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Roles of NAD$^+$ in Health and Aging

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NAD$^+$, the essential metabolite involved in multiple reactions such as the regulation of cellular metabolism, energy production, DNA repair, mitophagy and autophagy, inflammation, and neuronal function, has been the subject of intense research in the field of aging and disease over the last decade. NAD$^+$ levels decline with aging and in some age-related diseases, and reduction in NAD$^+$ affects all the hallmarks of aging. Here, we present an overview of the discovery of NAD$^+$, the cellular pathways of producing and consuming NAD$^+$, and discuss how imbalances in the production rate and cellular request of NAD$^+$ likely contribute to aging and age-related diseases including neurodegeneration. Preclinical studies have revealed great potential for NAD$^+$ precursors in promotion of healthy aging and improvement of neurodegeneration. This has led to the initiation of several clinical trials with NAD$^+$ precursors to treat accelerated aging, age-associated dysfunctions, and diseases including Alzheimer's and Parkinson's. NAD supplementation has great future potential clinically, and these studies will also provide insight into the mechanisms of aging.

THE DISCOVERY OF NAD$^+$

Nicotinamide adenine dinucleotide (NAD) can exist in two forms, the oxidized form, NAD$^+$, and the reduced form, NADH, which are coupled together and known as a "redox couple." While they are chemically similar, here we mainly focus on NAD$^+$. NAD$^+$ is an essential metabolite for life and health, as it participates in dozens of known cellular reactions that affect redox status, energy production, and metabolic homeostasis, in addition to having anti-inflammatory properties, and assisting in stem cell rejuvenation, autophagy/mitophagy, nuclear–mitochondrial communication, and cellular resilience and survival (Verdin 2015; Fang et al. 2016a,b; Lautrup

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et al. 2019). NAD^+ (named "cozymase" at that time) was purified by Arthur Harden and William John Young in 1906 (Harden and Young 1906) as an essential component in fermentation (Harden and Young 1906). Hans von Euler-Chelpin continued the work initiated by Harden and Young, reporting that the structure of NAD^+ is made up of two nucleotides. Combined, Harden, Young, and Euler-Chelpin showed that fermentation depends on NAD^+, and, in 1929, Arthur Harden and Hans von Euler-Chelpin were awarded the Nobel Prize. Dr. Hans von Euler-Chelpin stated in his Nobel lecture that "cozymase [NAD^+] is one of the most widespread and biologically important activators within the plant and animal world" (Euler 1930). Simultaneously, Otto Heinrich Warburg discovered the redox abilities of NAD^+ and NADH and their necessity for fermentation (Warburg and Christian 1936). In the 1940s, Arthur Kornberg discovered the reaction in which the precursor nicotinamide mononucleotide (NMN) is converted to NAD^+, and via this he found NAD synthetase (Kornberg 1948). He was awarded the Nobel Prize for his findings in 1950.

The investigations of the disease pellagra, now known to be caused by NAD^+ precursor deficiency, also contributed significantly to the knowledge about NAD^+ that we have today. Dr. Joseph Goldberger initially described pellagra as a nutritional deficiency, with dermatitis, diarrhea, dementia, and consequently death as the central characteristics of the disease. Conrad A. Elvehjem and C.K. Koehn conducted controlled experiments in dogs, which led to their discovery that nicotinic acid (NA), initially termed "anti-black tongue factor," was the mitigating factor in pellagra (Elvehjem et al. 1974). Later, Jack Preiss and Philip Handler connected NA to NAD^+ by clarifying the steps and enzymes in what is now referred to as the Preiss–Handler pathway, in which NA is metabolized by a three-step process to NAD^+ (Preiss and Handler 1958a,b).

Our current understanding of NAD^+ and its roles in cellular bioenergetics really began in the 1960s and 1970s with the identification of NAD^+-dependent reactions and proteins. Chambon, Weill, and Mandel described the process of poly ADP-ribosylation (PARP) as an NAD^+-dependent reaction (Chambon et al. 1963), initiating the field of PARP studies. Additionally, the identification of yeast SIR2 as NAD^+-dependent deacetylates by Guarente and colleagues revealed an additional group of NAD^+ consumers (Imai et al. 2000). In 2004, Brenner and colleagues identified the NAD^+ precursor nicotinamide riboside (NR) and uncovered the two-step process from NR to NAD^+ (Bieganowski and Brenner 2004). In recent years, interest in NAD^+ and its bioavailable precursors, including NR, NMN, and nicotinamide (NAM), has spiked. This review will summarize the knowledge gained from preclinical and clinical studies on the decline of NAD^+ during aging and the reasons for and consequences of reduced NAD. Furthermore, the identification and purification of NAD^+ precursors have opened the door to discovery of methods that can boost the level of intracellular and organismal NAD^+ levels, which will also be discussed here.

NAD^+-CONSUMING ENZYMES, BIOSYNTHETIC PATHWAYS, AND METABOLISM

NAD^+ is highly compartmentalized in different subcellular organelles, including in the nucleus, cytoplasm, and mitochondria. Mitochondria contain the largest subcellular pools of NAD^+ (Berger et al. 2005; Dölle et al. 2010; Nikiforov et al. 2011). While NAD^+ might be transported from cytoplasm to other subcellular organs (like mitochondria), it is generally believed that each pool of NAD^+ is independently regulated, involving subcellular specific localization of proteins involved in NAD^+ production and consumption (Berger et al. 2005; Gilley and Coleman 2010; Mayer et al. 2010).

NAD^+ is a fundamental molecule for several metabolic and cellular processes. It works as a redox coenzyme, because the conversion of NAD^+ to NADH is necessary for the citric acid cycle, β-oxidation, and glycolysis. Simultaneously, the oxidation of NADH to NAD^+ by complex I in the mitochondrial electron transport chain participates in ATP production and other essential metabolic processes. Major NAD^+-synthetic pathways, including the salvage pathway (including extracellular recycling and the newly

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discovered NADH pathway), the kynurenine pathway (de novo), and the Preiss–Handler pathway are discussed below.

The Four Classes of NAD+-Consuming Proteins

NAD^+ is a cosubstrate for at least four main classes of enzymes producing NAM as a byproduct of their consumption of NAD^+. These known NAD^+-consuming proteins include PARPs, the class III histone deacetylases sirtuins (SIRTs), ADP ribosyl-cyclases (CD38, CD73), and NADase sterile α and TIR motif-containing 1 (SARM1) (Lautrup et al. 2019; Covarrubias et al. 2021).

A central family of NAD^+ consumers are the PARPs, with PARP1 constituting the main PARP activity related to DNA damage (Rouleau et al. 2010; Fang et al. 2014). PARP1 is a key protein in the DNA damage response, locating sites with damaged DNA and using NAD^+ to generate long PAR chains (PARylation) on itself and on histones and other proteins, which acts as a scaffold to facilitate DNA repair (Fang et al. 2016a,b; Ray Chaudhuri and Nussenzweig 2017; Wilson et al. 2023a). PARP-mediated NAD^+ consumption has also been linked to the process of aging, pathological aging, and age-related diseases (more details below). The second class of NAD^+ consumers are the SIRTs. The mammalian SIRT family consists of seven mammalian SIRT proteins, which regulate many cellular processes including neuronal survival, metabolism, stress responses, and aging (Chalkiadaki and Guarente 2015; Covarrubias et al. 2021). Both the NAD^+-consuming PARPs and SIRTs are hyperactive in autophagy-deficient cells, and NAD^+ depletion contributes to death of autophagy-/mitophagy-deficient cells (Kataura et al. 2022). The past decade of research has revealed that NAD^+-consuming enzymes can directly regulate autophagy in cells, from early transcription events to late-stage autophagosome–lysosome fusion events (Wilson et al. 2023b).

The third group of NAD^+ consumers contains the ADP ribosyl cyclases CD38/CD157, which convert NAD^+ to cyclic ADP ribose (cADPR) and adenine diphosphate ribose (ADPR) under neutral pH conditions; in acidic conditions, they convert NAD^+ to nicotinamide adenine dinucleotide phosphate (NADP), and then to NA adenine dinucleotide phosphate (NAADP) (Reiten et al. 2021). CD38 and CD157 play important roles in the regulation of social behavior, calcium homeostasis, immune function, mitochondrial function, metabolism, and hormone secretion (Jin et al. 2007; Liu et al. 2008, 2017; Camacho-Pereira et al. 2016). Similarly, SARM1 has NADase properties, and produces NAM, cADPR, and ADPR while consuming NAD^+. SARM1 is primarily expressed in neurons where SARM1-mediated NAD^+ degradation promotes axon degeneration exclusively in damaged axons, as well as during neuronal morphogenesis and inflammation (Gerdts et al. 2015; Essuman et al. 2017; Murata et al. 2018; Figley and DiAntonio 2020). SARM1 functions as a metabolic sensor, which is activated by a large change in the NMN-to-NAD^+ ratio, and binding of NMN is required for injury-induced SARM1 activation and axon destruction (Figley et al. 2021). SARM1 is also expressed in immune cells (Panneerselvam et al. 2013; Gürtler et al. 2014; Zhao et al. 2019), but the underlying mechanism that SARM1 uses to regulate immunity is largely unexplored. Collectively, our current understanding of NAD^+ and its consuming enzymes highlights the vital importance of NAD^+ in life and health.

The Salvage Pathway and Related NAD+ Precursors

The salvage pathway generates NAD^+ from the precursor NAM or the upstream precursors NR or NMN as summarized in Figure 1A. The precursor NAM is produced during the degradation and consumption of NAD^+, and exogenous NAM, in addition to NR and NMN, arrive from a variety of foods including fruits, vegetables, milk, and meat (Chi and Sauve 2013; Mills et al. 2016; Fang et al. 2017; Covarrubias et al. 2021). The route of cell entry for NAD and the precursors mentioned here are still not completely understood. In specific cell types and conditions, NAD^+ can be transported into the cell via the transporter connexin 43 (Cx43) (Billington et al. 2008; Liu et al. 2018), whereas the smaller precursors, such as NAM, NMN, and NR, can enter the

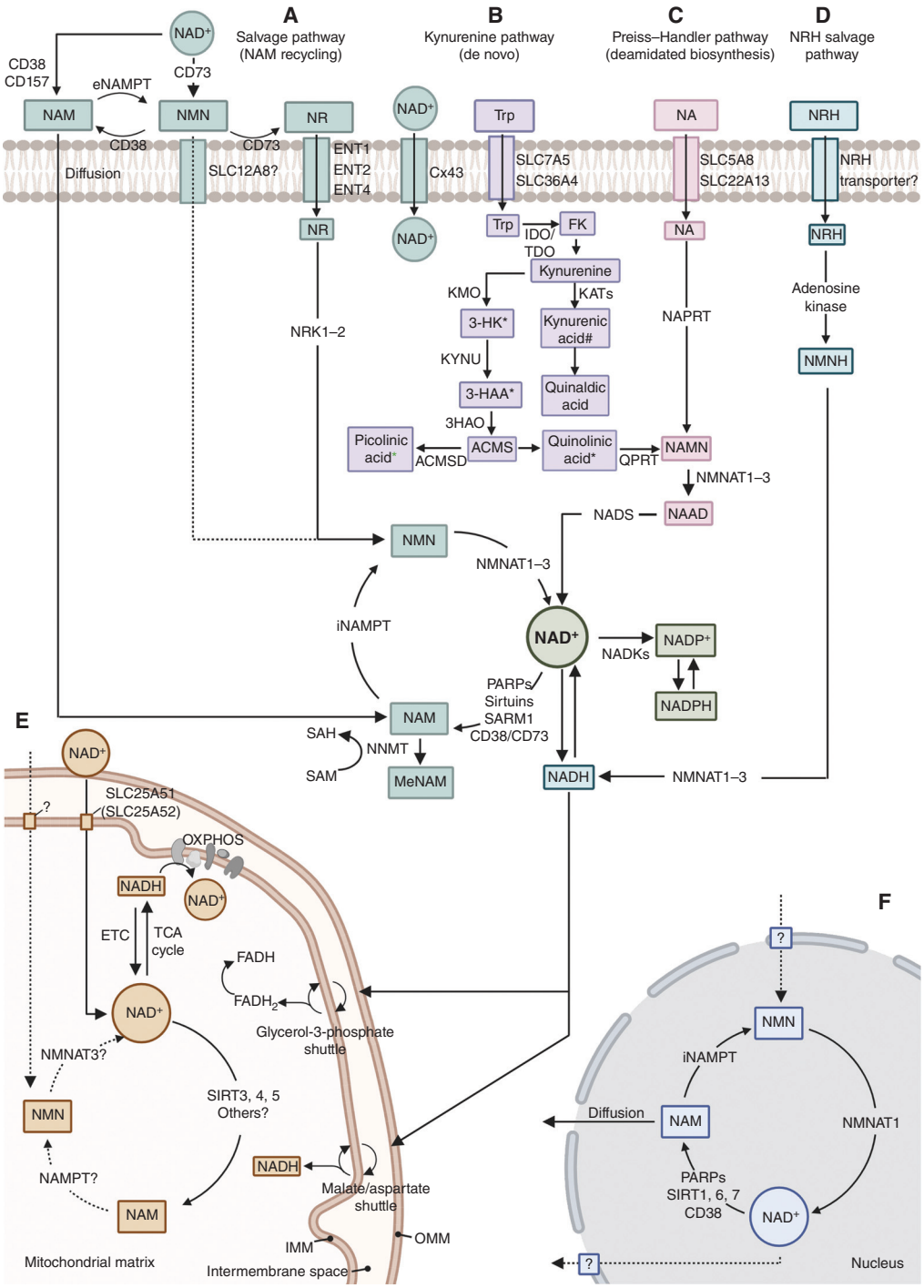

Figure 1. NAD^+ biosynthetic pathways and subcellular interactions. The biosynthesis of NAD^+ includes four pathways: the salvage pathway (*A*), the kynurenine pathway (*B*), the Preiss–Handler pathway (*C*), and the newly proposed dihydronicotinamide riboside (NRH) salvage pathway (*D*). The salvage pathway synthesizes NAD^+ from intracellular nicotinamide (NAM), extracellular NAD^+ and related metabolites (NAM, nicotinamide riboside [NR], nicotinamide mononucleotide [NMN]). Within the cell, NR is phosphorylated by nicotinamide riboside kinases (NRK1-2) to NMN. NMN is also produced from NAM by the intracellular NAMPT (iNAMPT). NAD^+ is generated from NMN through NMNAT1-3. (*Legend continues on following page.*)

cell via direct diffusion (NAM) or specific transporters (NR and NMN) (Grozio et al. 2013; Camacho-Pereira et al. 2016).

Within the cell, NAM is recycled to NMN by iNAMPT (Rongvaux et al. 2002; Ratajczak et al. 2016; Liu et al. 2018), followed by the conversion of NMN to NAD^+ by NMN adenylyl transferases 1-3 (NMNAT1-3). The NMNATs are involved in all three main NAD^+ synthesis routes converting either NMN or NAMN to NAD^+ (Salvage, Kynurenine, Preiss–Handler) (Fig. 1). They have different subcellular localizations, and the expression of NMNAT1 is tissue specific (Berger et al. 2005). NMNAT1 locates to the nucleus and is highly expressed in skeletal muscle, heart, kidney, liver, and pancreas (Emanuelli et al. 2001; Yalowitz et al. 2004). NMNAT2 is localized on the outer membrane of the Golgi apparatus and in the cytosol. NMNAT3 resides mainly in the mitochondria, although the activity responsible for converting NMN to NAD^+ within the mitochondria is still debated (Yalowitz et al. 2004; Berger et al. 2005; Yamamoto et al. 2016). Moreover, the expression and activity of NAMPT within the mitochondria may depend on many factors including cell types. In rat liver cells, NAMPT has been detected within the mitochondria (Yang et al. 2007), whereas experiments using immortalized cells show a lack of NAMPT inside the mitochondria (Pittelli et al. 2010). More studies are needed to understand the tissue- and cell-specific expression pattern of NAMPT and related enzymes.

Recently, the hydrogenated form of NR, NRH, was demonstrated to increase NAD^+ levels in cells in a more efficient way than using NR/NMN/NAM (Yang et al. 2019). The conversion of NRH to NAD^+ provides a potential novel entry point for boosting cellular NAD^+ (Fig. 1D; Yang et al. 2020). Adenosine kinase (AK), not NRK1 or NRK2, was demonstrated to convert NRH to the intermediate NMNH (Yang et al. 2020). It was demonstrated in vitro that the adenylation of NMNH to NADH by NNMATs followed by oxidation to NAD^+ is a likely route of action, but in vivo studies are needed to establish the mechanism of action and the physiological relevance (Yang et al. 2020; Ziegler and Nikiforov 2020). While the major advantage of using NRH is the

Figure 1. (*Continued*) (*B*) The kynurenine pathway produces NAD^+ from the amino acid tryptophan (Trp). Trp is converted to formylkynurenine (FK), which is then catalyzed into kynurenine, which is converted to either quinaldic acid or 3-hydroxykynurenine (3-HK), which by a four-step process is converted to nicotinic acid mononucleotide (NAMN), then following the remaining steps described in the (*C*) Preiss–Handler pathway. Some of the metabolites of the kynurenine pathway are neuroprotective (marked with #), and some are neurotoxic (marked with *). (*C*) The Preiss–Handler pathway describes the conversion of nicotinic acid (NA) to NAD^+ via a three-step process. NAMN is an intermediate both in the kynurenine pathway (*B*) and the Preiss–Handler pathway (*C*), and the enzymatic conversion of NAMN to NA adenine dinucleotide (NAAD) is by the NMNATs. Finally, NAD^+ synthase (NADS) synthesizes NAAD to NAD^+. (*D*) The recently demonstrated NRH salvage pathway uses dihydronicotinamide riboside (NRH), the reduced form of NR, to produce NAD^+. The pathway is still relatively unclarified, but the initial step is likely catalyzed by adenosine kinase (AK), providing NMNH from NRH. NMNH is suggested to be converted to NADH through NMNATs, finally being oxidized to NAD^+. (*E*) Mitochondrial NAD^+ homeostasis. NAD^+ is subcellular localized and regulated. SLC25A51, and in specific organs and likely specific cell types SLC25A52, are mammalian mitochondrial transporters for NAD^+. The cytosolic and mitochondrial NADH pools communicate indirectly through the transportation of reducing equivalents across the mitochondrial membranes by the glyceraldehyde 3-phosphate and malate-aspartate shuttles. NMNAT3 has been suggested to be an essential participant of the salvage pathway in mitochondria, but recent studies do not support the existence of an active mitochondrial NMNAT3 to synthesize NMN to NAD^+ within the mitochondria (Kory et al. 2020; Luongo et al. 2020). Within the mitochondrial matrix, NAD^+ is consumed by SIRT3-5, with NAM as a byproduct. NAM is potentially further converted to NMN through NAMPT, although the presence of NAMPT within the mitochondria is not fully understood (Yang et al. 2007; Pittelli et al. 2010). The existence of a mitochondrial NMN transporter is unknown. (*F*) Nuclear NAD^+ homeostasis. The nuclear transport of NAD^+ remains inconclusive, but nuclear pore-mediated NAD^+ diffusion has been speculated. Within the nucleus, the NAD^+ pathway involves iNAMPT, converting NAM, produced during the consumption of NAD^+, to NMN, and NMNAT1 synthesizing NAD^+ from NMN. (Figure is based on data in Reiten et al. 2021, and was created using BioRender.)

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efficient increase of cellular NAD^+, a major disadvantage is its low stability, which limits broad translational applications.

Kynurenine Pathway

The amino acid tryptophan (Trp) is metabolized to NAD^+ de novo via the kynurenine pathway (Fig. 1B). Trp is transported into the cell via the transmembrane solute carrier (SLC) transporters SLC7A5 and SLC36A4 (Pillai and Meredith 2011; Scalise et al. 2018), where it is catabolized via a two-step process to kynurenine, which can be converted to NAD^+, kynurenic acid, and xanthurenic acid. The relative contribution of the kynurenine pathway to NAD^+ levels is still not well understood, and most cells outside the liver/kidney do not express the enzymes necessary to produce NAD^+ from Trp (Liu et al. 2018; Covarrubias et al. 2021). However, evidence suggests that the kynurenine pathway plays an essential role in health. For example, the enzyme α-amino-β-carboxymuconate-ε-semialdehyde (ACMS) decarboxylase (ACMSD) inhibits spontaneous cyclization of ACMS in the kynurenine pathway. Pharmacologic or genetic inhibition of ACMSD enhances de novo NAD^+ synthesis, leading to improved mitochondrial function and tissue resilience after damage, especially in kidney and liver tissues (Katsyuba et al. 2018). The liver-dominant kynurenine pathway may also contribute to the generation of NAD^+ to detoxify ethanol in the liver: alcohol dehydrogenase complex catalyzes the reaction of ethanol and NAD^+ to form acetaldehyde, NADH, and H^+ (Edenberg 2000).

In addition to protection against exogenous damage to the kidney and liver, the kynurenine pathway is also linked to common neurodegenerative diseases such as Huntington's and Alzheimer's disease with more details in our recent review (Ogawa et al. 1992; Thevandavakkam et al. 2010; Campesan et al. 2011; Giil et al. 2017; Lautrup et al. 2019; González-Sánchez et al. 2020). The kynurenine pathway is involved in the generation of the neurotransmitters glutamate and acetylcholine, and it regulates N-methyl-D-aspartate receptor activity (Vécsei et al. 2013; Lautrup et al. 2019). Additionally, the intermediates of this pathway include both neurotoxic (3-hydroxykynurenine (3-HK), 3-hydroxyanthranilic acid (3-HAA), quinolinic acid, and free radicals) and neuroprotective metabolites (Trp, kynurenic acid, and picolinic acid) (Vécsei et al. 2013). These findings outline the importance of this de novo NAD^+ synthetic pathway in promoting tissue resilience and neuroprotection, among other defensive functions.

Preiss–Handler Pathway

The Preiss–Handler pathway, also called the deamidated biosynthesis pathway, synthesizes NAD^+ from the precursor NA via three steps (Fig. 1C). NA is transported into the cell via SLC5A8 and SLC22A13 (Gopal et al. 2005; Bahn et al. 2008). Within the cell, NA is converted to nicotinic acid mononucleotide (NAMN) by nicotinic acid phosphoribosyltransferase (NaPRT) (Houtkooper et al. 2010). Converging with the kynurenine pathway, NAMN is processed to nicotinic acid adenine dinucleotide (NAAD) by NMNAT1-3, and finally NAAD is metabolized to NAD^+ by NAD^+ synthase (NADS) (Zhang et al. 2003; Houtkooper et al. 2010). NR supplementation can increase the intracellular level of NAAD (Trammell et al. 2016a), connecting the Salvage and Preiss–Handler pathways, although the mechanism is yet to be understood.

CHANGES IN NAD^+ DURING AGING

Changes in NAD^+ during Normal Aging

During aging, the level of NAD^+ declines, and proteins involved in the biosynthesis and consumption of NAD^+ are altered (McReynolds et al. 2020). Multiple studies on mice have shown that NAD^+ levels decline in various tissues such as pancreas (Yoshino et al. 2011), kidney (McReynolds et al. 2021), white adipose tissue (Yoshino et al. 2011; Camacho-Pereira et al. 2016; McReynolds et al. 2021), spleen (Camacho-Pereira et al. 2016), skeletal muscle and muscle stem (Yoshino et al. 2011; Gomes et al. 2013; Camacho-Pereira et al. 2016; Zhang et al. 2016; Zou et al. 2020; McReynolds et al. 2021), urine-derived stem cells (Zou et al. 2020), liver (Mouchiroud et al. 2013; Camacho-Pereira et al. 2016; McReynolds et al.

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2021), skin (Massudi et al. 2012; Zou et al. 2020), and brain (Stein et al. 2014; Zhu et al. 2015), and an age-dependent decline of NAD^+ has also been reported in humans (Zhu et al. 2015; Chaleckis et al. 2016; Janssens et al. 2022) and nematodes (Mouchiroud et al. 2013; Fang et al. 2014, 2016a, b). Due to the low stability of NAD^+, noninvasive and in vivo methods to detect NAD^+ and its related metabolites are needed. An array of fluorescent NAD^+ or NAD^+/NADH sensors have been developed and used for detection of NAD during aging in zebrafish, mice, and human cells, with all showing an age-dependent decline in levels of NAD^+ (Zhao et al. 2011, 2015, 2016, 2018; Zou et al. 2020). Using a magnetic resonance (MR)-based in vivo NAD assay, the level of NAD^+, total NAD, and the NAD^+/NADH ratio were shown to decline and NADH to increase with age in the human brain (Zhu et al. 2015). Other studies on human tissue samples rely on enzymatic cycling assays or liquid chromatography mass spectrometry (LC-MS)-based analysis of NAD^+ and NAD(H) detection (Bernofsky and Swan 1973; Chaleckis et al. 2016; Clement et al. 2019; Sanchez-Roman et al. 2022). Since blood is easily accessible compared to other human tissues, researchers have sought to use blood to examine the age-related changes in NAD^+ as a biomarker of aging and disease (Fig. 2). Through use of LC-MS, it has been shown that the level of NAD^+ in both whole blood and plasma declines with age (Chaleckis et al. 2016; Clement et al. 2019). Additionally, a positive correlation has been observed between cognitive capacity and plasma levels of NAD^+/NADH in centenarians, which might be because NAD^+/NADH-consuming enzyme activities are working to decrease the oxidative DNA damage load (Sanchez-Roman et al. 2022). NAD^+ is a key regulator of DNA repair mechanisms, including base excision repair and homologous recombination. Reduced NAD^+ levels have been shown to impair DNA repair and increase DNA damage, which can contribute to an accumulation of the mutations and cellular dysfunctions generally associated with aging (Fang et al. 2017). Aging is also related to a significant decrease in NAD^+ levels in human skeletal muscle; the level of NAD^+ correlates with healthy aging (Janssens et al. 2022). Increased physical activity is associated with increased levels of NAD^+ in the skeletal muscle, whereas physical impairments in older people are associated with decreased NAD^+ levels when compared with normally active older individuals (Janssens et al. 2022).

Understanding why NAD^+ decreases with age remains incomplete, but studies on animal models have provided insight into the important players in age-dependent NAD^+ decline; age-related decreases are likely due to reduced production and increased consumption of NAD^+. Age-related NAD^+ depletion has been linked to increased nuclear DNA damage, which leads to increased activation of PARP1 (Mouchiroud et al. 2013; Fang et al. 2014, 2016a,b; Ryu et al. 2016; Zhang et al. 2016). High PARP1 activity will deplete the cell of NAD^+, thereby decreasing the activity of SIRT1 (and other SIRTs). This in turn results in increased acetylation of the transcription factor peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α) and mitochondrial dysfunction (Mouchiroud et al. 2013; Fang et al. 2014, 2016a,b; Ryu et al. 2016; Zhang et al. 2016). In addition, compromised mitophagy and mitochondrial unfolded protein response (UPR^{mt}), and mitochondrial dysfunction are consequences of NAD^+ depletion during aging and age-predisposed diseases (Fang et al. 2014, 2016a,b, 2019a,b; Ryu et al. 2016; Zhang et al. 2016). Depleted NAD^+ disrupts cellular metabolism, again leading to decreased cellular functions as shown in both neurons and glial cells, as wells as in stem cells (Ryu et al. 2016; Zhang et al. 2016; Fang et al. 2019b).

Depletion of NAD^+ has also been linked to age- and disease-related telomere shortening and dysfunction. In the telomere disorder dyskeratosis congenita, a rare genetic form of bone marrow failure where the marrow is unable to produce sufficient blood cells, decreased NAD^+ and imbalance of the NAD^+ metabolome was shown to be related to both telomere damage and cellular senescence (Sun et al. 2020). Moreover, NAD^+ was found to be reduced both due to the activity of PARP1, SIRT1 while the NAD^+ consumer CD38 (Sun et al. 2020), which is increased during aging in mice (Camacho-Pereira et al. 2016). CD38 is required for the age-associated decline in NAD^+ levels and age-related

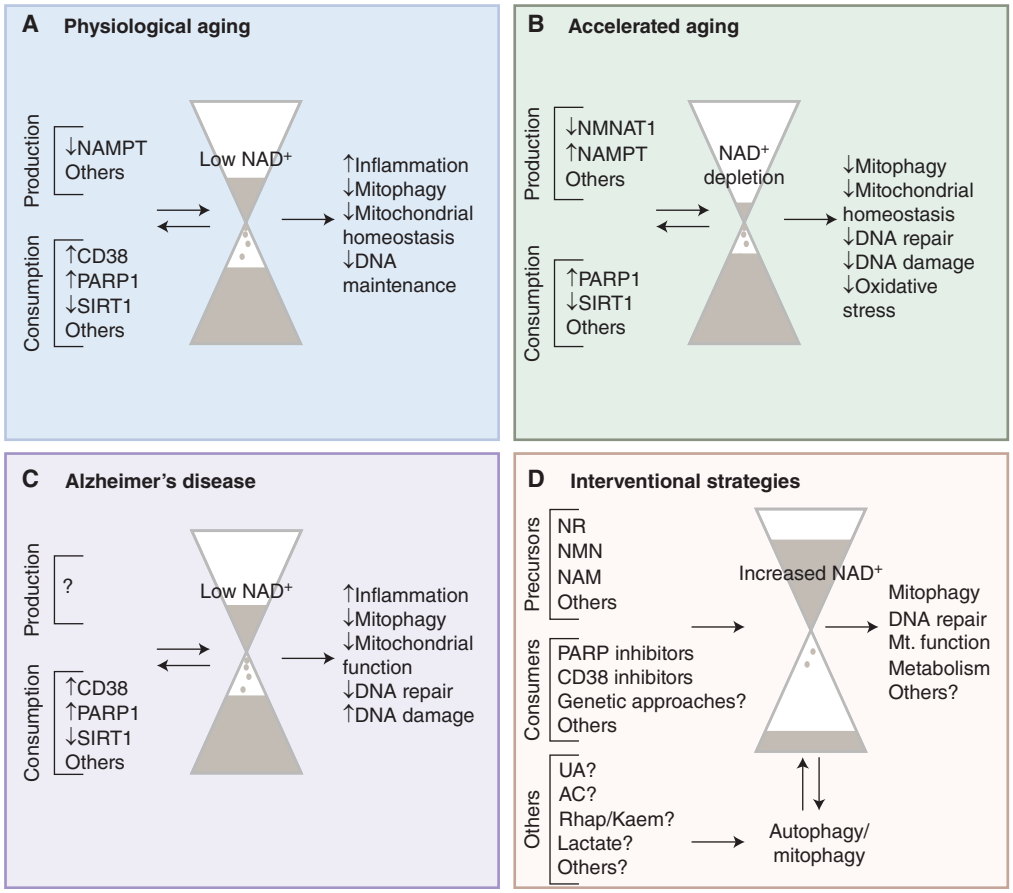

Figure 2. Mechanisms of reduced NAD^+ levels in aging and diseases, as well as interventional strategies. During aging and disease, the balance of the production and consumption of NAD^+ is altered, resulting in decreased levels of NAD^+. (*A–C*) The players involved during (*A*) normal aging, (*B*) accelerated aging, and (*C*) brain diseases such as Alzheimer's disease. (*D*) The known NAD^+ up-regulation strategies and outcomes. (UA) Urolithin A, (AC) actinonin, (Rhap) Rhapontigenin, (Kaem) Kaempferol.

mitochondrial dysfunction via a pathway that at least partially depends on the activity of the mitochondrial SIRT3 (Camacho-Pereira et al. 2016). A central role for CD38 in age-dependent NAD^+ depletion was also confirmed in activated macrophages from old mice, showing a hyperactivation of CD38, which resulted in lower levels of NAD^+ (Covarrubias et al. 2021).

Age-related decrease of NAD^+ levels may also be related to a decline in levels or activity of NAD^+-producing proteins (Yoshino et al. 2011; Fang et al. 2017). The level of NAMPT, which converts NAM to NMN and is referred to as the rate-limiting enzyme in the salvage pathway, decreases with age in mice (Yoshino et al. 2011; Stein and Imai 2014), and its expression has been linked to core circadian clock proteins and inflammation (Yaku et al. 2018). On the other hand, it was recently shown through isotope tracer experiments that the synthesis of NAD^+ across multiple tissues in mice was not altered, pointing at increased consumption as the primary driver for age-related decline of NAD^+ availability (McReynolds et al. 2021). Further studies are needed in animal models and in human tissues to further understand the mechanisms that deplete the aging cells and tissues of NAD^+.

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Changes of NAD+ Levels in Pathological Aging

In addition to normal aging, decreased NAD^+ levels have been observed in a number of accelerated aging disorders including Ataxia telangiectasia (A-T), Cockayne syndrome (CS), Werner syndrome (WS), and xeroderma pigmentosum group A (XPA) (Fang et al. 2014, 2016a,b, 2019a; Scheibye-Knudsen et al. 2014). A shared etiological feature of these conditions is the mutation of genes involved in DNA repair and genomic stability. Additionally, patients suffering from these diseases, except for WS, show severe neurodegeneration; lower levels of NAD^+ have been observed in *Caenorhabditis elegans* models and mouse brain samples of A-T, CS, WS, and XPA (Fang et al. 2014, 2016a,b; Scheibye-Knudsen et al. 2014). CS is caused by mutations in either the CS complementation group A (*CSA*) or group B (*CSB*) genes. Interestingly, the CS mouse models, $Csb^{m/m}$ and $Csa^{m/m}$, also showed reduced levels of NAD^+ and NAD^+/NADH ratio in the cochlea associated with reduced hearing in both CS mouse models due to disrupted ribbon synapses and outer hair cell loss (Okur et al. 2020a). These findings suggest that age-related hearing loss in normal aging might also be a consequence of a decline in NAD^+. In WS, the main characteristics of the patients include cancer, short stature, dyslipidemia, premature arthrosclerosis, and insulin-resistant diabetes (Takemoto et al. 2013; Oshima et al. 2017). In line with many of the features of WS being related to dysfunctional metabolism, primary fibroblasts, and blood samples from human WS patients showed a decreased level of NAD^+ and related metabolites compared to healthy control subjects (Fang et al. 2019a). Additionally, WS *C. elegans* models show a decrease in NAD^+ levels throughout the body, which likely contributes to compromised mitophagy, leading to disrupted fat metabolism, mitochondrial dysfunction, and decreased health span and life span (Fang et al. 2019a). As with normal aging, the explanation for the decline in NAD^+ levels might be found in an increased consumption, mainly by the DNA damage-activated PARP1, as well as decreased production of NAD^+.

In the above-mentioned premature aging diseases an increased activity of PARP1 and decreased activity of SIRT1 has been shown, and in A-T, XPA, and CS (Fang et al. 2014, 2016a,b, 2019a; Scheibye-Knudsen et al. 2014). These studies showed that reduction in NAD^+ led to mitochondrial dysfunction and compromised mitophagy, excessive PINK1 cleavage, and increased mitochondrial membrane potential, which in turn resulted in the accumulation of dysfunctional mitochondria, further contributing to oxidative stress and NAD^+ depletion, which contributes to a shortened life span, health span, and neurodegeneration (except for WS) (Fang et al. 2014, 2016a,b; Scheibye-Knudsen et al. 2014).

In WS, it was also shown that NMNAT1, responsible for the last step of the salvage pathway, was significantly reduced, while NAMPT was increased (Fang et al. 2019a). The latter might suggest a cellular compensatory feedback mechanism to increase NAD^+ (Fang et al. 2019a). To clarify the impact of the altered levels of NAD^+ biosynthesis-related proteins, and the direct consequences of NAD^+ depletion, more studies are required for both normal and pathological aging in different organisms, tissues, and cell types.

Changes in NAD+ Levels in Age-Associated Diseases with a Focus on AD

NAD^+ reduction is evident not only in normal and pathological aging, but also in age-related diseases, including diabetes (Yoshino et al. 2011; Mills et al. 2016), cardiovascular diseases (Diguet et al. 2018), kidney failure (Tran et al. 2016; Poyan Mehr et al. 2018; Morevati et al. 2021), and neurodegenerative diseases (Hou et al. 2019; Yulug et al. 2021a, 2023) with AD being the focus of this review. AD is one of the most common neurodegenerative diseases, and the prevalence of AD increases as the life expectancy of the global population increases. AD is a major global public health problem that needs to be addressed urgently (Livingston et al. 2020). The main clinical features of AD are cognitive deficits, mood swings, and behavioral problems. AD likely results from a combination of genetic and environmental risk factors, plus aging, with common pathological (and also possibly etiological) features being Aβ plaques, Tau aggregation and phosphorylation,

inflammation, DNA damage, mitochondrial dysfunction, and compromised autophagy/mitophagy (Goedert 2015; Canter et al. 2016; Fang et al. 2019b; Kobro-Flatmoen et al. 2021).

Recent studies support impairment of the NAD^+-mitophagy axis as a risk factor for, if not an independent cause of AD (Fang et al. 2019b; Xie et al. 2022). Accumulating evidence from rodent AD models suggests that NAD^+ deprivation and defects in NAD^+-dependent pathways are critical for AD pathogenesis (Hou et al. 2019). In mouse models of familial AD, decreased NAD^+ levels, and metabolic abnormalities in the brain have been described (Chambon et al. 1963; Dong and Brewer 2019; Hou et al. 2019). Additionally, rat cortical neurons stimulated with accumulating Aβ showed decreased NAD^+ levels (Liu et al. 2013). Transcriptomic analysis of postmortem brain tissues from AD patients and healthy controls revealed that mitochondrial dysfunction is involved in the underlying mechanism associated with AD (Yulug et al. 2021a), supporting the hypothesis of a compromised NAD^+-mitophagy axis in the development of AD (Kerr et al. 2017).

Moreover, it has previously been shown that reduced levels of NAD^+ resulted in disrupted cellular metabolism and mitochondrial dysfunction as well as compromised mitophagy in AD (Fang et al. 2019b; Hou et al. 2021). Also, heavily increased inflammation seen in the brain of AD patients and animal models has been linked to decreased NAD^+ (Hou et al. 2021). Preclinical studies of NAD^+ precursor-treated AD animal models have given insights into the consequences of NAD^+ and how NAD^+ might be a promising therapeutic target as discussed in the following section for both aging and age-associated diseases.

THE BENEFITS OF NAD^+ AUGMENTATION

NAD^+ Augmentation

Multiple approaches are effective at increasing cellular NAD^+ levels, such as caloric restriction, exercise, inhibiting NAD^+-consuming enzymes, overexpressing NAD^+ synthetic enzymes, or treating with NAD^+ precursors (Cheng et al. 2016; Fang et al. 2019b; Liu et al. 2019). Pharmacological up-regulation of cellular NAD^+ via NAD^+ precursors, such as the use of NR, NMN, or NAM, are in the current scientific spotlight. NR can increase NAD^+ levels in various model organisms as well as humans (Trammell et al. 2016a; Mitchell et al. 2018; Fang et al. 2019a; Hou et al. 2021). In humans, it has been shown that oral NR supplementation (1000 mg/d for 7 d) up-regulated NAD^+ levels up to 2.7-fold, and intermediate NAAD levels increased 45-fold in human blood (Trammell et al. 2016a). NMN supplementation can also increase intracellular NAD^+ levels in animal models (Blacher et al. 2015), and with the results of human clinical trials awaiting (see the section on Clinical Trials with NAD^+ Supplementation).

In mice, short-term (500 mg/kg/d for 14 d) (Fang et al. 2014) or long-term NR treatment (400 mg/kg/d in drinking water for 6 wk, or 570–590 mg/kg/d over 10 mo) had no detectable toxicity (Fang et al. 2016a,b; Frederick et al. 2016). Based on body weight, a dose of 570–590 mg/kg/d in mice is equivalent to 3.19–3.30 g/d for a human (Fang et al. 2016a,b). In rats, a study reported no adverse reactions at 300 mg/kg/d and the lowest NR dose that caused an observable adverse effect was 1000 mg/kg/d (Conze et al. 2016). NMN administration in wild-type C57BL/6N mice in either the short- (500 mg/kg/d, 7 d, IP) (Gomes et al. 2013) or long-term (100 mg/kg/d or 300 mg/kg/d, continuously for 12 mo), produced no signs of toxicity (Mills et al. 2016). Therefore, the low toxicity of NAD^+ precursors in mammals may make these good candidates for clinical intervention, and several studies examining the safety and toxicity of both NR and NMN are now ongoing (see the section on Clinical Trials with NAD^+ Supplementation). A detailed summary of both animal and clinical evidence of increased NAD^+ via NAD^+ precursors is available in Reiten et al. (2021).

Benefits of NAD^+ Augmentation to Normal Aging

How NAD^+ augmentation benefits normally aged individuals is still not clear, although several preclinical studies on age-predisposed diseases

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seem optimistic and have been providing ideas for the initial clinical studies on NAD^+ precursors (see the section Clinical Trials with NAD^+ Supplementation). In wild-type *C. elegans*, NR or NMN treatment increased the organismal level of NAD^+ and activated mitophagy resulting in an ~10% increase in life span (Mouchiroud et al. 2013; Li et al. 2014; Fang et al. 2016a,b, 2019a,b). Also, in wild-type mice, supplementation with NMN, NR, and NAM have shown beneficial effects (de Picciotto et al. 2016; Mills et al. 2016; Mitchell et al. 2018). In wild-type C57BL/6N mice, 12 mo of oral treatment with NMN ameliorated age-associated physiological decline including body weight gain, age-associated gene expression changes in key metabolic organs, and improvements in energy and lipid metabolism, insulin sensitivity, and physical activity (Mills et al. 2016). Furthermore, NMN treatment of old mice reversed vascular dysfunction and oxidative stress, likely via a pathway involving SIRT1 activation (de Picciotto et al. 2016; Tarantini et al. 2019). NAM treatment of wild-type mice has been shown to improve health span measures but not life span (Mitchell et al. 2018). NR treatment of wild-type C57BL/6N mice increased skeletal muscle NAD^+, thereby inducing anti-inflammatory pathways (Elhassan et al. 2019), the UPR^{mt} and synthesis of prohibitin 1 and 2, which rejuvenated muscle stem cells and improved muscle function in aged mice and in a muscular dystrophy mouse model (Ryu et al. 2016; Zhang et al. 2016). Additionally, NR treatment delayed senescence of neural and melanocytic stem cells and increased the life span of wild-type mice (Zhang et al. 2016). It has also been demonstrated that NAD^+ levels were increased after lactate treatment, which restored the mitochondrial function to that of young mice in a SIRT1-dependent manner (Gomes et al. 2013).

Aging is one of the major risk factors for multiple diseases, including obesity, diabetes, acute kidney failure, heart failure, and dementia (see below). In mice, NAM, NMN, or NR treatment has been shown to mitigate age- and high-fat diet-induced diabetes, obesity, and fatty liver disease by increasing the level of NAD^+ (Yoshino et al. 2011; Lee et al. 2015; Gariani et al. 2016; Trammell et al. 2016b; Uddin et al. 2016; Mitchell et al. 2018). After NAM or NMN treatment, the diabetic mice showed improved glucose tolerance, insulin sensitivity, and lipid profiles, and gene expression related to oxidative stress and inflammatory responses was restored (Yoshino et al. 2011; Mitchell et al. 2018). Furthermore, both murine heart failure models and cardiac biopsies from human patients with heart failure showed decreased levels of NAD^+, in addition to decreased protein levels of NAMPT (Diguet et al. 2018; Breton et al. 2020). Treating mice or isolated cardiomyocytes with NR attenuated heart failure and increased the level of NAD^+, NAAD, and related metabolites (Diguet et al. 2018). In mice, age-associated susceptibility to acute kidney injury in has also been shown to be rescued by NMN treatment via a pathway involving SIRT1 and its target PGC1α (Tran et al. 2016; Guan et al. 2017). Moreover, NR or NMN improved kidney function and prevented mitochondrial RNA/RIG-I-dependent inflammation during kidney injury (Doke et al. 2023). Combined, this preclinical data, in both in vitro and animal models, suggests that age-associated diseases might be improved by NAD^+ augmentation (Fig. 3A).

Benefits of NAD^+ Augmentation to Pathological Aging

In addition to biological aging, supplementation with the NAD^+ precursors NR and NMN have shown several benefits in accelerated aging diseases. Human XPA-deficient cells show compromised mitophagy, excessive PINK1 cleavage, and increased mitochondrial membrane potential. The mitochondrial abnormalities are likely due to hyperactive PARP-1 mediated decline of the NAD^+-SIRT1-PGC1α axis (Fang et al. 2014). Moreover, *C. elegans* models of XPA (*xpa*-1) also show mitochondrial dysfunction and compromised mitophagy, which at least partially contribute to the shortened life span and reduced health span of the worms. Confirming a central role of PARP1 hyperactivity in NAD^+ depletion in XPA, both PARP1 inhibition, NR, or NMN supplementation restored mitochondrial function and mitophagy, and in addition rescued life span defects in the XPA *C. elegans* model (Fang et al. 2014). Mitochondrial dysfunction, compro-

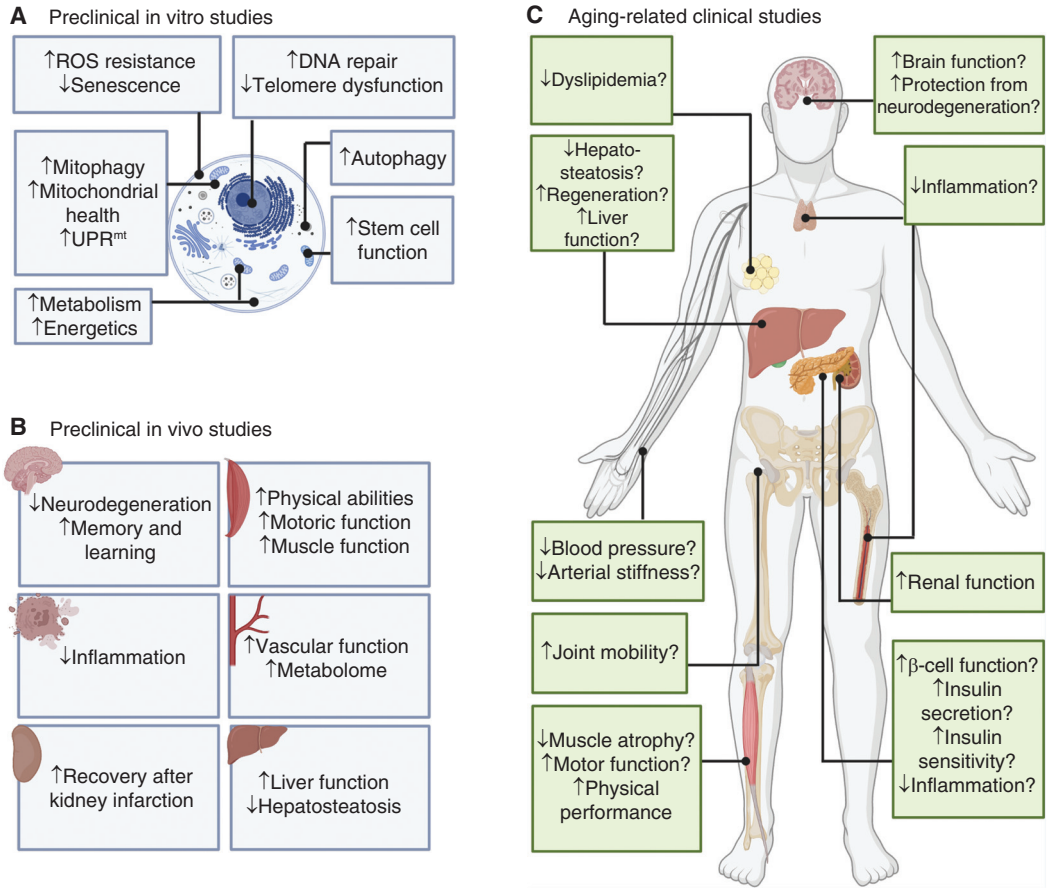

Figure 3. Effects of reported benefits of NAD^+ in different organs. (*A*) Reported preclinical in vitro studies showing the effects of treatment with nicotinamide (NAM), nicotinamide riboside (NR), or nicotinamide mononucleotide (NMN). (*B*) Reported preclinical in vivo studies showing the effects of treatment with NAM, NR, or NMN. (*C*) Aging-related features being analyzed in the completed, ongoing, and recruiting clinical trials for NAM, NR, or NMN. "?" represents the unknown effects of supplementation in humans due to lack of published data/results from clinical trials. (This figure was created using BioRender.)

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mised mitophagy, and life span defects are also seen in CS and A-T *C. elegans* models and can be at least partially rescued by PARP inhibition (Olaparib) or NAD^+ precursor treatment (NR or NMN) in low (micromolar) concentrations via mitophagy induction (Scheibye-Knudsen et al. 2014, 2016; Fang et al. 2016a,b). In $Atm^{-/-}$ mice and *atm-1* worms treatment with either NR, PARP1 inhibitor, or SIRT1 activator (SRT1720), shortened life span and health span was ameliorated through induction of mitophagy either through activation of the NAD^+-SIRTs axis, improved DCT-1 mediated mitophagy (thereby improving mitochondrial quality), and/or stimulation of DNA repair through activation of the nonhomologous end-joining repair (Ku70 and DNA-PKcs) (Fang et al. 2016a,b). In CS, NR supplementation or high-fat diet extended the life span and improved the health span of *C. elegans* (Fang et al. 2014; Okur et al. 2020a, b) and mouse models of CS (Scheibye-Knudsen et al. 2014; Okur et al. 2020a). $Csa^{m/m}$ and $Csb^{m/m}$ mice exhibit hearing deficiencies, which greatly resemble age-associated hearing loss, and short-term supplementation with NR (10 d) rescued this age-associated hearing deficiency (Okur et

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al. 2020a). Since the mechanism of hearing loss is similar in CS and age-related hearing loss, this provides ideas for therapeutic targets to ameliorate human age-related hearing loss.

Despite WS not being a neurodegeneration-related disease like the above-mentioned, studies suggest that impairment of the NAD^+-mitophagy-mitochondrial quality axis plays a role in disease etiology. Supplementation with NR or NMN extended life span and health span in *C. elegans* and *Drosophila melanogaster* models of WS (Fang et al. 2019a). In human WS cells and a *C. elegans* WS model (*wrn-1*(*gk99*)), NR supplementation increased BCL2/adenovirus E1B 19-kDa protein-interacting protein 3-like (NIX) and serine/threonine-protein kinase ULK1 (ULK-1)-dependent mitophagy, improved mitochondrial quality and fat metabolism, and decreased DNA damage and increased DNA repair (homologous recombination) in *C. elegans* (Fang et al. 2019a).

The above studies on premature aging diseases add to our current understanding of the communication between subcellular compartments and how the nuclear–mitochondrial cross talk affects both cellular and organismal health and aging (Fang et al. 2016a,b).

Benefits of NAD^+ Augmentation to AD

There is increasing evidence that NAD^+ supplementation is beneficial for the alleviation of cognitive impairment and pathological features of AD in animal models. NAD^+ augmentation inhibited AD pathogenesis and cognitive impairment in different AD animal models, including NAM-treated triple transgenic (3xTg) AD mice (Liu et al. 2013), NR-treated 3xTgAD/Polβ$^{+/-}$ mice, and APP/PS1 mice (Hou et al. 2018; Hou et al. 2021), and NMN-treated transgenic AD *C. elegans* models expressing neuronal $A\beta_{1-42}$ and neuronal Tau (Fang et al. 2019b). Additionally, treatment with combined metabolic activators (CMAs) consisting of NAD^+ precursors and glutathione precursors improved AD-associated pathology in an AD rat model (Yulug et al. 2021a). Moreover, it has been suggested that Aβ, p-Tau, neuroinflammation, and compromised mitophagy are the pathological occurrences or at least contributors to the development and progression of AD, and that NAD^+ supplementation can ameliorate AD (Fang et al. 2019b; Hou et al. 2021).

Two of the main pathological hallmarks of AD are accumulation and formation of extracellular Aβ plaques and intracellular formation of neurofibrillary tangles (NFTs) consisting of hyperphosphorylated Tau protein. Whether NAD^+ supplementation reduces Aβ pathology seems to be dependent on the animal model used (Gong et al. 2013; Liu et al. 2013). The reduced accumulation of Aβ in NAM- or NR-treated AD mice (APP/PS1 [Fang et al. 2019b] and Tg2576 [Gong et al. 2013]) may be due to reduced production of the pathological $A\beta_{1-42}$, possibly through activation of PGC-1α-regulated BACE1 (β-secretase) degradation; or it may be through enhancing microglia/astrocytes-based phagocytosis of Aβ plaques (Gong et al. 2013; Fang et al. 2019b). However, in 3xTgAD and 3xTg/Polβ$^{+/-}$ mice, no detectable effect was observed upon eliminating Aβ pathology (Hou et al. 2018; Fang et al. 2019b). NAD^+ augmentation has also been reported to reduce Tau pathology in AD models. NAD^+ supplementation has been demonstrated to inhibit Tau phosphorylation at different sites (Thr181, Ser202, Thr205, and Thr231), possibly by inhibiting the cyclin-dependent kinase 5 (Cdk5)-p25 complex activity (Green et al. 2008; Hou et al. 2018; Fang et al. 2019b).

Increasing NAD^+ has been shown to affect additional central cellular processes in AD animal models, such as mitochondrial function, mitophagy, DNA repair, inflammation, and senescence. Supplementation of AD mouse models with either NR or NMN improved mitochondrial function and induced mitophagic activity, leading to improvements in brain pathologies, neuronal function and survival, and inflammation (Hou et al. 2018; Fang et al. 2019b; Hou et al. 2021). Interestingly, specific mitophagy inducers, such as urolithin A and the antibiotic actinonin, have also been shown to ameliorate several AD features in both *C. elegans* and mouse models of AD (Fang et al. 2019b), indicating the benefits of stimulating the NAD^+-mitophagy axis in the treatment of AD. Additionally, through the use of artificial intelligence, we were able to identify novel mitophagy inducers: treatment with these restored memory and improved cognitive deficits in AD *C. elegans* and mouse models (Ai et al. 2022; Xie

et al. 2022). NAD^+ may ameliorate AD pathology by regulating lysosome and the ubiquitin-proteasome system (UPS) functions via improvement of mitophagic functions. NAD^+ augmentation via NR supplementation induced the UPS in the hippocampus and cortex of Tg2576 mice (Gong et al. 2013), and restored UPR^{mt} in APP/PS1 mice. More evidence supporting the function of the lysosome and the UPS in maintaining a healthy brain comes from studies showing that the toxic protein aggregates of Aβ and Tau inhibit the proteasome and autophagy or mitophagy, resulting in protein and mitochondrial accumulation primarily in the hippocampus and prefrontal cortex. Moreover NAD^+ supplementation reduces the pathological phenotypes of Aβ and p-Tau (Tseng et al. 2008; Fang et al. 2019b).

As we age, the levels of NAD^+ in our bodies gradually decline, and this has been linked to cellular senescence and aging. Cellular senescence is a state in which cells stop dividing and become irreversibly arrested, leading to tissue dysfunction and age-related diseases (López-Otín et al. 2023). Previously, it has been hypothesized that DNA damage is responsible for, or contributes to, the neuronal damage observed in AD, and oxidative stress may be the predominant type of DNA damage primarily repaired by base excision repair. In a base excision repair-deficient AD mouse model (3xTgAD/Polβ$^{+/-}$), and in the APP/PS1 model, NR supplementation reduced the amount of DNA damage as manifested by decreased staining of γH2AX (marking double-strand breaks [DSBs]) in the hippocampus (Hou et al. 2018, 2021). Studies have shown that boosting NAD^+ levels can delay the onset of cellular senescence and extend life span in various model organisms. NR supplementation also inhibited neuroinflammation and cellular senescence in AD mice, as well as in the accelerated aging *Atm*$^{-/-}$ mouse model, which shows severe cerebellar ataxia (Hou et al. 2018, 2021; Yang et al. 2021). The molecular mechanism behind this improvement is likely to include NAD^+-dependent induction of mitophagy, which cleans the cells of damaged mitochondria (Hou et al. 2021; Yang et al. 2021). Simultaneously, a NAD^+-dependent immune response, activated by NAD^+ augmentation, can lead to improved resolution of inflammation and phagocytic activity (Gong et al. 2013; Minhas et al. 2019) as well as inhibition of mitochondrial dysfunction–associated senescence (MiDAS) (Wiley et al. 2016). NAD^+ repletion inhibited the NLRP3 inflammasome and the key inflammatory response of cGAS-STING signaling (Fang et al. 2019b; Lautrup et al. 2019; Hou et al. 2021; Yang et al. 2021), providing a mechanism for the inhibitory effect of NAD^+ on inflammation and senescence. Senolytics are a type of drug that selectively target and kill senescent cells (Hou et al. 2019; López-Otín et al. 2023), and they could be combined with NAD supplementation to further reduce senescence.

NAD^+ augmentation can additionally restore neurogenesis in 3xTgAD and 3xTg/Polβ$^{+/-}$ mice by promoting the proliferation of neural precursor cells (Hou et al. 2018). NR and an allosteric activator of NAMPT, P7C3, have been reported to have neuroprotective abilities in mouse models of AD and PD, respectively (Pieper et al. 2005; De Jesús-Cortés et al. 2012; Hou et al. 2018). Consistent with this, studies of large-scale human brain samples demonstrated that hippocampal neurogenesis gradually decreases with the progression of AD (Moreno-Jiménez et al. 2019). The stem cell regenerative activity of NR and NMN likely also contributes to reducing hippocampus-dependent cognitive deficits seen in AD.

CLINICAL TRIALS WITH NAD^+ SUPPLEMENTATION

The preclinical trials presented above have led to numerous clinical trials aiming foremost at establishing the pharmacokinetics and toxicology of the NAD^+ precursors focusing on NR, NMN, and NAM, and next to test the effect on aging-related features, age-related diseases, and other diseases. Due to the recent extensive reviews on the clinical trials (Connell et al. 2019; Lautrup et al. 2019; Gilmour et al. 2020; Reiten et al. 2021), we will only present a short summary of the published data from the completed clinical trials related to aging and age-related diseases. Table 1 provides an overview of the clinical trials on NAD^+ precursors related to aging and disease. NR is orally bioavailable and tolerated, and supplementation with NR increased NAD^+ in

Table 1. A summary of clinical trials related to aging, pathological aging, and age-related diseases

NAD^+ precursors	Disease/condition	Dose administration	Duration of treatment	Demographics	Primary outcome and results	Status	NCT/UMIN/jRCT/PMID
NAM	AD	1500 mg BID	12 mo	Age: 50+ Sex: all	Change in p-Tau 231	Recruiting	NCT03061474
		1500 mg BID	6 mo	Age: 50–95 Sex: all	AD symptoms	Completed, results N/A	NCT00580931
	PD	100 mg BID	18 mo	Age: 35+ Sex: all	Inflammation and severity of PD symptoms	Recruiting	NCT03808961
NR	Aging and lipemia	250 mg BID	7 d	Age: 18–35 vs. 60–75 Sex: all	NAD^+ in blood Vasodilatory responsiveness Lipidemia Oxidative stress and inflammation	Completed, results N/A	NCT03501433
	AD	1000 mg/d	12 wk	Age: 55–89 Sex: all	Brain NAD^+ levels Brain redox state	Recruiting	NCT04430517
	PD	1000 mg/d	52 wk	Age: 18+ Sex: all	MDS-UPDRS NAD^+ metabolites in blood	Recruiting	NCT03568968
		500 mg BID	30 d	Age: 18+ Sex: all	PD-related patterns, neuronal metabolism, motor function	Completed, results N/A	NCT03816020
	A-T	25 mg/kg/d	4 mo	Age: 2+ Sex: all	Ataxia dysarthria, quality of life, laboratory parameters, intelligibility, and fatigue status	Enrolling, results N/A	NCT03962114
		300 mg/d	2 yr	Age: 3+ Sex: all	NAD^+ metabolome Patient well-being A-T characteristics and metabolism	Active, results N/A	NCT04870866
	WS	1000 mg/d	52 wk	Age: 20+ Sex: all	Safety WS characteristics and metabolism	Recruiting	jRCTs031190141
	Mild cognitive impairment	500 mg BID	12 wk	Age: 60–90 Sex: all	Cognitive scores from baseline	Recruiting	NCT03482167
		Dose escalation: 250 mg/d up to 1 g/d, then 1 g/d	4 wk + 6 wk (1 g/d)	Age: 65+ Sex: all	MoCA from baseline at 10 wk	Active, results N/A	NCT02942888
	Cognitive function, mood, and sleep	Crossover study with 300 mg/d and 1000 mg/d	8 wk	Age: 55+ Sex: all	Cognitive function, mood, and sleep	Completed, results N/A	NCT03562468

Continued

Table 1. *Continued*

NAD^+ precursors	Disease/condition	Dose administration	Duration of treatment	Demographics	Primary outcome and results	Status	NCT/UMIN/jRCT/ PMID
	Aging daily function and recovery	1200 mg/d	8 wk	Age: 60+ Sex: all	Cognitive function, mood, and daily activity	Not yet recruiting	NCT04078178
		1000 mg/d	3 wk	Age: 70–80 Sex: male	Skeletal muscle tissue NAD^+ levels and mitochondrial function	N/A	NCT02950441
		500 mg BID	6 wk	Age: 55–79 Sex: all	Results: NR is well tolerated in healthy middle-aged and older adults	Completed, published	NCT02921659 PMC29599478
		250 or 500 mg/d Basis (NR + PT)	8 wk	Age: 60–80 Sex: all	Safety and efficacy with regard to NAD^+ sustainability in elderly people	Completed, results submitted	NCT02678611
	Healthy elderly volunteers	500 mg BID	6 mo	Age: 65–80 Sex: female	Maximum oxygen uptake Muscle function, genes, and mitochondria Short Physical Performance Battery Muscle biopsy samples: respiration rate, immunoblot, and polymerase chain reaction (PCR) Bone metabolism	Recruiting	NCT03818802
	Frailty and sarcopenia	2x 250 mg BID	12 wk	Age: 65–85 Sex: all	Maximal oxygen uptake Muscle strength Gait speed	Not yet recruiting	NCT04691986
		500 mg NR/100 mg PT BID	45 d	Age: 55–80 Sex: all	Muscle regeneration in elderly	Completed, results N/A	NCT03754842
		500 mg NR/100 mg PT BID	90 d	Age: 65+	Safety and tolerance Posttraumatic fall/injury	Unknown	NCT03635411
	Hypertension in elderly	1000 mg/d	6 wk	Age: 65–105 Sex: all	Systolic blood pressure	Recruiting	NCT04112043
		500 mg BID	3 mo	Age: 50–79 Sex: all	Systolic blood pressure and arterial stiffness	Recruiting	NCT03821623
		1000 mg/d	3 mo	Age: 35–80 Sex: all	Arterial stiffness and elevated systolic blood pressure in patients with moderate-to-severe chronic kidney disease (CKD)	Recruiting	NCT04040959

Continued

Table 1. *Continued*

NAD^+ precursors	Disease/condition	Dose administration	Duration of treatment	Demographics	Primary outcome and results	Status	NCT/UMIN/jRCT/PMID
		Dose escalation: 500–2000 mg/d	14 d	Age: 18+ Sex: all	Whole blood NAD^+ levels Heart failure	Recruiting	NCT04528004
	ALS	1500 mg NR/300 mg PT or 1000 mg NR/200 mg PT	?	Age: 35+ Sex: all	Adverse effects Disease progression Overall survival	Recruiting	NCT04562831
		1500 mg NR/300 mg PT	?			Recruiting	NCT05095571
	Menopause	500 mg NR/100 mg PT/d	7 d	Age: 35+ Sex: female	Production of estradiol Change undesirable effects of menopause	Completed, results N/A	NCT04841499
NMN	Glucose metabolism disorders	500 mg/d	8 wk	Age: 55–75 Sex: female	Insulin sensitivity in skeletal muscle	Active	NCT03151239
		300 mg/d	16 wk	Age: 45–75 Sex: all	Muscle insulin sensitivity	Recruiting	NCT04571008
	Aging	300 mg/d	60 d	Age: 40–65 Sex: all	Cellular NAD^+ concentration in blood serum, physical performance, blood pressure	Completed, published	NCT04228640
		250 mg/d	12 wk	Age: 65+ Sex: male	Body composition in aging	Completed, published	UMIN000036321
		2% NMN, BID, crème	55 d	Age: 40–65 Sex: females	Wrinkles, fatigue, puffiness around eyes	Completed, results N/A	NCT04685096
	Safety in middle-aged and older individuals	300 mg or 600 or 900 mg/d	60 d	Age: 40–65 Sex: all		Completed, results N/A	NCT04823260
	Safety in middle-aged and older adults	250 mg/d	12 wk	Age: 40–65 Sex: all	Blood vessel conditions	Completed, published	UMIN000045205

(AD) Alzheimer's disease, (A-T) ataxia telangiectasia, (BID) twice a day, (NAM) nicotinamide, (NMN) nicotinamide mononucleotide, (NR) nicotinamide riboside, (PD) Parkinson's disease, (WS) Werner syndrome.

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healthy middle-aged and older adults (Trammell et al. 2016a; Martens et al. 2018). Some clinical studies showed NMN safely and effectively elevated NAD^+ metabolism in healthy middle-aged and older-aged adults (Huang 2022; Katayoshi et al. 2023). Currently, multiple studies of the safety, tolerance, and long-term effects of NMN have been completed; results are pending (jRCTs041200034, UMIN000039527, jRCTs041190080, UMIN000025739, UMIN000030609, UMIN000021309). NR, in combination with the polyphenol pterostilbene (PT), has been shown to increase NAD^+ levels by 90% in whole blood with no serious adverse events (Dellinger et al. 2017). Currently, the ongoing clinical trials related to aging focus on the effects of NR on cognitive function, daily activity and muscle function, mitochondrial function, hypertension, glucose, and fat metabolism. Several clinical trials are investigating the effects of NAD^+ precursor supplementation on age-related diseases such as heart failure, obesity and diabetes, and neurodegenerative diseases. Two clinical trials with obese and diabetic men have confirmed the bioavailability and safety of NR supplementation, but without seeing effects on weight, glucose intolerance, or insulin sensitivity (Trammell et al. 2016a; Dollerup et al. 2018). One recent clinical study showed that body weight, diastolic blood pressure, total cholesterol, low-density lipoprotein (LDL) cholesterol, and non-high-density lipoprotein cholesterol decreased significantly in the MIB-626 (NMN) group when compared to placebo (Pencina et al. 2023). Chronic NMN supplementation was well tolerated, caused no significant deleterious effect, and showed small but significant improvements in gait speed and performance in a grip test, suggesting alteration to muscle function in healthy older men (Igarashi et al. 2022) (UMIN000036321). One short-term therapeutic study using NR for up to 30 d showed no deleterious impact on methylation homeostasis (Gaare et al. 2023). CMA treatment has been shown to improve the cognitive function of PD patients (Yulug et al. 2021b) and of AD patients after 84 d of treatment compared to placebo (Yulug et al. 2021a). CMA treatment of AD patients resulted in increased plasma levels of NAM and related metabolites, among others, whereas tryptophan-related metabolites such as kynurenate, kynurenine, and tryptophan betaine decreased after CMA treatment (Yulug et al. 2023). Increased levels of tryptophan-related metabolites have previously been associated with increased neurodegeneration and impaired cognitive function (Guillemin et al. 2003; Ting et al. 2007; O'Farrell and Harkin 2017). Several clinical trials have started or are recruiting (Table 1) and looking at the potential of NAM or NR as preventative therapies for AD or PD, and the next years should reveal whether the effects on human patients are as promising as the preclinical data have been. Last, two clinical trials are ongoing examining the effects of NR on A-T patients (NCT04870866 and NCT03962114), and one on the effects of NR supplementation on WS patients in Japan (jRCTs031190141).

CONCLUSIONS AND FUTURE PERSPECTIVES

The hallmarks of aging include genomic instability, epigenetic alterations, telomere attrition, loss of proteostasis, mitochondrial dysfunction, cellular senescence, deregulated nutrient sensing, altered intercellular communication, stem cell exhaustion (Hou et al. 2019), disabled macroautophagy (Fang et al. 2017), chronic inflammation, and dysbiosis (López-Otín et al. 2023). Most of these pathways are strongly associated with decreased NAD^+ (Lautrup et al. 2019), and NAD^+ supplementation has been demonstrated to have beneficial effects on age-related diseases. However, there are still many challenges and shortcomings in the mechanistic insight. The detection methods are challenging due to the low stability of NAD^+ and some of its precursors and intermediates both physically and while crossing the gut system, and the detection of NAD^+, especially in the brain of human individuals remains a challenge. In vivo MIR scanning is now available (Zhu et al. 2015) and may provide insight into how NAD^+ levels change during aging and disease progression in live human patients. In line with the requirement for better detection methods comes a question on where the metabolites go and what they become. Iso-

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tope labeling or other tracing experiments would help to determine what actually happens to the supplements during and after uptake, how the NAD^+ and its precursors are transferred and to which tissues and/or cell types, and how they are being processed, among other questions.

Even though several studies have contributed to the knowledge we have today of the mechanisms underlying the effects seen after increasing NAD^+ with various precursors, more insight into the mechanisms is warranted. It would be interesting to continue the studies on the mechanisms downstream of NAD^+, and also to examine upstream pathways to better understand age- and disease-related declines.

Currently, both the preclinical and clinical studies with NAD^+ precursors (majorly NR and NMN) have shown a safe and nontoxic profile. However, the long-term effects are yet to be reported. It has been suggested that NAD^+ supplementation plays a role in the immune responses, and in cancer research it has been suggested that NAD^+ might increase the expression of inflammatory factors (Nacarelli et al. 2019). On the other hand, NAD^+ supplementation has been demonstrated to reduce inflammatory responses and the expression of inflammatory factors (Fang et al. 2019b; Hou et al. 2021). There is a continual need to further understand the impact of NAD^+ supplementation and its long-term effects. Ongoing clinical trials use short-term treatment, and it would be of interest and importance to evaluate long-term treatment with NAD^+ supplementation. It is also important to have larger studies involving more participants than the current ones. There are certain conditions where NAD^+ supplementation may want to be avoided, such as certain cancers (Demarest et al. 2019). The results from ongoing clinical trials will hopefully shed light on some of the missing knowledge regarding disease treatment, and hopefully the next decade of research will fill in some of the knowledge gaps in terms of both technical challenges and mechanisms.

COMPETING INTEREST STATEMENT

E.F.F. has an MTA with LMITO Therapeutics, Inc. (South Korea), a CRADA arrangement with ChromaDex (USA), and a commercialization agreement with Molecule AG/VITADAO, and is a consultant to Aladdin Healthcare Technologies (UK and Germany), the Vancouver Dementia Prevention Centre (Canada), Intellectual Labs (Norway), MindRank AI (China), and NYo3 (China).

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Roles of NAD$^+$ in Health and Aging

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