The balance between NAD⁺ biosynthesis and consumption in ageing

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ABSTRACT

Nicotinamide adenine dinucleotide (NAD⁺) is a vital coenzyme in redox reactions. NAD⁺ is also important in cellular signalling as it is consumed by PARPs, SARM1, sirtuins and CD38. Cellular NAD⁺ levels regulate several essential processes including DNA repair, immune cell function, senescence, and chromatin remodelling. Maintenance of these cellular processes is important for healthy ageing and lifespan. Interestingly, the levels of NAD⁺ decline during ageing in several organisms, including humans. Declining NAD⁺ levels have been linked to several age-related diseases including various metabolic diseases and cognitive decline. Decreasing tissue NAD⁺ concentrations have been ascribed to an imbalance between biosynthesis and consumption of the dinucleotide, resulting from, for instance, reduced levels of the rate limiting enzyme NAMPT along with an increased activity of the founding member poly ADPR polymerase 1 (PARP1). So far, 17 members of the PARP family have been identified, the majority of them potential target to ameliorate age-related diseases. The present review explores how ageing affects NAD⁺ metabolism and current approaches to reverse the age-dependent decline of NAD⁺.

1. Introduction

NAD⁺ is an essential molecule for all living organisms. NAD⁺ and its phosphorylated form nicotinamide adenine dinucleotide phosphate (NADP⁺) have a wide variety of functions, both in energy metabolism and in signalling pathways, and are involved in key cellular functions including replication, gene regulation and DNA repair (Nikiforov et al., 2015; Pollak et al., 2007). Originally, NAD and NADP were identified as redox coenzymes, with the capability to transfer electrons as part of the energy metabolism of the cell (Warburg and Griese, 1935). NAD-dependent electron transfer takes place in important metabolic pathways such as glycolysis and the Krebs cycle, making NAD a vital co-enzyme. NAD(P)-dependent electron transfer is carried out by a large group of enzymes known as dehydrogenases. These enzymes catalyse the reversible conversion between the oxidised forms of NAD(P), NAD⁺ and NADP⁺, and the reduced forms, NADH and NADPH, with the ratio between the two forms known as the redox state. Regulation of the redox state is important, not only for energy metabolism but also to provide cells with reducing species, such as NADPH, to prevent damage from free radicals (Agledal et al., 2010; Xiao et al., 2018; Ying, 2009). The redox state of NADP⁺/NADPH in the cell is shifted towards NADPH, providing a readily available pool of reducing species (Tischler et al., 1977). The NAD⁺/NADH ratio, on the other hand, is shifted towards NAD⁺ (Ju et al., 2014; Zhang et al., 2002).

In addition to its role as an electron carrier, NAD⁺ has been implicated as an important factor in a wide array of key cellular signalling pathways (Nikiforov et al., 2015; Berger et al., 2004; Houtkooper et al., 2010), and thus became subject of extensive research.

ADP-ribosylation is the transfer of an ADP-ribose (ADPR) moiety to a protein. This process was originally discovered in bacterial toxins (Moss and Vaughan, 1988). In these reactions, NAD⁺ serves as a substrate for enzymes cleaving NAD⁺ to add an ADPR moiety to an amino-acid acceptor. This transfer can be of a single ADPR moiety (mono-ADP ribosylation), or of multiple moieties by the formation of ADPR polymers (poly-ADP ribosylation) (Schreiber et al., 2006; Ame et al., 2004). In humans, mono- and poly-ADP ribosylation reactions are mainly catalysed by a family of enzymes referred to as the PARP family, based on the founding member poly ADPR polymerase 1 (PARP1). So far, 17 members of the PARP family have been identified, the majority of them mediating mono-ADP-ribosylation. Mono-ADP ribosylation has been found to play a role in the immune response, cell adhesion, the unfolded protein response and development of hippocampal neurons (Moss and Vaughan, 1988; Gute et al., 2017). Poly-ADP ribosylation has important functions in a wide variety of cellular processes (D’Amours et al.,...
Another function of NAD\(^+\) is NAD-dependent deacetylation, catalysed by sirtuins. The sirtuin protein family is homologous to the yeast silent information regulator 2 (SIR2), a protein found to increase life span in yeast by NAD-dependent deacetylation of telomere associated proteins (Denu, 2003). Homologues of the Sir2 protein are highly conserved in organisms ranging from archaea to humans, underlining their importance. Sirtuins deacetylate their targets by cleaving NAD\(^+\) and transferring the acetyl group onto ADPR. Thus, in sirtuin-mediated protein deacetylation, the acetyl group is transferred from a lysine residue onto ADPR producing O-acetyl-ADP-ribose (OAADPR) and nicotinamide (Nam) (Jackson and Denu, 2002). In humans, the Sir2 homolog SIRT1 has been found to affect transcriptional activity by deacetylating and inactivating TAFI68, a TATA box binding protein (Muth et al., 2001). Another target for sirtuin deacetylation is the tumour suppressor gene p53, with deacetylation of p53 inducing repression of p53-dependent transcription and apoptosis (Langley et al., 2002; Vaziri et al., 2001). Notably, sirtuins have been shown as essential for the regulation of transcriptional activity in many organisms, DNA repair and initiation of cell death pathways (Schreiber et al., 2006; Wang et al., 2011).

Most NAD\(^+\) in mammals is generated in the salvage pathway, in which Nam, stemming from NAD\(^+\)-dependent signalling reactions, is resynthesised into NAD\(^+\). Nam is converted to NMN by nicotinamide phosphoribosyltransferase (NAMPT), and the conversion of Nam to NAD\(^+\) is the rate limiting step in the salvage pathway. In the final step of NAD\(^+\) biosynthesis via the salvage pathway, Nam is further processed in the Preiss Handler pathway (Bender, 1983). Recently, it was demonstrated that PARP1-dependent activation of PARPs and sirtuins, regulating these reactions by negative feedback (Uehara et al., 2006; Avalos et al., 2005).

NAD\(^+\) also serves as a precursor for the calcium mobilising signalling molecules generated by CD38/157 and SARM1. The conversion of NAD\(^+\) to cyclic ADP-ribose (cADPR) occurs at neutral pH, while NADP\(^+\) is converted to NAADP at acidic pH, through base exchange, where the Nam moiety is exchanged with nicotinic acid (NA) (Guse, 2015). These products then act as potent calcium-mobilizing agents. Additionally, NAD\(^+\) is involved in the programmed axon degeneration pathway known as Wallerian degeneration, in which loss of activity of the NAD\(^+\) biosynthetic enzyme Nicotinamide Mononucleotide Adenyllyl Transferase 2 (NMNAT2) leads to accumulation of the NAD\(^+\) precursor nicotinamide mononucleotide (NMN). This, in turn, activates the NAD\(^+\) degrading enzyme SARM1, leading to NAD\(^+\) depletion and degradation of the axon (Essuman et al., 2017; Conforti et al., 2014; Gerdts et al., 2016). As Nam is an important NAD\(^+\) precursor as well as a product of NAD\(^+\) consuming reactions, it provides a link between regulation of NAD biosynthesis and NAD\(^+\) consuming reactions.

These non-redox functions of NAD\(^+\) involve cleavage of the dinucleotide to donate ADP or to generate second messenger molecules. As NAD\(^+\) is constantly consumed in these reactions, there is a need for continuous NAD\(^+\) biosynthesis. Imbalances between these NAD\(^+\) consumption and NAD\(^+\) biosynthesis may result in insufficient NAD\(^+\) supply, which has been linked to several diseases, such as neuropathy, autoimmune diseases, and cancer. Given the importance of NAD\(^+\) availability, it is interesting to note that NAD\(^+\) levels decrease with ageing, and recently, a strong link has emerged between age-related NAD\(^+\) decline and age-related diseases (Imai and Guarente, 2014; Verdin, 2015). In fact, enhancing NAD\(^+\) levels by supplementation with NAD\(^+\) precursors have been shown to be effective in increasing the life span of mice, yeast and C. elegans showing the therapeutic potential of modifying NAD\(^+\) levels (Rajman et al., 2018; Yoshino et al., 2018; Mouchiroud et al., 2018; Pang et al., 2016; Belenky et al., 2007). Efforts to manipulate cellular NAD\(^+\) levels mainly target the biosynthesis of NAD\(^+\), and, in recent years, the NAD\(^+\) biosynthetic pathways have been the subject of extensive study. In this review, we discuss how NAD\(^+\) biosynthesis and NAD\(^+\)-dependent processes are affected by ageing and provide an outlook on how NAD\(^+\) enhancing strategies can be utilised in reversing age-related NAD\(^+\) decline.

2. NAD\(^+\) biosynthesis

The constant degradation of NAD\(^+\) by enzymes involved in signalling necessitates a continuous resupply of the dinucleotide, and the in vivo half-life of NAD\(^+\) varies from 15 mins to 15 h depending on the tissue (Liu et al., 2018a). NAD\(^+\) can be synthesized from dietary precursors or recycled from NAD\(^+\) degradation products. Collectively the precursors, known as vitamin B3, are Nam, NMN, nicotinamide riboside (NR), nicotinic acid riboside (NAR). In addition, the biosynthetic intermediate NMN can serve as precursor. Moreover, quinoline acid (QA), a by-product of tryptophan catabolism, can be utilised to synthesise NAD\(^+\) de novo (Bogner and Bremer, 2006; Magni et al., 2008; Bender, 1983). An overview of established mammalian NAD biosynthetic pathways is provided in Fig. 1. Very recently, ditydroxycinnamidine riboside (NRH) was identified as a novel NAD\(^+\) precursor (Giroud-Gerbetant et al., 2019; Yang et al., 2020, 2019).

Apart from NMNATs, all mammalian NAD biosynthetic enzymes localise to the nucleus/cytosol, and are encoded by single genes, except for the two NRK isozymes (Chiarugi et al., 2012). The three different NMNAT isoforms are encoded by different genes and differ in their oligomeric state, catalytic properties and subcellular localisation. NMNAT1 is found in the nucleus, NMNAT2 is bound to the cytosolic face of the Golgi apparatus (Schweiger et al., 2001; Berger et al., 2005; Lau et al., 2010; Mayer et al., 2010). At least one splice variant of NMNAT3 encodes a mitochondrial matrix protein (Dolle et al., 2010). However, recent studies suggest NMNAT3 also to be localised to the mitochondrial intermembrane space or in endolysosomes (Berger et al., 2005; Davila et al., 2018; Nam et al., 2020).

The distribution of NAD\(^+\) biosynthetic enzymes to different organelles allows for the establishment of separate organellar NAD\(^+\) pools. The localisation of NMNAT1, 2, and 3 in the nucleus, cytoplasm and mitochondria, respectively, is of interest, since the majority of NAD\(^+\)-dependent metabolic pathways take place in these organelles (Liu et al., 2018a; Xu et al., 2011; Schwer et al., 2002; Anderson et al., 2017). Establishing distinct pools may allow the cell to regulate NAD\(^+\)-dependent metabolic pathways without affecting the whole cell by specifically altering the availability of the dinucleotide within an organelle. Differential regulation of NMNAT3 expression, which all use NMN to synthesise NAD\(^+\), may enable the cell to distribute NAD\(^+\) to the organelle where it is most needed in a given situation. Recently, it was demonstrated that PARP1-dependent activation of adipocyte differentiation genes is regulated by restricting NMN and, in turn, NAD\(^+\) from NMNAT1 in the nucleus by upregulation of NMNAT2 expression in the cytoplasm (Ryu et al., 2018).
3. Impaired NAD$^+$ biosynthesis in ageing

Age-related NAD$^+$ decline has partially been attributed to a lower rate of NAD$^+$ biosynthesis. The majority of NAD$^+$ is generated in the salvage pathway and lowered expression levels of NAMPT during ageing have been identified as a major factor. The expression of NAMPT at both mRNA and protein levels is reduced in multiple tissues in aged mice (Yoshino et al., 2011; Stein and Imai, 2014). This age-related decline in NAMPT expression leads to a reduction of NAD$^+$ levels in the affected tissues, which in turn will affect the activities of NAD$^+$-dependent redox reactions and signalling processes. For example, the decline in proliferation and self-renewal of neural stem/progenitor cells during ageing has been tied to the reduced expression of NAMPT and the ensuing diminished NAD$^+$ levels (Stein and Imai, 2014).

The exact mechanism of age-related NAMPT decline is dependent on both the tissue and context, and several factors have been reported to affect NAMPT levels during ageing. Alterations of the circadian rhythm have been shown to contribute to age associated NAMPT decline. The core complex of transcription factors for the circadian rhythm, CLOCK:BMAL1, regulates Nampt expression by binding to the Nampt promoter region (Nakahata et al., 2009; Peek et al., 2013; Ramsey et al., 2009). SIRT1, an NAD$^+$ dependent deacetylase, regulates the expression of circadian genes by oscillatory deacetylation of BMAL1 and PER2 (Asher et al., 2008; Nakahata et al., 2008). Thereby, SIRT1 affects the stringency of circadian gene expression, and inhibition of SIRT1 reduces the circadian amplitude. This interdependency demonstrates that NAD$^+$, NAMPT and SIRT1 exist as a regulatory feedback loop that partially controls the circadian rhythm. Ageing affects both the rhythmic quality and amplitude of the circadian rhythm (Hood and Amir, 2017). This may reduce the expression of Nampt which in turn will lead to lower NAD$^+$ levels. In the suprachiasmatic nucleus of the hypothalamus of aged mice, a region of the brain that controls circadian rhythms in vivo, both NAMPT and SIRT1 are significantly reduced when compared to young mice (Chang and Guarente, 2013).

Another possible mechanism of age-related NAMPT decline is chronic inflammation. Cellular, oxidative and environmental stressors that promote chronic inflammation increase with ageing in different metabolic tissues including adipose, skeletal muscle and liver (Kourtis and Tavernarakis, 2011). In affected tissues, inflammatory cytokines, which exacerbate cellular damage, are released. These cytokines are reported to decrease the expression of Nampt (Yoshino et al., 2011; Kralisch et al., 2005). Interestingly, two of these cytokines, TNF-α and IL-1β, inhibit CLOCK:BMAL1-mediated gene expression (Cavadini et al., 2007; Petrizilla et al., 2009). As a result, chronic inflammation and the degradation of the circadian rhythm during ageing may synergistically reduce NAMPT levels.

MicroRNA, specifically microRNA-34a, contributes to hepatic NAD$^+$ decline during ageing. MicroRNA-34a binds to the 3’ UTR region of
both Nampt and Sirt1 and suppresses their expression, which subsequently reduces the NAD\(^+\) biosynthetic capacity of the affected tissue (Choi et al., 2013; Li et al., 2011; Yamakuchi et al., 2008). It has been demonstrated that the hepatic levels of microRNA-34a increase with age (Choi et al., 2013).

In comparison to our understanding of how ageing affects NAMPT, very little is known about how ageing impacts de novo- and Preiss Handler mediated NAD\(^+\) biosynthesis. In particular, the role of NMNATs, which are the common enzymes for all the biosynthetic routes, remains elusive. In drosophila, which harbours only a single Nmnat gene, overexpression of NMNAT showed that oxidative stress biomarkers and ATP levels were improved. In addition, expression of longevity and mitochondrial related genes were increased (Liu et al., 2018b). This observation hints that NMNATs may affect the ageing process through their anti-oxidative stress and mitochondrial protection function. However, further research is needed to elucidate the role NMNATs may play in mammalian ageing.

4. NAD\(^+\) signalling and degradation in ageing

The expression and activity of several NAD\(^+\) consuming enzymes rise with age leading to increased consumption and lower levels of the dinucleotide. NAD\(^+\) is consumed by sirtuins, PARPs, CD38/157 and SARM1, and these enzymes compete for NAD\(^+\). Thereby, altering the activity or expression level of one enzyme affects the activity of the others (Fang et al., 2017).

In humans the sirtuin family is composed of seven members (SIRT1-SIRT7) which play a central role in several cellular metabolic processes related to health-span and longevity (Xanfi et al., 2012; Satoh et al., 2013). For instance, nuclear SIRT1, SIRT6 and SIRT7 are involved in DNA repair, while mitochondrial SIRT3, SIRT4 and SIRT5 and nuclear SIRT1 are involved in mitochondrial homeostasis (Carrico et al., 2018). Generally, sirtuins remove an acetyl group from lysine residues of target proteins. In addition, some sirtuins catalyse other reactions, such as, desuccinylation, demalonylation and fatty acid deacetylation (Choudhary et al., 2014). SIRT1 plays a key role in mitochondrial biogenesis by regulating the peroxisome proliferator activated receptor-gamma co-activator-Ialph (PGC-1α) (Amat et al., 2009), as well as the turn-over of defective mitochondria by mitophagy (Jang et al., 2012). Activation of the NAD\(^+\)/SIRT1-PGC-1α axis has therapeutic potential in neurodegenerative diseases, such as Huntington disease (Lloret and Schlesinger, 1997; Mizuguchi et al., 1995). In comparison CD157, a homolog of CD38, is expressed in endothelial cells and immune cells (Quarona et al., 2013). CD38 has emerged as one of the main drivers of NAD\(^+\) depletion during ageing suggesting that targeting its NAD\(^+\)ase activity may restore NAD\(^+\) levels. During ageing, CD38 expression increases in spleen, liver, and adipose tissue leading to lower NAD\(^+\) levels and mitochondrial dysfunction (Camacho-Pereira et al., 2016). Increased expression of CD38 has been linked to inflamminge and neurodegenerative disease. Accordingly, cellular senescence, which is an age-related proliferation arrest, activates the expression of CD38 in tissue-resident macrophages (Covarrubias et al., 2020; Chini et al., 2020). Likewise, proinflammatory cytokines and chemokines, released from senescent cells, increase the activity and the expression level of CD38 (Chini et al., 2019). Furthermore, there is data suggesting that CD38 expression increases with AD disease progression in a double transgenic mouse model expressing a chimeric mouse/human amyloid precursor protein (APP) with the Swedish mutation (APP\(\alpha 5\)) and a mutant presenilin 1 with the delta E9 (PS1\(\alpha 5\,\delta\)) compared to age-matched non-transgenic mice (Long et al., 2015). Similarly, an AD mouse model with CD38 knockout showed higher NAD\(^+\) levels, and a mild neurodegenerative phenotype with improved spatial learning (Buchheit et al., 2015). Compared to CD38, little is known about the role CD157 may play in ageing. However, increased levels of CD157 have been related to inflamminge, autoimmune disease and certain types of cancer (Covarrubias et al., 2020; Ortolan et al., 2019).

Previous studies have provided compelling evidence on the role of NAD\(^+\) consuming enzymes in ageing (Lautrup et al., 2019). However, further research is required to fully understand the underlying mechanisms of the age-associated activity changes of these enzymes, and the
interplay between NAD\(^+\) depletion and ageing.

5. Therapeutic reversal of ageing-related NAD\(^+\) decline

The importance of NAD\(^+\) in longevity, ageing and health span is well established and the levels of the dinucleotide can be regulated by both lifestyle choices and dietary means. The age-related NAD\(^+\) decline is due to an imbalance of NAD\(^+\) synthesis and NAD\(^+\) consumption capacities (Fig. 2). Several pharmacological approaches to address this imbalance have been established (Lautrup et al., 2019). To increase NAD\(^+\) levels, dietary supplementation with NAD\(^+\) precursors or activation of the rate limiting enzyme, NAMPT, has been explored (Gardell et al., 2019). De novo NAD\(^+\) biosynthesis can be stimulated by inhibition of \(\alpha\)-amino-\(\beta\)-carboxymuconate \(\varepsilon\)-semialdehyde decarboxylase (ACMSD) (Katsyuba et al., 2018). Alternatively, NAD\(^+\) consumption can be lowered by inhibition of CD38 and PARPs (Boslett et al., 2017).

In yeast and C. elegans beneficial effects of NAD\(^+\) precursors on the lifespan and healthspan have been firmly established (Mouchiroud et al., 2013; Fang et al., 2016; Belenky et al., 2007). Administration of low concentrations of Nam, \(\mu\)M range, to C. elegans increased lifespan (Mouchiroud et al., 2013). In contrast, higher doses of Nam, mM range, reduced lifespan (Schneisser et al., 2013; Gallo et al., 2004). Thereby, the effect of Nam on lifespan extension is unclear. This discrepancy might be due to the inhibitory effect of Nam at high concentrations on sirtuins and PARPs (Avalos et al., 2005; Saldeen et al., 2003). Another possible explanation is that high concentrations of Nam can deplete cells of S-adenosyl methionine (SAM), a vital substrate for methylation reactions, as Nam is methylated to N-methyl Nam (MNAM) and secreted (Pissios, 2017). This process, in turn, might affect DNA methylation and lead to aberrant gene expression. MNAM has also been associated with cardiac disease, PD and type 2 diabetes (Hwang and Song, 2020). Additionally, the positive impact of NR supplementation on the lifespan of both C. elegans and yeast is well established. The effect in C. elegans has been reported to be dependent on sirtuin activity in many studies. In C. elegans models of various age-related disorders, Cockayne syndrome, ataxia telangiectasia, xeroderma pigmentosum group A and Werner syndrome, supplementation with NR or NMN extended lifespan (Fang et al., 2016, 2014; Scheibye-Knudsen et al., 2014; Fang et al., 2019).

In mice, most studies have been performed using different disease models and relatively little is known how NAD\(^+\) precursor supplementation affects the longevity and healthspan of wild type mice. One study reported that long term administration of Nam has beneficial effects on the healthspan of mice fed a high fat diet. However, the lifespan was not affected (Mitchell et al., 2018). In another study supplementation with NR slightly increased the lifespan of old mice (Zhang et al., 2016). Long-term NMN supplementation, one year, starting at five months of age, enhanced physical activity, energy metabolism and insulin sensitivity with no signs of toxicity (Mills et al., 2016). In a mouse model of ataxia telangiectasia treatment with NMN increased NAD\(^+\) levels and lifespan (Fang et al., 2016). In another study, using a mouse model of muscular dystrophy supplementation with NR enhanced muscle stem cell function by regulating mitochondrial metabolism (Zhang et al., 2016; Ryu et al., 2016).

The promising results in mouse models have prompted several clinical trials in humans, and an overview of the ongoing clinical trials can be found on clinicaltrials.gov. The safety, bioavailability and NAD\(^+\) enhancing capacity of NR has been examined in two studies (Dellinger et al., 2018; Airhart et al., 2017). In one study the safety of supplementation with 250 or 500 mg NR daily for 60 days was assessed (Dellinger et al., 2018). In another study, participants were given 250 mg NR on day 1 and 2 and 1000 mg NR twice on day 7 and 8 and finally 1000 mg on day 9 (Airhart et al., 2017). In another study, supplementation with 1000 mg NR for 21 days elevated the muscle NAD\(^+\) metabolome, along with down-regulation of energy metabolism and mitochondrial pathways, while mitochondrial bioenergetics were unaffected. NR supplementation also reduced the levels of circulating metabolic intermediates including lactate, pyruvate, glucose and alanine. In addition, NR supplementation reduced blood pressure and improved endothelial function (Mitchell et al., 2018). NR supplementation has also been shown to improve muscle strength and power, and to increase muscular mass in older adults (Fang et al., 2016). In another study, supplementation with NMN increased muscle strength and power, and to increase muscular mass in older adults (Fang et al., 2016).

Fig. 2. Strategies for therapeutic reversal of age-dependent NAD\(^+\) decline. NAD\(^+\) levels are stable in healthy adults as the rate of biosynthesis and consumption is balanced. Ageing impairs NAD\(^+\) biosynthesis as the levels of NAMPT decrease and increase consumption as the activity of PARPs and CD38 rise. Different therapeutic interventions to stop the age-dependent NAD\(^+\) decline has been developed: The rate of NAD\(^+\) consumption can be decreased by inhibition of CD38/157 and PARPs and NAD\(^+\) biosynthesis can be increased by activation of NAMPT. Alternatively, De novo synthesis of NAD\(^+\) can be increased by inhibiting ACMSD. NAD\(^+\) levels can be restored and increased by supplementation with NAD\(^+\) precursors such as Nam, NR, NMN and NRH.
inflammatory cytokines (Ehassan et al., 2019). NR supplementation, 500 mg twice per day for 6 weeks, in healthy adults lowered blood pressure and reduced aortic stiffness but the results were not statistically significant. Other outcomes, such as physical activity, body fat, markers of exercise performance and glucose and insulin regulation did not improve (Martens et al., 2018). Another study explored the effect of NR supplementation, 1000 mg twice per day for 12 weeks, on insulin sensitivity and other metabolic parameters in obese, insulin-resistant men. The results showed that NR supplementation did not affect insulin sensitivity or glucose metabolism. No alterations in secondary outcomes were observed either (Dollerup et al., 2018). In these studies, the dosages and treatment regimens vary greatly, and future studies should focus on determining the best treatment regime and dosage. In comparison with NR, relatively little is known about the effect MN supplementation has in humans, but several clinical trials are ongoing. The safety of MN supplementation has been examined and doses up to 500 mg are considered safe in healthy males (Irie et al., 2020). In a recent study, MN supplementation, 250 mg daily for 10 weeks, increased muscle insulin sensitivity and insulin signalling in prediabetic women who are overweight or obese (Yoshino et al., 2021).}

Activation of NAD biosynthetic pathways also presents an intriguing way to counteract age-related NAD− decline. In particular, activation of NAMPT is of interest owing to its position as rate-limiting enzyme in the salvage pathway. Recently SBI-797812 was identified as a potent NAMPT activator which increased NMN production in vitro, in cells and in vivo, albeit only in the liver (Gardell et al., 2019). P7C3, a neuroprotective molecule, activates NAMPT and restores NAD+ levels in doxorubicin treated cells, hinting the molecule may be an interesting therapeutic for age-related disorders (Wang et al., 2014). Activation of de novo NAD+ biosynthesis by inhibition of ACSMD also increased the levels of the dinucleotide. The two ACMSD inhibitors TES-991 and TES-102524 increased NAD+ levels and SIRT1 activity, improving mitochondrial function in the brain, liver and kidney of mice (Katsyuba et al., 2018). Future work should elucidate the exact molecular mechanisms of these activators and inhibitors and evaluate their suitability for the treatment of ageing-associated deficiencies in humans.

Inhibition of NAD+ degrading enzymes has emerged as a powerful and promising approach to restore and increase levels of the dinucleotide during ageing. PARP1 inhibitors are commonly used as an adjunct to common cancer therapies as they make cells susceptible to DNA damage. However, PARP inhibition has also shown promise as an NAD+ enhancer in ageing, and in C. elegans inhibition of PARPs significantly extends lifespan (Mouchiroud et al., 2013). Notably, several CD38 inhibitors have been developed or are being developed, because the enzyme is one of the main drivers of age-related NAD− decline (Camacho-Pereira et al., 2016). In mice, luteolin, a flavonoid, inhibits CD38, increases NAD+ levels and protects the myocardium and endothelium following myocardial ischaemia (Boslett et al., 2017). In a clinical trial, luteolin had neuroprotective properties in children with autism (Taliou et al., 2013). Apigenin, another flavonoid inhibitor of CD38, increased NAD+ levels in human cells and mouse tissues (Escande et al., 2013). It also improved lipid and glucose homeostasis in a mouse model of obesity (Escande et al., 2013). In a more recent study apigenin was found to not only inhibit CD38 but also lower the expression level of the enzyme. In the kidneys of diabetic rats apigenin increased the intracellular ratio of NAD+/NADH and mitochondrial antioxidative enzyme activity was enhanced (Ogura et al., 2020). The compound 78c, a derivative of 4-aminoquinoline, inhibits CD38 and in mice increased the NAD+ levels in heart, liver and muscle (Haffner et al., 2015). Treatment of mice with 78c prevents NAD− decline during ageing, and old mice treated with the compound exhibited improved muscle function, reduced DNA damage and ameliorated metabolic dysfunction (Tarrago et al., 2018). It has also been shown that 78c protects against postischemic endothelial and cardiac myocyte injury in mice (Boslett et al., 2019). DSRM-3716 was recently identified as potent inhibitor of SARM1 NADase activity. The compound phenocopied SARM1 depletion and protected axons from degeneration induced by axotomy or mitochondrial dysfunction. Additionally, DSRM-3716 rescued rotenone treated axons that already had entered the metablastic state (Hughes et al., 2021). Before any of these inhibitors are used in clinical trials, their mechanism of action should be elucidated. In addition, it will be important to determine if they cause any deleterious side effects.

6. Conclusion and future perspectives

NAD+ has in the past decade emerged as a key component in healthy ageing and longevity. Numerous excellent studies have demonstrated that there is an age-dependent reduction of NAD+ levels. This decrease is partially due to an imbalance between NAD+ biosynthesis and degradation, as the biosynthetic capacity is decreased, whereas NAD+ degradation increases during ageing. Therapeutic augmentation of NAD+ levels improves the health span and lifespan and alleviates the symptoms of various age-related pathologies in animal models. Supplementation with NR has shown some promise in clinical trials and numerous other trials are ongoing or planned. NMN supplementation clinical trials have only been recently initiated, but their results will be of great interest. Future work and clinical trials should also focus on activators and inhibitors of NAD+ biosynthesis and NAD+ degradation, respectively, as work in mice models have revealed encouraging effects.

Despite the considerable advances that have been made in the past decade, several questions remain unanswered. Perhaps, among the most pressing questions, the molecular mechanisms and pathways linking NAD+ and ageing have so far remained elusive and not understood. Furthermore, the long-term effects of MNM and NR supplementation are not known, this is of importance since NAD+ augmentation may also promote tumour growth in animal models.

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