Received: 24 June 2020 | Revised: 5 August 2020 | Accepted: 7 August 2020

DOI: 10.1111/apha.13551

REVIEW ARTICLE

ACTA PHYSIOLOGICA

Nicotinamide adenine dinucleotide: Biosynthesis, consumption and therapeutic role in cardiac diseases

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Funding information
This work was supported by a grant to FAZ and MR from the American University of Beirut Faculty of Medicine (MPP–320095), and to FAZ by Centre

Abstract

Nicotinamide adenine dinucleotide (NAD) is an abundant cofactor that plays crucial roles in several cellular processes. NAD can be synthesized de novo starting with tryptophan, or from salvage pathways starting with NAD precursors like nicotinic acid (NA), nicotinamide (NAM) or nicotinamide riboside (NR), referred to as niacin/B_3 vitamins, arising from dietary supply or from cellular NAD catabolism. Given the interconversion between its oxidized (NAD^+) and reduced form (NADH), NAD participates in a wide range of reactions: regulation of cellular redox status, energy metabolism and mitochondrial biogenesis. Plus, NAD acts as a signalling molecule, being a cosubstrate for several enzymes such as sirtuins, poly-ADP-ribose-polymerases (PARPs) and some ectoenzymes like CD38, regulating critical biological processes like gene expression, DNA repair, calcium signalling and circadian rhythms. Given the large number of mitochondria present in cardiac tissue, the heart has the highest NAD levels and is one of the most metabolically demanding organs. In several models of heart failure, myocardial NAD levels are depressed and this depression is caused by mitochondrial dysfunction, metabolic remodelling and inflammation. Emerging evidence suggests that regulating NAD homeostasis by NAD precursor supplementation has therapeutic efficiency in improving myocardial bioenergetics and function. This review provides an overview of the latest understanding of the different NAD biosynthesis pathways, as well as its role as a signalling molecule particularly in cardiac tissue. We highlight the significance of preserving NAD equilibrium in various models of heart diseases and shed light on the potential pharmacological interventions aiming to use NAD boosters as therapeutic agents.

National de la Recherche Scientifique (CNRS) #103507/103487; The American University of Beirut Faculty of Medicine (MPP–320145), Seed grant #100410 and Collaborative Research Stimulus (CRS) # 103556, and to MM from ANR (Agence Nationale pour la Recherche) grant NAD-Heart #ANR-17-CE17-0015-01 and Fondation de France grant #0075811.

KEYWORDS

cardiac diseases, cardiac metabolism, COVID-19, inflammation, mitochondria, NAD, redox potential, transcription factors

1 | NAD$^+$: REDOX COFACTOR AND SIGNALLING MOLECULE

In 1906, the British scientists, William Young and Sir Arthur Harden discovered an unidentified factor that they called *"conferment"* responsible for alcoholic fermentation.[1] In 1929-1930, the German biochemist Hans von Euler-Chelpin, identified this heat-stable factor as a nucleotide sugar phosphate.[2] Six years later, the German scientist Otto Heinrich Warburg demonstrated the function of the nucleotide coenzyme in hydride transfer and identified the nicotinamide portion as the site of redox reactions[3] and its structure was established in 1950 by Pricer and Kornberg.[4] It is only later in the century that signalling pathways consuming NAD as a substrate were discovered. NAD is the key coenzyme for energy metabolism redox reactions. In its reduced form NADH, it is the principal contributor of electrons to the respiratory chain.

The alteration of NAD homeostasis is becoming widely studied in the context of both ageing and cardiac diseases.[5] NAD is reduced to NADH during the oxidation of glucose and fatty acids. Two NADH are generated by glycolysis and converted back into NAD$^+$ under anaerobic conditions by the lactate dehydrogenase enzyme or under aerobic condition, by the malate-aspartate and glycerol-3-phosphate shuttle, which is responsible for transferring reducing equivalents to mitochondria. Pyruvate, the end-product of glycolysis, can be further oxidized and contribute to the Krebs cycle after decarboxylation by the pyruvate dehydrogenase complex, yielding one more NADH and one acetyl-coenzyme A. The mitochondrion is the site of fatty acid β-oxidation and production of a flavine-adenine-2 ($FADH_2$) molecule, an NADH molecule and acetyl-coenzyme-A for each cleavage cycle of two carbon atoms. Mitochondrial oxidative phosphorylation via the Krebs cycle generates three NADH molecules and one $FADH_2$ molecule, making reduced NADH the main electron donor to the respiratory chain.

NAD also serves as a precursor of nicotinamide adenine dinucleotide phosphate (NADP) via phosphorylation mediated by the cytosolic and mitochondrial NAD kinases or through interconversion between NADH and NADPH by the mitochondrial nicotinamide nucleotide transhydrogenase, the latter playing a key role in the balance between mitochondrial energy output and antioxidant capacities.[6] Of note, as a cofactor, NAD is recycled between its reduced and oxidized form (NAD$^+$ and NADH) without alteration in the total NAD pool. In a very different way, the oxidized form NAD$^+$ can be consumed as a substrate by different enzymes like the sirtuins (SIRTs),[7] the PARPs [8] or the cyclic ADP-ribose synthases (cADPRSs) like CD38[9] that cleave the N-glycosidic bond between the nicotinamide and the ADP-ribose moieties.[5] This net consumption of NAD$^+$ is compensated for by de novo and salvage synthesis pathways (Figure 1), maintaining therefore a balanced pool under normal physiological conditions.

2 | BIOSYNTHESIS PATHWAYS

NAD can be produced by two routes: de novo and salvage pathways. De novo biosynthesis of NAD starts with dietary tryptophan (Trp).[10] In the heart, this pathway contributes a slight fraction to the total cellular NAD pool.[11] The salvage pathways on the other hand constitute a major source of NAD biosynthesis in all tissues and employ vitamin B_3 molecules as precursors, including nicotinic acid (NA), nicotinamide (NAM) and nicotinamide riboside (NR), provided from diet. NAM is also derived from NAD catabolism through enzymes that utilize NAD as substrate in order to accomplish their functions (Figure 1).[12-14]

2.1 | De novo NAD synthesis

Trp is converted into NAD through an eight-step pathway known as the de novo pathway. Trp is initially transformed into N-formylkynurenine by the rate-limiting enzyme tryptophan 2,3-dioxygenase (TDO) or indoleamine 2,3-dioxygenase (IDO). N-formylkynurenine is subsequently turned into α-amino-β-carboxymuconate-ε-semialdehyde (ACMS) by a succession of four enzymatic reactions. Being unstable, ACMS undergoes a total oxidation to CO_2 and H_2O or a spontaneous cyclization, subsequently producing the nicotinic acid mononucleotide (NAMN) precursor, quinolinic

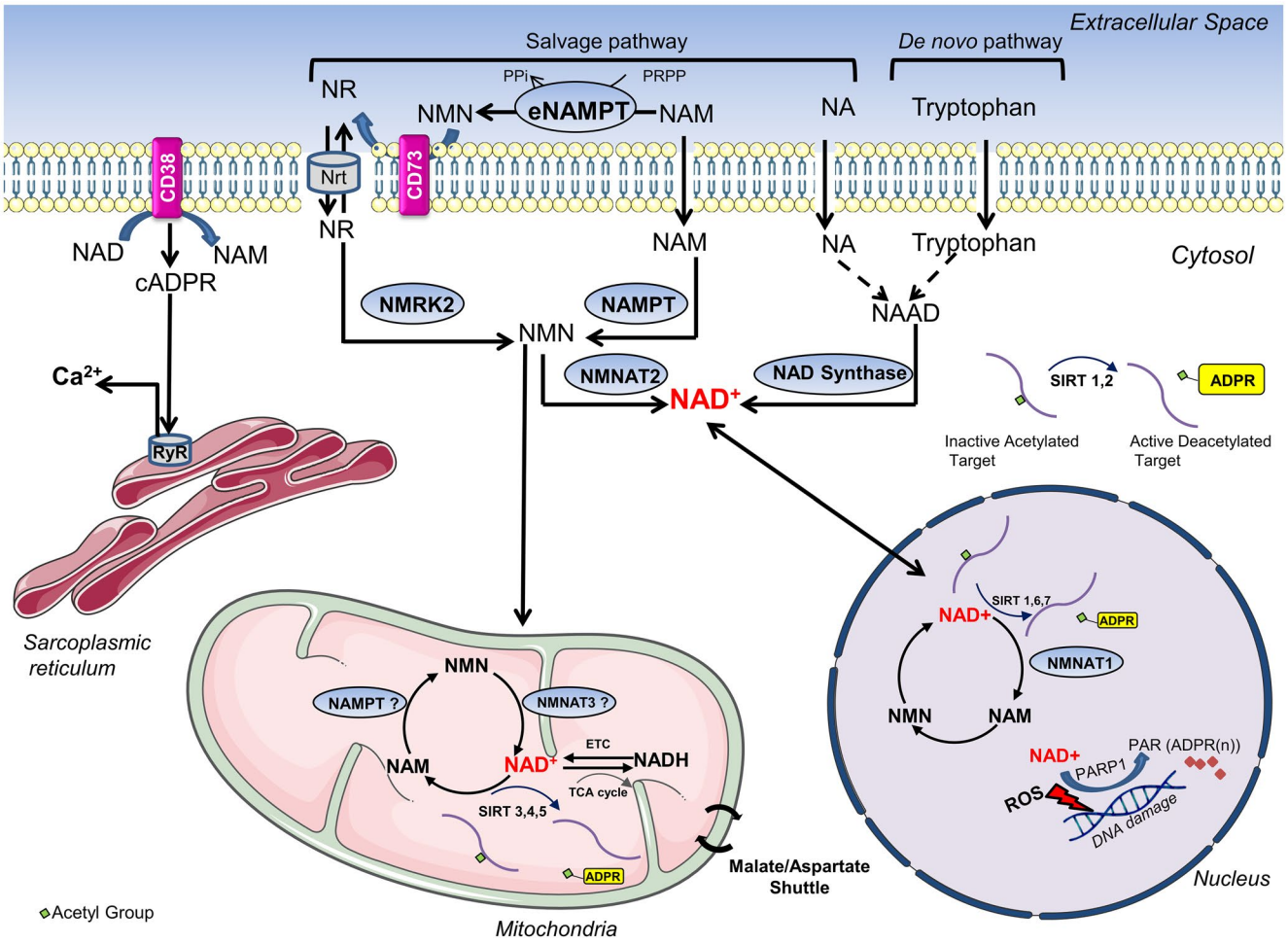

FIGURE 1 Nicotinamide adenine dinucleotide (NAD) biosynthesis pathways and signalling in the cardiomyocyte. The bodily NAD contributions derive either from the de novo pathway (from tryptophan) or from salvage pathways (from B3 vitamins: nicotinic acid [NA], nicotinamide riboside [NR] and nicotinamide [NAM]). In cardiac tissue, the greater proportion of NAD^+ is produced via salvage pathways. NR is turned into nicotinamide mononucleotide (NMN) under the phosphorylation action of nicotinamide riboside kinase 2 (NMRK2) enzyme. The rate-limiting step in NAD biosynthesis from NAM is the transfer of a phosphoribosyl residue from 5-phosphoribosyl-1-pyrophosphate (PRPP) to NAM catalysed by nicotinamide phosphoribosyltransferase (NAMPT) to produce NMN. NMN is then converted into NAD^+ by cytosolic nicotinamide mononucleotide adenylyltransferease 2 (NMNAT 2), mitochondrial NMNAT3 and nuclear NMNAT1. Additionally, extracellular NMN and NAD can be turned into NR and NAM by membrane clusters of differentiation 73 (CD73) and 38 (CD38), respectively, and subsequently taken up by NMRK2 and NAMPT. CD38 cleaves NAD^+ to create cyclic ADPR (cADPR) and ADPR second messengers. These second messengers induce calcium (Ca^{2+}) mobilization from outer and intracellular stores, mainly the sarcoplasmic reticulum, through the activation of the ryanodine receptor (RyR). Several reactions transform NA into NAAD and NAAD is converted into NAD^+ by NAD synthase. Sirtuins (SIRTs) exert their deacetylation activity only in the presence of NAD^+ as a substrate and convert it into NAM. SIRTs 3, 4 and 5 are located in the mitochondria, SIRT 2 is located in the cytosol and SIRTs 1, 6 and 7 are located in the nucleus. Poly-ADP-ribose polymerases (PARPs) exert their poly-ADP-ribosylation activity only in the presence of NAD^+ as a substrate for their reaction and convert it into NAM (PARP-1 is located in the nucleus). The resulting NAM from SIRTs and PARPs is recycled via the NAD^+ salvage pathway

acid (Qa). The conversion of Qa into NAMN is catalysed by quinolinate phosphoribosyl transferase (QPRT) using alpha-D-5-phosphoribosyl-1-pyrophosphate (PRPP) as the sugar phosphate backbone.[15] NAMN is then fused with an ATP to form the nicotinic acid adenine dinucleotide (NAAD) by the ubiquitous enzymes nicotinamide mononucleotide adenylyltransferases (NMNAT).[16] In the final step, NAAD is amidated to NAD in an ATP-dependent reaction via glutamine-dependent NAD synthase activity.[17]

2.2 | Salvage pathways

The salvage pathways involve catalytic conversions into NAD from three main NAD precursors: In one, NA and PRPP are converted into NAMN by nicotinic acid phosphoribosyl transferase (NAPT), which is subsequently converted into NAD^+ by the actions of NMNAT and NADS enzymes, so joining at the end of the de novo pathway, which is the reason why the pathways, from Trp and from

NA can also be grouped as the deamidated precursors pathway.[18] In a second pathway, NAM is turned into nicotinamide mononucleotide (NMN) by the activity of nicotinamide phosphoribosyl transferase (NAMPT). NAMPT carries a PRPP backbone to the NAM fraction. It is noteworthy that NAMPT is a principal enzyme for cellular NAD pool regeneration given that NAM is the common end-product of most of the enzymatic reactions hydrolysing NAD (Sirtuins, PARPs, CD38, Bst1 and SARM1). NMN is then converted into NAD^+ by nicotinamide mononucleotide adenylyltransferase (NMNAT). NMNAT 1, 2 and 3 are clearly active in the heart.[19-21] Extracellular NA and NAM[16] are membrane permeable and can freely enter the cytosol. In the third pathway, NR reaches the salvage pathway via equilibrate nucleoside transporters (ENTs)[16] and is phosphorylated into NMN by nicotinamide riboside kinases 1 and 2 (NMRK1/2). NMN is finally fused to ATP by the NMNAT enzymes into NAD.[22] NMRK2 is specific for cardiac and skeletal muscle and its expression level is found to be strongly upregulated in several models of cardiomyopathies related to mutations in serum response factor,[23] *lamin-A*,[24] *Idh2*[25] and *PGC1α*.[26] NR is naturally found in cow milk [13,27] and NR supplementation was shown to boost cellular NAD levels in mice.[28,29]

NMN, the end-product of NAMPT and NMRK reactions, has been proposed as an alternative precursor available to the cells when administrated in the cell culture medium or through injection. As with most phosphorylated nucleotides, it is not supposed to be stable in plasma and if present, it is only at low concentrations. Several studies showed that extracellular NMN has to be dephosphorylated by the CD73 ectonucleotidase to be converted into NR before cellular internalization in hepatocytes,[22] muscle cells[30] and neurons.[31] In the mouse small intestine, *Slc12a8*, belonging to the *SLC12* gene family of the electroneutral cation–chloride co-transporters, has been identified as a specific NMN transporter allowing minute entry of this precursor into the cells..[32] As some aspects of the analytical method used for this demonstration is a matter of debate among experts,[33,34] further research is needed to clarify the role and relevance of this transporter.

3 | SIGNALLING PATHWAYS CONSUMING NAD^+

In addition to being a cofactor of redox reactions, NAD is a signalling molecule used as a substrate in enzymatic reactions that share in common the property of irreversibly cleaving NAD into NAM and ADP ribose moieties. The three major families of enzymes that cleave NAD are as follows: SIRTs, PARPs and cADPRSs, like CD38 and Bst1. These enzymes act as metabolic sensors with significant influence on cardiac metabolism, function and ageing.[35]

3.1 | Sirtuins

SIRTs are NAD^+-dependent deacetylases similar to the silent information regulator in yeast, initially isolated in a screening for silencing factors.[36] Subsequent studies revealed that Sir2 operates as a histone deacetylase.[37] Hence, SIRTs are enzymes that consume NAD and release NAM and O-acetyl ADP ribose with the deacetylated substrate.[38,39] Subsequent studies revealed that SIRTs are important regulators of several cellular processes including organism lifespan,[40,41] fat mobilization in human cells,[42] cellular response to stress[43] and apoptosis.[44] As of now, seven SIRTs homologues have been discovered with ubiquitous expression. Cell biological studies have demonstrated different subcellular compartments for the SIRTs (Table 1).

SIRT1 and SIRT2 are found in both nucleus and cytosol, SIRT3, 4 and 5 are mitochondrial and SIRT6 and 7 are nuclear.[63] All exhibit a deacetylase activity with the exception of SIRT4, which is an ADP-ribosyl transferase. SIRT5 also exhibits an enzymatic activity as a desuccinylase and demalonylase; SIRT6 as a demyristoylase, depalmitoylase and ADP-ribosyl transferase.

SIRT1— Fasting, exercise and low glucose availability activate nuclear SIRT1.[64] SIRT1 modulates the acetylation level of several transcription factors and, thus, their activity. Transcription factors regulated by SIRT1 act as key metabolic regulators and include the peroxisome proliferator-activated receptors (PPARs), PPAR coactivator-1 (PGC-1), p53 and the FOXO family, and participate in responding to oxidative stress and autophagy.[65] In the cardiac tissue, SIRT1 overexpression is protective.[47,66] However, at high expression levels, SIRT1 is associated with development of hypertrophic cardiomyopathy and damaged or reduced levels of mitochondria, as evidenced by lower levels of NAD, ATP, citrate synthase activity and expression of PPAR-γ co-activator 1α.[47] Recently, we showed with a cardiac-specific inducible murine knockout model that SIRT1 is required to protect the heart against pressure overload-induced cardiac dysfunction and notably mitochondrial dysfunction.[67]

Doulamis et al carried out a study in 81 patients with coronary artery disease scheduled for open-heart surgery. ELISA performed on serum from these patients revealed that SIRT1 levels released from dead cardiomyocytes, correlated with a history of hypertension. The index of low SIRT1 correlated with patient history of MI. Thus, SIRT1 levels could be a potential prognostic tool for MI incidence in patients.[68]

Although in the failing heart Nampt maintains cardiac function and metabolism, its overexpression is harmful in pressure overload. This was attributed in part to excessive SIRT1 activation under these conditions with Nampt and SIRT1 cooperatively suppressing mitochondrial function and ATP production.[69] In contrast, a recent study showed that exercise training-based cardiac rehabilitation (ET-CR)

TABLE 1 Sirtuins: location, physiological role and changes in cardiac diseases

Sirtuin	Location	Physiological role	Changes in expression in cardiac diseases
Sirtuin 1	Nucleus Cytosol	Deacetylase	↑ MI,[45] but ↓ activity[46] ↑ Following pressure overload[47,48] ↑ Phenylephrine-induced hypertrophy[49]
Sirtuin 2	Nucleus Cytosol	Deacetylase	↓ Angiotensin II–induced hypertrophy[50]
Sirtuin 3	Mitochondria (Nucleus)	Deacetylase	↓ Following MI[51] ↑ Following pressure overload or hypertrophic agonists[52,53] ↓ ROS- and obesity-related heart failure[54,55]
Sirtuin 4	Mitochondria	ADP-ribosyl transferase	↓ Myocardial ischaemia reperfusion[56] ↑ Angiotensin II- or pressure overload-induced hypertrophy[57]
Sirtuin 5	Mitochondria	Deacetylase Desuccinylase Demalonylase	
Sirtuin 6	Nucleus	Deacetylase Demyristoylase Depalmitoylase ADP-ribosyl transferase	↓ Cardiac hypertrophy following angiotensin II[58] or phenylephrine[59] ↓ Transverse aortic constriction-induced heart failure[60]
Sirtuin 7	Nucleus	Deacetylase	↑ After pressure overload[61] ↑ Myocardial infarction[62]

programs lead to SIRT1 activation and anti-oxidant capacity, which in return leads to a positive feedback on NAD synthesis via the salvage pathway.[70]

SIRT2—SIRT2 is abundant in energetic tissues like the heart, the brain and adipose tissues.[71] SIRT2 is important for chromosome stability during mitoses,[72] and inhibits adipogenesis via deacetylating FOXO1. SIRT2 can also inhibit the inflammatory response in mice via deacetylating p65 and inhibiting the activity of NF-κB.[73] Furthermore, Liu et al showed in a recent study that SIRT2 has a mitochondrial function, as well as an autophagy/mitophagy function.[74] On the other hand, SIRT2 deficiency promotes fibrosis and cardiac hypertrophy through impairment of AMP-activated protein kinase (AMPK) activity, known to sense energy metabolism.[50] AMPK is also a repressor of protein synthesis counteracting the process of cardiac hypertrophy.[75]

SIRT3-5—SIRT3, SIRT4 and SIRT5 act as mitochondrial stress sensors by modulating the activity of enzymes important in energy metabolism.[76] SIRT3 is known to extend lifespan and protect mitochondrial function by regulating the acetylation level of several mitochondrial target proteins, such as optic atrophy 1 (OPA1), a pro-fusion protein of the inner mitochondrial membrane.[77] Other mitochondrial enzymes include manganese superoxide dismutase, ornithine transcarbamylase, long-chain acyl-CoA dehydrogenase, acetyl-CoA synthetase 2 and isocitrate dehydrogenase 2.[78,79]

Following MI, SIRT3 expression levels decrease.[51] Moreover, Porter et al showed that 7-month-old SIRT3 heterozygous mice developed larger MI sizes and worsened cardiac dysfunction compared to wild-type mice in a Langendorff MI model.[80] SIRT3 overexpression protects the myocardium from hypertrophic remodelling. SIRT3 mainly targets mitochondrial enzymes like acetyl-coenzyme-1-synthetase, activated when deacetylated and NAD supplementation blocks angiotensin II–induced cardiac hypertrophy via SIRT3 activation.[81,82] A role for SIRT3 was also demonstrated in regulating mitochondrial dynamics (fusion/fission).[83] Another recent study indicates that suppression of SIRT3 promotes development of cardiomyocyte hypertrophy and metabolic impairment.[84] In another study, it was shown that in mice lacking SIRT3, OXPHOS enzymes are hyperacetylated, ATP and NAD levels are diminished and these mice are hypersensitive to aortic constriction, ostensibly as a result of activation of CypD, a mitochondrial permeability transition pore regulator.[53,85]

In a mouse model of hypertrophy, NAD treatment was able to block the hypertrophic response, resulting in a decreased heart-to-body weight ratio, myocyte cross-sectional area, fibrosis and left ventricular wall thickness. However, in SIRT3-KO mice, these protective effects were not observed. In summary, this study showed that exogenous NAD activates the SIRT3-LKB1-AMP-activated kinase pathway and

blocks cardiac hypertrophy.[82] It is clear that SIRT3's function is linked to the metabolic condition of the cell. A previous study revealed that decreased NAD levels in failing hearts were associated with mitochondrial protein hyperacetylation and decreased complex-II respiratory function as a result of reduced catalytic function of SIRT3.[86] Of note, a recent study demonstrated that the acetylation of some enzymes targeted by SIRT3 is not always causally linked to their activity.[87] Besides directly regulating enzymatic activity, lysine acetylation/de-acetylation can interact with other modes of post-translational modification to affect various aspects of cellular signalling, including protein–protein interactions and protein cellular localization and stability.

Unlike other SIRTs, SIRT4 is not a deacetylase, it functions as an ADP-ribosyltransferase on histones and bovine serum albumin.[88] It has been shown in the context of hyopoxia that SIRT4 prevents apoptosis in H9c2 cardiomyocytes.[89] SIRT5 has desuccinylation, demalonylation and deglutarylation activities on mitochondrial proteins. The underlying post-translational modifications in addition to acetylation are collectively known as acylation and result from the abundant presence of acetyl-coA in the mitochondrial matrix.[90] Numerous studies have shown hundreds of SIRT5 substrates. SIRT5 plays key roles in keeping metabolic equilibrium of the cell; it is implicated in metabolic processes, including glycolysis, TCA cycle, FAO, the ETC, ketone body formation and ROS detoxification. SIRT5 is also important for cardiac health. For instance, *Sirt5*-KO mice have decreased survival following transverse aortic constriction (TAC) and develop exaggerated hypertrophy 4 weeks following TAC with decreased ejection fraction and impaired oxidative metabolism.[91]

SIRT6,7—SIRT6 is associated with chromatin and involved in genomic stability, glucose homeostasis and inflammation,[92-94] Evidence also supports a role for SIRT6 in stimulating poly-ADP-ribosylation activity of PARP1 during oxidative stress-induced DNA damage.[95] Additional evidence implies that SIRT6 is essential for the heart. SIRT6 expression and activity is found to be decreased in both human and mouse failing hearts.[21,96] Plus, 8-12 weeks following SIRT6 deficiency, mice develop concentric cardiac hypertrophy. Moreover, in response to angiotensin II, NAD levels are decreased as well as the deacetylase activity of SIRT6,[97] while SIRT6 overexpression blocks the associated cardiac hypertrophy.[96]

Acetylation may also inhibit glucose uptake in cardiac cells, contributing to insulin resistance, metabolic inflexibility and cardiomyocyte dysfunction under conditions of diabetic cardiomyopathy.[98,99] Notably, mice overexpressing SIRT6 are protected from obesity and insulin resistance when fed high fat or high sucrose diets. This also includes protection from mitochondrial fragmentation associated with SIRT3 downregulation.[100] Similarly, SIRT7 plays a crucial nuclear role related to ribosomal biogenesis, regulating both transcription and RNA elongation.[101] SIRT7 expression correlates with cell growth, being highly expressed in metabolically active organs.[102] SIRT7-deficient mice have shorter lifespan, extensive fibrosis, cardiac hypertrophy and inflammatory cardiomyopathy.[103]

3.2 | PARPs

PARPs are NAD consuming enzymes that transfer ADP ribose from NAD^+ to proteins. PARP1 and PARP2 are the most widely studied PARPs being ubiquitous nuclear proteins and mainly activated by DNA damage and leading to the recruitment of DNA repair proteins.[104] PARPs can link with many transcription factors specific to cardiac and skeletal muscle, including TEF-1 and MyoD that are involved in regulating the activation of muscle genes.[105] Although PARPs are important pathophysiological modulators related to DNA repair during cell injury, their prolonged overactivation is detrimental by affecting the intracellular NAD^+ pool, which may lead to NAD^+ depletion and cell death.[106] For instance, PARP1 overexpression is of importance in cell death, myocardial fibrosis and damage, which is reduced by NAD supplementation.[107] Moreover, the inhibition of PARP1 is protective against tachypacing and contractile dysfunction in atrial cardiomyocytes caused by oxidative stress and DNA breaks. Often, highly active PARP1 is found in patients with atrial fibrillation who have remarkable DNA damage.[108] In the context of MI, PARP1 inhibition protects against ischaemic myocardial damage by reducing apoptosis, attenuating cardiac fibrosis and promoting autophagy regulatory mechanisms.[109,110]

Inhibition of PARPs can be cardioprotective.[111] In response to starvation, PARP1 is activated, which stimulates the accumulation of FOXO3a in the nucleus and its binding to promoters of target genes related to autophagy. When autophagy is stimulated, mitochondrial metabolism becomes impaired and cardiomyocytes die.[112] PARP2 is the second well-identified family of PARPs. It has been shown that PARP2 inhibition protects cardiomyocytes from angiotensin II–induced hypertrophy via SIRT1 activation.[113]

3.3 | CD38

CD38 ectoenzyme cleaves NAD to generate cyclic ADP ribose, a second messenger in calcium signalling. CD38 exists in two conformations: a glycosylated type II membrane protein with a catalytic C-terminus facing outward and a non-catalytical N-terminus facing inside the cell,[114] and a second conformation which is a non-glycosylated form with the catalytic site facing the cytosol.[115] CD38 has a substrate

preference for $NADP^+$ over NAD^+. CD38 is located in the cardiac endothelium.[116] Following ischaemic MI, the inhibition of CD38 prevents NADPH expenditure and preserves endothelium-dependent relaxation and nitric oxide generation. Thus, activation of CD38 is an important cause of post-ischaemic endothelial dysfunction. As a result, therapeutic agents maintaining NADPH levels by restoring them or preventing their reduction could be promising for the treatment of coronary syndrome and MI.[116] In cardiomyocytes, CD38 produces NAADP and CADPR (Ca^{2+} mobilizing messengers). These messengers remarkably contribute to the activation of Ca^{2+} transients by β-adrenoceptor signaling.[117]

CD38 knockout mice display a 30-fold increase in cellular NAD^+ levels.[118] Experiments performed on CD38 knockout mice showed that hearts exhibited remarkable protection against ischaemia/reperfusion (I/R) with preserved NADP(H) and glutathione levels, increased recovery of left ventricular contractile function, decreased myocyte enzyme release and decreased infarct size.[119] Wang et al conducted a study in CD38 knockout mice fed a high-fat diet, which showed that CD38 deficiency decreased fatty acid content and increased intracellular NAD^+ concentrations. They performed in vitro studies to better understand the mechanism of these protective effects. Indeed, in vitro knockdown of CD38 attenuated ROS production and lipid synthesis following oleic acid treatment. Furthermore, mitochondrial SIRT3 expression with its target genes FOXO3 and SOD2 were markedly upregulated in H9c2 cell line after oleic acid stimulation. In summary, CD38 deficiency protected the myocardium from high-fat diet-induced oxidative stress via activating the SIRT3/FOXO3 pathway.[120] Interestingly, increase in this condition and inhibitors of CD38 help maintain NAD levels.[121]

This dual role of NAD, as a coenzyme of energy metabolism and as a substrate consumed by different enzymes, places this metabolite at the centre of various signalling pathways that can be recruited in the context of a pathological cardiac stress and in cardiac remodelling.

4 | NAD DEPLETION

NAD depletion has been documented in the context of several cardiac pathologies, including cardiac hypertrophy, dilated cardiomyopathy (DCM) and MI.

4.1 | Cardiac hypertrophy

NAD depletion was found to be associated with pathologic cardiac hypertrophy.[82] Several mechanisms could be responsible for the depletion of NAD during pathologic hypertrophy. Oxidative stress is one possibility, since overstimulation of cells induces oxidative stress, which in turn activates PARP1. PARP1 forms poly(ADP-ribose) polymers while consuming NAD and this leads to NAD depletion.[122] Extracellular NAD levels are lower than NAD intracellular concentrations, which leads to a loss of cellular NAD upon the opening of Cx43 channels especially under stress conditions.[123] Reduced NAMPT levels also cause reduced NAD production, in hearts stimulated with hypertrophic agonists.[124]

4.2 | Dilated cardiomyopathy

Our previous work also showed in a DCM mouse model (Serum Response Factor heart knockout) a decrease in myocardial NAD levels along with a decrease in NAMPT expression levels.[23] Furthermore, it has been shown in the heart of mouse and human cardiomyopathy owing to lamin A/C gene mutation that the NAD salvage pathway is altered.[125] Severe oxidative stress can result in increased NAD turnover caused by increased activity of NAD-consuming enzymes such as PARPs and/or decreased activity of NAD salvage pathways, resulting in depletion of intracellular NAD levels. Complex I deficiency in mice, decreased the NAD^+/NADH ratio and subsequently inhibited SIRT3 activity, which led to protein hyperacetylation and sensitization of the mitochondrial permeability transition pore (mPTP) to opening.[126]

4.3 | Myocardial infarction

Myocardial levels of NAD^+ were found to be significantly reduced in a mouse model of MI when compared to the control group. This drop was accompanied by a reduced fuel oxidative flux, diminished ATP synthesis and a decrease in the complex II respiration rate.[86]

5 | IMPORTANCE OF NAD REPLENISHMENT

The therapeutic effects of NAD have recently gained attention since raising NAD levels is now considered a promising treatment for several diseases. Araki et al showed that adding NAD to neurons after mechanical damage stunted axonal degeneration.[127] Likewise, NAD administration intranasally markedly reduced brain damage in a rat model of transient focal ischaemia.[128] Moreover, Vaur et al showed that NR supplementation in the cortex reduces brain damage caused by NMDA injection,[129] further highlighting the therapeutic significance of NAD. As for its role in cardiovascular diseases, Pillai et al showed that NAD can potentially block hypertrophy.[82] Further studies have shown that NAD^+

supplementation protected H9c2 cells against hypoxia via the SIRT1-p53 pathway.[130]

Recently, attention has been focused on approaches for activation of cardiac signalling pathways that inhibit hypertrophy. In this regards, severe oxidative stress can result in the depletion of NAD, preventing cells from carrying out energy-dependent functions and defence mechanisms owing to the loss of cell-survival factors dependent on NAD, such as sirtuins. In vitro experiments, together with gene knockout and transgenic mouse models indicated that the antihypertrophic actions of exogenous NAD involved activation of the SIRT3-LKB1-AMPK signalling pathway, thereby blocking the pro-hypertrophic action of mTOR and Akt1. Furthermore, SIRT3 stimulation was shown to reduce ROS levels and subsequent Akt1 signalling, thus, blocking cardiac hypertrophy.[53,82]

The importance of replenishment of NAD pools has been established in multiple disease scenarios. For instance, Picotto et al showed that treatment of old mice with NMN reverses age-related aortic stiffening, oxidative stress, collagen deposition and elastin fragmentation by activating SIRT1.[131] Apigenin belonging to flavones subclass increases tissue NAD^+ levels and enhances glucose and lipid balance in obese mice by augmenting SIRTs 1 and 3 activities.[132,133]

The effects of NR administration on cardiac function were studied in a murine model of lamin A/C gene *LMNA* cardiomyopathy. Vignier et al showed that oral administration of NR increases cardiac protein PARylation and markedly improves NAD cellular content as well as left ventricular structure and function.[125] NR administration was also tested for effects on heart failure. As demonstrated previously, in the failing heart, NAD^+ levels fall along with a drop in the expression of NAMPT enzyme that recycles the nicotinamide precursor, and an increase in NMRK2 that phosphorylates the NR precursor. This switch is also in evidence in human failing heart biopsies. Diguet et al showed that NR efficiently rescues NAD^+ synthesis and attenuates heart failure development in mice by stabilizing NAD^+ levels in the failing heart, indicating that NR could be useful for treating heart failure.[23] NR also prolonged the lifespan of mice with iron deficiency–induced heart failure and improved mitochondrial and cardiac function.[134] In addition, dietary NR supplementation and the subsequent replenishment of NAD^+ stores improved heart function in a mouse model of muscular dystrophy with cardiomyopathy (Duchenne),[135] and reduced cardiomyocyte death and contractile dysfunction in mice subjected to pressure overload.[136]

Regarding NAMPT, it has been shown that overexpressing NAMPT with NMN injection (500 mg/kg, i.p.) prior to ischaemia or repetitive administration just before or during reperfusion markedly protects against pressure overload and I/R injury.[137] In addition, the important effects of NAMPT and NMN on cardiac function were demonstrated in Friedreich's ataxia cardiomyopathy model, where SIRT3 mediates NMN-induced improvements in metabolic cardiac function.[138]

6 | PHARMACOLOGICAL STRATEGIES TO BOOST NAD LEVELS

Owing to its potential therapeutic relevance and being a critical cofactor and signalling molecule, NAD^+ has garnered much attention recently. NAD^+ imbalance is considered a hallmark in the pathogenesis of cardiac disorders. Several approaches are being explored in order to boost NAD^+ levels through NAD precursors supplementation,[139] NAD biosynthetic enzymes activation[140] and NAD depletion inhibition.[141]

6.1 | NAD precursors supplements

NAD precursors include niacin, NR, NMN, NAM and NA. Daily ingestion of 15 mg niacin has been shown to have several health effects, among which are decreasing the risk of MI and cardiovascular diseases.[142] Pharmacological approaches are the most common applications to effectively upregulate NAD^+ levels. NR and NMN are the best molecules for animal experiments and clinical trials because they are soluble and orally bioavailable.

NMN was found to be cardioprotective against I/R, when delivered acutely at reperfusion. This cardioprotection was mediated in part by the stimulation of glycolytic flux, with an enhancement of ATP synthesis during ischaemia and an increase in acidosis during reperfusion.[137,143] Increased acidosis would be protective by blocking opening of the mitochondrial permeability transition pore (mPTP) during reperfusion, thereby attenuating cell death. In their study, Nadtochiy and colleagues also show that this cardioprotection is insensitive to SIRT1 inhibition that alkalinize cells. This alkalinization was not sufficient to counter the acidification caused by NMN. Consequently, boosting NAD levels might be beneficial as a result of changes in cellular pH.[143]

However, NMN is a phosphorylated compound more expensive to synthesize and it does not enter cells intact, but rather is dephosphorylated by CD73 into NR, at least in muscle cells.[30] For instance, NR has shown more benefit than NA and NAM in enhancing NAD^+ levels.[29] The ability of NA to boost NAD^+ levels has been demonstrated lately.[144] However, NA use is limited by flushing[145,146] and NAM by its inhibition of SIRTs at elevated doses.[147] The absence of side effects associated with NR makes it the most favourable NAD precursor. Brenner et al conducted the first 8-week randomized, double-blind, placebo-controlled clinical trial on healthy men and women.[29,148] They showed that consumption of NR significantly increases

blood NAD within 2 weeks in a dose-dependent manner, and this increase was maintained throughout the study. No flushing was recorded. These data were corroborated by others showing excellent safety and efficacy of NR as an NAD precursor.[149] Altogether these studies give a basic comprehension of the consequences of NR supplementation for human physiological functions.

NA reduces the synthesis of low-density lipoprotein cholesterol (LDL-C) by several means, including a likely direct reduction in liver cholesterol synthesis, as well as receptor-mediated inhibition of free fatty acid release from body fat and suppression of liver apolipoprotein C3 expression resulting in reduced VLDL-C production.[28,150] NA also increases beneficial high-density lipoprotein cholesterol (HDL-C) by several means. Besides flushing, an unpleasant side effect of NA is itching. These are caused by receptor-mediated prostaglandin production that can be mitigated somewhat by taking an aspirin or a non-steroidal anti-inflammatory drug (NSAID) 30 minutes prior.[150] A lower incidence of unpleasant side effects is associated with extended-release formulations. NAM is much less likely to cause flushing or itching, but does not lower cholesterol or exhibit beneficial effects on plasma fats.[151] Generally, mild-to-moderate side effects are associated with NR, although it was reported to cause unexpectedly high levels of NAAD in human peripheral blood mononuclear cells (PBMC) and in mouse liver and heart.[29] At least in rodents, NR is more effective in boosting NAD^+ than NAM or NA. A discussion of the tissue-specific ligand activities of NA, NAM and NR can be found elsewhere.[152]

6.2 | NAD biosynthetic enzyme activation

An alternative emerging option to increase NAD^+ levels is to directly activate its biosynthesis. Several enzymes are currently under consideration. For instance, activating the rate-limiting enzyme NAMPT, using a NAMPT-activating compound such as SBI-797812 succeeded in elevating liver NAD^+ levels.[153] Another example is NMNATs. These are attractive targets being involved in both de novo and salvage pathways.[154]

6.3 | NAD^+ depletion inhibitors

The third strategy to boost NAD^+ concentrations is by inhibiting NADases (PARPs and CD38). For example, CD38 is inhibited by very low concentrations of flavonoids such as apigenin.[133] As for PARP inhibitors, recently, they are being approved for the treatment of cancer (niraparib, olaparib, rucaparib, talazoparib and veliparib).[155] Others like XAV939, which is a PARP5 inhibitor, are able to boost NAD^+ levels.[156]

7 | THE RELATIONSHIP BETWEEN NAD LEVELS, ENZYMES SYNTHESIZING OR CONSUMING NAD^+ AND INFLAMMATION

NAD^+ roles extend beyond that of a coenzyme. As shown in Figure 2, NAD^+ links cellular metabolism status to inflammation and immune response. For instance, a recent study demonstrated that inhibition of PARP, the NAD-dependent enzyme, in a rat model of MI, protects against ischaemic myocardial damage by reduction in apoptosis and inflammation.[109] Furthermore, sirtuins can be regulators of inflammation. For instance, nuclear SIRT1 acts in association with PPAR-α to protect the heart from inflammation by inhibiting expression of pro-inflammatory cytokine monocyte chemoattractant protein-1 (MCP-1) in neonatal cardiomyocytes and blocked the activation of NF-κB as a result of exposure to phenylephrine.[157] Mice lacking SIRT7 have also been reported to undergo a reduction in lifespan and develop cardiac hypertrophy with increased inflammatory macrophages and cytokine levels (IL-12 and IL-13).[103]

As previously mentioned, inside the cell NAMPT is involved in the NAD salvage pathway. Outside the cell eNAMPT, also known as visfatin, acts as a pro-inflammatory cytokine, promoting development of cardiac hypertrophy and adverse cardiac remodelling with increased activation of mitogen-activated protein kinases, namely, JNK1, p38 and ERK,[158] although others have proposed that eNAMPT derived from monocytes promoted myocardial adaptation to pressure overload.[159] Serum NAMPT levels correlate with circulating inflammatory markers (IL-6, CRP and MCP-1).[160] In MI, circulating NAMPT levels and intracellular expression in macrophages and monocytes are enhanced.[161] Plasma NAMPT levels are more pronounced in coronary artery diseases compared to healthy controls.[160,162]

Notably, NAMPT has been associated with macrophage polarization. Inhibition of intracellular NAMPT was shown to attenuate M1 polarization of human macrophages, whereas neutralizing extracellular NAMPT reduced M2 polarization, as well as expression levels of IL-1ra, IL-4, IL-10 and IL-13.[160] Moreover, adding NAD^+ to cultured macrophages increased M2 polarization and the expression levels of IL-1ra and IL-10, which are anti-inflammatory (with no effect on M1). Somewhat consistent with these findings are the recent observations that intracellular NAMPT and NAD^+ appear to be necessary for activation of the major protein component of the inflammasome (NLRP3) of human primary monocytes, which are precursors of macrophages.[163] Their contribution was attributed to the maintenance of TLR4 signal transduction, which is critical as a priming signal for the NLRP3 inflammasome, specifically their importance for TLR4-induced phosphorylation of several downstream proteins in the MyD88-dependent signal pathway. SIRT2 has been

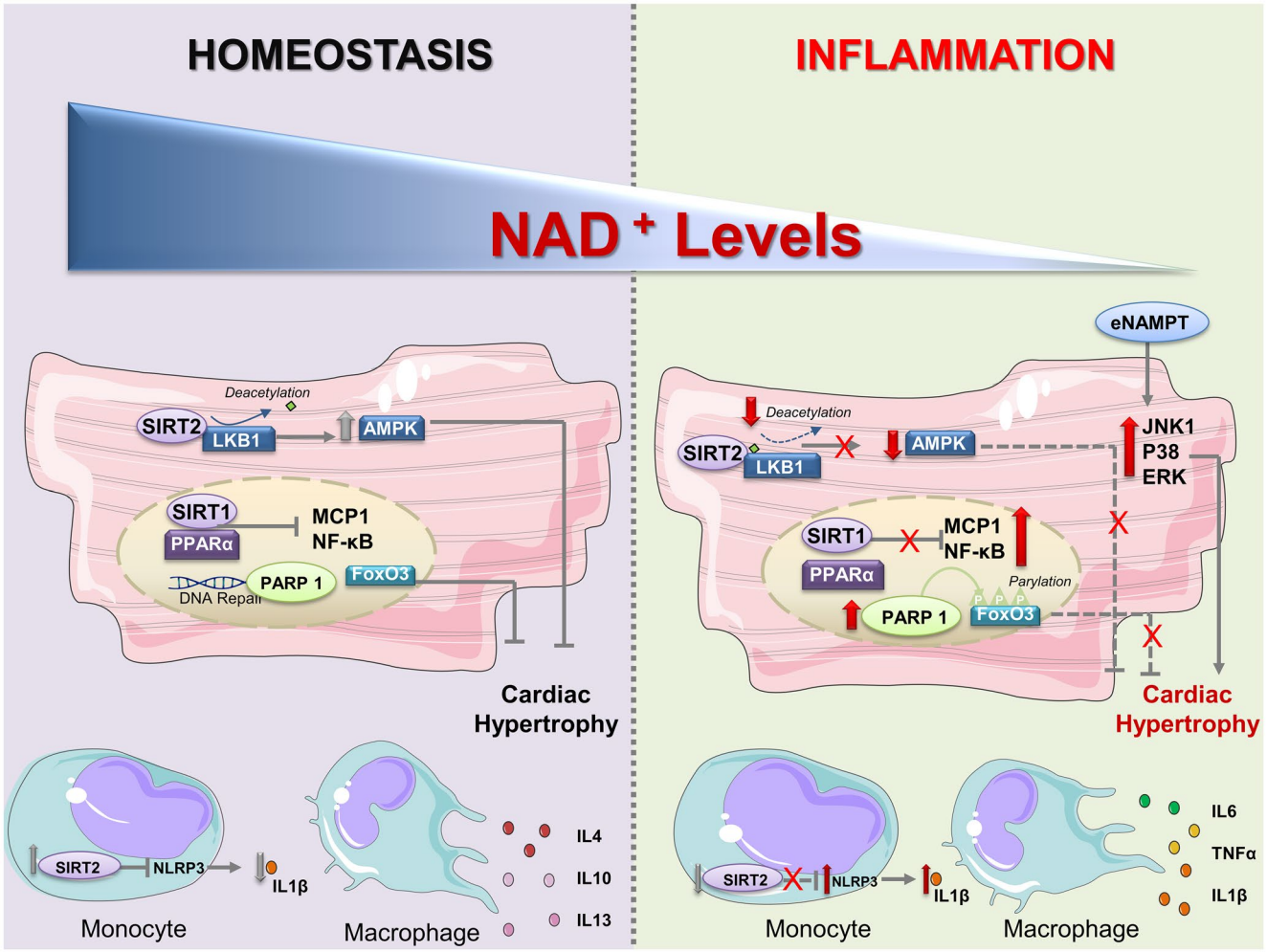

FIGURE 2 Diagram summarizing the main reported actions of enzymes involved in NAD biosynthesis or signalling pathways in relation to inflammation and NAD levels. Left Panel: In healthy conditions (NAD homeostasis), cytoplasmic SIRT2 promotes the activity of AMPK by deacetylating upstream LKB1. In the nucleus, SIRT1 acts in association with PPAR-α to protect the heart from inflammation by inhibiting the expression of MCP-1 and NF-κB. Nuclear PARP-1 functions as a DNA damage sensor that binds to both single- and double-stranded DNA breaks leading to DNA repair. FoxO3 transcription factor blocks hypertrophy. In monocytes, nicotinamide adenine dinucleotide (NAD^+) activates SIRT2, which inhibits the activation of NLRP3 inflammasome and decreases the production of pro-IL-1β. Right Panel: In stress conditions (low NAD levels), eNAMPT acts as a pro-inflammatory cytokine, promoting the development of cardiac hypertrophy with increased activation of MAPKs: JNK1, p38 and ERK. In the nucleus, SIRT1 promotes inflammation by inhibiting the expression of MCP-1 and NF-κB. Nuclear PARP-1 activity is upregulated, and is partly responsible for the drop in NAD levels. PARP-1 exacerbates cardiac hypertrophy partially by poly(ADP-ribosyl)ation of FoxO3. In monocytes, low NAD^+ levels deactivate SIRT2, which activates NLRP3 inflammasome activation and increase pro-IL-1β production. Loss of QPRT activity (lower NAD levels) elevates lactate, pentose phosphate pathway intermediates and pro-inflammatory TCA intermediates that favour production of interleukin-1β (IL-1β) and IL-6 over anti-inflammatory cytokines. Abbreviations: DNA, deoxyribonucleic acid; ERK, extracellular signal-regulated kinase; FoxO3, forkhead box O-3 transcription factor; JNK1, c-Jun N-terminal kinase-1; LKB1, liver kinase B1; MAPKs, mitogen-activated protein kinases; MCP-1, monocyte chemoattractant protein-1; NAD, nicotinamide adenine dinucleotide; NF-κB, nuclear factor-κB; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; p38, p38 mitogen-activated protein kinase; PARP 1, poly(ADP-ribose) polymerase 1; Poly (ADP-ribose) polymerase-1; PPARα, peroxisome proliferator-activated receptor alpha; SIRT1, Sirtuin 1

shown to protect the heart from cardiac hypertrophy stimuli by promoting the activity of AMPK by deacetylating its upstream kinase LKB1.[50]

Plasma NAMPT levels were evaluated in patients with ST elevation MI, at the time of being admitted to the hospital. These levels were higher than in controls and this elevation correlated with elevated levels of cardiac enzymes (creatine kinase, troponin I).[164] NR is also known to reduce obesity-related inflammation and is highly involved in inflammatory arthritis given the fact that NAMPT expression is elevated in the serum of a mouse model of arthritis. In addition, APO866, a specific competitive NAMPT inhibitor, efficiently reduced the severity and the progression of arthritis and decreased NAD^+ in inflammatory cells and TNF-α

levels.[165,166] Furthermore, a recent study showed that oral NR administration augments the aged human skeletal muscle NAD^+ metabolome and induces the release of circulating anti-inflammatory cytokines.[167]

Finally, a recent pre-clinical study showed that infection of certain cell lines with SARS-CoV-2, the virus responsible for COVID-19, destabilizes NAD^+ synthesis and utilization.[168] Cellular NAD^+ levels drop, leading to a decline in SIRT activity. The NLP3 inflammasome, usually controlled by SIRT, becomes hyperactivated, causing pulmonary fibrosis,[169] a COVID-19 characteristic. Thus, preserving balanced NAD^+ levels may attenuate COVID-19 symptoms.

Altogether, these studies show a complex role of NAD in inflammation with both pro and contra arguments on the necessity to modulate NAD levels during this process, and whether it should be increased or decreased would depend on the context and the period of inflammation.

8 | NAD PRECURSORS IN CLINICAL TRIALS

Although many NAD^+ boosters succeeded in several murine models, only a few have made it to clinical trials, such as the NAD^+ precursor, NA (niacin). NA significantly lowers LDL and increases HDL and is commercially available under the name of Niacor.[170] There is also Acipimox, an NA analogue, which has been shown to boost NAD levels and mitochondrial oxidative capacity.[31] Despite the fact that NA lowers LDL by raising NAD^+ levels, human cholesterol improvement is still unsettled as Tunaru et al previously demonstrated that NA mediates its anti-lipolytic effect via its receptors PUMA-G and HM74.[171] However, evidence with NR would suggest that reduced cholesterol arises from increased cellular NAD^+ levels and subsequent activation of SIRT1 in the liver, which influences the activity of transcription factors and cofactors linked to cholesterol homeostasis.[28]

Clinical trials involving NR supplementation are currently ongoing and have shown that orally administered NR is well tolerated with no adverse events.[29,149] In particular, Tramell and colleagues performed a randomized, double-blind study on 12 subjects and showed that only one dose of 1000 mg of NR was sufficient to increase NAD^+.[29] In addition, in a non-randomized trial, oral administration of NR resulted in a dose-dependent increase in NAD^+.[149] Martens et al showed in a randomized, double-blind, crossover clinical trial that chronic NR supplementation is well tolerated and efficient in stimulating NAD^+ metabolism in healthy middle-aged and older adults.[172] They also demonstrated that NR supplementation tended to reduce systolic blood pressure and aortic stiffness, major risk indicators of cardiovascular health status.[172] Martens et al not only assessed the effects of NR on cardiovascular parameters but also on other physiological functions. However, no change was found in energy expenditure and no enhancement in control glycaemia or insulin sensitivity.[172] A clinical trial to evaluate the advantage of NR in systolic heart failure is in progress with the aim of examining the effects of NR administration on several outcomes, including systolic and diastolic functions of the left ventricle.

Other clinical trials examined the effects of NRPT, a combination of NR and pterostilbene, a polyphenol found in blueberries.[173] Dellinger et al in the first-in-human clinical trial, performed on 120 healthy adults, showed that repeated NRPT doses are safe and efficient in chronically increasing NAD^+ levels without unfavourable effects.[173] NRPT significantly increased the amount of NAD^+ in a dose-dependent manner by approximately 40% in the NRPT(1x) group who received the recommended NRPT dose and around 90% in the NRPT(2x) group who received a double dose.[173] However, as noted in a letter to Clinical Nutrition, pterostilbene was reported to raise low-density lipoprotein ("bad") cholesterol in people in a dose-dependent manner,[174] an effect also present in the NPRT study.[173]

Other more recent NAD precursors are currently being tested in clinical trials. Despite the fact that the preliminary results so far in small human clinical trials look encouraging, much still remains to be done. From a practical point of view there are a lot of issues to consider regarding NAD boosters such as the best delivery method, the choice of optimal and safe doses, the distribution of NAD in different tissues and their uptake into the cell. All of this is needed to realize the desired efficacy in specific diseases.

9 | CONCLUSION AND PERSPECTIVE

Although the discovery of NAD has become history, there is still much to learn about its pharmacology and role in human diseases. Numerous studies have shown that NAD homeostasis is required for normal cardiac function. The concept of increasing NAD levels as a therapeutic strategy has been demonstrated to be beneficial in pre-clinical models of several cardiovascular pathologies, including DCM.[23] Various pharmacological strategies may be employed to boost NAD levels, including NAD precursor supplements, NAD biosynthetic enzyme activation and NAD depletion inhibitors. In order to broadly use NAD boosters, the safety profile and fundamental aspects of this coenzyme should be studied substantially. Several natural and synthetic NAD precursors have been tested in humans, or are currently undergoing clinical assessment. What we know so far is that NAD^+ boosters are relatively safe, but

the question remains as to how likely is it to translate their therapeutic promise to humans.

ACKNOWLEDGEMENTS

GWB acknowledges the Department of Pharmacology and Toxicology at the University of Mississippi Medical Center.

CONFLICT OF INTEREST

The authors declare no competing financial interests and have nothing to disclose.

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How to cite this article: Tannous C, Booz GW, Altara R, et al. Nicotinamide adenine dinucleotide: Biosynthesis, consumption and therapeutic role in cardiac diseases. *Acta Physiol*. 2021;231:e13551. https://doi.org/10.1111/apha.13551