THE EMBO JOURNAL

# Review

Focus: Metabolism

# Modulating NAD$^+$ metabolism, from bench to bedside

Elena Katsyuba & Johan Auwerx*

## Abstract

Discovered in the beginning of the 20$^{th}$ century, nicotinamide adenine dinucleotide (NAD$^+$) has evolved from a simple oxido-reductase cofactor to being an essential cosubstrate for a wide range of regulatory proteins that include the sirtuin family of NAD$^+$-dependent protein deacylases, widely recognized regulators of metabolic function and longevity. Altered NAD$^+$ metabolism is associated with aging and many pathological conditions, such as metabolic diseases and disorders of the muscular and neuronal systems. Conversely, increased NAD$^+$ levels have shown to be beneficial in a broad spectrum of diseases. Here, we review the fundamental aspects of NAD$^+$ biochemistry and metabolism and discuss how boosting NAD$^+$ content can help ameliorate mitochondrial homeostasis and as such improve healthspan and lifespan.
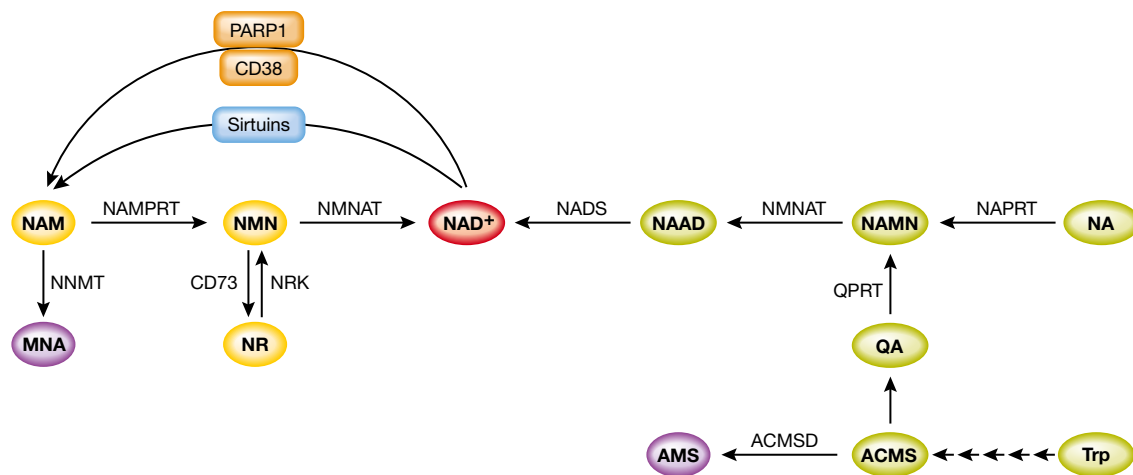
## Introduction

The first cofactor ever described, nicotinamide adenine dinucleotide (NAD$^+$), was discovered by the British biochemists Arthur Harden and William John Young in 1906 (Harden & Young, 1906). They observed that adding boiled yeast extracts to non-boiled yeast extracts significantly accelerated alcoholic fermentation, suggesting that the boiled yeast fraction contained something capable of promoting the fermentation reaction. They named this heat-stable, but yet unidentified factor, "cozymase". Almost 25 years later, Hans von Euler-Chelpin established the chemical composition of the cozymase as an adenine, a reducing sugar group and a phosphate (von Euler & Myrbäck, 1930). Finally, in 1936, Otto Heinrich Warburg discovered the capability of the cozymase to transfer hydride from one molecule to another and identified nicotinamide base as the site of redox reactions (Warburg & Christian, 1936). Together with its reduced counterpart, NADH, NAD$^+$ has since been known for being involved in reactions that required the transfer of electrons from one molecule to another. As such, the redox couple NAD$^+$/NADH has been reported to participate in numerous reactions requiring an electron exchange, such as glycolysis, pyruvate-to-lactate and pyruvate-to-acetyl-CoA interconversions, β-oxidation, citric acid cycle (TCA cycle), and oxidative phosphorylation. Moreover, addition of a phosphate to the adenosine ribose of NAD$^+$ by NAD$^+$ kinases (NADKs) leads to a formation of nicotinamide adenine dinucleotide phosphate (NADP$^+$). NADP$^+$ and its reduced form, NADPH, play a key role in cellular defense against oxidative stress, as well as in the synthesis of fatty acids, cholesterol, and DNA. Detailed description of the physiological roles of the NADP$^+$/NADPH redox couple is reviewed elsewhere (Ying, 2008). Although the role of NAD$^+$ in redox reactions is now rather well understood, NAD$^+$ biology underwent a renaissance when NAD$^+$ was reported to influence the activity of the sirtuins (Imai et al, 2000), a family of NAD$^+$-dependent deacylases, implicated in the regulation of metabolism and mitochondrial function (Haigis & Sinclair, 2010; Houtkooper et al, 2012). Besides sirtuins, other enzymes, such as the poly ADP-ribose polymerase (PARP) protein family and the cyclic ADP-ribose (cADPR) synthases, such as CD38 and CD157, are currently known to require NAD$^+$ as a cosubstrate to perform their function. The dependence of these important metabolic enzymes on NAD$^+$ levels provides an attractive possibility to modulate their activity and thereby achieve health benefits and has led to an increased interest in NAD$^+$ metabolism over the last decade. The therapeutic potential of NAD$^+$ boosting techniques to activate the sirtuins has now been explored in a large spectrum of preclinical disease models that mimic rare genetic disorders, such as the Cockayne syndrome, as well as pandemic-like contemporary diseases, such as obesity or non-alcoholic fatty liver disease (NAFLD). The near future will hopefully see these studies translate from the bench to the bedside.

## Biosynthesis of NAD$^+$

Intracellular NAD$^+$ can be produced through either de novo synthesis or via salvage pathways from precursor molecules, naturally occurring vitamins: nicotinamide (NAM), nicotinic acid (NA), and nicotinamide riboside (NR) (Bogan & Brenner, 2008; Houtkooper et al, 2010) (Fig 1). The NAD$^+$ de novo synthesis pathway starts from the amino acid tryptophan (Bender, 1983; Houtkooper et al,

Laboratory of Integrative and Systems Physiology, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland
*Corresponding author. Tel: +41 216939522; E-mail: admin.auwerx@epfl.ch

**Figure 1. Pathways modulating NAD$^+$ content in mammals.**
Intermediates of the amidated and deamidated routes are depicted in yellow and green, respectively. NAD$^+$-consuming enzymes competing with sirtuins for NAD$^+$ availability are depicted in orange. Purple color indicates metabolites not recycled in the NAD$^+$ synthesis pathway.

2010) and most likely takes place in the cytosol, since all the enzymes catalyzing the different steps of this process are localized there (Houtkooper *et al*, 2010).

NAD$^+$ synthesis from NAM requires only two steps: NAM gets first converted by nicotinamide phosphoribosyltransferase (NAMPRT) into NAM mononucleotide (NMN), which in its turn leads to the production of NAD$^+$ in a reaction catalyzed by nicotinamide mononucleotide adenylyltransferase (NMNAT) (Fig 1). Three different isoforms of NMNAT have been reported, each of them possessing a specific subcellular localization: NMNAT1 is a nuclear enzyme (Emanuelli *et al*, 2001; Yalowitz *et al*, 2004), NMNAT2 is located in the cytosol and Golgi apparatus (Yalowitz *et al*, 2004; Berger *et al*, 2005), while NMNAT3 was detected in the cytosol and mitochondria (Zhang *et al*, 2003; Berger *et al*, 2005; Yang *et al*, 2007). NR also gets converted into NMN by nicotinamide riboside kinase (NRK) (Bieganowski & Brenner, 2004). Mammals possess two isoforms of NRK: an ubiquitously expressed NRK1 and NRK2, whose expression was mainly detected in heart, skeletal muscle, brown adipose tissue (BAT), and liver (Bogan & Brenner, 2008). Interestingly, it has been recently reported that NRK1 is required for NAD$^+$ synthesis not only from the exogenously administered NR, but also NMN (Ratajczak *et al*, 2016). Both NAM and NR operate via the "amidated" route to produce NAD$^+$ (Fig 1).

Nicotinic acid, in its turn, initiates the "deamidated" route (Fig 1). Conversion of NA into NA mononucleotide (NAMN) constitutes the first step of this route, which most often is referred as the Preiss–Handler pathway (Preiss & Handler, 1958). The NMNATs recognize both NAMN and NMN as substrates; however, in the case of NAMN the conversion results in NA adenine dinucleotide (NAAD), and therefore, one additional step, catalyzed by NAD synthetase (NADS), is required to produce NAD$^+$ (Fig 1). Interestingly, it has been recently reported that NR leads to the production of NAAD via a yet-unknown mechanism (Trammell *et al*, 2016a).

The *de novo* NAD$^+$ synthesis pathway, which converts tryptophan into NAD$^+$, consists of eight steps. The first reaction of this pathway constitutes of a conversion of tryptophan into N-formylkynurenine, which in mammals can be catalyzed by two different enzymes: tryptophan-2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO). This conversion is considered to be the first rate-limiting step for the pathway. TDO is the major contributor to NAD$^+$ biosynthesis in liver, while IDO is ubiquitously expressed in extrahepatic tissues, with the highest activity detected in lung, intestine, and spleen (Yamazaki *et al*, 1985; Kudo & Boyd, 2000). TDO is induced by tryptophan and glucocorticoids (Comings *et al*, 1995), while IDO is induced by inflammatory stimuli (Yoshida & Hayaishi, 1978; Yoshida *et al*, 1979; Takikawa *et al*, 1986; Heyes *et al*, 1992; Reinhard, 1998; Sanni *et al*, 1998; Daubener & MacKenzie, 1999). N-formylkynurenine gets converted by formamidase (KFase) into kynurenine. Kynurenine in its turn leads to 3-OH kynurenine in a reaction catalyzed by kynurenine 3-hydroxylase (K3H). Kynureninase (Kyase) then forms 3-hydroxyanthranilate, which gets transformed into α-amino-β-carboxymuconate-ε-semialdehyde (ACMS) by 3-hydroxyanthranilate 3,4-dioxygenase (3HAO). The formation of this unstable ACMS constitutes a branching point of the *de novo* NAD$^+$ synthesis pathway (Bender, 1983; Houtkooper *et al*, 2010). ACMS can either undergo cyclization forming quinolinic acid (QA), which is then converted by quinolinate phosphoribosyltransferase (QPRT) into NAMN and from this point fuses with the Preiss–Handler pathway to produce NAD$^+$ (Fig 1). Otherwise, the carbon group of ACMS can be removed, which either leads to the production of picolinic acid or is directed to total oxidation to $CO_2$ and $H_2O$. While the cyclization of ACMS is a spontaneous reaction, the transformation of ACMS into α-amino-β-muconate-ε-semialdehyde (AMS) is catalyzed by the enzyme α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase (ACMSD) (Fig 1).

## Preferential source for NAD$^+$ production

The existence of different pathways leading to NAD$^+$ production raises questions on the relative importance of each pathway and which of them possess the highest potential to boost NAD$^+$ levels. The preferable precursor for NAD$^+$ production within the organism is hence still a matter of debate. There is evidence that NAM possesses a higher NAD$^+$ boosting capability when compared to NA in different organs in mice (Collins & Chaykin, 1971, 1972; Mori *et al*, 2014; Yang *et al*, 2014). Additionally, in human plasma, levels of NAM were reported to be fivefold higher than NA levels (Jacobson *et al*, 1995). However, several other studies claim the opposite: NA is a more effective NAD$^+$ precursor than NAM (Ijichi *et al*, 1966; Hagino *et al*, 1968; Lin & Henderson, 1972; Williams *et al*, 1985; Jackson *et al*, 1995; Hara *et al*, 2007). It is important to mention that in Mori *et al* (2014) the authors quantified the activity of NMNAT and NADS; therefore, the comparison was rather made between the "deamidated" (e.g., from NA) and "amidated" route, which includes both NAM and NR. And even if the authors of this study claim that NAM is the main precursor for NAD$^+$ synthesis, the possibility of a significant contribution of other precursors using the amidated NAD$^+$ biosynthesis route (e.g., NR) cannot be discounted. In support of this, a very recent study showed that NR has a greater capacity over NA and NAM to boost hepatic NAD$^+$ levels (Trammell *et al*, 2016a). It is also important to mention that both NA and NAM have reported side effects, whereas no adverse effects are currently reported for NR. NA activates the G protein-coupled receptor, GPR109A and causes flushing, characterized by vasodilation and a burning sensation (Benyo *et al*, 2006). While NAM raises health concerns for treatment of diabetic patients, as high doses of NAM can be hepatotoxic (Knip *et al*, 2000).

As for tryptophan, its administration to humans has been used as treatment for pain, sleep disorders, depression, hyperactivity, and bulimia (Richard *et al*, 2009). No severe adverse effects have been reported for tryptophan administration, even with doses going as high as 20 g/day in schizophrenic patients (Sidransky, 2001). A large number of reviews attribute a marginal role to the *de novo* NAD$^+$ synthesis pathway. However, a solid support for this claim is lacking. One of the studies frequently cited to sustain this point of view reports that tryptophan alone is not sufficient to maintain the physiological NAD$^+$ concentration of the cell (Nikiforov *et al*, 2011). However, this conclusion was exclusively based on the observation that supplementation with tryptophan is not sufficient to protect cells from the death induced by NAMPRT inhibitor FK866 and no NAD$^+$ quantification was performed in this study. In addition, some studies show that, at least in the liver, tryptophan constitutes the preferable substrate for NAD$^+$ production. Rat primary hepatocytes, treated with NA, NAM, or tryptophan, were reported to use exclusively tryptophan for their NAD$^+$ biosynthesis, even though they were still able to take up NA and NAM from the culture medium (Bender & Olufunwa, 1988). Administration of tryptophan, NA, or NAM to rats showed that tryptophan resulted in the highest hepatic NAD$^+$ concentrations (Bender *et al*, 1982). Moreover, it has been shown that in rat liver, NA and NAM have a very limited capacity for NAD$^+$ production, probably due to the saturation of the involved phosphoribosyltransferases, whereas no such limitations were detected

for the NAD$^+$ synthesis from tryptophan (Williams *et al*, 1950; Bender *et al*, 1982; McCreanor & Bender, 1986).

## NAD$^+$ consuming enzymes

Sirtuin proteins require NAD$^+$ as a cosubstrate for their activity. A detailed description of their role in the regulation of metabolism and aging is beyond the scope of this review, but has been extensively covered elsewhere (Haigis & Sinclair, 2010; Satoh *et al*, 2011; Houtkooper *et al*, 2012; Chang & Guarente, 2014). Besides sirtuins, two different protein families are well known to use NAD$^+$ as a cofactor for their enzymatic activities. These include the PARPs and the cADPR synthases, CD38 and CD157. PARPs are involved in DNA repair, maintenance of genomic integrity, and cell death, with PARP1 accounting for more than 85% of NAD$^+$ consumption of this protein family (Bai & Canto, 2012). cADPR, which is generated by CD38 and CD157, is a signaling molecule that controls intracellular calcium fluxes. The catalytic efficiency of CD38 is significantly higher than that of CD157 (Quarona *et al*, 2013). While CD38 expression was initially considered to be limited to the immune system, it was later found to be ubiquitously distributed. CD38 is an important NAD$^+$ consumer, as its loss of function (LOF) in mice led up to a 30-fold increase in NAD$^+$ levels in different tissues (Barbosa *et al*, 2007). Combined increases in PARP1 (Braidy *et al*, 2011; Mouchiroud *et al*, 2013) and CD38 (Camacho-Pereira *et al*, 2016) activities upon aging were reported to cause age-associated reduction in NAD$^+$ content. For more extensive coverage of these NAD$^+$ consuming enzymes, we refer the readers to a few reviews on PARPs (Bai & Canto, 2012; Canto *et al*, 2013; Jubin *et al*, 2016) and cADPR synthases (Malavasi *et al*, 2008; Quarona *et al*, 2013).

## Regulation of NAD$^+$ content

### Regulation of NAD$^+$ content by diet and aging

An increase of NAD$^+$ levels followed by sirtuin activation is observed in situations of energy deficit, such as fasting (Rodgers *et al*, 2005; Chen *et al*, 2008; Cantó *et al*, 2010), calorie restriction (CR) (Qin *et al*, 2006; Chen *et al*, 2008; Cantó *et al*, 2010) or low glucose feeding (Fulco *et al*, 2008), and exercise (Canto *et al*, 2009; Cantó *et al*, 2010; Costford *et al*, 2010). On the contrary, multiple studies reported that high-fat (HF)/high-fat high-sucrose (HFHS) feeding diminishes NAD$^+$ content in liver (Yoshino *et al*, 2011; Gariani *et al*, 2016, 2017; Trammell *et al*, 2016b), skeletal muscle (Canto *et al*, 2012), BAT (Canto *et al*, 2012), and white adipose tissue (WAT) (Yoshino *et al*, 2011). Orotic acid administration (Fukuwatari *et al*, 2002) or feeding a methionine-/choline-deficient (MCD) diet (Gariani *et al*, 2017) also leads to the concomitant appearance of liver fat accumulation and a drop in hepatic NAD$^+$ levels. A recent study, however, reported that administration of a HF diet for 11 weeks led to an increase in NAD$^+$ content in mouse liver, accompanied by enhanced Sirt1 activity (Penke *et al*, 2015). The stimulation of the NAD$^+$ biosynthesis could represent an initial compensatory attempt to maintain energy homeostasis (Penke *et al*, 2015; Drew *et al*, 2016), whereas more prolonged exposure to HF diet reduces NAD$^+$ content, resulting in functional damage (Drew *et al*, 2016). Interestingly, in *Saccharomyces cerevisiae* NAD$^+$

content is affected by the carbon source used: Yeast grown on ethanol contain practically double the amount of NAD$^+$ compared to yeast grown on glucose (Agrimi *et al*, 2011).

Finally, a decrease in NAD$^+$ content was also reported to be associated with aging in *Caenorhabditis elegans*, mice, rats, and humans (Braidy *et al*, 2011; Yoshino *et al*, 2011; Massudi *et al*, 2012; Gomes *et al*, 2013; Mouchiroud *et al*, 2013; Camacho-Pereira *et al*, 2016; Guan *et al*, 2017).

### Regulation by circadian rhythm

Circadian rhythm is another important regulator of NAD$^+$ content. The key regulators of the mammalian circadian clock machinery are the transcription factors CLOCK and BMAL1, which act together as a heterodimer. Their transcriptional targets, PER and CRY, form a negative feedback loop by repressing CLOCK-BMAL1 activity. Hepatic NAD$^+$ levels oscillate in a diurnal manner (Nakahata *et al*, 2009; Ramsey *et al*, 2009). Mice with LOF mutations in the circadian activator genes, *Clock* and *Bmal1*, show reduced NAD$^+$ content, while NAD$^+$ levels were elevated in mice with mutations in the clock repressor genes *Cry1* and *Cry2* (Ramsey *et al*, 2009). The circadian clock-controlled expression of *Namprt* is thought to be responsible for this fine-tuning of the NAD$^+$ availability (Nakahata *et al*, 2009; Ramsey *et al*, 2009). Importantly, clock-driven oscillations of NAD$^+$ were claimed to regulate the activity of both SIRT1 (Nakahata *et al*, 2009; Ramsey *et al*, 2009) and SIRT3 (Peek *et al*, 2013). It is possible that modulation of the redox state of NAD$^+$ can reciprocally impact on the circadian clock. *In vitro* NADH was shown to enhance binding of the CLOCK-BMAL1 heterodimer to DNA, whereas NAD$^+$ was inhibiting this process (Rutter *et al*, 2001). On their turn, the NAD$^+$-dependent enzymes SIRT1, SIRT6, and PARP1 were reported to control the circadian clock machinery via post-translational modifications of the core clock transcription factors and via regulation of their transcription (Asher *et al*, 2008, 2010; Chang & Guarente, 2013; Masri *et al*, 2014).

## NAD⁺ boosting strategies

NAD$^+$ levels can be increased either by promoting its synthesis—by enhancing the enzymes involved in NAD$^+$ biosynthesis or administration of NAD$^+$ precursor molecules—or by limiting its consumption. Supplementation with NA, NAM, NR, NMN, or tryptophan can increase NAD$^+$ content (Canto *et al*, 2015). Overexpressing or activating enzymes catalyzing the rate-limiting steps of NAD$^+$ biosynthesis also are efficient to boost NAD$^+$ levels (Araki *et al*, 2004; Sasaki *et al*, 2006; Hsu *et al*, 2009; Wang *et al*, 2014a; Williams *et al*, 2017). However, the translational potential of this approach may be lower compared to the other strategies.

Pharmacological or genetic inhibition of non-sirtuin NAD$^+$ consumers, such as PARP-1 or CD38 (Fig 1), can help to preserve NAD$^+$ levels for sirtuin activation (Aksoy *et al*, 2006a; Barbosa *et al*, 2007; Bai *et al*, 2011; Pirinen *et al*, 2014), especially in situations when non-sirtuin NAD$^+$ consumers are overactivated (Bai *et al*, 2011; Braidy *et al*, 2011; Mouchiroud *et al*, 2013; Fang *et al*, 2014; Mukhopadhyay *et al*, 2014; Ryu *et al*, 2016; Gariani *et al*, 2017). For instance, DNA damage is known to cause a dramatic decline in NAD$^+$ intracellular levels, which is due to PARP activation (Berger, 1985), and overexpression of CD38 in cells leads to a

~35% decrease in NAD$^+$ levels (Hu *et al*, 2014). On the contrary, *Cd38*$^{-/-}$ and *PARP-1*$^{-/-}$ mice have increased NAD$^+$ content in different organs (Aksoy *et al*, 2006a,b; Young *et al*, 2006; Bai *et al*, 2011).

Increased nicotinamide methyl transferase (*NNMT*) expression was reported in obesity and type 2 diabetes (Lee *et al*, 2005; Yaguchi *et al*, 2005; Salek *et al*, 2007; Kraus *et al*, 2014). NNMT is the enzyme catalyzing the transformation of NAM into methylnicotinamide (MNA) (Fig 1) and is highly expressed in liver and adipose tissue (Aksoy *et al*, 1994; Riederer *et al*, 2009). Inhibiting NNMT in these tissues should increase NAD$^+$ content, since NAM would not be degraded but exclusively reconverted into NAD$^+$ (Fig 1). In line, the knockdown of *NNMT* increased NAD$^+$ levels in adipose tissue, but not in the liver (Kraus *et al*, 2014).

Resveratrol, a natural polyphenol found in red wine, activates AMPK and thereby increases NAD$^+$ levels (Fulco *et al*, 2008; Canto *et al*, 2009; Price *et al*, 2012; Desquiret-Dumas *et al*, 2013), which promotes sirtuin activation.

## Therapeutic potential of NAD⁺

### Pellagra

Pellagra, a disease that was epidemic in the XVII–XIX centuries in several rural areas of Europe and the United States (Bender, 1983), owes its name to the Italian "pelle" = skin and "agra" = sour or rough, which describes its most noticeable feature. Otherwise, it is also called the disease of "the three Ds": Diarrhea, dermatitis, and dementia, which, if untreated, can lead to the fourth "D", i.e. death. In 1915, Joseph Goldberger demonstrated that pellagra was not an infectious disease, as previously thought, but is due to poor nutrition and could be prevented by consumption of fresh meat and milk (Bender, 1983). Administration of both NAM and NA (Elvehjem *et al*, 1937), and later tryptophan (Krehl *et al*, 1945) were also reported to prevent pellagra.

### Aging

Sirtuins are well-known longevity regulators, and their decreased function with age might at least be partially explained by a systemic decline in NAD$^+$ levels upon aging (Mouchiroud *et al*, 2013) [reviewed in (Imai & Guarente, 2014; Menzies *et al*, 2016)]. Rising NAD$^+$ content, followed by sirtuin activation, has been reported to increase lifespan in yeast (Lin *et al*, 2004; Belenky *et al*, 2007; Easlon *et al*, 2008), worms (Mouchiroud *et al*, 2013), and mice (Zhang *et al*, 2016). Administration of NR, NMN, or NAM recovered NAD$^+$ content and protected against aging-related complications, such as mitochondrial dysfunction (Gomes *et al*, 2013; Mouchiroud *et al*, 2013; Mills *et al*, 2016), decline in physical performance (Mills *et al*, 2016; Zhang *et al*, 2016) and muscle regeneration (Zhang *et al*, 2016), arterial dysfunction (de Picciotto *et al*, 2016), decline in vision (Lin *et al*, 2016; Mills *et al*, 2016), including glaucoma (Williams *et al*, 2017), and age-associated insulin resistance (Mills *et al*, 2016).

The most striking benefits of NAD$^+$ supplementation on aging were observed in several rare diseases linked to abnormal DNA repair that are typified by accelerated aging, such as the Cockayne syndrome group B (CSB), xeroderma pigmentosum group A (XPA), or ataxia-telangiectasia (A-T). In a mouse model of CSB, neurons

show mitochondrial defects, which have an impact on the cerebellum and inner ear. Administration of PARP inhibitors or the NAD$^+$ precursor, NR, to $csb^{-/-}$ animals attenuated many of the phenotypes of CSB and restored altered mitochondrial function in their neurons (Scheibye-Knudsen *et al*, 2014). Another DNA damage repair disorder is XPA, which is also characterized by mitochondrial alterations and reduced NAD$^+$-SIRT1 signaling due to the overactivation of PARP1 (Fang *et al*, 2014). Treatment with NAD$^+$ precursors, NR and NMN, or with the PARP inhibitor, Olaparib, rescued the XPA phenotype in cells and worms. Similar observations of increased PARylation, NAD$^+$ depletion, and mitochondrial dysfunction were made in mouse and worm models of another progressive neurodegenerative disease, A-T (Fang *et al*, 2016). Restoring the NAD$^+$/SIRT1 pathway, by NR and NMN administration to *C. elegans* and mice, improved A-T neuropathology (Fang *et al*, 2016).

### Metabolic disorders

The importance of NAD$^+$ as a metabolic regulator has been demonstrated by its efficacy to attenuate many features of the metabolic syndrome, a cluster of pathologies including insulin resistance, fatty liver, dyslipidemia, and hypertension, with increased risk of developing type 2 diabetes and heart failure. Different approaches aiming to raise NAD$^+$ levels were shown to provide protection against obesity, such as (i) inhibition of NAD$^+$ consumers, PARPs (Bai *et al*, 2011; Gariani *et al*, 2017) and CD38 (Barbosa *et al*, 2007), (ii) administration of NAD$^+$ precursors, such as NR (Canto *et al*, 2012; Gariani *et al*, 2016; Trammell *et al*, 2016b) or NMN (Yoshino *et al*, 2011), (iii) or inhibition of NNMT (Kraus *et al*, 2014). NAD$^+$ boosting was also efficient to improve glucose homeostasis in obese, prediabetic, and T2DM animals (Barbosa *et al*, 2007; Bai *et al*, 2011; Yoshino *et al*, 2011; Canto *et al*, 2012; Kraus *et al*, 2014; Gariani *et al*, 2016, 2017; Trammell *et al*, 2016b). Likewise, reestablishing NAD$^+$ levels with NR or PARP inhibitors also protected from non-alcoholic steatohepatitis (NASH) (Gariani *et al*, 2016, 2017; Mukhopadhyay *et al*, 2017) as well as alcoholic steatohepatitis (ASH) (Mukhopadhyay *et al*, 2017).

### Muscle function

Increase in muscle NAD$^+$ content, resulting from NR administration or PARP inhibition, improved muscle function and exercise capacity in mice (Canto *et al*, 2012; Pirinen *et al*, 2014), including in aged animals (Zhang *et al*, 2016). Interestingly, muscular dystrophy is characterized by a dramatic drop in NAD$^+$ in the muscle (Ryu *et al*, 2016). NR administration to the *mdx* mouse, a model for muscular dystrophy, improved muscle function by enhancing bioenergetics, attenuating inflammation and fibrosis (Ryu *et al*, 2016), as well as, by favoring regeneration and preventing the exhaustion and senescence of muscle stem cells, typical to the *mdx* mice (Zhang *et al*, 2016).

The beneficial effects of improving muscle bioenergetics are also illustrated in models of mitochondrial myopathies. Increasing muscle NAD$^+$ levels by the administration of NR or a PARP inhibitor preserved muscle function in two different models of mitochondrial myopathy (Cerutti *et al*, 2014; Khan *et al*, 2014). Similar benefits on mitochondrial myopathy were seen with the AMPK agonist, AICAR (Viscomi *et al*, 2011), which may at least in part be due to the recovery of NAD$^+$ content upon AMPK activation.

### Cardiac function

Exposing the heart to different types of stresses was reported to result in a decline in cardiac NAD$^+$ content (Pillai *et al*, 2005, 2010; Karamanlidis *et al*, 2013; Yamamoto *et al*, 2014). For instance, cardiomyocyte hypertrophy is characterized by a drop in cellular NAD$^+$ levels. Supplementation with NAD$^+$ was hence protective against cardiac hypertrophy in mice, and these anti-hypertrophic effects were in part attributed to the activation of SIRT3 (Pillai *et al*, 2010).

Cardiac ischemia is another condition causing a steep decrease in NAD$^+$ levels. NMN administration protected the mice from ischemic injury via the recovery of cardiac NAD$^+$ content and subsequent SIRT1 activation (Yamamoto *et al*, 2014). Similarly, cardiac-specific overexpression of NAMPRT in mice increased NAD$^+$ content and reduced the extent of myocardial infarction and apoptosis in response to prolonged ischemia and ischemia/reperfusion (Hsu *et al*, 2009). Maintaining NAD$^+$ levels in pressure-overloaded hearts is crucial for myocardial adaptation and protection from heart failure, as demonstrated by NMN administration to mice treated with the NAMPRT inhibitor FK866 (Yano *et al*, 2015) and to cardiac-specific mitochondrial complex I-deficient mice (Lee *et al*, 2016). In a mouse model of heart failure caused by iron deficit, reconstituting NAD$^+$ content also improved mitochondrial quality, protected cardiac function, and increased lifespan (Xu *et al*, 2015). Similarly, NR administration improved cardiac function in aged *mdx* mice, which, like muscular dystrophy patients, display cardiomyopathy (Ryu *et al*, 2016).

### Renal function

Multiple studies demonstrated the loss of SIRT1 and SIRT3 activity as a key feature of kidney dysfunction, including kidney abnormalities linked with aging (Koyama *et al*, 2011; Zhuo *et al*, 2011; Morigi *et al*, 2015; Ugur *et al*, 2015; Guan *et al*, 2017). Acute kidney injury (AKI) is characterized by a reduction in NAD$^+$ content and NAMPRT expression (Morigi *et al*, 2015; Ugur *et al*, 2015). Promoting NAD$^+$ synthesis via NAM or NMN supplementation was reported to mitigate AKI in ischemia/reperfusion- and cisplatin-induced mouse models of AKI (Tran *et al*, 2016; Guan *et al*, 2017). Furthermore, administration of the AMPK agonist, AICAR, which positively impacts on NAD$^+$ levels (Canto *et al*, 2009), was protective against cisplatin-induced AKI in SIRT3-dependent manner (Morigi *et al*, 2015). Although no NAD$^+$ quantification was performed in this particular study, the involvement of SIRT3, as well as the increase in *Namprt* expression detected upon AICAR administration, points toward a potential increase in NAD$^+$ levels (Morigi *et al*, 2015). Kidney mesangial cell hypertrophy is also characterized by a depletion of NAD$^+$ content (Zhuo *et al*, 2011) and restoring intracellular NAD$^+$ levels via supplementation with exogenous NAD$^+$ prevented its onset by activating SIRT1 and SIRT3 (Zhuo *et al*, 2011).

### Neurodegeneration

NAD$^+$ boosting has also been shown to be neuroprotective. Raising NAD$^+$ levels protects against neuronal death induced by ischemic brain (Klaidman *et al*, 2003; Sadanaga-Akiyoshi *et al*, 2003; Kabra *et al*, 2004; Feng *et al*, 2006; Kaundal *et al*, 2006; Zheng *et al*, 2012) or spinal cord injuries (Xie *et al*, 2017). Axonal degeneration is considered as an early pathological mechanism in

this type of neurodegeneration. An accumulating amount of data indicates that axonal degeneration is not only limited to ischemic brain and spinal cord injuries, but constitutes a hallmark process, preceding neuronal death, in a much larger spectrum of disease states, including traumatic brain injury, inflammatory disorders, like multiple sclerosis, and degenerative disorders, such as Alzheimer's and Parkinson's diseases (Lingor *et al*, 2012; Johnson *et al*, 2013). Degenerating axons show a decrease in NAD$^+$ content (Wang *et al*, 2005; Gerdts *et al*, 2015), while replenishing NAD$^+$ by supplementing NAM (Wang *et al*, 2005), NR and NMN (Sasaki *et al*, 2006), and high doses of NAD$^+$ (Araki *et al*, 2004), or over-expressing enzymes involved in NAD$^+$ biosynthesis (Araki *et al*, 2004; Sasaki *et al*, 2006) delayed axonal degeneration. In line with this, supplementation with NAM, NMN, or NR was neuroprotective in rodent models of Alzheimer disease (Qin *et al*, 2006; Gong *et al*, 2013; Liu *et al*, 2013; Turunc Bayrakdar *et al*, 2014; Wang *et al*, 2016a), and supplementation with NAM or LOF of PARP were protective in *Drosophila* models of Parkinson's disease (Lehmann *et al*, 2017).

NAD$^+$ depletion is also involved in the neurodegeneration induced by highly toxic misfolded prion protein (Zhou *et al*, 2015). Replenishment of intracellular NAD$^+$ stocks, either by providing NAD$^+$ or NAM, rescued the neurotoxic effects of protein aggregates (Zhou *et al*, 2015). Importantly, restoring NAD$^+$ content is not exclusively protecting neurons, since it has also been reported to prevent the death of astrocytes (Alano *et al*, 2004).

P7C3, a compound that enhances neurogenesis (Pieper *et al*, 2010) and that was neuroprotective in mouse models of Parkinson's disease (De Jesus-Cortes *et al*, 2012), amyotrophic lateral sclerosis (Tesla *et al*, 2012) and brain injury (Yin *et al*, 2014), was subsequently identified as an NAMPRT activator (Wang *et al*, 2014a). Therefore, the beneficial effects of P7C3 on neuron preservation seem at least in part to be due to a NAMPRT-mediated increase in NAD$^+$ levels (Wang *et al*, 2014a).

Nicotinamide riboside supplementation recovered depressed sensory and motor neuron conduction velocities and thermal insensitivity in T2DM mice (Trammell *et al*, 2016b) and alleviated chemotherapy-induced peripheral neuropathy in rats (Hamity *et al*, 2017), indicating that NAD$^+$ also is beneficial in the peripheral neuronal system.

NAD$^+$ boosting was also able to protect mice from loss of vision and hearing (Shindler *et al*, 2007; Brown *et al*, 2014). Intravitreal injections of NR in mice attenuated optic neuritis in a dose-dependent manner (Shindler *et al*, 2007). Even if no NAD$^+$ quantification was performed in this study, SIRT1 activity was necessary for the neuroprotective effects of NR, since the protection was blunted in the presence of sirtinol, a SIRT1 inhibitor (Shindler *et al*, 2007). Furthermore, systemic administration of NAM and overexpression of Nmnat1 had spectacular effects on vision in DBA/2J mice, which are prone to glaucoma (Williams *et al*, 2017). Noise exposure results in degeneration of the neurons innervating the cochlear hair cells. Increase in NAD$^+$ levels induced by NR administration prevented against noise-induced hearing loss and neurite degeneration (Brown *et al*, 2014). In line with this, CR was shown to protect against cochlear cell death and aging-associated hearing loss in a Sirt3-dependent manner (Someya *et al*, 2010). It is therefore tempting to speculate that this improvement could also be associated with increased NAD$^+$ levels upon

CR, though no direct measurements of NAD$^+$ levels were performed in this study.

## Future challenges and perspectives

Although it was thought that the NAD$^+$ biosynthetic pathways were entirely understood, we still continue to discover new actors of NAD$^+$ metabolism. For instance, whereas the conversion of NR into NMN by NRK was established some time ago (Bieganowski & Brenner, 2004), the opposite reaction, that is, the transformation of NMN into NR by CD73, was only recently shown to occur in humans (Garavaglia *et al*, 2012). Another example is the recent description that NR not only induced NAAD concentrations by 45-fold, but that it is also a direct biosynthetic precursor of NAAD (Trammell *et al*, 2016a). The biochemical basis of this conversion cannot be explained with our current state of knowledge, as NAD$^+$ synthetase, which catalyzes the transformation of NAAD into NAD$^+$, works unidirectionally (Bieganowski *et al*, 2003; Wojcik *et al*, 2006) and can therefore not catalyze the reverse reaction from NAD$^+$ into NAAD.

Additionally, it is possible that we still ignore some functions that NAD$^+$ might accomplish within the cell. For instance, very recently NAD$^+$ was found to be linked to RNA in bacteria (Chen *et al*, 2009). By forming a cap at the 5′-terminus of bacterial RNA molecule, it is not only increasing its stability (Cahova *et al*, 2015), but also serves as non-canonical initiation nucleotides for *de novo* transcription initiation (Bird *et al*, 2016). Initially thought to be prokaryote-specific, this RNA modification appears to be also conserved in eukaryotic systems (Jiao *et al*, 2017; Walters *et al*, 2017). Similarly to bacteria, in eukaryotic cells NAD$^+$ addition seems to occur during transcription initiation (Bird *et al*, 2016; Walters *et al*, 2017). Intriguingly, a subset of eukaryotic non-coding RNAs have also been reported to possess a NAD$^+$-cap. Since these RNAs are formed exonucleolytically, NAD$^+$ cap addition in their case would occur post-transcriptionally (Jiao *et al*, 2017). Oppositely to prokaryotes, in mammalian cells the NAD$^+$ cap was reported to rather promote mRNA decay (Jiao *et al*, 2017). The full physiological significance of NAD$^+$-capping is yet to be discovered. It is, however, tempting to speculate that the proportion of cellular mRNA possessing NAD$^+$ cap might be influenced by intracellular NAD$^+$ content and thus by the energy state of the cell.

Devising better NAD$^+$ quantification methods is a critical challenge in the field. Measurements based on UV–Vis methods are less accurate and sensitive than mass spectrometry methods (Trammell & Brenner, 2013). Moreover, accurate NAD$^+$ quantification in different subcellular compartments is challenging due the complexity of subcellular fractionations and the NAD$^+$ isolation procedures. Over the last few years, a new generation of NAD$^+$ biosensors was developed, allowing NAD$^+$ quantification in intact cells as well as within specific subcellular compartments (Hung *et al*, 2011; Bilan *et al*, 2014; Cambronne *et al*, 2016). Further development and wider application of these biosensors combined with strategies to explore the kinetics of NAD$^+$ biosynthesis and metabolism, using flux studies, will hence be important for future research. Besides, according to a recent study NADP and NADPH were more significantly deregulated in T2DM and obesity than NAD$^+$ and less correctable by NR

supplementation (Trammell *et al*, 2016b). Monitoring of the entire NAD$^+$ metabolome could hence help our further understanding of its role in metabolism, which might extend far beyond NAD$^+$–sirtuin or NAD$^+$–PARP axis.

As reviewed here, manipulations of NAD$^+$ concentrations have demonstrated multiple beneficial effects in a large spectrum of diseases in animal models (Fig 2). Translating these effects into clinical benefits now becomes one of the main challenges. The fact that the long-term administration of the NAD$^+$ precursor molecules showed no deleterious effects in animals should be considered promising. As such, administration of NMN for 12 months demonstrated no toxicity in mice (Mills *et al*, 2016). Similarly, administration of NR to mice for a duration of 5–6 months (Gong *et al*, 2013), 10 months (Zhang *et al*, 2016), and 12 months (Tummala *et al*, 2014) showed no obvious adverse effects. Moreover, 2016 was marked by the first report on the effects of NR in humans, showing that the oral administration of NR led to a dose-dependent increase in NAD$^+$ levels in blood in healthy volunteers (Trammell *et al*, 2016a). Another NAD$^+$ precursor, NAM, has also been already tested in humans and protected β-cell function in type 1 diabetes patients (Olmos *et al*, 2006) and even though the clinical trials for NAM as a treatment for type 1 diabetes failed, no adverse effects of NAM were detected (Gale *et al*, 2004; Cabrera-Rode *et al*, 2006). Furthermore, a slow release form of NA (acipimox) was effective in inducing mitochondrial activity in skeletal muscle of type 2 diabetic patients (van de Weijer *et al*, 2015).

In theory, all this bodes well for the use of NAD$^+$ precursors in the clinic. The fact that many of these NAD$^+$ precursors are rightfully considered vitamins (which are generally regarded as safe (GRAS)), and that they are widely available to the public at large, however, also poses some issues. Despite the many health benefits that are inferred from their use in animal disease models, these NAD$^+$ precursors need still to undergo rigorous clinical testing in diseases setting, before one can recommend their widespread use. Therefore, some caution is required, so that the overuse or improper use in uncontrolled settings does not hamper their clinical development as nutra- or pharmaceutical agents.

It will also be important to identify which pharmacological strategies aiming to boost NAD$^+$ content would be the most appropriate in patients. Various monoclonal antibodies targeting CD38 have been developed as a treatment for hematological malignancies, some of them being in preclinical, and some even in late clinical, studies (van de Donk *et al*, 2016). Many flavonoids were reported to inhibit human CD38 at low micromolar concentrations
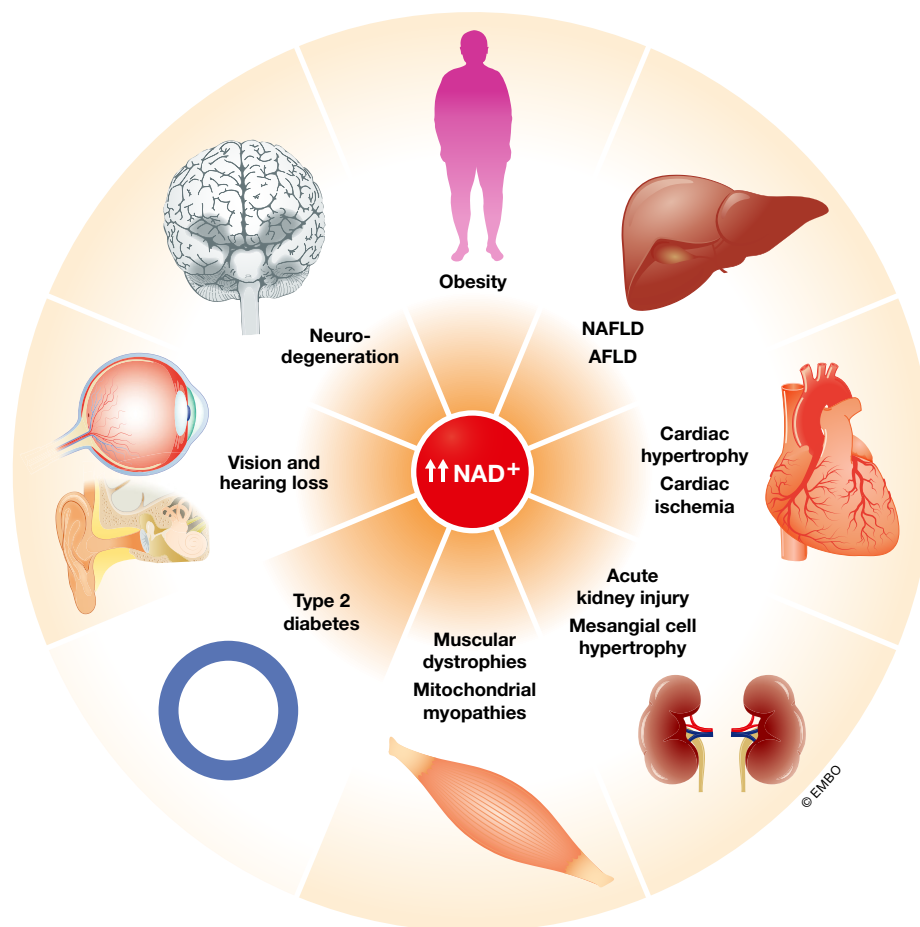


**Figure 2. Therapeutic potential of NAD$^+$ boosting in humans based on findings in animal studies.**
NAFLD, non-alcoholic fatty liver disease; AFLD, alcoholic fatty liver disease.

(Kellenberger *et al*, 2011; Escande *et al*, 2013), some of them showing promising therapeutic effects in mice (Escande *et al*, 2013; Boslett *et al*, 2017). Thiazoloquin(az)olinones have recently been described as potent CD38 inhibitors, able to elevate NAD$^+$ in plasma, liver, and muscle in mice (Haffner *et al*, 2015). Moreover, several reports have disclosed different small molecules blocking CD38 activity at low micromolar concentrations (Zhou *et al*, 2012; Moreau *et al*, 2013; Swarbrick *et al*, 2014; Wang *et al*, 2014b; Becherer *et al*, 2015). Testing and exploring their therapeutic potential in animal models is now just a matter of time.

Several PARP inhibitors are currently either marketed (Olaparib) or undergoing advanced clinical trials for the treatment of cancers in patients with BRCA mutations (Wang *et al*, 2016b). The drawback of these PARP inhibitors is that none of them is selective for a specific PARP family member (Wang *et al*, 2016b). For instance, the clinically approved compound, Olaparib, inhibits PARP-1, PARP-2, PARP-3, and PARP-4 (Wahlberg *et al*, 2012), increasing chances of adverse effects. As a case in point, the loss of both PARP-1 and PARP-2 in mice was reported to cause embryonic lethality (Menissier de Murcia *et al*, 2003), while a T cell-specific deficiency in both them leads to highly aggressive lymphomas (Navarro *et al*, 2017). Moreover, all current PARP inhibitors are genotoxic (Ito *et al*, 2016), which raises concerns about their use for non-oncologic indications. Indeed, some side effects that could be tolerated in case of the treatment of life-threatening diseases such as advanced cancers (Brown *et al*, 2016) may not be tolerated for non-oncologic indications. All these evidences indicate that the development of safer and more selective PARP inhibitors is necessary.

The growing literature on the beneficial effects of raising and maintaining NAD$^+$ levels in different disease models and the high evolutionary conservation of the NAD$^+$–sirtuin signaling axis suggests that strategies that increase cellular NAD$^+$ content may have a preventive and/or therapeutic potential in a large number of human diseases (Fig 2). With the first reports on human trials of various NAD$^+$ boosting techniques that start to appear, we are entering in the exciting era of NAD$^+$ therapeutics. While there is no certitude that NAD$^+$ boosting will be able to extend lifespan in humans, such strategies definitely possess the potential to delay age-associated physiological decline, and therefore, we predict that they will be useful to manage aging-related diseases and extend healthspan.

## Conflict of interest

JA is a founder and SAB member of Mitobridge, a company that develops NAD$^+$ boosting therapeutics.

## References

Agrimi G, Brambilla L, Frascotti G, Pisano I, Porro D, Vai M, Palmieri L (2011) Deletion or overexpression of mitochondrial NAD$^+$ carriers in *Saccharomyces cerevisiae* alters cellular NAD and ATP contents and affects mitochondrial metabolism and the rate of glycolysis. *Appl Environ Microbiol* 77: 2239–2246

Aksoy S, Szumlanski CL, Weinshilboum RM (1994) Human liver nicotinamide N-methyltransferase. cDNA cloning, expression, and biochemical characterization. *J Biol Chem* 269: 14835–14840

Aksoy P, Escande C, White TA, Thompson M, Soares S, Benech JC, Chini EN (2006a) Regulation of SIRT 1 mediated NAD dependent deacetylation: a novel role for the multifunctional enzyme CD38. *Biochem Biophys Res Comm* 349: 353–359

Aksoy P, White TA, Thompson M, Chini EN (2006b) Regulation of intracellular levels of NAD: a novel role for CD38. *Biochem Biophys Res Comm* 345: 1386–1392

Alano CC, Ying W, Swanson RA (2004) Poly(ADP-ribose) polymerase-1-mediated cell death in astrocytes requires NAD$^+$ depletion and mitochondrial permeability transition. *J Biol Chem* 279: 18895–18902

Araki T, Sasaki Y, Milbrandt J (2004) Increased nuclear NAD biosynthesis and SIRT1 activation prevent axonal degeneration. *Science* 305: 1010–1013

Asher G, Gatfield D, Stratmann M, Reinke H, Dibner C, Kreppel F, Mostoslavsky R, Alt FW, Schibler U (2008) SIRT1 regulates circadian clock gene expression through PER2 deacetylation. *Cell* 134: 317–328

Asher G, Reinke H, Altmeyer M, Gutierrez-Arcelus M, Hottiger MO, Schibler U (2010) Poly(ADP-ribose) polymerase 1 participates in the phase entrainment of circadian clocks to feeding. *Cell* 142: 943–953

Bai P, Cantó C, Oudart H, Brunyánszki A, Cen Y, Thomas C, Yamamoto H, Huber A, Kiss B, Houtkooper RH, Schoonjans K, Schreiber V, Sauve AA, Menissier-de Murcia J, Auwerx J (2011) PARP-1 inhibition increases mitochondrial metabolism through SIRT1 activation. *Cell Metab* 13: 461–468

Bai P, Canto C (2012) The role of PARP-1 and PARP-2 enzymes in metabolic regulation and disease. *Cell Metab* 16: 290–295

Barbosa MT, Soares SM, Novak CM, Sinclair D, Levine JA, Aksoy P, Chini EN (2007) The enzyme CD38 (a NAD glycohydrolase, EC 3.2.2.5) is necessary for the development of diet-induced obesity. *FASEB J* 21: 3629–3639

Becherer JD, Boros EE, Carpenter TY, Cowan DJ, Deaton DN, Haffner CD, Jeune MR, Kaldor IW, Poole JC, Preugschat F, Rheault TR, Schulte CA, Shearer BG, Shearer TW, Shewchuk LM, Smalley TL Jr, Stewart EL, Stuart JD, Ulrich JC (2015) Discovery of 4-amino-8-quinoline carboxamides as novel, submicromolar inhibitors of NAD-hydrolyzing enzyme CD38. *J Med Chem* 58: 7021–7056

Belenky P, Racette FG, Bogan KL, McClure JM, Smith JS, Brenner C (2007) Nicotinamide riboside promotes Sir2 silencing and extends lifespan via Nrk and Urh1/Pnp1/Meu1 pathways to NAD$^+$. *Cell* 129: 473–484

Bender DA, Magboul BI, Wynick D (1982) Probable mechanisms of regulation of the utilization of dietary tryptophan, nicotinamide and nicotinic acid as precursors of nicotinamide nucleotides in the rat. *Br J Nutr* 48: 119–127

Bender DA (1983) Biochemistry of tryptophan in health and disease. *Mol Aspects Med* 6: 101–197

Bender DA, Olufunwa R (1988) Utilization of tryptophan, nicotinamide and nicotinic acid as precursors for nicotinamide nucleotide synthesis in isolated rat liver cells. *Br J Nutr* 59: 279–287

Benyo Z, Gille A, Bennett CL, Clausen BE, Offermanns S (2006) Nicotinic acid-induced flushing is mediated by activation of epidermal langerhans cells. *Mol Pharmacol* 70: 1844–1849

Berger NA (1985) Poly(ADP-ribose) in the cellular response to DNA damage. *Radiat Res* 101: 4–15

Berger F, Lau C, Dahlmann M, Ziegler M (2005) Subcellular compartmentation and differential catalytic properties of the three

human nicotinamide mononucleotide adenylyltransferase isoforms. *J Biol Chem* 280: 36334–36341

Bieganowski P, Pace HC, Brenner C (2003) Eukaryotic NAD$^+$ synthetase Qns1 contains an essential, obligate intramolecular thiol glutamine amidotransferase domain related to nitrilase. *J Biol Chem* 278: 33049–33055

Bieganowski P, Brenner C (2004) Discoveries of nicotinamide riboside as a nutrient and conserved NRK genes establish a Preiss-Handler independent route to NAD$^+$ in fungi and humans. *Cell* 117: 495–502

Bilan DS, Matlashov ME, Gorokhovatsky AY, Schultz C, Enikolopov G, Belousov VV (2014) Genetically encoded fluorescent indicator for imaging NAD(+)/NADH ratio changes in different cellular compartments. *Biochim Biophys Acta* 1840: 951–957

Bird JG, Zhang Y, Tian Y, Panova N, Barvik I, Greene L, Liu M, Buckley B, Krasny L, Lee JK, Kaplan CD, Ebright RH, Nickels BE (2016) The mechanism of RNA 5′ capping with NAD$^+$, NADH and desphospho-CoA. *Nature* 535: 444–447

Bogan KL, Brenner C (2008) Nicotinic acid, nicotinamide, and nicotinamide riboside: a molecular evaluation of NAD$^+$ precursor vitamins in human nutrition. *Annu Rev Nutr* 28: 115–130

Boslett J, Hemann C, Zhao YJ, Lee HC, Zweier JL (2017) Luteolinidin protects the postischemic heart through CD38 inhibition with preservation of NAD (P)(H). *J Pharmacol Exper Ther* 361: 99–108

Braidy N, Guillemin GJ, Mansour H, Chan-Ling T, Poljak A, Grant R (2011) Age related changes in NAD$^+$ metabolism oxidative stress and Sirt1 activity in wistar rats. *PLoS One* 6: e19194

Brown KD, Maqsood S, Huang JY, Pan Y, Harkcom W, Li W, Sauve A, Verdin E, Jaffrey SR (2014) Activation of SIRT3 by the NAD(+) precursor nicotinamide riboside protects from noise-induced hearing loss. *Cell Metab* 20: 1059–1068

Brown JS, Kaye SB, Yap TA (2016) PARP inhibitors: the race is on. *Br J Cancer* 114: 713–715

Cabrera-Rode E, Molina G, Arranz C, Vera M, Gonzalez P, Suarez R, Prieto M, Padron S, Leon R, Tillan J, Garcia I, Tiberti C, Rodriguez OM, Gutierrez A, Fernandez T, Govea A, Hernandez J, Chiong D, Dominguez E, Di Mario U *et al* (2006) Effect of standard nicotinamide in the prevention of type 1 diabetes in first degree relatives of persons with type 1 diabetes. *Autoimmunity* 39: 333–340

Cahova H, Winz ML, Hofer K, Nubel G, Jaschke A (2015) NAD captureSeq indicates NAD as a bacterial cap for a subset of regulatory RNAs. *Nature* 519: 374–377

Camacho-Pereira J, Tarrago MG, Chini CC, Nin V, Escande C, Warner GM, Puranik AS, Schoon RA, Reid JM, Galina A, Chini EN (2016) CD38 dictates age-related NAD decline and mitochondrial dysfunction through an SIRT3-dependent mechanism. *Cell Metab* 23: 1127–1139

Cambronne XA, Stewart ML, Kim D, Jones-Brunette AM, Morgan RK, Farrens DL, Cohen MS, Goodman RH (2016) Biosensor reveals multiple sources for mitochondrial NAD(+). *Science* 352: 1474–1477

Canto C, Gerhart-Hines Z, Feige JN, Lagouge M, Noriega L, Milne JC, Elliott PJ, Puigserver P, Auwerx J (2009) AMPK regulates energy expenditure by modulating NAD$^+$ metabolism and SIRT1 activity. *Nature* 458: 1056–1060

Canto C, Houtkooper RH, Pirinen E, Youn DY, Oosterveer MH, Cen Y, Fernandez-Marcos PJ, Yamamoto H, Andreux PA, Cettour-Rose P, Gademann K, Rinsch C, Schoonjans K, Sauve AA, Auwerx J (2012) The NAD (+) precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet-induced obesity. *Cell Metab* 15: 838–847

Cantó C, Jiang LQ, Deshmukh AS, Mataki C, Coste A, Lagouge M, Zierath JR, Auwerx J (2010) Interdependence of AMPK and SIRT1 for metabolic

adaptation to fasting and exercise in skeletal muscle. *Cell Metab* 11: 213–219

Canto C, Sauve AA, Bai P (2013) Crosstalk between poly(ADP-ribose) polymerase and sirtuin enzymes. *Mol Aspects Med* 34: 1168–1201

Canto C, Menzies KJ, Auwerx J (2015) NAD(+) metabolism and the control of energy homeostasis: a balancing act between mitochondria and the nucleus. *Cell Metab* 22: 31–53

Cerutti R, Pirinen E, Lamperti C, Marchet S, Sauve AA, Li W, Leoni V, Schon EA, Dantzer F, Auwerx J, Viscomi C, Zeviani M (2014) NAD(+)-dependent activation of Sirt1 corrects the phenotype in a mouse model of mitochondrial disease. *Cell Metab* 19: 1042–1049

Chang HC, Guarente L (2013) SIRT1 mediates central circadian control in the SCN by a mechanism that decays with aging. *Cell* 153: 1448–1460

Chang HC, Guarente L (2014) SIRT1 and other sirtuins in metabolism. *Trends Endocrinol Metab* 25: 138–145

Chen D, Bruno J, Easlon E, Lin SJ, Cheng HL, Alt FW, Guarente L (2008) Tissue-specific regulation of SIRT1 by calorie restriction. *Genes Dev* 22: 1753–1757

Chen YG, Kowtoniuk WE, Agarwal I, Shen Y, Liu DR (2009) LC/MS analysis of cellular RNA reveals NAD-linked RNA. *Nat Chem Biol* 5: 879–881

Collins PB, Chaykin S (1971) Comparative metabolism of nicotinamide and nicotinic acid in mice. *Biochem J* 125: 117P

Collins PB, Chaykin S (1972) The management of nicotinamide and nicotinic acid in the mouse. *J Biol Chem* 247: 778–783

Comings DE, Muhleman D, Dietz G, Sherman M, Forest GL (1995) Sequence of human tryptophan 2,3-dioxygenase (TDO2): presence of a glucocorticoid response-like element composed of a GTT repeat and an intronic CCCCT repeat. *Genomics* 29: 390–396

Costford SR, Bajpeyi S, Pasarica M, Albarado DC, Thomas SC, Xie H, Church TS, Jubrias SA, Conley KE, Smith SR (2010) Skeletal muscle NAMPT is induced by exercise in humans. *Am J Physiol Endocrinol Metab* 298: E117–E126

Daubener W, MacKenzie CR (1999) IFN-gamma activated indoleamine 2,3-dioxygenase activity in human cells is an antiparasitic and an antibacterial effector mechanism. *Adv Exp Med Biol* 467: 517–524

De Jesus-Cortes H, Xu P, Drawbridge J, Estill SJ, Huntington P, Tran S, Britt J, Tesla R, Morlock L, Naidoo J, Melito LM, Wang G, Williams NS, Ready JM, McKnight SL, Pieper AA (2012) Neuroprotective efficacy of aminopropyl carbazoles in a mouse model of Parkinson disease. *Proc Natl Acad Sci USA* 109: 17010–17015

Desquiret-Dumas V, Gueguen N, Leman G, Baron S, Nivet-Antoine V, Chupin S, Chevrollier A, Vessieres E, Ayer A, Ferre M, Bonneau D, Henrion D, Reynier P, Procaccio V (2013) Resveratrol induces a mitochondrial complex I-dependent increase in NADH oxidation responsible for sirtuin activation in liver cells. *J Biol Chem* 288: 36662–36675

van de Donk NW, Janmaat ML, Mutis T, Lammerts van Bueren JJ, Ahmadi T, Sasser AK, Lokhorst HM, Parren PW (2016) Monoclonal antibodies targeting CD38 in hematological malignancies and beyond. *Immunol Rev* 270: 95–112

Drew JE, Farquharson AJ, Horgan GW, Williams LM (2016) Tissue-specific regulation of sirtuin and nicotinamide adenine dinucleotide biosynthetic pathways identified in C57Bl/6 mice in response to high-fat feeding. *J Nutr Biochem* 37: 20–29

Easlon E, Tsang F, Skinner C, Wang C, Lin SJ (2008) The malate-aspartate NADH shuttle components are novel metabolic longevity regulators required for calorie restriction-mediated life span extension in yeast. *Genes Dev* 22: 931–944

Elvehjem CA, Madden RJ, Strong FM, Woolley DW (1937) Relation of nicotinic acid and nicotinic acid amide to canine black tongue. *J Am Chem Soc* 59: 1767–1768

Emanuelli M, Carnevali F, Saccucci F, Pierella F, Amici A, Raffaelli N, Magni G (2001) Molecular cloning, chromosomal localization, tissue mRNA levels, bacterial expression, and enzymatic properties of human NMN adenylyltransferase. *J Biol Chem* 276: 406–412

Escande C, Nin V, Price NL, Capellini V, Gomes AP, Barbosa MT, O'Neil L, White TA, Sinclair DA, Chini EN (2013) Flavonoid apigenin is an inhibitor of the NAD⁺ ase CD38: implications for cellular NAD⁺ metabolism, protein acetylation, and treatment of metabolic syndrome. *Diabetes* 62: 1084–1093

von Euler H, Myrbäck K (1930) Co-Zymase. XVII. *Hoppe-Seyler's Zeitschrift für physiologische Chemie* 190: 93–100

Fang EF, Scheibye-Knudsen M, Brace LE, Kassahun H, SenGupta T, Nilsen H, Mitchell JR, Croteau DL, Bohr VA (2014) Defective mitophagy in XPA via PARP-1 hyperactivation and NAD(+)/SIRT1 reduction. *Cell* 157: 882–896

Fang EF, Kassahun H, Croteau DL, Scheibye-Knudsen M, Marosi K, Lu H, Shamanna RA, Kalyanasundaram S, Bollineni RC, Wilson MA, Iser WB, Wollman BN, Morevati M, Li J, Kerr JS, Lu Q, Waltz TB, Tian J, Sinclair DA, Mattson MP *et al* (2016) NAD⁺ replenishment improves lifespan and healthspan in ataxia telangiectasia models via mitophagy and DNA repair. *Cell Metab* 24: 566–581

Feng Y, Paul IA, LeBlanc MH (2006) Nicotinamide reduces hypoxic ischemic brain injury in the newborn rat. *Brain Res Bull* 69: 117–122

Fukuwatari T, Morikawa Y, Sugimoto E, Shibata K (2002) Effects of fatty liver induced by niacin-free diet with orotic acid on the metabolism of tryptophan to niacin in rats. *Biosci Biotechnol Biochem* 66: 1196–1204

Fulco M, Cen Y, Zhao P, Hoffman EP, McBurney MW, Sauve AA, Sartorelli V (2008) Glucose restriction inhibits skeletal myoblast differentiation by activating SIRT1 through AMPK-mediated regulation of Nampt. *Dev Cell* 14: 661–673

Gale EA, Bingley PJ, Emmett CL, Collier T, European Nicotinamide Diabetes Intervention Trial Group (2004) European Nicotinamide Diabetes Intervention Trial (ENDIT): a randomised controlled trial of intervention before the onset of type 1 diabetes. *Lancet* 363: 925–931

Garavaglia S, Bruzzone S, Cassani C, Canella L, Allegrone G, Sturla L, Mannino E, Millo E, De Flora A, Rizzi M (2012) The high-resolution crystal structure of periplasmic Haemophilus influenzae NAD nucleotidase reveals a novel enzymatic function of human CD73 related to NAD metabolism. *Biochem J* 441: 131–141

Gariani K, Menzies KJ, Ryu D, Wegner CJ, Wang X, Ropelle ER, Moullan N, Zhang H, Perino A, Lemos V, Kim B, Park YK, Piersigilli A, Pham TX, Yang Y, Ku CS, Koo SI, Fomitchova A, Canto C, Schoonjans K *et al* (2016) Eliciting the mitochondrial unfolded protein response by nicotinamide adenine dinucleotide repletion reverses fatty liver disease in mice. *Hepatology* 63: 1190–1204

Gariani K, Ryu D, Menzies KJ, Yi HS, Stein S, Zhang H, Perino A, Lemos V, Katsyuba E, Jha P, Vijgen S, Rubbia-Brandt L, Kim YK, Kim JT, Kim KS, Shong M, Schoonjans K, Auwerx J (2017) Inhibiting poly ADP-ribosylation increases fatty acid oxidation and protects against fatty liver disease. *J Hepatol* 66: 132–141

Gerdts J, Brace EJ, Sasaki Y, DiAntonio A, Milbrandt J (2015) SARM1 activation triggers axon degeneration locally via NAD(+) destruction. *Science* 348: 453–457

Gomes AP, Price NL, Ling AJY, Moslehi JJ, Montgomery MK, Rajman L, White JP, Teodoro JS, Wrann CD, Hubbard BP, Mercken EM, Palmeira CM, de Cabo R, Rolo AP, Turner N, Bell EL, Sinclair DA (2013) Declining NAD(+)

induces a pseudohypoxic state disrupting nuclear-mitochondrial communication during aging. *Cell* 155: 1624–1638

Gong B, Pan Y, Vempati P, Zhao W, Knable L, Ho L, Wang J, Sastre M, Ono K, Sauve AA, Pasinetti GM (2013) Nicotinamide riboside restores cognition through an upregulation of proliferator-activated receptor-gamma coactivator 1alpha regulated beta-secretase 1 degradation and mitochondrial gene expression in Alzheimer's mouse models. *Neurobiol Aging* 34: 1581–1588

Guan Y, Wang SR, Huang XZ, Xie QH, Xu YY, Shang D, Hao CM (2017) Nicotinamide mononucleotide, an NAD⁺ precursor, rescues age-associated susceptibility to AKI in a sirtuin 1-dependent manner. *J Am Soc Nephrol* 28: 2337–2352

Haffner CD, Becherer JD, Boros EE, Cadilla R, Carpenter T, Cowan D, Deaton DN, Guo Y, Harrington W, Henke BR, Jeune MR, Kaldor I, Milliken N, Petrov KG, Preugschat F, Schulte C, Shearer BG, Shearer T, Smalley TL Jr, Stewart EL *et al* (2015) Discovery, synthesis, and biological evaluation of Thiazoloquin (az)olin(on)es as potent CD38 inhibitors. *J Med Chem* 58: 3548–3571

Hagino Y, Lan SJ, Ng CY, Henderson LM (1968) Metabolism of pyridinium precursors of pyridine nucleotides in perfused rat liver. *J Biol Chem* 243: 4980–4986

Haigis MC, Sinclair DA (2010) Mammalian sirtuins: biological insights and disease relevance. *Annu Rev Pathol* 5: 253–295

Hamity MV, White SR, Walder RY, Schmidt MS, Brenner C, Hammond DL (2017) Nicotinamide riboside, a form of vitamin B3 and NAD⁺ precursor, relieves the nociceptive and aversive dimensions of paclitaxel-induced peripheral neuropathy in female rats. *Pain* 158: 962–972

Hara N, Yamada K, Shibata T, Osago H, Hashimoto T, Tsuchiya M (2007) Elevation of cellular NAD levels by nicotinic acid and involvement of nicotinic acid phosphoribosyltransferase in human cells. *J Biol Chem* 282: 24574–24582

Harden A, Young WJ (1906) The alcoholic ferment of yeast-juice. *Proc Biol Sci* 77: 405–420

Heyes MP, Saito K, Jacobowitz D, Markey SP, Takikawa O, Vickers JH (1992) Poliovirus induces indoleamine-2,3-dioxygenase and quinolinic acid synthesis in macaque brain. *FASEB J* 6: 2977–2989

Houtkooper RH, Canto C, Wanders RJ, Auwerx J (2010) The secret life of NAD⁺: an old metabolite controlling new metabolic signaling pathways. *Endocr Rev* 31: 194–223

Houtkooper RH, Pirinen E, Auwerx J (2012) Sirtuins as regulators of metabolism and healthspan. *Nat Rev Mol Cell Biol* 13: 225–238

Hsu CP, Oka S, Shao D, Hariharan N, Sadoshima J (2009) Nicotinamide phosphoribosyltransferase regulates cell survival through NAD⁺ synthesis in cardiac myocytes. *Circ Res* 105: 481–491

Hu Y, Wang H, Wang Q, Deng H (2014) Overexpression of CD38 decreases cellular NAD levels and alters the expression of proteins involved in energy metabolism and antioxidant defense. *J Proteome Res* 13: 786–795

Hung YP, Albeck JG, Tantama M, Yellen G (2011) Imaging cytosolic NADH-NAD(+) redox state with a genetically encoded fluorescent biosensor. *Cell Metab* 14: 545–554

Ijichi H, Ichiyama A, Hayaishi O (1966) Studies on the biosynthesis of nicotinamide adenine dinucleotide. 3. Comparative *in vivo* studies on nicotinic acid, nicotinamide, and quinolinic acid as precursors of nicotinamide adenine dinucleotide. *J Biol Chem* 241: 3701–3707

Imai S, Armstrong CM, Kaeberlein M, Guarente L (2000) Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* 403: 795–800

Imai S, Guarente L (2014) NAD⁺ and sirtuins in aging and disease. *Trends Cell Biol* 24: 464–471

Ito S, Murphy CG, Doubrovina E, Jasin M, Moynahan ME (2016) PARP inhibitors in clinical use induce genomic instability in normal human cells. *PLoS One* 11: e0159341

Jackson TM, Rawling JM, Roebuck BD, Kirkland JB (1995) Large supplements of nicotinic acid and nicotinamide increase tissue NAD⁺ and poly(ADP-ribose) levels but do not affect diethylnitrosamine-induced altered hepatic foci in Fischer-344 rats. *J Nutr* 125: 1455−1461

Jacobson EL, Dame AJ, Pyrek JS, Jacobson MK (1995) Evaluating the role of niacin in human carcinogenesis. *Biochimie* 77: 394−398

Jiao X, Doamekpor SK, Bird JG, Nickels BE, Tong L, Hart RP, Kiledjian M (2017) 5′ end nicotinamide adenine dinucleotide cap in human cells promotes RNA decay through DXO-mediated deNADding. *Cell* 168: 1015−1027.e10

Johnson VE, Stewart W, Smith DH (2013) Axonal pathology in traumatic brain injury. *Exp Neurol* 246: 35−43

Jubin T, Kadam A, Jariwala M, Bhatt S, Sutariya S, Gani AR, Gautam S, Begum R (2016) The PARP family: insights into functional aspects of poly(ADP-ribose) polymerase-1 in cell growth and survival. *Cell Prolif* 49: 421−437

Kabra DG, Thiyagarajan M, Kaul CL, Sharma SS (2004) Neuroprotective effect of 4-amino-1,8-napthalimide, a poly(ADP ribose) polymerase inhibitor in middle cerebral artery occlusion-induced focal cerebral ischemia in rat. *Brain Res Bull* 62: 425−433

Karamanlidis G, Lee CF, Garcia-Menendez L, Kolwicz SC, Suthammarak W, Gong G, Sedensky MM, Morgan PG, Wang W, Tian R (2013) Mitochondrial complex I deficiency increases protein acetylation and accelerates heart failure. *Cell Metab* 18: 239−250

Kaundal RK, Shah KK, Sharma SS (2006) Neuroprotective effects of NU1025, a PARP inhibitor in cerebral ischemia are mediated through reduction in NAD depletion and DNA fragmentation. *Life Sci* 79: 2293−2302

Kellenberger E, Kuhn I, Schuber F, Muller-Steffner H (2011) Flavonoids as inhibitors of human CD38. *Bioorg Med Chem Lett* 21: 3939−3942

Khan NA, Auranen M, Paetau I, Pirinen E, Euro L, Forsström S, Pasila L, Velagapudi V, Carroll CJ, Auwerx J, Suomalainen A (2014) Effective treatment of mitochondrial myopathy by nicotinamide riboside, a vitamin B3. *EMBO Mol Med* 6: 721−731

Klaidman L, Morales M, Kem S, Yang J, Chang ML, Adams JD Jr (2003) Nicotinamide offers multiple protective mechanisms in stroke as a precursor for NAD⁺, as a PARP inhibitor and by partial restoration of mitochondrial function. *Pharmacology* 69: 150−157

Knip M, Douek IF, Moore WP, Gillmor HA, McLean AE, Bingley PJ, Gale EA, European Nicotinamide Diabetes Intervention Trial Group (2000) Safety of high-dose nicotinamide: a review. *Diabetologia* 43: 1337−1345

Koyama T, Kume S, Koya D, Araki S, Isshiki K, Chin-Kanasaki M, Sugimoto T, Haneda M, Sugaya T, Kashiwagi A, Maegawa H, Uzu T (2011) SIRT3 attenuates palmitate-induced ROS production and inflammation in proximal tubular cells. *Free Radic Biol Med* 51: 1258−1267

Kraus D, Yang Q, Kong D, Banks AS, Zhang L, Rodgers JT, Pirinen E, Pulinilkunnil TC, Gong F, Wang YC, Cen Y, Sauve AA, Asara JM, Peroni OD, Monia BP, Bhanot S, Alhonen L, Puigserver P, Kahn BB (2014) Nicotinamide N-methyltransferase knockdown protects against diet-induced obesity. *Nature* 508: 258−262

Krehl WA, Teply LJ, Sarma PS, Elvehjem CA (1945) Growth-retarding effect of corn in nicotinic acid-low rations and its counteraction by tryptophane. *Science* 101: 489−490

Kudo Y, Boyd CA (2000) Human placental indoleamine 2,3-dioxygenase: cellular localization and characterization of an enzyme preventing fetal rejection. *Biochim Biophys Acta* 1500: 119−124

Lee YH, Nair S, Rousseau E, Allison DB, Page GP, Tatarranni PA, Bogardus C, Permana PA (2005) Microarray profiling of isolated abdominal subcutaneous adipocytes from obese vs non-obese Pima Indians: increased expression of inflammation-related genes. *Diabetologia* 48: 1776−1783

Lee CF, Chavez JD, Garcia-Menendez L, Choi Y, Roe ND, Chiao YA, Edgar JS, Goo YA, Goodlett DR, Bruce JE, Tian R (2016) Normalization of NAD⁺ redox balance as a therapy for heart failure. *Circulation* 134: 883−894

Lehmann S, Loh SH, Martins LM (2017) Enhancing NAD⁺ salvage metabolism is neuroprotective in a PINK1 model of Parkinson's disease. *Biol Open* 6: 141−147

Lin LF, Henderson LM (1972) Pyridinium precursors of pyridine nucleotides in perfused rat kidney and in the testis. *J Biol Chem* 247: 8023−8030

Lin SJ, Ford E, Haigis M, Liszt G, Guarente L (2004) Calorie restriction extends yeast life span by lowering the level of NADH. *Genes Dev* 18: 12−16

Lin JB, Kubota S, Ban N, Yoshida M, Santeford A, Sene A, Nakamura R, Zapata N, Kubota M, Tsubota K, Yoshino J, Imai S, Apte RS (2016) NAMPT-mediated NAD(+) biosynthesis is essential for vision in mice. *Cell Rep* 17: 69−85

Lingor P, Koch JC, Tonges L, Bahr M (2012) Axonal degeneration as a therapeutic target in the CNS. *Cell Tissue Res* 349: 289−311

Liu D, Pitta M, Jiang H, Lee JH, Zhang G, Chen X, Kawamoto EM, Mattson MP (2013) Nicotinamide forestalls pathology and cognitive decline in Alzheimer mice: evidence for improved neuronal bioenergetics and autophagy procession. *Neurobiol Aging* 34: 1564−1580

Malavasi F, Deaglio S, Funaro A, Ferrero E, Horenstein AL, Ortolan E, Vaisitti T, Aydin S (2008) Evolution and function of the ADP ribosyl cyclase/CD38 gene family in physiology and pathology. *Physiol Rev* 88: 841−886

Masri S, Rigor P, Cervantes M, Ceglia N, Sebastian C, Xiao C, Roqueta-Rivera M, Deng C, Osborne TF, Mostoslavsky R, Baldi P, Sassone-Corsi P (2014) Partitioning circadian transcription by SIRT6 leads to segregated control of cellular metabolism. *Cell* 158: 659−672

Massudi H, Grant R, Braidy N, Guest J, Farnsworth B, Guillemin GJ (2012) Age-associated changes in oxidative stress and NAD⁺ metabolism in human tissue. *PLoS One* 7: e42357

McCreanor GM, Bender DA (1986) The metabolism of high intakes of tryptophan, nicotinamide and nicotinic acid in the rat. *Br J Nutr* 56: 577−586

Menissier de Murcia J, Ricoul M, Tartier L, Niedergang C, Huber A, Dantzer F, Schreiber V, Ame JC, Dierich A, LeMeur M, Sabatier L, Chambon P, de Murcia G (2003) Functional interaction between PARP-1 and PARP-2 in chromosome stability and embryonic development in mouse. *EMBO J* 22: 2255−2263

Menzies KJ, Zhang H, Katsyuba E, Auwerx J (2016) Protein acetylation in metabolism—metabolites and cofactors. *Nat Rev Endocrinol* 12: 43−60

Mills KF, Yoshida S, Stein LR, Grozio A, Kubota S, Sasaki Y, Redpath P, Migaud ME, Apte RS, Uchida K, Yoshino J, Imai SI (2016) Long-term administration of nicotinamide mononucleotide mitigates age-associated physiological decline in mice. *Cell Metab* 24: 795−806

Moreau C, Liu Q, Graeff R, Wagner GK, Thomas MP, Swarbrick JM, Shuto S, Lee HC, Hao Q, Potter BV (2013) CD38 structure-based inhibitor design using the N1-Cyclic inosine 5′-diphosphate ribose template. *PLoS One* 8: e66247

Mori V, Amici A, Mazzola F, Di Stefano M, Conforti L, Magni G, Ruggieri S, Raffaelli N, Orsomando G (2014) Metabolic profiling of alternative NAD biosynthetic routes in mouse tissues. *PLoS One* 9: e113939

Morigi M, Perico L, Rota C, Longaretti L, Conti S, Rottoli D, Novelli R, Remuzzi G, Benigni A (2015) Sirtuin 3-dependent mitochondrial dynamic

improvements protect against acute kidney injury. *J Clin Invest* 125: 715–726

Mouchiroud L, Houtkooper RH, Moullan N, Katsyuba E, Ryu D, Canto C, Mottis A, Jo YS, Viswanathan M, Schoonjans K, Guarente L, Auwerx J (2013) The NAD(+)/sirtuin pathway modulates longevity through activation of mitochondrial UPR and FOXO signaling. *Cell* 154: 430–441

Mukhopadhyay P, Rajesh M, Cao Z, Horváth B, Park O, Wang H, Erdelyi K, Holovac E, Wang Y, Liaudet L, Hamdaoui N, Lafdil F, Haskó G, Szabo C, Boulares AH, Gao B, Pacher P (2014) Poly (ADP-ribose) polymerase-1 is a key mediator of liver inflammation and fibrosis. *Hepatology* 59: 1998–2009

Mukhopadhyay P, Horvath B, Rajesh M, Varga ZV, Gariani K, Ryu D, Cao Z, Holovac E, Park O, Zhou Z, Xu MJ, Wang W, Godlewski G, Paloczi J, Nemeth BT, Persidsky Y, Liaudet L, Hasko G, Bai P, Boulares AH *et al* (2017) PARP inhibition protects against alcoholic and non-alcoholic steatohepatitis. *J Hepatol* 66: 589–600

Nakahata Y, Sahar S, Astarita G, Kaluzova M, Sassone-Corsi P (2009) Circadian control of the NAD+ salvage pathway by CLOCK-SIRT1. *Science* 324: 654–657

Navarro J, Gozalbo-Lopez B, Mendez AC, Dantzer F, Schreiber V, Martinez C, Arana DM, Farres J, Revilla-Nuin B, Bueno MF, Ampurdanes C, Galindo-Campos MA, Knobel PA, Segura-Bayona S, Martin-Caballero J, Stracker TH, Aparicio P, Del Val M, Yelamos J (2017) PARP-1/PARP-2 double deficiency in mouse T cells results in faulty immune responses and T lymphomas. *Sci Rep* 7: 41962

Nikiforov A, Dolle C, Niere M, Ziegler M (2011) Pathways and subcellular compartmentation of NAD biosynthesis in human cells: from entry of extracellular precursors to mitochondrial NAD generation. *J Biol Chem* 286: 21767–21778

Olmos PR, Hodgson MI, Maiz A, Manrique M, De Valdes MD, Foncea R, Acosta AM, Emmerich MV, Velasco S, Muniz OP, Oyarzun CA, Claro JC, Bastias MJ, Toro LA (2006) Nicotinamide protected first-phase insulin response (FPIR) and prevented clinical disease in first-degree relatives of type-1 diabetics. *Diabetes Res Clin Pract* 71: 320–333

Peek CB, Affinati AH, Ramsey KM, Kuo HY, Yu W, Sena LA, Ilkayeva O, Marcheva B, Kobayashi Y, Omura C, Levine DC, Bacsik DJ, Gius D, Newgard CB, Goetzman E, Chandel NS, Denu JM, Mrksich M, Bass J (2013) Circadian clock NAD⁺ cycle drives mitochondrial oxidative metabolism in mice. *Science* 342: 1243417

Penke M, Larsen PS, Schuster S, Dall M, Jensen BA, Gorski T, Meusel A, Richter S, Vienberg SG, Treebak JT, Kiess W, Garten A (2015) Hepatic NAD salvage pathway is enhanced in mice on a high-fat diet. *Mol Cell Endocrinol* 412: 65–72

de Picciotto NE, Gano LB, Johnson LC, Martens CR, Sindler AL, Mills KF, Imai S, Seals DR (2016) Nicotinamide mononucleotide supplementation reverses vascular dysfunction and oxidative stress with aging in mice. *Aging Cell* 15: 522–530

Pieper AA, Xie S, Capota E, Estill SJ, Zhong J, Long JM, Becker GL, Huntington P, Goldman SE, Shen CH, Capota M, Britt JK, Kotti T, Ure K, Brat DJ, Williams NS, MacMillan KS, Naidoo J, Melito L, Hsieh J *et al* (2010) Discovery of a proneurogenic, neuroprotective chemical. *Cell* 142: 39–51

Pillai JB, Isbatan A, Imai S, Gupta MP (2005) Poly(ADP-ribose) polymerase-1-dependent cardiac myocyte cell death during heart failure is mediated by NAD⁺ depletion and reduced Sir2alpha deacetylase activity. *J Biol Chem* 280: 43121–43130

Pillai VB, Sundaresan NR, Kim G, Gupta M, Rajamohan SB, Pillai JB, Samant S, Ravindra PV, Isbatan A, Gupta MP (2010) Exogenous NAD blocks cardiac

hypertrophic response via activation of the SIRT3-LKB1-AMP-activated kinase pathway. *J Biol Chem* 285: 3133–3144

Pirinen E, Canto C, Jo YS, Morato L, Zhang H, Menzies KJ, Williams EG, Mouchiroud L, Moullan N, Hagberg C, Li W, Timmers S, Imhof R, Verbeek J, Pujol A, van Loon B, Viscomi C, Zeviani M, Schrauwen P, Sauve AA *et al* (2014) Pharmacological inhibition of poly(adp-ribose) polymerases improves fitness and mitochondrial function in skeletal muscle. *Cell Metab* 19: 1034–1041

Preiss J, Handler P (1958) Biosynthesis of diphosphopyridine nucleotide. I. Identification of intermediates. *J Biol Chem* 233: 488–492

Price NL, Gomes AP, Ling AJ, Duarte FV, Martin-Montalvo A, North BJ, Agarwal B, Ye L, Ramadori G, Teodoro JS, Hubbard BP, Varela AT, Davis JG, Varamini B, Hafner A, Moaddel R, Rolo AP, Coppari R, Palmeira CM, de Cabo R *et al* (2012) SIRT1 is required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function. *Cell Metab* 15: 675–690

Qin W, Yang T, Ho L, Zhao Z, Wang J, Chen L, Zhao W, Thiyagarajan M, MacGrogan D, Rodgers JT, Puigserver P, Sadoshima J, Deng H, Pedrini S, Gandy S, Sauve AA, Pasinetti GM (2006) Neuronal SIRT1 activation as a novel mechanism underlying the prevention of Alzheimer disease amyloid neuropathology by calorie restriction. *J Biol Chem* 281: 21745–21754

Quarona V, Zaccarello G, Chillemi A, Brunetti E, Singh VK, Ferrero E, Funaro A, Horenstein AL, Malavasi F (2013) CD38 and CD157: a long journey from activation markers to multifunctional molecules. *Cytometry B Clin Cytom* 84: 207–217

Ramsey KM, Yoshino J, Brace CS, Abrassart D, Kobayashi Y, Marcheva B, Hong HK, Chong JL, Buhr ED, Lee C, Takahashi JS, Si Imai, Bass J (2009) Circadian Clock feedback cycle through NAMPT-mediated NAD⁺ biosynthesis. *Science* 324: 651–654

Ratajczak J, Joffraud M, Trammell SA, Ras R, Canela N, Boutant M, Kulkarni SS, Rodrigues M, Redpath P, Migaud ME, Auwerx J, Yanes O, Brenner C, Canto C (2016) NRK1 controls nicotinamide mononucleotide and nicotinamide riboside metabolism in mammalian cells. *Nat Commun* 7: 13103

Reinhard JF Jr (1998) Altered tryptophan metabolism in mice with herpes simplex virus encephalitis: increases in spinal cord quinolinic acid. *Neurochem Res* 23: 661–665

Richard DM, Dawes MA, Mathias CW, Acheson A, Hill-Kapturczak N, Dougherty DM (2009) L-Tryptophan: basic metabolic functions, behavioral research and therapeutic indications. *Int J Tryptophan Res* 2: 45–60

Riederer M, Erwa W, Zimmermann R, Frank S, Zechner R (2009) Adipose tissue as a source of nicotinamide N-methyltransferase and homocysteine. *Atherosclerosis* 204: 412–417

Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P (2005) Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. *Nature* 434: 113–118

Rutter J, Reick M, Wu LC, McKnight SL (2001) Regulation of clock and NPAS2 DNA binding by the redox state of NAD cofactors. *Science* 293: 510–514

Ryu D, Zhang H, Ropelle ER, Sorrentino V, Mazala DA, Mouchiroud L, Marshall PL, Campbell MD, Ali AS, Knowels GM, Bellemin S, Iyer SR, Wang X, Gariani K, Sauve AA, Canto C, Conley KE, Walter L, Lovering RM, Chin ER *et al* (2016) NAD⁺ repletion improves muscle function in muscular dystrophy and counters global PARylation. *Sci Transl Med* 8: 361ra139

Sadanaga-Akiyoshi F, Yao H, Tanuma S, Nakahara T, Hong JS, Ibayashi S, Uchimura H, Fujishima M (2003) Nicotinamide attenuates focal ischemic brain injury in rats: with special reference to changes in nicotinamide and NAD⁺ levels in ischemic core and penumbra. *Neurochem Res* 28: 1227–1234

Salek RM, Maguire ML, Bentley E, Rubtsov DV, Hough T, Cheeseman M, Nunez D, Sweatman BC, Haselden JN, Cox RD, Connor SC, Griffin JL (2007) A metabolomic comparison of urinary changes in type 2 diabetes in mouse, rat, and human. *Physiol Genomics* 29: 99–108

Sanni LA, Thomas SR, Tattam BN, Moore DE, Chaudhri G, Stocker R, Hunt NH (1998) Dramatic changes in oxidative tryptophan metabolism along the kynurenine pathway in experimental cerebral and noncerebral malaria. *Am J Pathol* 152: 611–619

Sasaki Y, Araki T, Milbrandt J (2006) Stimulation of nicotinamide adenine dinucleotide biosynthetic pathways delays axonal degeneration after axotomy. *J Neurosci* 26: 8484–8491

Satoh A, Stein L, Imai S (2011) The role of mammalian sirtuins in the regulation of metabolism, aging, and longevity. *Handb Exp Pharmacol* 206: 125–162

Scheibye-Knudsen M, Mitchell SJ, Fang EF, Iyama T, Ward T, Wang J, Dunn CA, Singh N, Veith S, Hasan-Olive MM, Mangerich A, Wilson MA, Mattson MP, Bergersen LH, Cogger VC, Warren A, Le Couteur DG, Moaddel R, Wilson DM III, Croteau DL et al (2014) A high-fat diet and NAD(+) activate Sirt1 to rescue premature aging in cockayne syndrome. *Cell Metab* 20: 840–855

Shindler KS, Ventura E, Rex TS, Elliott P, Rostami A (2007) SIRT1 activation confers neuroprotection in experimental optic neuritis. *Invest Ophthalmol Vis Sci* 48: 3602–3609

Sidransky H (2001) *Tryptophan: biochemical and health implications*. Boca Raton, FL: CRC Press

Someya S, Yu W, Hallows WC, Xu J, Vann JM, Leeuwenburgh C, Tanokura M, Denu JM, Prolla TA (2010) Sirt3 mediates reduction of oxidative damage and prevention of age-related hearing loss under caloric restriction. *Cell* 143: 802–812

Swarbrick JM, Graeff R, Zhang H, Thomas MP, Hao Q, Potter BV (2014) Cyclic adenosine 5′-diphosphate ribose analogs without a "southern" ribose inhibit ADP-ribosyl cyclase-hydrolase CD38. *J Med Chem* 57: 8517–8529

Takikawa O, Yoshida R, Kido R, Hayaishi O (1986) Tryptophan degradation in mice initiated by indoleamine 2,3-dioxygenase. *J Biol Chem* 261: 3648–3653

Tesla R, Wolf HP, Xu P, Drawbridge J, Estill SJ, Huntington P, McDaniel L, Knobbe W, Burket A, Tran S, Starwalt R, Morlock L, Naidoo J, Williams NS, Ready JM, McKnight SL, Pieper AA (2012) Neuroprotective efficacy of aminopropyl carbazoles in a mouse model of amyotrophic lateral sclerosis. *Proc Natl Acad Sci USA* 109: 17016–17021

Trammell SA, Brenner C (2013) Targeted, LCMS-based metabolomics for quantitative measurement of NAD(+) metabolites. *Comput Struct Biotechnol J* 4: e201301012

Trammell SA, Schmidt MS, Weidemann BJ, Redpath P, Jaksch F, Dellinger RW, Li Z, Abel ED, Migaud ME, Brenner C (2016a) Nicotinamide riboside is uniquely and orally bioavailable in mice and humans. *Nat Commun* 7: 12948

Trammell SA, Weidemann BJ, Chadda A, Yorek MS, Holmes A, Coppey LJ, Obrosov A, Kardon RH, Yorek MA, Brenner C (2016b) Nicotinamide riboside opposes type 2 diabetes and neuropathy in mice. *Sci Rep* 6: 26933

Tran MT, Zsengeller ZK, Berg AH, Khankin EV, Bhasin MK, Kim W, Clish CB, Stillman IE, Karumanchi SA, Rhee EP, Parikh SM (2016) PGC1alpha drives NAD biosynthesis linking oxidative metabolism to renal protection. *Nature* 531: 528–532

Tummala KS, Gomes AL, Yilmaz M, Graña O, Bakiri L, Ruppen I, Ximénez-Embún P, Sheshappanavar V, Rodriguez-Justo M, Pisano DG, Wagner EF, Djouder N (2014) Inhibition of *de novo* NAD(+) synthesis by oncogenic URI Causes liver tumorigenesis through DNA damage. *Cancer Cell* 26: 826–839

Turunc Bayrakdar E, Uyanikgil Y, Kanit L, Koylu E, Yalcin A (2014) Nicotinamide treatment reduces the levels of oxidative stress, apoptosis, and PARP-1 activity in Abeta(1–42)-induced rat model of Alzheimer's disease. *Free Radical Res* 48: 146–158

Ugur S, Ulu R, Dogukan A, Gurel A, Yigit IP, Gozel N, Aygen B, Ilhan N (2015) The renoprotective effect of curcumin in cisplatin-induced nephrotoxicity. *Ren Fail* 37: 332–336

Viscomi C, Bottani E, Civiletto G, Cerutti R, Moggio M, Fagiolari G, Schon EA, Lamperti C, Zeviani M (2011) *In vivo* correction of COX deficiency by activation of the AMPK/PGC-1alpha axis. *Cell Metab* 14: 80–90

Wahlberg E, Karlberg T, Kouznetsova E, Markova N, Macchiarulo A, Thorsell AG, Pol E, Frostell A, Ekblad T, Oncu D, Kull B, Robertson GM, Pellicciari R, Schuler H, Weigelt J (2012) Family-wide chemical profiling and structural analysis of PARP and tankyrase inhibitors. *Nat Biotechnol* 30: 283–288

Walters RW, Matheny T, Mizoue LS, Rao BS, Muhlrad D, Parker R (2017) Identification of NAD+ capped mRNAs in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* 114: 480–485

Wang J, Zhai Q, Chen Y, Lin E, Gu W, McBurney MW, He Z (2005) A local mechanism mediates NAD-dependent protection of axon degeneration. *J Cell Biol* 170: 349–355

Wang G, Han T, Nijhawan D, Theodoropoulos P, Naidoo J, Yadavalli S, Mirzaei H, Pieper AA, Ready JM, McKnight SL (2014a) P7C3 neuroprotective chemicals function by activating the rate-limiting enzyme in NAD salvage. *Cell* 158: 1324–1334

Wang S, Zhu W, Wang X, Li J, Zhang K, Zhang L, Zhao YJ, Lee HC, Zhang L (2014b) Design, synthesis and SAR studies of NAD analogues as potent inhibitors towards CD38 NADase. *Molecules* 19: 15754–15767

Wang X, Hu X, Yang Y, Takata T, Sakurai T (2016a) Nicotinamide mononucleotide protects against beta-amyloid oligomer-induced cognitive impairment and neuronal death. *Brain Res* 1643: 1–9

Wang YQ, Wang PY, Wang YT, Yang GF, Zhang A, Miao ZH (2016b) An update on poly(ADP-ribose)polymerase-1 (PARP-1) inhibitors: opportunities and challenges in cancer therapy. *J Med Chem* 59: 9575–9598

Warburg O, Christian W (1936) Pyridine, the hydrogen transfusing component of fermentative enzymes. *Helv Chim Acta* 19: 79–88

van de Weijer T, Phielix E, Bilet L, Williams EG, Ropelle ER, Bierwagen A, Livingstone R, Nowotny P, Sparks LM, Paglialunga S, Szendroedi J, Havekes B, Moullan N, Pirinen E, Hwang JH, Schrauwen-Hinderling VB, Hesselink MK, Auwerx J, Roden M, Schrauwen P (2015) Evidence for a direct effect of the NAD+ precursor acipimox on muscle mitochondrial function in humans. *Diabetes* 64: 1193–1201

Williams JN, Feigelson P, Elvehjem CA (1950) Relation of tryptophan and niacin to pyridine nucleotides of tissue. *J Biol Chem* 187: 597–604

Williams GT, Lau KM, Coote JM, Johnstone AP (1985) NAD metabolism and mitogen stimulation of human lymphocytes. *Exp Cell Res* 160: 419–426

Williams PA, Harder JM, Foxworth NE, Cochran KE, Philip VM, Porciatti V, Smithies O, John SW (2017) Vitamin B3 modulates mitochondrial vulnerability and prevents glaucoma in aged mice. *Science* 355: 756–760

Wojcik M, Seidle HF, Bieganowski P, Brenner C (2006) Glutamine-dependent NAD+ synthetase. How a two-domain, three-substrate enzyme avoids waste. *J Biol Chem* 281: 33395–33402

Xie L, Wang Z, Li C, Yang K, Liang Y (2017) Protective effect of nicotinamide adenine dinucleotide (NAD+) against spinal cord ischemia-reperfusion injury via reducing oxidative stress-induced neuronal apoptosis. *J Clin Neurosci* 36: 114–119

Xu W, Barrientos T, Mao L, Rockman HA, Sauve AA, Andrews NC (2015) Lethal cardiomyopathy in mice lacking transferrin receptor in the heart. *Cell Rep* 13: 533–545

Yaguchi H, Togawa K, Moritani M, Itakura M (2005) Identification of candidate genes in the type 2 diabetes modifier locus using expression QTL. *Genomics* 85: 591–599

Yalowitz JA, Xiao S, Biju MP, Antony AC, Cummings OW, Deeg MA, Jayaram HN (2004) Characterization of human brain nicotinamide 5′-mononucleotide adenylyltransferase-2 and expression in human pancreas. *Biochem J* 377: 317–326

Yamamoto T, Byun J, Zhai P, Ikeda Y, Oka S, Sadoshima J (2014) Nicotinamide mononucleotide, an intermediate of NAD+ synthesis, protects the heart from ischemia and reperfusion. *PLoS One* 9: e98972

Yamazaki F, Kuroiwa T, Takikawa O, Kido R (1985) Human indolylamine 2,3-dioxygenase. Its tissue distribution, and characterization of the placental enzyme. *Biochem J* 230: 635–638

Yang H, Yang T, Baur JA, Perez E, Matsui T, Carmona JJ, Lamming Dudley W, Souza-Pinto NC, Bohr VA, Rosenzweig A, de Cabo R, Sauve Anthony A, Sinclair DA (2007) Nutrient-sensitive mitochondrial NAD+ levels dictate cell survival. *Cell* 130: 1095–1107

Yang SJ, Choi JM, Kim L, Park SE, Rhee EJ, Lee WY, Oh KW, Park SW, Park C-Y (2014) Nicotinamide improves glucose metabolism and affects the hepatic NAD-sirtuin pathway in a rodent model of obesity and type 2 diabetes. *J Nutr Biochem* 25: 66–72

Yano M, Akazawa H, Oka T, Yabumoto C, Kudo-Sakamoto Y, Kamo T, Shimizu Y, Yagi H, Naito AT, Lee JK, Suzuki J, Sakata Y, Komuro I (2015) Monocyte-derived extracellular Nampt-dependent biosynthesis of NAD(+) protects the heart against pressure overload. *Sci Rep* 5: 15857

Yin TC, Britt JK, De Jesus-Cortes H, Lu Y, Genova RM, Khan MZ, Voorhees JR, Shao J, Katzman AC, Huntington PJ, Wassink C, McDaniel L, Newell EA, Dutca LM, Naidoo J, Cui H, Bassuk AG, Harper MM, McKnight SL, Ready JM *et al* (2014) P7C3 neuroprotective chemicals block axonal degeneration and preserve function after traumatic brain injury. *Cell Rep* 8: 1731–1740

Ying W (2008) NAD+/NADH and NADP+/NADPH in cellular functions and cell death: regulation and biological consequences. *Antioxid Redox Signal* 10: 179–206

Yoshida R, Hayaishi O (1978) Induction of pulmonary indoleamine 2,3-dioxygenase by intraperitoneal injection of bacterial lipopolysaccharide. *Proc Natl Acad Sci USA* 75: 3998–4000

Yoshida R, Urade Y, Tokuda M, Hayaishi O (1979) Induction of indoleamine 2,3-dioxygenase in mouse lung during virus infection. *Proc Natl Acad Sci USA* 76: 4084–4086

Yoshino J, Mills KF, Yoon MJ, S-i Imai (2011) Nicotinamide mononucleotide, a key NAD(+) intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice. *Cell Metab* 14: 528–536

Young GS, Choleris E, Lund FE, Kirkland JB (2006) Decreased cADPR and increased NAD+ in the Cd38-/- mouse. *Biochem Biophys Res Commun* 346: 188–192

Zhang X, Kurnasov OV, Karthikeyan S, Grishin NV, Osterman AL, Zhang H (2003) Structural characterization of a human cytosolic NMN/NAMN adenylyltransferase and implication in human NAD biosynthesis. *J Biol Chem* 278: 13503–13511

Zhang H, Ryu D, Wu Y, Gariani K, Wang X, Luan P, D'Amico D, Ropelle ER, Lutolf MP, Aebersold R, Schoonjans K, Menzies KJ, Auwerx J (2016) NAD(+) repletion improves mitochondrial and stem cell function and enhances life span in mice. *Science* 352: 1436–1443

Zheng CB, Han J, Xia WL, Shi ST, Liu JR, Ying WH (2012) NAD(+) administration decreases ischemic brain damage partially by blocking autophagy in a mouse model of brain ischemia. *Neurosci Lett* 512: 67–71

Zhou Y, Ting KY, Lam CM, Kwong AK, Xia J, Jin H, Liu Z, Zhang L, Cheung Lee H, Zhang L (2012) Design, synthesis and biological evaluation of noncovalent inhibitors of human CD38 NADase. *ChemMedChem* 7: 223–228

Zhou M, Ottenberg G, Sferrazza GF, Hubbs C, Fallahi M, Rumbaugh G, Brantley AF, Lasmezas CI (2015) Neuronal death induced by misfolded prion protein is due to NAD+ depletion and can be relieved *in vitro* and *in vivo* by NAD+ replenishment. *Brain* 138: 992–1008.

Zhuo L, Fu B, Bai X, Zhang B, Wu L, Cui J, Cui S, Wei R, Chen X, Cai G (2011) NAD blocks high glucose induced mesangial hypertrophy via activation of the sirtuins-AMPK-mTOR pathway. *Cellular Physiol Biochem* 27: 681–690