a crucial enzyme in this pathway because of its ability to convert NRH to reduced nicotinamide mononucleotide (NMNH). Consequently, this distinct pharmacokinetic attribute of NRH may be concomitant with the promising NAD⁺ boosting capabilities displayed following NRH administration in preclinical studies (Yang et al., 2020). Subsequent to the conversion from NRH to NMN, NMN is converted to NADH through NMNAT1–3, and then oxidised to NAD⁺ as the final step (Yang et al., 2020). Interestingly, the NRH salvage pathway may converge with the classical salvage pathway, possibly through NRH quinone oxidoreductase 2 (NQO2)-mediated oxidations of NRH to NR (Yang et al., 2020). Furthermore, it is possible that NMNH may enter the classical salvage pathway through conversion to NMM, however the potential mechanism of this action is not yet known (Yang et al., 2019). Collectively, the NRH salvage pathway is likely a new approach to elevate cellular NAD⁺, while how this pathway interacts with the other three NAD⁺ synthetic pathways remain elusive.

3. The multifunctional NAD⁺: redox and metabolic homeostasis, energy production, and cell signalling pathways

3.1. Innate characteristics of NAD⁺

The classical cellular functions of NAD⁺ are the maintenance of redox homeostasis, and bioenergetics. Whereas the NAD⁺/NADH ratio at physiological level is essential for maintaining metabolic homeostasis through the catabolisation of carbohydrates, proteins and lipids, the NAD⁺/NADPH ratio provides the reductive milieu necessary for the synthesis of fatty acids, nucleotides and amino acids (Gilmour et al., 2020). NADPH converts glutathione to its reduced form, and functions as a substrate for NADPH oxidase, providing support in the resistance against reactive oxygen species (ROS). This wide range of functions is made possible by the innate characteristics of NAD⁺ and the de novo generation of the associated phosphorylated form, NADP⁺, through NAD⁺ kinases (NADKs). Overall, this suggests an intricate and essential interaction between the NAD⁺/NADH and NAD⁺/NADPH ratios (Xiao et al., 2018) to maintain cellular homeostasis. It provides an explanation as to why imbalances may cause severe side effects and why conditions concomitant with attenuated NAD⁺ levels may benefit greatly from NAD⁺ restoration. Cells regulate bioenergetics via modulating NAD⁺/NADH ratios in the subcellular compartments. In normal condition, NAD⁺ and NADH are used in oxidative reactions during glycolysis, the TCA cycle, and OXPHOS. In bioenergetically demanding conditions, a reduction in the hepatic NAD⁺/NADH ratio induces a shift towards a cellular environment favouring gluconeogenesis and ketone body synthesis (Gilmour et al., 2020). To note, NAD⁺ is fractionised in nucleus, cytoplasm, and mitochondria, and there are subcellular channels that control cellular NAD⁺ homeostasis (Fig. 1E, F). Recent studies show the SLC25A51 transporter transfers NAD⁺ across mitochondrial membranes (Girardi et al., 2020; Kory et al., 2020; Luongo et al., 2020) (Fig. 1E). In summary, there is a sophisticated and precisely controlled cellular system regulating subcellular NAD⁺ levels, supplying adequate energy levels through glycolysis, TCA, and OXPHOS, as well as adapting to intracellular and extracellular changes.

3.2. NAD⁺ consumers and metabolism

In addition to its roles in redox homeostasis and bioenergetics, NAD⁺ is necessary for cell survival and signalling, regulation of transcription and calcium homeostasis. NAD⁺ is known as a co-substrate for several types of enzymes, such as the class III histone deacetylases enzymes the sirtuins (SIRTs, in mammals SIRT1–7), DNA repair signalling Poly-ADP-ribose polymersases (PARPs), and other proteins such as CD38, CD157, and NADase sterile α and TIR motif-containing 1 (SARM1) (Lautrup et al., 2019). NAD⁺ is enzymatically catalysed into NAM in reactions mediated by these proteins (Bock et al., 1965; Imai et al., 2000; Rouleau et al., 2010; Schuber and Lund, 2009), and these proteins control broad and diverse cellular pathways in an NAD⁺-dependent manner.

SIRTs are deacetylases, converting NAD⁺ to NAM and 2/3-o-acetyl-ADP-ribose in the process of removing acyl groups from lysine
residues on target proteins (Imai et al., 2000). There are seven SIRTs in mammals, specific to different subcellular compartments. Whereas SIRT1, SIRT6 and SIRT7 are mainly located in the nucleus, SIRT2 is cytoplasmic and SIRT3–5 are mitochondrial (Chalkiadaki and Guarente, 2015; Verdin, 2015b). The nuclear sirtuins are particularly associated with deacetylating histones, transcription factors and transcription coactivators, thereby regulating the transcription of genes involved in metabolism, mitochondrial homeostasis, stem cell rejuve-
nation, as well as both tumour growth or inhibition (Imai and Guarente, 2014; Motta et al., 2004; Ramsey et al., 2008). Among the seven SIRTs, some of the key discoveries have related to SIRT1, SIRT6, and SIRT3. SIRT1 regulates mitochondrial homeostasis through mitochondrial biogenesis via deacetylation of the transcription coactivator peroxisome proliferator-activated receptor-gamma coactivators (PGC-1a) (Rodgers et al., 2005), and the clearance of damaged mitochondria via mitophagy (Fang et al., 2014). While SIRT6 acts as a longevity protein in rodents via enhancing DNA repair and other mechanisms (Kaidi et al., 2010; Mao et al., 2011; Tian et al., 2019), deficiencies in this molecule result in developmental retardation (Zhang et al., 2016). Additionally, mitochondrial SIRT3 plays an important role in maintaining a healthy brain via mediating adaptive responses to exercise, as well as responding to metabolic and excitatory challenges (Cheng et al., 2016; Kaidi et al., 2010).

The NAD+-consuming PARPs, a total of 17 members in mammals, play important roles in DNA repair, and the regulation of transcription and metabolism, among other tasks. A true PARP is defined as having two capacities: a) to transfer the first ADP-ribose moiety from NAD+ to a recipient protein, preferably to lysine/glutamate residues, and b) to sequentially add multiple ADP-ribose units to the preceding ones to extend the poly(ADP-ribose) chains (Rouleau et al., 2010). Based on these two criteria and the current evidence, PARP1, PARP2 and PARP5a (tankyrase 1) are true PARPs, while the others are likely mono-ADP-ribosyltransferases (Leung, 2017; Rouleau et al., 2010). In response to age-dependent accumulation of DNA damage, PARPs consume more NAD+ resulting in reduced cellular/tissue NAD+ (Fang et al., 2016a; Mouchiroud et al., 2013b). To note, excessive PARP-1 activation initiates cell apoptotic death termed ‘parthanatos’ via the mitochondrial release and nuclear translocation of apoptosis-inducing factor (AIF) (Andrabi et al., 2014; Wang et al., 2016). By extrapolation, a reduced NAD+-mitophagy axis may play a role in parthanatos as repressed mitophagy leads to the accumulation of damaged mitochondria to release AIF.

The ADP ribosyl cyclases (CD38/CD157) are NADases that convert NAD+ to cyclic ADP ribose (cADPR) and ADP ribose (ADPR) in neutral pH conditions; in acid conditions, NAD first converts to NADP, and then to nicotinic acid adenine dinucleotide phosphate (NAADP). CD38 and CD157 play important roles in the modulation of social behaviour, calcium homeostasis, immunity, mitochondrial homeostasis, metabolism and even hormone secretion (Adebamjo et al., 1999; Camacho-Pereira et al., 2016; Jin et al., 2007). The mechanisms driving CD38 regulation of calcium homeostasis are complex, and fluctuations in intracellular calcium storages correlate with variations in the concentration of cADPR, ADPR and NADP+-derived NAADP (Ying, 2008). As the CD38 levels increase with age, possibly compounded by the PARP1 driven NAD+ depletion, interruptions to calcium signalling may occur. Indeed, perturbed calcium signalling has been recognised as one of the ten hallmarks of brain ageing (Mattson and Arumugam, 2018). Recent research in mice has found that in the prod Amn stage, in healthy and M1-like macrophages, there was an age-dependent hyper-activation of CD38, which leads to NAD+ depletion (Covarrubias et al., 2020a). Macrophages are normally classified into pro-inflammatory M1-like and naïve or M2 anti-inflammatory macrophages; during ageing, there is an increase of the percentage of M1 type. Mechanistically, the age-associated increase of senescent cells in visceral white adipose tissue and liver, characterised by irreversible cell cycle stagnation and the acquisition of a pro-inflammatory senescence-associated secretory phenotype (SASP), enhanced CD38-dependent NADase activity (Covarrubias et al., 2020a). Thus, as stressors accumulate with age, excessive release of SASP-linked pro-inflammatory cytokines may lead to a disrupted equilibrium between M1 and M2 macrophages, CD38-induced NAD+ depletion and a state of low-grade chronic inflammation collectively known as “inflammageing” (Covarrubias et al., 2020a, 2021). Similar to CD38/CD157, SARM1 exhibits NADase properties and provides NAM, cADPR and ADPR from the conversion of NAD+, SARM1 is activated by an increase in the ratio of NMN/NAD+ with NMN binding a prerequisite for injury-induced SARM1 activation and axon destruction (Figley et al., 2021).

In summary, the most well-known cellular NAD+-consuming enzymes are SIRTs, PARPs, the ADP ribosyl cyclases (CD38/CD157), and SARM1. While each of them uses NAD+ for designated physiological functions, in normal conditions such as aging and exogenous damage (e.g., injury-induced axon degeneration), hyperactivation of some of these NAD+-consuming enzymes such as PARP and SARM1 and CD38 could result in detrimental effects caused by NAD+ depletion.

### 3.3. Pathological consequences of depleted NAD+

Although NAD+ depletion occurs in various tissues during ageing (Fang et al., 2014; Mouchiroud et al., 2013b; Zhu et al., 2015), here we would like to focus on the brain. In the ageing brain, there are multiple cellular changes associated with NAD+ depletion that may disrupt metabolism, bioenergetics and overall homeostasis (Lautrup et al., 2019). The most prominent of these are termed the ‘Hallmarks of Brain Ageing’, comprising 1) Impaired DNA repair, 2) aberrant neuronal network activity, 3) stem cell exhaustion, 4) glial cell activation and inflammation, 5) impaired adaptive stress response signalling, 6) dys-regulated neuronal calcium homeostasis, 7) oxidative damage, 8) mitochondrial dysfunction, and 9) impaired molecular waste disposal (Mattson and Arumugam, 2018). Replenishment of the exhausted NAD+ pools by NAD+ precursor supplementation in these cases has shown varying degrees of success in regards to these hallmarks, as summarised recently (Lautrup et al., 2019). In addition to biological ageing, NAD+ depletion also happens in and is likely a driver of a group of premature ageing diseases associated with neurodegeneration, including xero-derma pigmentosum group A (XPA), Cockayne syndrome (CS), and Ataxia Telangiectasia (A-T) (Fang et al., 2016a, 2014). A shared aetiological feature of these diseases is mutation of genes involved in DNA repair, a cellular process responsible for maintenance of genomic stability. While there are likely many molecular mechanisms that drive neurodegeneration in these diseases, one pathway is hyper-PARYlation-induced impairment of the NAD+–mitophagy axis, resulting in accumulation of damaged mitochondria, as reviewed elsewhere (Fang et al., 2016b). Furthermore, reduced NAD+ and / or impairment of the NAD+ synthetic pathways have been reported in Alzheimer’s disease (AD), and other common neurodegenerative diseases, such Parkinson’s disease (PD), Huntington’s disease (HTD), and amyotrophic lateral sclerosis (ALS). Interestingly, impairment of the NAD+–mitophagy axis is likely a common cause/risk factor of these diseases (Lautrup et al., 2019).

While the four NAD+ coenzymes (NAD+, NADH, NADP+, NADPH) are the central catalysts in metabolism and redox homeostasis, our understanding of NAD+ has been extended to include participation in tissue function, and organismal longevity. While the balance of NAD+ generation and consumption is well-regulated in young and healthy individuals, NAD+ is reduced in biological ageing, accelerated ageing, and a series of age-predisposed diseases, including AD. Thus, there is a correlation of NAD+ depletion in ageing and disease; the proportional contribution of NAD+ depletion as a ‘bystander’ of ageing and these age-predisposed diseases remain to be determined.
4. Effects of NAD\textsuperscript{+} restoration on health and disease

Genetic approaches, such as ubiquitous or tissue-specific knockout of PARP1 and CD38, and pharmacological methods, such as the use of NR, NMN, NAM, increase cellular NAD\textsuperscript{+} and exhibit broad health benefits. The in vitro restoration of NAD\textsuperscript{+} mitigates bioenergetic impairment, improves mitochondrial biogenesis, restores the cellular protective capabilities against ROS, reduces DNA damage, promotes DNA repair, stimulates neuronal regeneration, as well as prohibits cellular senescence and improves stemness; the evidences were summarized elsewhere (Covarrubias et al., 2021; Fang et al., 2017). NAD\textsuperscript{+} augmentation also ameliorates systemic and organic dysfunction and improves healthspan and/or lifespan in the preclinical models, such as round-worm Caenorhabditis elegans, fruit fly Drosophila melanogaster, and mice (Covarrubias et al., 2020b; Fang et al., 2016a, 2014; Gomes et al., 2013; Mouchiroud et al., 2013b; Vannini et al., 2019; Wiley et al., 2016). Compared with genetic approaches to knockout NAD\textsuperscript{+}-consuming enzymes to achieve cellular NAD\textsuperscript{+} preservation, practically pharmacological supplementation of NAD\textsuperscript{+} precursors are easy to achieve (Fig. 2A). In view of the broad health benefits of NAD\textsuperscript{+} precursors, there are more than 30 clinical trials on the use of NR or NMN currently listed on the NIH Clinical Trials database (clinicaltrials.gov). Many of the Phase I clinical trials are focused on bioavailability and safety (see Table 1). Further information outlining the proposed effects of NAD\textsuperscript{+} augmentation is described in Fig. 2B-C. Here, we will elaborate in detail on two topics: NAD\textsuperscript{+} precursors in healthspan and longevity, and safety and potential side effects.

4.1. The use of NAD\textsuperscript{+} precursors in diseases

Manipulating NAD\textsuperscript{+} homeostasis through pharmacologic approaches has been the subject of many pre-clinical and some clinical studies in recent years (Fang et al., 2019a, a; Fang et al., 2014; Mitchell et al., 2018; Mouchiroud et al., 2013b; Zhang et al., 2016). Most of these studies have included the precursors NA, NAM, NMN and/or NR. Here we will focus on NR, NMN, and NRH.

Two precursors that have been extensively studied are NMN and NR. Preclinical studies using NMN have shown broad health benefits, covering the reversion of vascular dysfunction and oxidative stress, suppression of age-associated body weight gain, promotion of physical activity, improvement of insulin sensitivity, amelioration of the plasma lipid profile and mitigation of other age-associated phenotypes in mice (de Picciotto et al., 2016; Mills et al., 2016; Yoshino et al., 2011). NMN treatment leads to increased longevity and improved health in xpa-1 nematodes (Fang et al., 2014). In a C. elegans model of XPA, NMN was shown to extend lifespan by 117 % compared to control worms (Fang et al., 2014). Other studies using NMN for the treatment of metabolic, neurodegenerative and accelerated aging diseases have shown NMN supplementation increased NAD\textsuperscript{+} levels leading to amelioration of disease pathologies, such as mitochondrial dysfunction, as reviewed elsewhere (Katsuya et al., 2020; Yoshino et al., 2018). Mitophagy, the mechanism by which damaged mitochondria are targeted and degraded by lysosomes, is important for mitochondrial homeostasis and healthy longevity (Aman et al., 2021). That NMN/NR induces mitophagy was first reported in 2014 (Fang et al., 2014) and since been reported in both in vitro and in vivo in different models by many other laboratories (Lynch et al., 2020; Sun et al., 2020; Vannini et al., 2019). A detailed summary covering how NMN/NR induces mitophagy/autophagy is available elsewhere, covering transcriptional, translational, and post-translational regulation of genes/proteins involved in mitophagy/autophagy process (Fang, 2019). Impeded mitophagy has also been suggested to be a contributing etiological factor for accelerated ageing in WS and neurodegeneration in AD (Fang et al., 2019a, b). Enhancing mitophagy through NMN supplementation might be an effective strategy for the treatment of cognitive deficits in AD (Fang et al., 2019b). A very recent clinical study showed that NMN increased muscle insulin sensitivity in prediabetic women (Yoshino et al., 2021), although criticism on the experimental design on whether it was an effectively randomised trial or not, arose (Brenner, 2021).

NR displays great pharmacological potential, as it may increase healthy longevity in neurodegenerative and accelerated ageing diseases. NR increased life- and/or healthspan in a similar fashion to NMN when administered to C. elegans, D. melanogaster, or mouse models of a series of premature ageing diseases, such as XPA, CS, AT, and WS (Fang et al., 2019a, a; Fang et al., 2014; Okur et al., 2020). While there is a likelihood of the involvement of different NAD\textsuperscript{+}-dependent pathways, improved DNA repair (e.g., non-homologous DNA end joining), ameliorates the impact of telomere dysfunction (Sun et al., 2020), and mitophagy-dependent mitochondrial homeostasis are among the mechanisms contribute to the health benefits by NAD\textsuperscript{+} (Fang et al., 2016a; Verdin, 2015a). In addition to improving outcome in premature ageing diseases, NR could inhibit high-fat-induced side effects. NR supplementation mitigated oxidative metabolism, and prevented body-weight gain in HFD-fed mice, as well as improving insulin sensitivity and increased mitochondrial content in skeletal muscle (Canto et al., 2012). A recent clinical trial of NR supplementation (1000 mg twice daily for 12 weeks) in nondiabetic, obese men did not find any significant impact on β- and alpha-cell secretion, insulin sensitivity and the overall levels of pancreas hormones and incretins in NR treated patients (Dollerup et al., 2019); a potential reason could be the groups were not randomised for hepatic lipids (Brenner, 2021). It is suggested that a combination of NR plus exercise may maximise any clinical benefit of NR in the future clinical trials (Fluharty and Brenner, 2020). To note, parallel studies in cells, worms, and mice suggest NR and NMN show similar effects in elevating NAD\textsuperscript{+} and in the extension of lifespan in the model systems used (Fang et al., 2016a, 2014).

NRH is a potent NAD\textsuperscript{+} precursor. NRH was discovered to be an upstream reductant used by the enzyme NQO2 in the process of converting quinones to dihydroquinones (Megarity et al., 2014; Riches et al., 2017). In cell culture system, NRH was more efficient and potent at elevating cellular NAD\textsuperscript{+} than NR (Yang et al., 2019). In AML12 hepatocytes treated with NRH, it was observed that the cells would need about a 50 times higher concentration of NR in order to enhance the NAD\textsuperscript{+} levels to a similar degree as NRH (Giroud-Gerbetant et al., 2019); to note, there is also a possibility that different cells may respond differently to NR and NRH, and similar comparison experiments need to be done in other cell types. In mice, while both NR and NRH displayed a dose dependent increase in hepatic NAD\textsuperscript{+} levels when intraperitoneally (IP) administered, NRH injections induced markedly higher hepatic NAD\textsuperscript{+} levels than NR (Giroud-Gerbetant et al., 2019). This effect of NAD\textsuperscript{+} boosting by NRH was also shown in muscle and kidney, as it significantly increased the NAD\textsuperscript{+} levels in both tissues at 250 mg/kg and 500 mg/kg, whereas NR failed to provide a significant increase of NAD\textsuperscript{+} levels in muscle tissue at 250 mg/kg (Giroud-Gerbetant et al., 2019). In kidney tissue, there was no substantial difference in NAD\textsuperscript{+} increase between NR and NRH at 500 mg/kg injections (Giroud-Gerbetant et al., 2019). This might ascribed to the high renal NRK1 expression relative to other tissues, suggesting that the superiority of NRH may be tissue-specific in vivo (Giroud-Gerbetant et al., 2019). These findings suggest that NRH may be the most potent precursor discovered to date. Further studies using different systems are required to confirm these reports and to provide data on safety and bioavailability.

4.2. Cautions on the clinical use of NAD\textsuperscript{+} precursors: safety and potential side effects

Results from clinical trials suggest NA, NAM, NR, and NMN are safe within the dose tested (Table 1), but cautions exist. First, NAD\textsuperscript{+} and the NAD\textsuperscript{+} synthetic pathway could be either pro-cancer or anti-cancer in different conditions. Altered levels of NAD\textsuperscript{+} and related NAD\textsuperscript{+} synthetic enzymes occur in different cancer cells, such as breast and lung, and there are anti-cancer drugs/drug candidates that target different NAD\textsuperscript{+}
Fig. 2. NAD⁺ boosting strategies and its benefits at cellular, animal, and human levels. (A) NAD⁺ boosting strategies. Potential non-pharmacological NAD⁺ boosting strategies comprise lifestyle changes, such as improving habitual exercise patterns, establishing a healthy diet and maintaining consistent mealtimes and sleeping patterns. “?” represents unknown information. Pharmacological NAD⁺ boosting strategies include inhibitors of NAD⁺ consumers, modulators of rate-limiting NAD⁺ biosynthetic enzymes, and NAD⁺ precursor supplementation. (B) Potential benefits of NAD⁺ supplementation at cellular and animal levels. (C) Potential benefits of NAD⁺ supplementation at human levels. “?” represent these uncertainties/potential advantageous effects of NAD⁺ augmentation. See text for details including references.
Table 1
A partial summary of clinical trials of NAD+ precursors and their efficacy in healthy elderly and in individuals with diseases.

| NAD+ precursors | Disease/Condition | Dose administration | Duration of treatment | Demographics | Primary outcome | Phase | Status and results | References/NCT |
|-----------------|-------------------|---------------------|-----------------------|--------------|----------------|-------|-------------------|----------------|
| F-A             |                   | Single dose 4 g/day  | 1 year                | Age: 18+     | - Efficacy of daily doses of NAM in slowing disease progression | Phase 2 | Not yet recruiting | NCT03761511 |
|                 |                   | or highest dose     |                       | Sex: All     | - Safety of NAM- treatment in F-A patients |       |                   |                 |
|                 |                   | tolerated with a    |                       |              | - Upregulation of |       |                   |                 |
|                 |                   | minimum of 2 g/     |                       |              |  Frataxin in F-A patients |       |                   |                 |
|                 |                   | day, PO             |                       |              | - Change in p-tau 231 |       |                   |                 |
|                 |                   | Dose-escalation of 2-8 g, PO | Up to 9 weeks | Age: 18+     | - Effects of NAM on AD symptoms | Phase 2 | Recruiting, no results | NCT03061474 |
|                 |                   | 1500 mg BID, PO     | 12 months             | Age: 50+     | - Effects of NAM on inflammation |       |                   |                 |
|                 |                   | 1500 mg BID, PO     | 6 months              | Age: 50–95   | - Effects of NAM on severity of PD symptoms |       |                   |                 |
|                 |                   | 100 mg BID, PO      | 18 months             | Sex: All     | - Effect of NR on NAD+ in blood |       | Recruiting, no results | NCT03808961 |
|                 |                   | 250 mg/day, BID, PO | 7 days                | Age: 18–65   | - Effect of NR on NAD+ metabolites in blood |       | Recruiting, no results | NCT03501433 |
|                 |                   | 900 mg BID, PO      | 3 months              | Sex: All     | - Effect of NR on PD related patterns, neurometabolic profile and motoric function |       | Recruiting, no results | NCT03816020 |
|                 |                   | 1000 mg/day, PO     | 12 weeks              | Age: 18+     | - Effect of NR on PD related patterns, neurometabolic profile and motoric function |       | Recruiting, no results | NCT03816020 |
|                 |                   | 1000 mg/day, PO     | 52 weeks              | Age: 18+     | - Effect of NR on PD related patterns, neurometabolic profile and motoric function |       | Recruiting, no results | NCT03816020 |
|                 |                   | 500 mg BID          | 30 days               | Sex: All     | - Effect of NR on PD related patterns, neurometabolic profile and motoric function |       | Recruiting, no results | NCT03816020 |
| NR              |                   | 25 mg/kg/day        | 4 months              | Sex: All     | - Effect of NR on PD related patterns, neurometabolic profile and motoric function |       | Recruiting by invitation, no results | NCT03962114 |
| A-T             |                   | Doses given by body |                      | Age: 10–40   | - Effects of NR in combination with exercise on aerobic capacity and glucose homostasis |       | Recruiting, no results | NCT04192136 |
|                 |                   | weight:             |                       |              | - Effects of NR in combination with exercise on aerobic capacity and glucose homostasis |       |                   |                 |
|                 |                   | 1  >72 kg: 900 mg/ day, PO |                  |              | - Effects of NR in combination with exercise on aerobic capacity and glucose homostasis |       |                   |                 |
|                 |                   | 2  >48 kg and ≤72 kg: 600 mg/day, PO |              |              | - Effects of NR in combination with exercise on aerobic capacity and glucose homostasis |       |                   |                 |
|                 |                   | 3  24 ≤48 kg: 300 mg/day, PO |                 |              | - Effects of NR in combination with exercise on aerobic capacity and glucose homostasis |       |                   |                 |
|                 |                   | 12 weeks            |                       | Sex: All     | - Effects of NR on MoCA from baseline at 10 weeks |       | Recruiting, no results | NCT02942888 |
|                 |                   | 2 × 250 mg BID      | 12 weeks              | Age: 60–90   | - Effects of NR on MoCA from baseline at 10 weeks |       | Recruiting, no results | NCT03482167 |
|                 |                   | 10 weeks            |                       | Sex: All     | - Effects of NR on MoCA from baseline at 10 weeks |       | Recruiting, no results | NCT03482167 |
|                 | Mild cognitive     | Dose escalation of 250 mg/day every week to 1 g/day (4 weeks) than 1 g/day (6 weeks) |              | Age: 60+     | - Effects of different doses of NR on |       | Recruiting, no results | NCT03562468 |
|                 | impairment         | 12 weeks            |                       | Sex: All     | - Effects of different doses of NR on |       | Recruiting, no results | NCT03562468 |
|                 | Cognitive function | Crossover study with 300 mg/day, 8 weeks |              | Age: 55+     | - Effects of different doses of NR on |       | Recruiting, no results | NCT03562468 |

(continued on next page)
synthetic enzymes (e.g., NAMPT) as reviewed recently (Demarest et al., 2019). The NAD⁺ precursors also show anticancer capacities, for example in the treatment of skin cancer (Chen et al., 2015). Such differences indicate the complexity of NAD⁺ precursors in cancer development and treatment, highlighting the need for further detailed investigation with focus on cancer types and stages of the diseases. Further, while NAD⁺ augmentation is considered neuroprotective

| Table 1 (continued) |
|---------------------|
| NAD⁺ precursors     | Disease/Condition | Dose administration | Duration of treatment | Demographics | Primary outcome                                                                 | Phase | Status and results | References/NCT |
| NAM (Nicotinamide)  | Chemotherapy induced peripheral neuropathy | PO and 1000 mg/day, PO | 84 days | Age: 18 – 85, Sex: All | Cognitive function, mood, and sleep, Effects of NR on quality of life, neuropathy, nerve fibre density and corneal sensitivity | 2      | Recruiting, no results | NCT04112641 |
| NMN (NAD⁺)          | Ageing daily function and recovery | 2 × 250 mg BID | 8 weeks | Age: 60+, Sex: All | Effects of NR on cognitive function, mood and daily activity, Effects of NR on skeletal muscle tissue NAD⁺ levels and mitochondrial function | N/A    | Not yet recruiting, no results | NCT04078178 |
| NMN (NAD⁺)          | 1000 mg/day | 3 weeks | Age: 70 – 80, Sex: Male | Effects of NR on muscle function, genes and mitochondria | 2 | N/A | NCT02950441 |
| NMN (NAD⁺)          | 500 mg BID, PO | 6 weeks | Age: 55 – 79, Sex: All | Incidents of treatment emergent adverse events | Phase 1 and 2 | Completed, NR is well tolerated in healthy middle-aged and older adults | NCT02921659 |
| NMN (NAD⁺)          | Peripheral diabetic neuropathy | 2 × 250 mg BID, PO | 6 months | Age: 30 – 80, Sex: All | Effects of NR on intraepidermal nerve fiber density, Effects of NR on max oxygen uptake, Effects of NR on muscle function, genes and mitochondria, Effects of NR on Short Physical Performance Battery | Phase 1 and 2 | Recruiting, no results | NCT03685253 |
| NMN (NAD⁺)          | Healthy elderly volunteers | 500 mg BID, PO | 6 months | Age: 65 – 80, Sex: Female | Muscle biopsy samples: Respiration rate, immunoblot and PCR, Effects of NR on Bone metabolism | N/A | Recruiting, no results | NCT03818802 |
| NMN (NAD⁺)          | Mild concussion | 3 × 250 mg capsule, BID, PO | 84 days | Age: 18+, Sex: All | Effects of NR on brain NAD⁺ levels, Effects of NR on muscle recovery after exercise in people with Li-Fraumeni syndrome, Effects of NR on mitochondrial function in skeletal muscle | N/A | Recruiting, no results | NCT02721537 |
| NMN (NAD⁺)          | - Week 0: 250 mg | 12 weeks | Age: 18 – 70, Sex: All | Effects of NR on exercise in people with Li-Fraumeni syndrome | Phase 1 and 2 | Completed, NR might rescue mitochondrial function in fibroblasts of Li-Fraumeni syndrome patients | NCT03789175 |
| NMN (NAD⁺)          | - Week 1: 500 mg | 8 weeks washout | Age: 18 – 70, Sex: All | Effects of NR on mitochondrial function in skeletal muscle | N/A | Recruiting, no results | NCT03818802 |
| NMN (NAD⁺)          | - Week 2: 750 mg | at week 24 through 30 if positive response | Sex: All | Effects of NR on mitochondrial function in skeletal muscle | N/A | Recruiting, no results | NCT04691986 |
| NMN (NAD⁺)          | - Week 7: 1000 mg | - | - | - | - | - | - |
| NMN (NAD⁺)          | Frailty and sarcopenia | 2 × 250 mg BID, PO, capsule | 12 weeks | Age: 65 – 85, Sex: All | Maximal oxygen uptake, Muscle strength | N/A | Not yet recruiting, no results | NCT04691986 |
| NMN (NAD⁺)          | Hypertension in elderly | 1000 mg/day, PO | 6 weeks | Age: 65 – 105, Sex: All | Systolic blood pressure, Effects of NR on systolic blood pressure and arterial stiffness | Phase 1 | Recruiting, no results | NCT04112043 |
| NMN (NAD⁺)          | 500 mg BID | 3 months | Age: 50 – 79, Sex: All | Systolic blood pressure and arterial stiffness | Phase 2 | Recruiting, no results | NCT03821623 |
| NMN (NAD⁺)          | Glucose metabolism disorders | Two capsules with a total of 250 mg/day | 8 weeks | Age: 55 – 75, Sex: Female | Intraepidermal nerve fibres, Effects of NR on cellular NAD⁺ concentration in blood serum, Maximal oxygen uptake, Muscle strength | N/A | Recruiting, no results | NCT04571008 |
| NMN (NAD⁺)          | Two capsules with a total of 300 mg/day | 16 weeks | Age: 45 – 75, Sex: All | Maximal oxygen uptake, Muscle strength | N/A | Recruiting, no results | NCT04571008 |
| NMN (NAD⁺)          | Ageing | 300 mg/day, PO | 60 days | Age: 40 – 65, Sex: All | Maximal oxygen uptake, Muscle strength | N/A | Recruiting, no results | NCT04228640 |
| NMN (NAD⁺)          | Parkinson’s disease | 300 mg/day, PO | 60 days | Age: 65 – 85, Sex: All | Maximal oxygen uptake, Muscle strength | N/A | Recruiting, no results | NCT04691986 |
some NAD⁺ metabolites could be neurotoxic in specific conditions. In the kynurenine pathways, kynurenic and picolinic acid are neuroprotective, but 3-HK, 3-HAA, and quinolinic acid are neurotoxic (Fig. 1A). Furthermore, excessive bioaccumulation of methylated NAM (MeNAM) may cause neuronal death (Mori et al., 2012), and higher intracellular NNM/NAD⁺ ratio is a driver of injury-induced SARM1-dependent axon degeneration (Figley et al., 2021).

NA is a well-established pharmacological drug, utilised clinically in the treatment of pellagra and dyslipidaemia for many years, so the mapping of the side-effects following NA administration is more detailed in comparison to other precursors (DiPalma and Thayer, 1991). A very common side-effect of NA is flushing, typically occurring immediately after injection and lasting for 1–2 min (DiPalma and Thayer, 1991). This side-effect may be accompanied by pruritus, skin rashes and a sensation similar to that of a menopausal hot flush (DiPalma and Thayer, 1991).

Additionally, when high doses of NA are administered, acute symptoms like gastrointestinal distress, general vasodilatation and moderate increase in liver enzymes might occur (DiPalma and Thayer, 1991). When administered over time, the flushing symptoms prove to be transient, transitioning into abdominal pain, vomiting, diarrhoea, peptic ulceration, icterus and heart arrhythmias (DiPalma and Thayer, 1991). Finally, slow-releasing and high doses of NA, corresponding to 3 g/day or more, have been shown to induce a reversible hepatotoxicity in humans (Haslam et al., 1984; Winter and Boyer, 1973). While NA is general safe, side effects exist (Hwang and Song, 2020). These side effects include epigenetic changes, impeded bioenergetics, potential carcinogenesis and GI disturbances (nausea, vomiting, diarrhoea) (Braidy and Liu, 2020).

NNM is well-tolerated at lower doses, but high dose administration may be detrimental. In mice, a 12-month long-term administered of NNM in the water did not result in any deleterious effects (Mills et al., 2016). In healthy Japanese men, a single oral dose of up to 500 mg NNM was found to be well tolerated and safe with no significant side-effects (Irie et al., 2020). Moreover, several issues with the study design (no placebo group, only one dose tested), strongly argue for more clinical trials with NNM to rigorously identify therapeutic and toxic dose ranges and to include both males and females, as well as healthy and disease states (Irie et al., 2020). Some potential side-effects of NNM have been proposed, especially concomitant with high dose administration, such as hepatic pressure and cancer growth as reviewed elsewhere (Yoshino et al., 2018).

Compared with NMM, there are more finished clinical trials on the safety and bioavailability data on NR (https://clinicaltrials.gov/ and Table 1). One clinical trial on the time and dose-dependent effects of NR on blood NAD⁺ metabolism in humans, and revealed that NR was bioavailable, safe and well tolerated in humans in doses up to 1000 mg (Trammell et al., 2016). A randomised clinical study from 2018 on the clinical bioavailability of NR concluded that NR displayed few side-effects and may be considered safe if administered in a dose of 1000 mg/day (Martens et al., 2018). Speaking of NRH, it is generally safe in clinical bioavailability of NR concluded that NR displayed few adverse effects (Giroud-Gerbetant et al., 2019; Yang et al., 2019). A single doses of intraperitoneally-injected NRH at 1000 mg/kg and orally 10 mg/day (Martens et al., 2018). A very common side-effect of NRH is flushing, typically occurring immediately after injection and lasting for 1–2 min (DiPalma and Thayer, 1991). This side-effect may be accompanied by pruritus, skin rashes and a sensation similar to that of a menopausal hot flush (DiPalma and Thayer, 1991).

Additionally, when high doses of NR are administered, acute symptoms like gastrointestinal distress, general vasodilatation and moderate increase in liver enzymes might occur (DiPalma and Thayer, 1991). When administered over time, the flushing symptoms prove to be transient, transitioning into abdominal pain, vomiting, diarrhoea, peptic ulceration, icterus and heart arrhythmias (DiPalma and Thayer, 1991). Finally, slow-releasing and high doses of NR, corresponding to 3 g/day or more, have been shown to induce a reversible hepatotoxicity in humans (Haslam et al., 1984; Winter and Boyer, 1973). While NR is general safe, side effects exist (Hwang and Song, 2020). These side effects include epigenetic changes, impeded bioenergetics, potential carcinogenesis and GI disturbances (nausea, vomiting, diarrhoea) (Braidy and Liu, 2020).

The large amount of work completely looking into the potential use of NAD⁺ for clinical treatment of disease in recent years illuminates the vast potential of NAD⁺ replenishment in mitigation of different diseases, neurodegeneration, ageing phenotypes and overall in promoting health and longevity. It is likely that there is a hierarchy of NAD⁺ precursors, concordant with the degree of efficacy displayed by the different compounds to replenish cellular NAD⁺ levels. Among all the known NAD⁺ precursors, NRH is likely the most potent NAD⁺ booster. Potential immediate concerns on the use of NRH are low stability (sensitive to oxygen and moister) and the generation of oxidative radicles, thus further safely and bioavailability data of NRH in animals and humans are necessary. Moreover, we need to establish the relationship between NAD⁺ precursor, pharmacokinetics and their efficacy for the treatment of different diseases, such as metabolic and neurodegenerative diseases, and its roles in promoting healthspan in the human population. Further, the anti-viral activities of NAD⁺ precursors on Zika virus (Yang et al., 2021) and SARS-CoV-2 (COVID-19) (Heer et al., 2020) were reported recently, and it would be exciting to explore further on the additional molecular mechanisms and to perform possible clinical trials when sufficient preclinical data available. Although a lot of work still remains, the potential benefits of boosting NAD⁺ may hold great therapeutic potential for the human population.

Declaration of Competing Interest
E.F.F. has CRADA arrangement with ChromaDex, and is consultant to Aladinn Healthcare Technologies, Vancouver Dementia Prevention Centre, Intellectual Labs, and MindRank AI Ltd.

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