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NAD+ Metabolism in Cardiac Health, Aging, and Disease

Mahmoud Abdellatif, MD, PhD; Simon Sedej, PhD; Guido Kroemer, MD, PhD

ABSTRACT: Nicotinamide adenine dinucleotide (NAD+) is a central metabolite involved in energy and redox homeostasis as well as in DNA repair and protein deacetylation reactions. Pharmacological or genetic inhibition of NAD+-degrading enzymes, external supplementation of NAD+ precursors, and transgenic overexpression of NAD+-generating enzymes have wide positive effects on metabolic health and age-associated diseases. NAD+ pools tend to decline with normal aging, obesity, and hypertension, which are all major risk factors for cardiovascular disease, and NAD+ replenishment extends healthspan, avoids metabolic syndrome, and reduces blood pressure in preclinical models. In addition, experimental elevation of NAD+ improves atherosclerosis, ischemic, diabetic, arrhythmogenic, hypertrophic, or dilated cardiomyopathies, as well as different modalities of heart failure. Here, we critically discuss cardiomyocyte-specific circuitries of NAD+ metabolism, comparatively evaluate distinct NAD+ precursors for their preclinical efficacy, and raise outstanding questions on the optimal design of clinical trials in which NAD+ replenishment or supraphysiological NAD+ elevations are assessed for the prevention or treatment of major cardiac diseases. We surmise that patients with hitherto intractable cardiac diseases such as heart failure with preserved ejection fraction may profit from the administration of NAD+ precursors. The development of such NAD+-centered treatments will rely on technological and conceptual progress on the fine regulation of NAD+ metabolism.

Key Words: cardiomyopathy ■ heart failure ■ human ■ NAD ■ nicotinamide ■ nicotinamide mononucleotide ■ obesity

Nicotinamide adenine dinucleotide (NAD) is essential for the metabolism of eukaryotic cells. The capacity of NAD to shuttle electrons between its oxidized (NAD+) and reduced (NADH) forms is indispensable for oxidation-reduction reactions that capture or liberate cellular energy in the form of ATP. Beyond its role in energy metabolism, NAD+ has also been recognized as a pivotal signaling molecule and a rate-limiting substrate of multiple enzymes involved in DNA repair, epigenetic regulation, posttranslational modifications, and metabolic adaptation to changing nutritional states.[1]

Over the period of the last decade, a growing repertoire of studies transformed our understanding of NAD+ biology and its pathophysiological implications.[2,3] In this regard, experimental strategies for NAD+ repletion can delay several hallmarks of aging and simultaneously suppress the manifestation of age-related diseases in rodent models.[4–7] On the basis of these observations, NAD+ precursors harbor promise as antiaging drugs, igniting renewed interest in the metabolism and pleiotropic action of NAD+. However, in spite of the accumulating preclinical evidence in favor of the broad health-improving effects of NAD+ enhancers,[8–10] only few clinical trials have been performed in humans.

In the context of cardiovascular morbidity, emerging preclinical evidence indicates that increasing cellular NAD+ content might represent a promising therapeutic avenue.[11–13] In support of this idea, disrupted NAD+ metabolism is increasingly considered as an amendable cardiovascular risk factor.[6,14] In fact, the pathogenesis of various chronic cardiovascular diseases has been consistently shown to coincide with perturbations of NAD+ homeostasis.[15–17] The cardiovascular system is particularly vulnerable to such dysregulation in NAD+ metabolism because of the high energy demand of the heart.[18] Specifically, depletion of intracellular NAD+ impairs mitochondrial fatty acid β-oxidation and oxidative phosphorylation, underscoring that adequate NAD+

Correspondence to: Simon Sedej, PhD, Auenbruggerplatz 15, 8036 Graz, Austria; or Guido Kroemer, MD, PhD, 15 rue de l'Ecole de Médecine, Paris 75006, France. Email simon.sedej@medunigraz.at or kroemer@orange.fr

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Nonstandard Abbreviations and Acronyms

cADPR	cyclic ADP-ribose
CD38	cyclic ADP-ribose synthase
CD73	ecto-5'-nucleotidase
DCM	dilated cardiomyopathy
FXN	frataxin encoding gene
HFpEF	heart failure with preserved ejection fraction
HFrEF	heart failure with reduced ejection fraction
I/R	ischemia/reperfusion
Lmna	lamin A/C encoding gene
L-NAME	N-nitro-L-arginine methyl ester
Me-NAM	methyl-nicotinamide
NA	nicotinic acid
NAD+	nicotinamide adenine dinucleotide, oxidized form
NADH	nicotinamide adenine dinucleotide, reduced form
NAM	nicotinamide
NaMN	nicotinic acid mononucleotide
NAMPT	nicotinamide phosphoribosyltransferase
NMN	nicotinamide mononucleotide
NMNAT	nicotinamide mononucleotide adenylytransferase
NR	nicotinamide riboside
NRK	nicotinamide riboside kinase
PARP	poly(ADP-ribose) polymerase
PBMCs	peripheral blood mononuclear cells
PKC	protein kinase C
SIRT	sirtuin deacetylase
SRF	serum response factor
TAC	transverse aortic banding

availability is critical for the maintenance of myocardial bioenergetic efficiency and, thus, normal pump function. Diminution of the cardiovascular NAD+ pool below a critical threshold also entails major dysfunctions at the cellular level. These include, but are not limited to, deregulated nutrient sensing, epigenetic and gene dysregulation, autophagy impairment, and low-grade inflammation, all of which can independently fuel the development of cardiovascular disease.[1]

In this in-depth review, we discuss the current understanding of NAD+ metabolism, and how impaired NAD+ homeostasis aggravates common cardiometabolic risk factors, thus favoring cardiovascular morbidity. We then delve into the therapeutic potential of different NAD+-based therapies against prevalent cardiovascular maladies, ranging from ischemic, hypertrophic, arrhythmogenic, diabetic, and dilated cardiomyopathies to heart failure. Last, we present unfolding clinical evidence in favor of the utility of NAD+ precursors in cardiovascular medicine.

NAD+ METABOLISM IN THE HEART AND CIRCULATION

Cardiomyocytes accumulate NAD+ mostly within their mitochondria,[19] where the bulk of cellular oxidation-reduction reactions occur. However, NAD+ is also present in the cytosol and nucleus, where NAD+-derived metabolites and NAD+-dependent enzymes contribute to various cellular functions.[1] In recent years, significant progress has been made in the understanding of tissue-specific NAD+ synthesis, transport, and catabolism.

NAD+ Biosynthesis

Although the liver and, to a lesser extent, the kidney can synthesize NAD+ from the amino acid tryptophan through the kynurenine pathway, the majority of organs, including the heart, lack the enzymes necessary for the de novo biosynthesis of NAD+ (Figure 1A). Instead, cardiac cells generate NAD+ from preformed pyridine moieties such as nicotinamide. Nicotinamide is intracellularly available as an end-product of nonoxidative NAD+ catabolism and, thus, represents a readily available substrate for NAD+ production by NAMPT (nicotinamide phosphoribosyltransferase), the main rate-limiting enzyme in the NAD+ salvage pathway (Figure 2). However, intracellular recycling of NAD+ is not unlimited, because nicotinamide is also regularly metabolized and excreted in urine. Therefore, dietary intake of NAD+ precursors, such as nicotinamide, nicotinic acid (NA), and nicotinamide riboside (NR)—collectively known as vitamin B3—is required to sustain organismal NAD+ homeostasis. These NAD+ precursors are intracellularly converted to NAD+ through the amidated or deamidated pathways (Figure 2). Explicitly, NAMPT and NMRKs (NR kinases) convert nicotinamide and NR, respectively, into nicotinamide mononucleotide (NMN) via the amidated pathway, whereas NA enters the deamidated pathway to form NA mononucleotide. Both NMN and NA mononucleotide are subsequently used as substrates for NAD+ production in the reaction catalyzed by NMN adenylytransferases.

Different NAD+ precursors might vary in their efficacy to replenish NAD+, both in a tissue- and context-dependent manner. With respect to the human heart, gene expression data (Figure 1A) indicate that NAD+ biosynthetic enzymes of the amidated pathway are much more abundant than those of the deamidated pathway. Indeed, the amidated pathway accounts for 99.3% of cardiac NAD+ stores.[20] The protein expression of NMRKs and NAMPT appears to be highly context-dependent. Under physiological conditions, NMRKs are not detectable by protein immunoblotting,[21] suggesting that NAMPT might be the sole rate-limiting enzyme for NAD+ biosynthesis expressed in the healthy

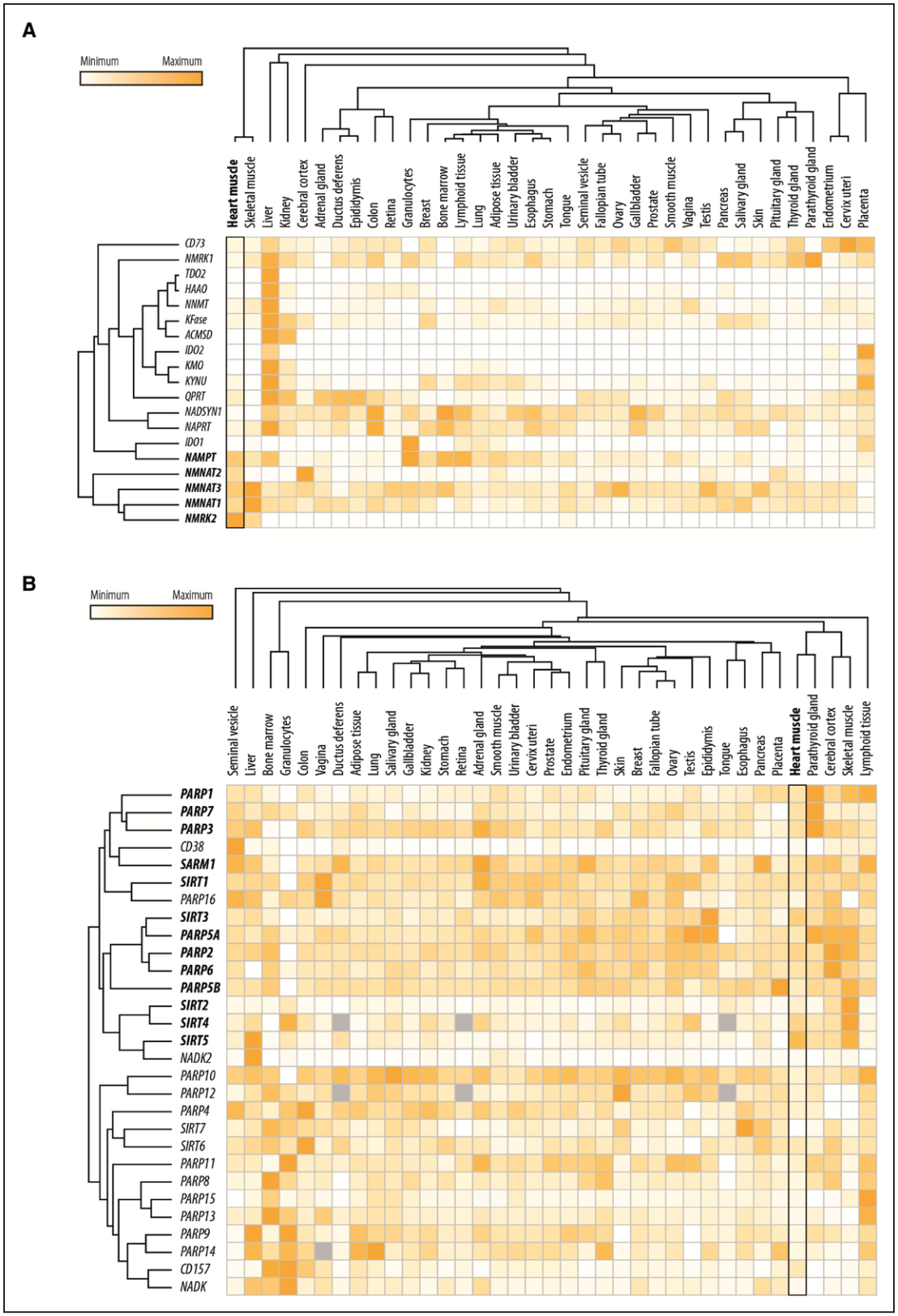

Figure 1. Gene expression levels of the enzymes involved in NAD+ metabolism.
Shown are the relative gene expression levels of the enzymes involved in (**A**) the deamidated and amidated pathways of NAD+ biosynthesis as well as (**B**) those involved in NAD+ catabolism in the heart and other organs/tissues. The data were retrieved from The Human Protein Atlas (http://www.proteinatlas.org/) and subsequently subjected to hierarchical clustering in Morpheus (https://software.broadinstitute.org/morpheus). Data not available in the atlas are represented by gray boxes. NAD+ indicates nicotinamide adenine dinucleotide, oxidized form.

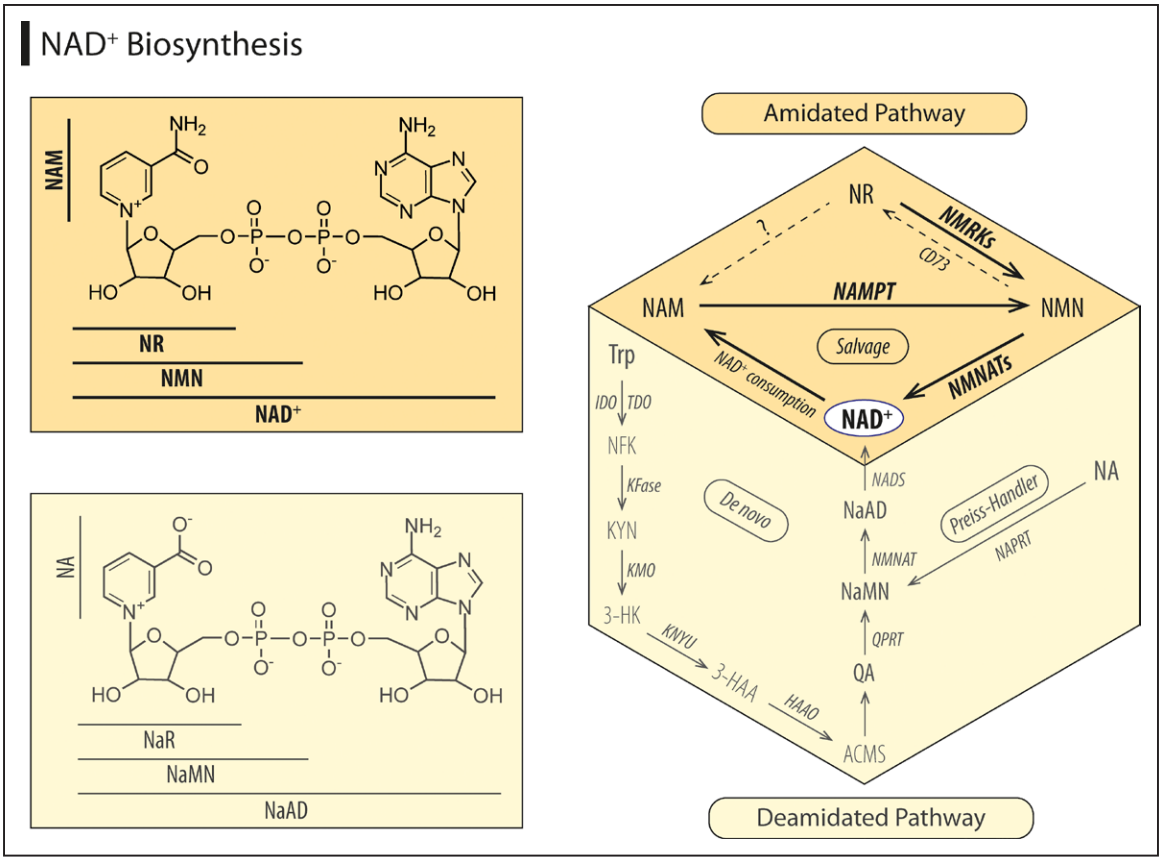

Figure 2. Biosynthetic pathways of NAD+.
Because the heart lacks the enzymes necessary for the de novo biosynthesis of NAD+ from the amino acid tryptophan (Trp), cardiac cells instead salvage NAD+ from preformed pyridine moieties, such as nicotinamide (NAM), nicotinic acid (NA), or nicotinamide riboside (NR)—collectively known as vitamin B3. These NAD+ precursors are metabolized to NAD+ through the amidated pathway, where NAM phosphoribosyltransferase (NAMPT) and NR kinases (NMRKs) convert NAM and NR, respectively, into nicotinamide mononucleotide (NMN), whereas NA is introduced to the Preiss-Handler pathway to form NA mononucleotide (NaMN). Both NMN and NaMN are subsequently used as substrates for NAD+ production in the reaction catalyzed by NMN adenylytransferase (NMNAT). Highlighted in dark color are the enzymes and precursors that are sufficiently expressed in the heart (Figure 1A). 3-HAA indicates 3-hydroxyanthranilic acid; 3-HK, 3-hydroxykynurenine; ACMS, 2-amino-3-carboxymuconate-6-semialdehyde; CD73, ecto-5′-nucleotidase; HAAO, 3-hydroxyanthranilic acid dioxygenase; IDO, indoleamine-2,3-dioxygenase; KFase, kynurenine formamidase; KMO, kynurenine 3-monooxygenase; KYN, kynurenine; KYNU, kynureninase; NAAD, NA adenine dinucleotide; NAD+, nicotinamide adenine dinucleotide, oxidized form; NADS, NAD synthetase; NAPRT, NA phosphoribosyltransferase; NFK, N-formylkynurenine; QA, quinolinic acid; QPRT, quinolinate phosphoribosyltransferase; and TDO, tryptophan-2,3-dioxygenase.

heart. Hence, it is conceivable that nicotinamide is the primary source of cardiac NAD+ under physiological conditions, especially because nicotinamide is the most abundant NAD+ precursor in the circulation (2000 nmol/L of nicotinamide versus 7 nmol/L of NR).[21,22] In support of this notion, acute NR administration fails to increase cardiac NAD+ content in healthy mice, contrasting with its ability to enhance hepatic NAD+ levels.[23] By contrast, dilated cardiomyopathy (DCM) is associated with reduced NAMPT expression and NMRK upregulation, and thus, NR effectively replenishes cardiac NAD+.[16,24,25]

NAD+ Transport and Delivery

NAD+ cannot cross the plasma membrane by passive diffusion because of its hydrophilicity, positive charge, and molecular size. Therefore, mammalian cells import NAD+ precursors for intracellular NAD+ synthesis.[26] Among these, nicotinamide and NA are the smallest and most membrane-permeant molecules.[26–28] NR is transported into cells through the equilibrative nucleoside transporter family members.[29] As for NMN, initial evidence suggested that it must be dephosphorylated to NR by NT5E (ecto-5'-nucleotidase, best known as CD73) before entering the cell. In fact, deletion of NMRKs, which are required for NR conversion to NAD+, limits the ability of NMN to elevate intracellular NAD+ levels.[21,30] More recently, however, the cation/chloride cotransporter SLC12A8 has been recognized as a specific (intestinal) NMN transporter.[31] Hence, future in vivo studies are required to further clarify the transport mechanisms of NAD+ and its precursors in the cardiovascular system, including the recently discovered mammalian mitochondrial NAD+ transporter, SLC25A51/MCART1.[32–34]

Another area of intense investigation concerns the biochemical conversions that NAD+ precursors undergo

in vivo before they reach the heart or other peripheral tissues. Recent reports suggest that orally administered NAD+ precursors are subjected to extensive first-pass metabolism in the gastrointestinal tract, liver, and later on in the circulation.[22,35] Stable isotope tracing revealed that both nicotinamide and NR are converted by the gut microbiota into NA, which is subsequently incorporated into the intestinal and hepatic NAD+ pools through the deamidated pathway.[35] In fact, detectable NAD+ metabolites in the circulation appeared to be generated from both the deamidated and amidated pathways, indicating that oral nicotinamide and NR supplementation stimulates both pathways in vivo. Another mouse study reported that orally delivered NR and NMN are almost entirely converted to nicotinamide before reaching the circulation.[22] Other studies also reported that intraperitoneal injection of NR and NMN is followed by a surge in the circulating levels of nicotinamide.[21,23,36] Even intravenous administration of NR and NMN results within minutes in a substantial increase in nicotinamide plasma levels, suggesting an instantaneous conversion of NR and NMN into nicotinamide within the extracellular space.[22] In support of this notion, NR is quickly degraded to nicotinamide when added to murine plasma in vitro,[21] whereas intravenously injected NMN is barely detectable in the circulation.[22] Although these findings indicate that different NAD+ precursors are converted into nicotinamide, available evidence argues against the idea that all these molecules exert identical effects. For instance, as opposed to nicotinamide, NR efficiently restores NAD+ levels and cardiac function in genetic models of DCM.[24] On the flip side, NR failed to improve human skeletal muscle mitochondrial defects,[37] whereas NA convincingly restored NAD+ levels and improved mitochondrial myopathy in patients.[38]

Taken together, the metabolism of NAD+ precursors appears to be much more complex than initially anticipated, especially in extrahepatic tissues. Hence, systematic studies should perform head-to-head comparisons of different NAD+ precursors with respect to their galenic and disease-specific properties.

NAD+ Consumption

Steady-state levels of NAD+ are determined not only by its biosynthesis but also by the rate of its utilization. Three classes of enzymes, SIRTs (the sirtuin family of deacetylases), PARPs (poly(ADP-ribose) polymerases), and cADPR (cyclic ADP-ribose) synthases, are known to consume NAD+, resulting in its net catabolism to nicotinamide (Figure 3).

Mammalian SIRTs are composed of a family of 7 members (SIRT1–7), which are operative in different cellular compartments, including the nucleus (SIRT1, SIRT6, and SIRT7), cytoplasm (SIRT2), and mitochondria (SIRT3, SIRT4, and SIRT5). Sirtuins are energy sensors that use NAD+ as a cosubstrate to regulate cellular metabolism.[39] However, the Michaelis constant values of different sirtuins vary significantly,[2] indicating that NAD+ concentration does not equally affect the activity of different sirtuins. Indeed, SIRT2, SIRT4, SIRT5, and SIRT6 operate at subphysiological Michaelis constant values, and thus, their activity is not rate-limited by NAD+, whereas SIRT1 and SIRT3 are highly dependent on NAD+ bioavailability.[2] In fact, available evidence indicates that NAD+-replenishing interventions mediate many of their effects in the cardiovascular system through an increase in the activity of SIRT1 and SIRT3.[40]

At variance with sirtuins, all PARPs are active at rather low levels of NAD+; however, under conditions of increased DNA damage, PARPs may consume significant amounts of NAD+, which then become rate-limiting.[22,41] The PARP family is composed of 16 enzymes in mice and 17 in humans, among which PARP1 and PARP2 are considered key DNA damage responders, and thus are required for DNA repair and stability. In contrast, little is known about the function of other PARP family members, as well as their contribution to global or compartment-specific NAD+ consumption.

Another class of NAD+-consuming enzymes is constituted by the cyclic ADP-ribose synthases, including CD38 and its homolog BST1 (bone marrow stromal cell antigen 1, best known as CD157). These ectoenzymes are mainly expressed by immune cells and apparently consume significant amounts of NAD+ to the extent that they are also referred to as NADases.[42] Although under normal conditions, high expression of CD38 is only evident in tissues with abundant immune cell populations (Figure 1B), aging and pathological conditions associated with elevated immune cell infiltration cause higher expression of CD38 in various tissues.[43,44] Notably, cADPR—the product of NAD+ consumption by CD38—is known to regulate calcium homeostasis, and thus might affect cardiomyocyte excitation-contraction coupling.[45]

Taken together, different enzymes consume NAD+ at different rates and in a cell type- and context-dependent fashion. Although most of the NAD+-using enzymes are expressed in the heart (Figure 1B), their relative contribution to net cardiac NAD+ catabolism remains to be elucidated. To this end, for a comprehensive overview of the (patho-)physiological cardiovascular role of these enzymes, we refer the readers to excellent reviews focusing on sirtuins,[14,40,46,47] PARPs,[48,49] or CD38.[50]

NAD+ AND CARDIOVASCULAR RISK FACTORS

Epidemiological as well as preclinical studies suggest that old age and obesity are among the most important factors that erode health at all levels, including in the cardiovascular system.[51,52]

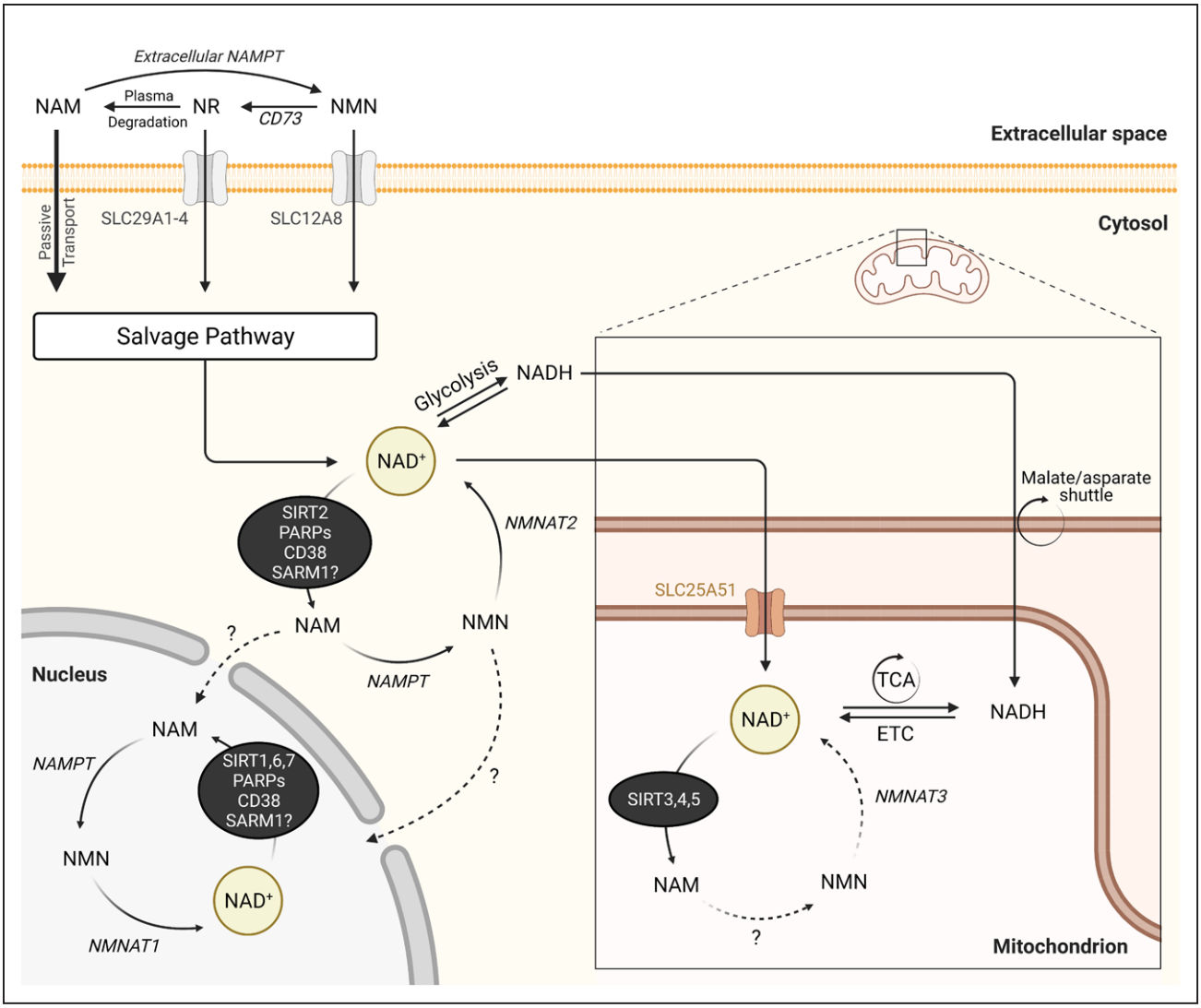

Figure 3. NAD+ catabolic pathways.
After their uptake, NAD+ precursors are converted to NAD+ in different subcellular compartments including the mitochondria, cytosol, and nucleus. Accordingly, generated NAD+ controls cellular redox reactions, but also NAD+-dependent enzymes. The latter are responsible for the net catabolism of NAD+ and are composed of 3 classes: SIRTs (the sirtuin family of deacetylases), PARPs (poly(ADP-ribose) polymerases), and the cADPRs (cyclic ADP-ribose) synthases. In addition, SARM1 (sterile α and Toll/interleukin-1 receptor motif-containing 1) has an intrinsic NADase activity, thereby cleaving NAD+ and producing NAM as an end product. ETC indicates electron transport chain; NAD+, nicotinamide adenine dinucleotide, oxidized form; NAM, nicotinamide; NAMPT, nicotinamide phosphoribosyltransferase; NMN, nicotinamide mononucleotide; NMNAT, nicotinamide mononucleotide adenylytransferase; NR, nicotinamide riboside; and TCA, tricarboxylic acid cycle. This figure was created with BioRender.com.

NAD+ Dysregulation in Aging

Intracellular NAD+ concentrations decline with age in various tissues and species, including in humans.[10,42–44,53–56] Specifically in the heart, the decline in NAD+ content varies significantly between species and studies, reporting a 0% to 65% reduction in 2-year-old rodents.[57–59] Steady-state NAD+ concentration might decline because of a progressive decay in NAD+ biosynthesis, increased activity of NAD+ degradation enzymes, or a combination of both. On NAD+ biosynthesis, downregulation of NAMPT has been implicated in the age-related decline of NAD+ concentration. However, although this has been documented for multiple tissues/organs,[60] it is still unknown whether this also occurs in the heart. Alternatively, the age-related decline in circulating levels of extracellular NAMPT that was documented in mice and humans might indirectly affect systemic NAD+ levels.[56]

A growing body of evidence implicates CD38 as a major culprit in age-related NAD+ decline in mammals.[42–44] Thus, CD38-deficient aged mice exhibit increased NAD+ content in various tissues.[42] Similarly, a specific CD38 inhibitor reverses age-related NAD+ degradation and improves several aspects of health, including cardiac function in aged mice.[57] It is interesting that inhibiting CD38 was found to increase NAD+ through an NMN-dependent mechanism, suggesting that in addition

to NAD+, NMN is an alternative substrate for CD38.[43] Indeed, because of its unique cellular localization with the catalytic site toward the extracellular space, CD38 exhibits an ectoenzymatic activity that degrades circulating NMN in vivo.[42] As such, coadministration of NAD+ precursors and CD38 antagonists might be more efficacious than CD38 inhibition alone for delaying cardiac aging.[57] Because CD38 is predominantly expressed in immune cells,[61] it might contribute to a variable extent to the decline of NAD+ content, depending on the abundancy of tissue-resident immune cells.[57] Indeed, the progressive accumulation of senescent cells that secrete proinflammatory cytokines has been shown to elevate CD38 tissue levels and hence to promote the age-associated diminution of NAD+ and NMN.[43,44] Because murine and human cardiomyocytes are subjected to senescence,[62] it is plausible that CD38 might be also involved in the age-related NAD+ decline in the heart, despite the seemingly negligible transcriptional expression of CD38 under baseline/healthy conditions (Figure 1B).

PARPs reportedly consume NAD+ to repair age-related DNA damage in aging tissues.[63] By contrast, PARP inhibition replenishes NAD+ in aged organisms and prevents premature aging caused by deficient DNA repair in several mouse models.[41,64,65] In the myocardium, maintaining DNA stability is crucial for normal cellular functions, especially in the postmitotic cardiomyocytes. Given their limited regenerative capacity,[66] these long-lived cells are exposed to accumulating metabolic and oxidative damage throughout their lifetimes, which ultimately causes DNA damage and PARP activation, thereby reducing NAD+ concentration in the aging heart.[58] Activated PARPs might consume significant amounts of NAD+ to fuel the DNA surveillance and repair machinery. However, in the case of genotoxic stress and metabolic collapse caused by excessive DNA damage and NAD+ depletion, respectively, excessive PARP activation might ignite cell death pathways, as reported for a mouse model of heart failure induced by pressure overload.[67]

Exogenous NAD+ supplementation or overexpression of either NAMPT or NMN adenylyltransferase restores cellular NAD+ levels and prevents cardiac myocyte death in vitro,[67] suggesting that increased NAD+ bioavailability or synthesis can counterbalance increased NAD+ consumption. In support of this notion, late-in-life dietary intake of nicotinamide delays the hallmarks of cardiac aging in C57BL/6 mice, including reduced cardiac hypertrophy and diastolic dysfunction.[15] Along similar lines, oral NMN supplementation to aged mice elicits geroprotective effects on the vasculature by improving aortic stiffness in association with increased arterial SIRT1 activation and reduced vascular oxidative stress.[68] Another study reported improved cerebromicrovascular circulation and neurovascular coupling in NMN-treated aged mice.[69] Several extracardiac metabolic benefits were also reported for aged mice treated with NMN, nicotinamide, or NR.[9,60,70] Intriguingly, the benefits of NAD+ precursors on mammalian healthspan do not necessarily correlate with parallel gains in lifespan.[9,71] However, a report suggested that late-in-life NR administration modestly extends longevity.[10]

Taken together, a large body of evidence supports that NAD+-regenerative strategies have a large antiaging potential that extends to the cardiovascular system. However, supraphysiological levels of cardiac NAD+ are not necessarily advantageous, because embryonic overexpression of NAMPT reportedly leads to cardiac hypertrophy in young (6-month-old) mice.[72] NAD+ precursors are likely to be beneficial because they avoid the age-related NAD+ overconsumption caused by inflammation (CD38) and DNA damage (PARP1). This assertion has been recently substantiated in an elegant study by McReynolds and colleagues.[59] The authors performed NAD+ flux measurements in 25-month-old mice, revealing that a modest and tissue-specific decline in NAD+ is explained by increased NAD+ degradation rather than impaired NAD+ production.[59]

NAD+ Dysregulation in Obesity

Similar to other cell types, the energy state of cardiomyocytes is reflected by the cellular NAD+/NADH ratio, and the failure to maintain the NAD+ pool is sufficient to cause metabolic imbalance, energy deficit, and functional decompensation.[73] In this context, it is important to note that the metabolic changes that occur in cardiac NAD+ metabolism depend not only on the severity of the cardiomyopathy but also on the comorbidities, in particular obesity, diabetes, and hypertension.[73] A plausible explanation for how obesity might induce NAD+ decline resides in the associated subclinical inflammatory state.[74] Such a sterile (systemic) inflammation might downregulate NAMPT expression, thereby reducing the NAD+ salvage pathway activity in multiple tissues and organs,[60] and possibly also the heart. In support of this hypothesis, mice with reduced NAD+ content in their fat depots because of adipocyte-specific NAMPT deletion display local (adipose tissue) inflammation but also a severe multiorgan insulin resistance with a 50% reduction of insulin-induced glucose uptake in the heart, which can be rescued with NMN.[75] Consistently, intraperitoneal injection of NMN stimulates NAD+ biosynthesis that can reinstate blood glucose control in obese wild-type mice,[60] and in mice with systemic *Nampt* haplodeficiency.[76] Other studies using alternative NAD+ precursors, including nicotinamide and NR, have substantiated such metabolic benefits.[9,77–79] Intriguingly, nicotinamide-mediated metabolic benefits associate with increased, not decreased, SIRT1 expression.[9,79] This observation has been reproduced in the heart,[15] and is likely mediated by methyl-nicotinamide–induced inhibition of SIRT1 proteolysis.[80]

Besides stimulating NAD+ biosynthesis, targeting NAD+ degradation pathways may improve several physiological and metabolic aspects of diet-induced obesity. For example, mice with *Parp1* or *Cd38* deficiency, and mice treated with PARP or CD38 inhibitors, exhibit improved glucose and lipid homeostasis,[42,57,64,81,82] as well as exercise capacity.[83] Increased NAD+ content in response to these interventions correlates with an increased deacylase activity of SIRT1 and SIRT3 that results in enhanced mitochondrial biogenesis, oxidative phosphorylation, and energy expenditure.[42,64] Moreover, the elevation of intracellular NAD+ levels occurring after CD38 deletion protects the mouse heart from high-fat diet (HFD)–induced oxidative stress via activating the SIRT3/FOXO3-mediated antioxidative stress pathway.[84]

NAD+ Dysregulation in Hypertension

Considering that hypertension is intimately linked to aging and obesity, which both are associated with NAD+ deficiency, NAD+ metabolism has emerged as a potential therapeutic target for hypertension. Indeed, NAMPT expression was found to be downregulated in clinical and experimental hypertension.[85] In contrast, systemic NAMPT overexpression protected mice from angiotensin II–induced hypertension.[85] Along the same lines, increased NAD+ biosynthesis upon nicotinamide supplementation lowers systolic blood pressure in N-nitro-L-arginine methyl ester–treated mice, *eNOS*$^{-/-}$ mice, and *Dahl* salt-sensitive rats.[15,86] Although the precise mechanisms underlying such antihypertensive nicotinamide effects are elusive, reduced inflammation has been suggested to be involved.[86] Taking into account that nicotinamide is generally regarded safe in humans, it merits further evaluation as an adjuvant therapy of hypertension. Indeed, a recent pilot study showed that supplementation of the alternative NAD+ precursor NR for 6 weeks causes a mild reduction in blood pressure and aortic stiffness in healthy middle-aged and older adults.[87] Future trials must examine the effects of NAD+ on patients with hypertension in whom the reduction of blood pressure might be more important.

TARGETING NAD+ METABOLISM IN EXPERIMENTAL MODELS OF CARDIOVASCULAR DISEASE

As the interest in NAD+ metabolism increased, so did the number of studies that examined NAD+ and its precursors role in a wide range of cardiovascular disorders (Table 1).

Atherosclerotic and Other Vascular Diseases

NA (also commonly referred to as niacin) has long been known for its lipid-lowering ability.[107] However, the addition of niacin to standard lipid-lowering therapy using statins failed to confer additional benefits to high-risk cardiovascular patients.[108–110] Available preclinical evidence on the role of NAD+ in atherosclerosis is also inconclusive. For example, pharmacological inhibition of systemic NAMPT activity evokes an atheroprotective effect in WT mice,[111] whereas global overexpression of NAMPT exacerbates atherosclerosis in apolipoprotein E–deficient (*ApoE*$^{-/-}$) mice.[112] By contrast, leukocyte-restricted overexpression of NAMPT effectively protects from Western diet–induced atherosclerotic plaques in low-density lipoprotein receptor–deficient (*Ldlr*$^{-/-}$) mice, correlating with marked anti-inflammatory effects.[113] In light of these inconsistent findings, future studies should explore the effects of cell type–specific overexpression or deletion of NAMPT on atherogenesis and hyperlipidemia.

Beyond its lipid-lowering action, NA exerts potent anti-inflammatory effects, including in human endothelial and immune cells.[114,115] Accordingly, NAD+ replenishment using NA decreases immune cell infiltration and matrix degradation, leading to reduced formation of abdominal aortic aneurysms in mice subjected to calcium chloride or angiotensin II infusion.[88] Nicotinamide, which arguably has no detectable lipid-lowering effect,[116] also confers protective effects against abdominal aortic aneurysms.[88] Nicotinamide-treated mice showed enhanced SIRT1 activity, and coadministration of the SIRT1 inhibitor EX527 abolished the vasoprotective effects of nicotinamide.[88]

Ischemic Cardiomyopathy

Early evidence obtained from experimental models of cardiac ischemia/reperfusion (I/R) indicates that cardiac NAD+ levels decline in response to I/R injury in mice and dogs.[89,90,117] It is interesting that myocardial NAD+ deficiency in dogs was evident in both ischemic and nonischemic cardiac regions, whereas restoring NAD+ levels by intravenous administration of NAD+ improved myocardial bioenergetics.[89,90] In mice, myocardial NAD+ depletion is detectable as early as 15 minutes after I/R. By contrast, genetic ablation of PARPs preserves myocardial NAD+ content, restricts myocardial infarct size, and attenuates the resulting proinflammatory response.[117] Along similar lines, CD38-deficient mice, which have a preserved NAD+ pool, are resistant to myocardial ischemia and I/R injuries.[118–120] Pharmacological inhibition of CD38 also suppresses I/R-induced myocardial infarction and promotes recovery of cardiac function.[121] Consistently, aminobenzamide (3-AB), a pharmacological PARP inhibitor, reproduces such cardioprotective effects in rats subjected to I/R or myocardial infarction.[122,123] However, extended PARP inhibition reverts these benefits, perhaps because of the suppression of PARP-dependent DNA repair.[124]

Table 1. List of Cardiovascular Disorders for Which There Is Direct Preclinical Evidence for the Therapeutic Potential of Increased NAD+ Biosynthesis

Condition	Intervention/dosage	Animal model	Effects	Proposed mechanisms	Reference
Aortic aneurysm	Niacin (0.3% wt/vol in the drinking water) NAM (0.4% wt/vol in the drinking water)	Mice subjected to calcium chloride or angiotensin II infusion	- Decreased immune cell infiltration and matrix degradation - Reduced abdominal aortic aneurysms formation	- Reduced inflammation and SIRT1 activation - Causality testing: coadministration of the SIRT1 inhibitor EX-527 abolished the vasoprotective effects of NAM	88
Ischemic cardiomyopathy	Cardiomyocyte-specific overexpression of NAMPT	A mouse model of myocardial ischemia-reperfusion injury	- Enhanced ATP levels - Attenuated apoptotic cell death - Reduced myocardial infarction size	- Improved bioenergetics - Autophagy induction	17
	NAD+ (0.5 mg/kg BW, IV)	A dog model of myocardial ischemia-reperfusion injury	- Elevated myocardial content of creatine phosphate and ATP/ADP ratio	- Improved bioenergetics	89,90
	NAD+ (10–20 mg/kg BW, IV)	A rat model of myocardial ischemia-reperfusion injury (in vivo or ex vivo)	- Reduced accumulation of ischemic succinate - Reduced infarct size (in vivo) And cardiac dysfunction (ex vivo)	- Reduced oxidative stress	91,92
	NAD+ (20 mg/kg BW, IV)	A swine model of myocardial ischemia-reperfusion injury	- Reduced measures of myocardial necrosis, fibrosis, and stiffness - Enhanced recovery of cardiac function	- Reduced inflammation	93
	NAM (enriched diet; 0.5 g/kg)	A rat model of ex vivo myocardial ischemia-reperfusion injury	- Decreased myocardial infarction size	- Reduced oxidative stress (in vitro)	94,95
	NR (100 mg/kg BW, oral gavage)	A mouse model of myocardial ischemia-reperfusion injury	- Improved ejection fraction and reduced infarct size	Not available	96
	NMN (100 mg/kg BW, injected IP every other day)	An aged rat model of ex vivo myocardial ischemia-reperfusion injury	- Restored NAD+/NADH ratio - Reduced infarct size - Preserved cardiac function	- Reduced mitochondrial membrane potential and ROS levels	97
	NMN (500 mg/kg BW, intraperitoneal injected 30 min before ischemia induction or repeatedly during and within 24 h of reperfusion)	A mouse model of myocardial ischemia reperfusion injury	- Reduced infarct size - Improved systolic function	- SIRT1 activation and reduced FoxO1 hyperacetylation - Causality testing: NMN failed to reduce the infarct size in SIRT1-KO mice	98
Diabetic cardiomyopathy	Cardiomyocyte-specific overexpression of NAMPT	HFD-fed mice	- Reduced cardiac hypertrophy and fibrosis - Improved diastolic function	- Reduced oxidative stress through preserved NADPH/NADP+ and GSH/GSSG+ ratios - Autophagy induction - Causality testing: pharmacological inhibition of NAD kinase abolishes the cardioprotective effects of NAMPT overexpression	99
		Streptozotocin-treated mice with cardiac-specific *Ndufs4* knockout	- Elevated NAD+/NADH ratio - Restored cardiac function	- Reduced oxidative stress and protein hyperacetylation	100
Hypertrophic cardiomyopathy	Cardiomyocyte-specific overexpression of NAMPT	A mouse model of isoproterenol-induced cardiac hypertrophy	- Reduced cardiac hypertrophy - Improved cardiac function	- Restored NAD redox balance and reduced mitochondrial protein hyperacetylation	101
	NAD+ (1 mg/kg BW daily, infused from IP implanted osmotic pumps)	A mouse model of agonist (isoproterenol or angiotensin II)–induced cardiac hypertrophy	- Reduced cardiac hypertrophy and fibrosis - Improved markers of heart failure, including ANP and BNP	- Activation of SIRT3 - Causality testing: the anti-hypertrophic effect of NAD+ was absent in *SIRT3*$^{-/-}$ mice	102

(*Continued*)

Table 1. Continued

Condition	Intervention/dosage	Animal model	Effects	Proposed mechanisms	Reference
	NMN (500 mg/kg BW, IP every 3 d)	A mouse model of isoproterenol-induced cardiac hypertrophy	- Reduced hypertrophy and fibrosis - Enhanced cardiac function	- SIRT1 activation and reduced oxidative stress - Causality testing: the protective effects of NMN were partly antagonized by a SIRT1 inhibitor (sirtinol) in vitro	103
	NAM (80 mg/L in the drinking water)	A rat model of arteriovenous fistula-induced volume overload	- Reduced hypertrophy - Enhanced endothelial function and cardiac relaxation	- Reduced oxidative stress	104
	Cardiomyocyte-specific overexpression of NAMPT	A mouse model of transverse aortic constriction-induced pressure overload	- Failed to sustain myocardial NAD+ content - No benefits on hypertrophy or fibrosis - Exacerbated cardiac dysfunction	Not available	105
	NMN (500 mg/kg BW, IP every 3 d)	A mouse model of transverse aortic constriction-induced pressure overload	- Reduced hypertrophy - Improved contractile function and no ventricular dilation - Reduced lung congestion	- Restored NAD redox balance and reversed mitochondrial protein hyperacetylation	101
	NR (450 mg/kg BW daily, dietary supplementation)	A mouse model of transverse aortic constriction-induced pressure overload	- Partly preserved systolic function	Not available	16
Dilated cardiomyopathy	NR (400 mg/kg BW daily, dietary supplementation)	*Lmna* mutant mice	- Enhanced cardiac contractility, reduced wall thinning and attenuated ventricular dilation - Improved survival	Not available	24
	NR (400 mg/kg BW daily, dietary supplementation)	*SRF* mutant mice	- Improved cardiac function - Reduced ventricular dilation	- Occur in absence of clear in vivo changes in NAD redox balance, mitochondrial respiration or protein acetylation - In vitro testing suggested increased glycolysis	16
	NMN (500 mg/kg BW, IP twice weekly)	A mouse model of Friedreich's ataxia cardiomyopathy	- Improved load-independent measures of cardiac contractility and compliance	- Improved myocardial bioenergetics and efficiency - Causality testing: SIRT3 ablation attenuates the benefits of NMN in vivo	25
HFpEF	NAM (450 mg/kg BW daily in the drinking water)	Male and female ZSF1 obese rats with metabolic syndrome and HFpEF	- Reduced hypertrophy - Improved diastolic function, exercise and cardiopulmonary functional capacity - Reduced lung congestion	- Improved myocardial and skeletal muscle bioenergetics due to rewiring of general metabolism. - Deacetylation of diastole-regulating proteins, namely titin and SERCA2a	15
	NR (400 mg/kg BW daily, dietary supplementation)	A mouse model of HFpEF induced by HFD+L-NAME	- Reduced cardiac remodeling - Improved diastolic function and exercise capacity - Reduced lung congestion	- Improved mitochondrial fatty acid oxidation through reduced VLCAD hyperacetylation	106
	Pharmacological NAMPT activation by P7C3-A20 (10 mg/kg BW, IP 5 times per week)	A mouse model of HFpEF induced by HFD+L-NAME	- Improved diastolic function	Not available	106

HFD indicates high-fat diet; HFpEF, heart failure with preserved ejection fraction; IP, intraperitoneal; IV, intravenous; L-NAME, N[ω]-nitro-l-arginine methyl ester; NAD+, nicotinamide adenine dinucleotide, oxidized form; NAM, nicotinamide; NAMPT, nicotinamide phosphoribosyltransferase; NMN, nicotinamide mononucleotide; NR, nicotinamide riboside; SIRT, sirtuin deacetylase; and VLCAD, very long-chain acyl-CoA dehydrogenase.

As an alternative to inhibiting NAD+-consuming enzymes, increasing NAD+ biosynthesis has emerged as a strategy for elevating NAD+. Indeed, mice with cardiomyocyte-specific overexpression of NAMPT are protected from the cardiac decline in NAD+ and ATP pools, and show reduced myocardial infarction in vivo.[17] In rats,

intravenous injection of NAD+ reduces I/R-induced myocardial infarction in a dose-dependent manner.[91] Intraperitoneal injection of NAD+ also reduces cardiac dysfunction induced by I/R injury ex vivo, an effect that occurs in concert with reduced ischemic accumulation of succinate and reactive oxygen species.[92] In a swine model of cardiac I/R, intravenous NAD+ injection before reperfusion significantly reduced signs of myocardial necrosis, fibrosis, and inflammation, ameliorated myocardial metabolism, and promoted the recovery of cardiac function.[93] It is interesting that the cardioprotective effects of NAD+ are tied to the reactivation of autophagic flux.[17,125] However, whether this causally contributes to myocardial recovery from I/R in vivo remains to be tested. This is particularly important because autophagy reportedly plays a dual role in I/R.[126,127]

Dietary nicotinamide administration to mice decreased myocardial infarction in response to an ex vivo I/R protocol.[94] This finding is intriguing considering that NAMPT is downregulated at the protein and mRNA levels in response to ischemia or I/R.[17] Thus, despite reduced NAMPT expression, the NAD+ salvage pathway might, at least in part, preserve its activity.[22] In fact, cardiac NAD+ levels appear to be only modestly reduced in haploinsufficient NAMPT mice, where NAMPT protein expression is reduced by 50%.[17] Alternatively, oral nicotinamide administration might bypass NAMPT through microbiota-mediated conversion to other precursors such as NA, which can regenerate NAD+ through the deamidated pathway.[35] Further research using isotope tracing–based flux measurements and enzyme activity assays should explore these possibilities.

Oral administration of other NAD+ precursors also improves cardiac function and myocardial remodeling in mice subjected to I/R. For instance, mice treated with NR exhibit higher ejection fraction and smaller infarct size after I/R.[96] Similarly, intraperitoneal injection of NMN to aged rats prevents the decline in the NAD+/NADH ratio in response to myocardial I/R ex vivo. NMN-treated rats also display smaller infarct size, preserved cardiac function, intact mitochondrial membrane potential, and reduced reactive oxygen species levels.[97] Along similar lines, mice receiving NMN manifest restored myocardial NAD+ and cardioprotection against I/R in vivo.[98] This positive effect is evident when NMN is injected before ischemia induction or later, during reperfusion. Of note, NMN failed to exert similar cardioprotective effects in SIRT1-deficient mice, indicating that SIRT1 might mediate NMN-induced cardioprotection in I/R.[98]

Collectively, mounting preclinical evidence indicates that genetic and pharmacological interventions to supplement NAD+ exert marked cardioprotective effects, not only against ischemia but also during subsequent reperfusion injury. Hence, it is tempting to speculate that patients with coronary artery syndrome—who are typically at increased risk of myocardial infarction—might benefit more from emerging NAD+-regenerative therapies than from niacin.

Diabetic Cardiomyopathy

Mice with HFD-induced diabetic cardiomyopathy have been recently shown to exhibit an impaired NAD redox balance and a lower ratio of reduced to oxidized form of nicotinamide adenine dinucleotide phosphate (NADPH/NADP+), indicating reduced antioxidant detoxification.[99] In contrast, mice with cardiomyocyte-specific overexpression of NAMPT avoid HFD-induced oxidative stress and preserve normal ratios of NADPH/NADP+ and reduced to oxidized glutathione (GSH/GSSG). Furthermore, despite unaltered weight gain and hyperglycemia, NAMPT overexpression protects HFD-fed mice from cardiac hypertrophy, fibrosis, inflammation, and the cardinal sign of diabetic cardiomyopathy, diastolic dysfunction.[99] Mechanistically, pharmacological inhibition of NAD kinase, the enzyme that converts NAD+ to NADP+, abolished the cardioprotective effects of NAMPT overexpression. However, NAMPT heterozygous mice (which are particularly vulnerable to diastolic dysfunction induced by HFD) showed no further deterioration in the NAD+/NADP+ ratio,[99] suggesting that the NAD+-to-NADP+ conversion cannot be the sole mechanism involved. In fact, in vitro experiments with cardiomyocytes revealed that SIRT1, but not SIRT3, is required for the protective effects of NAMPT.[99] Furthermore, the redox imbalance of NAD and protein hyperacetylation might be involved, as demonstrated in mice lacking the mitochondrial complex I subunit (*Ndufs4*).[100] In support of this notion, NAMPT overexpression restored the NAD+/NADH ratio, reversed protein hyperacetylation, and protected *Ndufs4* mice from diabetic cardiomyopathy.[100]

The inhibition of NAD+-consuming enzymes has been examined for the treatment of diabetic cardiomyopathy. For instance, the PARP1 inhibitor INO1001 was tested in angiotensin II–treated obese and diabetic leptin–resistant (*db/db*) mice with diabetic cardiomyopathy.[128] PARP1 inhibition improved signs of cardiac hypertrophy, fibrosis, inflammation, and oxidative stress. Unfortunately, NAD+ amounts were not measured in this study, which nonetheless demonstrated that SIRT1 was required for the beneficial effects of INO1001, at least in vitro.[128] Regardless, in CD38-deficient mice, increased NAD+ levels coincide with enhanced cardiac metabolism and reduced oxidative stress on HFD feeding.[84] Although functional cardiac phenotyping was not performed in these mice,[84] preliminary data suggest improved diastolic function in HFD-fed mice with reduced CD38 activity.[129]

In sum, recent studies suggest that upregulation of NAD+ biosynthesis or reduction of its catabolism exert beneficial effects against diabetic cardiomyopathy. Future research efforts will need to follow a more translational approach using NAD+ precursors and address the question whether systemic NAD+ replenishment acts

through cardiomyocyte-autonomous or other cell non-autonomous (likely extracardiac) effects to improve diabetic cardiomyopathy.

Arrhythmogenic Cardiomyopathies

Deranged cardiac metabolism in general, and perturbations of NAD+ metabolism in particular, may affect the function of cardiac ion channels. Mouse studies revealed that a reduced NAD+/NADH ratio alters the expression and conductance of cardiac sodium channel $Na_v1.5$ through NADH-dependent protein kinase C activation.[130–132] Consistently, increased intracellular NADH reduces sodium currents in vitro and increases the risk of ventricular tachycardia in wild-type mouse hearts ex vivo.[131] Conversely, addition of NAD+ to isolated mouse hearts with $Na_v1.5$ channel haploinsufficiency reduces the risk of ventricular tachycardia.[131] NAD+ administration also completely restores sodium currents in hypertensive deoxycorticosterone acetate (DOCA)-salt mice with nonischemic cardiomyopathy, while improving conduction velocity in human failing hearts,[133] indicating that the antiarrhythmic properties of NAD+ are clinically relevant.

In addition to the NAD redox state, NAD+-dependent enzymes may mediate the antiarrhythmic effects of NAD+. For instance, NAD+ modulates sodium current via SIRT1-dependent deacetylation of $Na_v1.5$ channels both in vitro and in vivo.[134] In line with this, cardiomyocyte-specific SIRT1-deficient mice, which exhibit increased $Na_v1.5$ acetylation, have decreased sarcolemmal expression of $Na_v1.5$ channels, causing abnormalities in cardiac conduction and premature death through arrhythmia.[134] It is important to note that the arrhythmogenic phenotype of mice with cardiac SIRT1 deficiency recapitulates human cardiac arrhythmias resulting from a loss of function of $Na_v1.5$.[134] Given the clinical importance of $Na_v1.5$ in modulating the propensity for arrhythmia, a recent study evaluated the effect of NR and nicotinamide supplementation on $Na_v1.5$ function.[135] Unlike nicotinamide, an equimolar concentration of NR increases peak sodium current in a protein kinase C–dependent manner and reduces the late sodium current in neonatal rat ventricular cardiomyocytes through both acetylation-dependent and -independent mechanisms. Initial in vivo results from healthy lightly anesthetized mice also show that NR supplementation improves cardiac electrophysiology, as indicated by a shorter corrected QT interval.[135] Thus, future in vivo testing in clinically relevant arrhythmia models may provide the grounds for the therapeutic use of NAD+ precursors against inherited or acquired arrhythmia.

In humans, a recent study found that cardiomyocytes of patients with atrial fibrillation show substantial DNA damage, which was associated with PARP1 activation.[136] In agreement with this observation, tachypacing was demonstrated to impair contractile function in different experimental atrial fibrillation models by inducing DNA damage, PARP1 hyperactivation, and subsequent NAD+ depletion.[136] Conversely, PARP1 inhibition replenished NAD+ levels, reduced oxidative stress–induced DNA damage, and improved cardiomyocyte contractility,[136] indicating that PARP1 inhibition can reverse the progression of atrial fibrillation. Likewise, pharmacological inhibition of CD38 and CD157 prevents ouabain-induced Ca^{2+} overload and arrhythmias in vivo.[137] Knockout (KO) of *CD38* or administration of the CD38 inhibitor SAN-4825 also reduces isoproterenol-induced arrhythmias.[138] In stark contrast, however, NAD+-induced recovery of sodium currents can be hindered by the CD38 antagonist pelargonidin, raising the possibility that baseline CD38-mediated signaling is required for the antiarrhythmic actions of NAD+.[133] Thus, interventions targeting CD38 in the setting of arrhythmia may need to strike a delicate balance between limiting CD38 hyperactivity while sustaining its baseline functionality.

Taken together, reduced levels and impaired redox balance of the cardiac NAD+ pool negatively affect the electric activity of the heart. Nonetheless, the exploration of dysregulated NAD+ homeostasis in arrhythmogenic cardiomyopathy is still in its infancy, requiring further research.

Pathological Cardiac Hypertrophy

Unlike exercise-induced physiological cardiac hypertrophy that is linked to increased NAD+, pathological cardiac hypertrophy is associated with NAD+ decline in mice subjected to aortic constriction or treatment with hypertension-inducing drugs.[16,102] This effect was attributed to limited NAD+ biosynthesis on the basis of reduced NAMPT expression.[16,102] Cardiomyocyte NAD+ levels are also reduced on coincubation with the prohypertrophic agent phenylephrine in vitro.[102] On the contrary, exogenous NAD+ supplementation restores NAD+ levels and NAMPT expression in mice, which show reduced cardiac hypertrophy and fibrosis and improved serological markers of heart failure.[102] Mechanistically, the cardioprotective effects of NAD+ are detectable in *SIRT1*$^{+/-}$ but not in *SIRT3*$^{-/-}$ mice, suggesting a causal role for SIRT3 in mediating the antihypertrophic effects of NAD+, at least in isoproterenol-induced pathological hypertrophy.[102] More recently, SIRT7 has been also implicated in the cardioprotective effects of NMN, at least in vitro.[139] NMN also improves isoproterenol-induced cardiac hypertrophy, fibrosis, and dysfunction in vivo.[103] Similarly, nicotinamide protects from cardiac hypertrophy in a rat model of arteriovenous fistula–induced volume overload.[104] In both models, the antihypertrophic effects of these NAD+ precursors are linked to suppressed oxidative stress. Along similar lines, pharmacological inhibition of PARP1 prevents left ventricular hypertrophy in spontaneously hypertensive *Dahl* rats,[140] whereas CD38 KO renders mice more resilient to agonist-induced pathological hypertro-

phy.[141] It is interesting that increasing NAD+ levels by NAMPT overexpression in cardiomyocytes is sufficient to protect from isoproterenol-induced cardiac hypertrophy, dysfunction, and dilation.[101] However, another study showed that *Nampt*$^{+/-}$ mice (lacking a copy of the *Nampt* gene) are also resilient to agonist-induced hypertrophy.[72] Here, NAMPT depletion was not cardiac-specific, and NAD+ levels were not reported.[72] Thus, further research elucidating the precise function of NAMPT, especially beyond NAD+ production in the heart, is needed to reconcile these apparently disparate observations.

NAD+ replacement therapy has also been tested in a more aggressive form of pathological hypertrophy, induced by transverse aortic constriction (TAC). Mice subjected to such pressure overload initially develop hypertrophic cardiomyopathy, which progresses toward heart failure and ventricular dilation. Intraperitoneal administration of NMN to these mice significantly reduces hypertrophy, improves contractile function, and suppresses ventricular dilation and lung congestion.[101] These effects are accompanied by a restored NAD redox balance and suppressed mitochondrial protein hyperacetylation.[101] NR has also been tested in TAC mice, but failed to improve the NAD+/NADH ratio.[16] Accordingly, dietary NR supplementation did not reduce pathological cardiac hypertrophy or survival in these mice, although it modestly attenuated the decline in ejection fraction. A possible explanation for such a discrepancy between NR and NMN could reside in the route, dose, or timing of administration. That said, cardiomyocyte-specific NAMPT overexpression also yielded controversial results in mice subjected to TAC. In fact, NAMPT transgenic mice display exacerbated contractile dysfunction at 4 weeks after TAC.[105] It is important to note, however, that NAMPT transgenic mice do not preserve high cardiac NAD+ on pressure overload, which might explain the lack of cardioprotection. It would be interesting to see whether supplementation with the NAMPT substrate nicotinamide would mitigate the consequences of TAC in such NAMPT-overexpressing mice. On the flip side, in support of a protective role of NAMPT against pressure overload, *Nampt*$^{+/-}$ mice with reduced cardiac NAD+ levels are more susceptible to TAC-induced cardiomyopathy than both NAMPT transgenic and WT mice.[105] Unlike NAMPT-overexpressing mice, *Nampt*$^{+/-}$ mice display systolic dysfunction within 2 weeks of TAC, and by 4 weeks, they already develop pulmonary congestion, indicative of heart failure.[105]

In sum, NAD+ metabolism is clearly dysregulated in pathological cardiac hypertrophy. The effects of NAD+-targeted interventions are heavily influenced by the underlying cause of hypertrophy (TAC versus agonist-induced), the precursor used (NMN versus NR), and the treatment regimen (preventive versus therapeutic), calling for more systematic studies that should focus on a realistic (therapeutic and pharmacological) setting.

Dilated Cardiomyopathy

DCM, which is characterized by a progressive decline in cardiac contractility and ventricular dilation, is a leading cause of heart failure with reduced ejection fraction. Although DCM commonly occurs as a complication of ischemic cardiac demise (discussed in Ischemic Cardiomyopathy above), it can also develop because of genetic (nonischemic) causes.[142] Thus, NAD+ metabolism has been also examined in genetic mouse models of DCM.

One such genetic model is the *Lmna* mutant mouse, which harbors a mutation in the lamin A/C encoding gene.[143,144] The onset of DCM in *Lmna* mice correlates with a manifest decline in the cardiac NAD+ pool.[24] Consistently, NAMPT expression—on both a transcriptional and a protein level—is reduced, whereas that of NMRK2 is increased not only in the mouse model but also in patients with *Lmna* mutations.[24] Accordingly, dietary NR supplementation replenished NAD+ levels, partially rescued the deleterious cardiac phenotype, and delayed premature mortality in *Lmna* mutant mice. Furthermore, the use of a more therapeutic approach—in which NR treatment was initiated when cardiac function already started to decline—prevented further cardiac deterioration in these mutant mice. In contrast, intraperitoneal nicotinamide administration failed to increase NAD+ levels or to confer any significant cardioprotective effects in *Lmna* mutants.[24] This work strongly suggests that distinct NAD+ precursors are not equivalent in their therapeutic potency. Whether these differences may be explained by dosage, route of administration, pharmacokinetics, or potential off-target effects (outside of the increase in NAD+ pools) remains to be investigated.[24]

Similar to *Lmna* mutants, mice harboring a cardiac KO of the *Srf* gene coding for serum response factor (*SRF*) develop DCM and display cardiac NAD+ deficiency coupled to reduced expression of *Nampt* and a multifold increase in *Nmrk2* mRNA species.[16] Accordingly, intraperitoneal injections of nicotinamide failed to increase NAD+, whereas oral or intraperitoneal administration of NR efficiently restored cardiac NAD+ abundance. Importantly, NR supplementation to *SRF* mutant mice prevented cardiac dysfunction and dilation. It is interesting to note, the cardioprotective effects of NR are not associated with any alterations in NAD+/NADH ratio, mitochondrial respiration, or SIRT1/3 activation in these mice. In fact, NR-treated *SRF* mice showed increased acetylation of the nuclear SIRT1 targets, FOXO1 and p53.[16] Hence, future studies should elucidate the mechanisms through which NR exerts its cardioprotective actions in the setting of DCM induced by *Srf* or *Lmna* mutations.

Another DCM model is the Friedreich's ataxia cardiomyopathy mouse model, which is induced by KO of *frataxin* (FXN).[145] After developing an initial hypertrophic cardiomyopathy phenotype, FXN-KO mice typically develop progressive DCM and heart failure with reduced ejection fraction.[145] At variance with *Lmna* and

Srf mutants, steady-state levels of NAD+ are not reduced in FXN-KO mice.[25] However, *Nampt* transcripts are reduced, suggesting that NAD+ salvage reserve might be compromised.[25] In support of this notion, bypassing NAMPT by supplementing NMN to FXN-KO mice elevates NAD+ levels and preserves cardiac function.[25] Specifically, NMN improves load-independent measures of cardiac contractility and compliance in FXN-KO mice. NMN supplementation also improves myocardial efficiency and bioenergetics in FXN-KO hearts.[25] This effect is coupled to reduced glycolytic flux and lactate accumulation, characteristic of heart failure in FXN-KO hearts. SIRT3 appears to be mechanistically involved in the cardioprotective effects of NMN, because double KO mice lacking FXN and SIRT3 are less protected by NMN than FXN-KO mice.[25] That said, NMN did not reduce global mitochondrial protein lysine hyperacetylation in FXN-KO mice, and SIRT3 ablation unexpectedly attenuated some of the deleterious features of Friedreich's ataxia cardiomyopathy, including left ventricular wall thinning, myocardial stiffness, and glycolytic intermediates accumulation. Thus, SIRT3 activation does not appear to be involved in all cardioprotective effects of NMN in FXN-KO mice.[25]

Taken together, these studies show that NMN and NR hold promise for the treatment of DCM and related heart failure with reduced ejection fraction. However, the available evidence remains ambiguous about how these NAD+ precursors modulate protein (de)acetylation in DCM. Given that patients with Friedreich's ataxia show increased *FXN* mRNA expression and protein concentration in response to high-dose nicotinamide,[146] future randomized trials should also evaluate the clinical outcomes of nicotinamide in these patients.[147]

Heart Failure With Preserved Ejection Fraction

Heart failure with preserved ejection fraction (HFpEF), which affects half of heart failure patients, still lacks evidence-based therapies.[148] Given its ever-growing prevalence, along with a poor prognosis that is on par with several forms of cancers,[148] HFpEF is considered one of the most pressing unmet medical needs. In this regard, 2 recent studies have provided strong evidence that NAD+ metabolism might be a promising actionable target to treat HFpEF.[15,106] The first study demonstrated that human HFpEF is associated with reduced cardiac NAD+ content.[15] Similarly, ZSF1 obese rats, a model of HFpEF and hyperphagia-induced metabolic syndrome,[149–151] exhibit low NAD+ levels both in the heart and liver. It is remarkable that cardiac NAMPT expression was preserved both in humans and rats with HFpEF, suggesting that low steady-state NAD+ is attributable to increased NAD+ consumption or low circulating levels of the NAMPT substrate nicotinamide, which was reduced both in rats and patients.[15] Indeed, nicotinamide supplementation to ZSF1 obese rats restores cardiac and hepatic NAD+ concentrations while reducing cardiac hypertrophy and end-diastolic pressure, ameliorating relaxation and passive myocardial stiffness, thus improving diastolic dysfunction, the hallmark of HFpEF. Accordingly, nicotinamide reduced lung congestion and enhanced exercise and cardiopulmonary functional capacity. Mechanistically, nicotinamide ameliorated myocardial and skeletal muscle bioenergetics, correlating with reduced adiposity and a metabolic shift from glycolysis toward fatty acid β-oxidation. Because nicotinamide also improves diastolic function in nonobese rodent models of aging and hypertension,[15] it has been proposed that nicotinamide might exert cardiac-specific effects in addition to its (noncell autonomous) effects on general metabolism. Indeed, acetylproteome analysis of the heart revealed that 2 diastole-regulating proteins, titin and SERCA2a, were deacetylated upon nicotinamide supplementation.[15] In vitro assays corroborate the functional relevance of this effect because deacetylating titin and SERCA2a by recombinant SIRT1 protein improves cardiomyocyte passive tension and intracellular Ca^{2+} cycling.[15,152] Further research efforts must determine which among the multiple titin acetylation sites that are modulated by nicotinamide is responsible for improving cardiomyocyte elasticity. Similarly, cardiac acetylome analyses by mass-spectrometric proteomics did not reveal any clear alterations in global protein acetylation in nicotinamide-treated rats in vivo. Future research should therefore focus on specific molecular targets of protein (de)acetylation, a procedure that might challenge the premise that hyperacetylation per se threatens metabolic resilience in the myocardium.[153]

The second preclinical HFpEF study examined whether established HFpEF induced by the combination of HFD and chronic treatment with the NO synthase inhibitor N-nitro-L-arginine methyl ester can be reversed by administering NR.[106,154] Indeed, after the development of HFpEF, dietary NR supplementation restored NAD+ abundance, and improved cardiac remodeling as well as diastolic function, exercise capacity, and lung congestion.[106] Again, the cardioprotective effects of increasing NAD+ did not correlate with any changes in total mitochondrial protein acetylation. However, targeted protein analysis revealed reduced acetylation of very long-chain acyl-CoA dehydrogenase in NR-treated mice, coinciding with improved palmitoylcarnitine-mediated mitochondrial respiration.[106] Of note, the NAMPT activator, P7C3-A20, also increases NAD+, reproducing the cardiac functional benefits of NR in the absence of any improvements in obesity or insulin sensitivity in HFpEF mice.[106] At odds with the previous study in which NAMPT protein levels were preserved in HFpEF,[15] the hearts of both mice and patients with HFpEF exhibited reduced levels of *NAMPT* mRNA.[106] These observations suggest a reduced reserve capacity to upregulate NAMPT and NAD+ biosynthesis in HFpEF.

Taken together, NAD+ supplementation using nicotinamide or NR improves cardiac diastolic function through several mutually nonexclusive mechanisms. Pending further confirmation in preclinical models, HFpEF may be considered as a promising indication for NAD+ replacement therapies.

NAD+ SUPPLEMENTATION IN HUMANS

Historically, the most extensively studied NAD+ precursor in humans is NA, which is commonly referred to as niacin. It is interesting that niacin was the first hypolipidemic agent shown to reduce mortality in humans.[155] However, coadministration of niacin and statins failed to additively reduce residual cardiovascular risk in patients.[108–110] Recently, the effect of niacin on lipid control and cardiovascular risk was reexamined in a meta-analysis of 119 clinical trials.[156] The authors found that niacin, administered as a monotherapy without statins, reduces the risk of cardiovascular events, including acute coronary syndrome, stroke, and revascularization. However, they concluded that the available evidence is rather limited because only 17 trials adequately examined the effect of niacin on lipid control and cardiovascular risk.[156] Regardless, because niacin causes unpleasant flushing, is less potent in increasing NAD+ levels than other precursors,[23] and might worsen survival in statin-receiving patients,[157,158] the interest moved toward other NAD+ enhancers.

Clinical trials that evaluated NAD+ boosting strategies other than niacin mostly focused on NR. Although these trials generally included small numbers of subjects, they demonstrated that NR supplementation is safe and does not elicit obvious adverse side effects, but does increase whole blood NAD+ levels.[23,87,159] In fact, oral NR administration for 5 to 9 days (escalating dose up to 1 g twice a day from day 3) increases NAD+ levels, improves mitochondrial respiration, and attenuates proinflammatory cytokine gene expression in peripheral blood mononuclear cells of hospitalized patients with advanced heart failure with reduced ejection fraction.[160] Along similar lines, NR enhances mitochondrial function and attenuates the activation of the NLRP3 inflammasome in circulating leukocytes extracted from healthy subjects.[161] In addition, oral NR (1 g daily) reduces circulating levels of IL-5 and IL-6 in the skeletal muscle from healthy elderly volunteers,[162] while inducing minor improvements in body composition and sleeping metabolic rate in healthy overweight or obese men and women.[163] However, not all studies support the therapeutic potential of dietary NR supplementation. For example, no remarkable improvement has been observed in skeletal muscle mitochondria,[37] insulin sensitivity, or whole-body glucose metabolism in obese, insulin-resistant men treated with NR (1 g twice daily).[164] Thus, the effect of NR supplementation on mitochondrial health and systemic glucose homeostasis in humans remains uncertain.

In contrast with NR, only a handful of trials using NMN have been completed so far. The first human NMN study performed on healthy Japanese men reported that oral administration of NMN (100, 250, or 500 mg) is safe and triggers an increase in circulating nicotinamide metabolites.[165] Although this study did not report changes in steady-state NAD+ levels, it confirmed that NMN does not cause acute adverse effects. More recently, another pilot study examined the safety and efficacy of chronic NMN administration in overweight or obese postmenopausal women with prediabetes.[166] This trial demonstrated that NMN supplementation (250 mg daily for 10 weeks) increases NAD+ levels in circulating PMBCs while improving insulin sensitivity as well as skeletal muscle insulin signaling. However, NMN did not reduce body weight or ameliorate body composition, skeletal muscle strength, or mitochondrial respiration. Although the salutary effects of NMN on insulin and glucose homeostasis appear more appealing than those of NR,[163,164] future head-to-head comparisons must confirm these differences in 1 single clinical trial.

Nicotinamide is another safe NAD+ precursor, which, in contrast with NA/niacin, does not cause flushing.[22] Indeed, nicotinamide is well-tolerated at relatively high doses for months or even years of chronic administration.[167] For instance, 12 months of oral nicotinamide supplementation (1 g daily) is safe and efficient for the prophylaxis of nonmelanoma skin carcinomas.[168] In another rather large-scale trial, nicotinamide was safely administered for 5 years at a dose of 1.2 g/m^2 daily (up to a maximum of 3 g daily) to individuals at risk of type 1 diabetes, although without any clinical efficacy.[169] It is more important to note that nicotinamide administration (1 or 3 g daily for 3 days) to patients undergoing cardiac surgery reduced the levels of the cardiac injury marker troponin T.[170] Moreover, observational studies indicate that a diet enriched in nicotinamide (and NA) is associated with lower blood pressure and a reduced risk of cardiac-specific mortality in humans.[15]

A common denominator of all these studies is that oral administration of different NAD+ precursors is safe and tolerable, and augments NAD+ or its metabolites, although to a varying extent. In Table 2, we provide a comprehensive overview on currently ongoing trials dealing with natural NAD+ precursors and their possible effects on cardiovascular-relevant end points. These clinical trials have been inspired by rodent studies demonstrating that increasing intracellular NAD+ levels may improve cardiovascular diseases. Nonetheless, several practical issues with relation to the administration of NAD+-regenerative therapeutics will need to be overcome, such as how to best deliver NAD+ precursors (for instance by slow-release capsules, releasing their content in the ileum rather than in the stomach), at which dose and time of the day, taking into account chronobiological fluctuations of NAD+.[171] Alternative pharmacological strategies that elevate cellular NAD+

Table 2. List of Ongoing Clinical Trials Testing the Safety and Efficacy of NAD+ Precursors Against Cardiovascular or Related End Points

Precursor	Regimen/dosage	Disease/target population	Study design	Trial phase	Estimated enrollment	Follow-up	Study outcome(s)	NAD+ measurement	Estimated completion	Trial acronym and identifier
NAM	3 g/d on the day of surgery and postsurgical days 1 and 2	Patients undergoing on-pump cardiac surgery	Randomized, placebo-controlled, double-blind trial	2	304	3 mo	Prevention of cardiac surgery–associated acute kidney injury	…	June 2024	NACAM NCT04750616
	2.5 g/d	Women with early-onset preeclampsia	Single group, open-label trial	2	25	7 d	Changes in mean blood pressure	…	July 2020	NCT03419364
Niacin	Not specified	HFrEF	Randomized, single (participant)–blind trial with crossover design	2	12	…	Effects on cardiac function and mixed venous oxygen saturation	…	December 2022	KETO-COX NCT04703361
	Dose escalation up to 2 g/d	Healthy individuals	Single group, open-label trial	2	24	16 wk	Changes in lipoprotein composition and function as well as vascular compliance	…	July 2020	NCT02322203
NR	Dose escalation up to 2 g/d	Patients with HFrEF scheduled for elective LVAD surgery	Randomized, placebo-controlled, double-blind trial	1	40	…	Effects on myocardial mitochondrial function and morphology, protein and epigenetic modifications, as well as inflammatory markers in the heart and circulation	Myocardial and whole blood levels	August 2024	NRII NCT04528004
	1 g/d	Hypertension	Randomized, placebo-controlled, double-blind trial	1	74	6 wk	Changes in systolic blood pressure and arterial stiffness	…	May 2021	The NEET Trial NCT04112043
	1 g/d	Patients with moderate to severe chronic kidney disease	Randomized, placebo-controlled, double-blind trial	2	118	3 mo	Changes in aortic stiffness and arterial blood pressure	PBMCs	September 2024	NCT04040959
	1 g/d	(Pre)hypertensive middle-aged and older adults (SBP: 120–139 mm Hg)	Randomized, placebo-controlled, double-blind trial	2	118	3 mo	Changes in systolic blood pressure and arterial stiffness	Whole blood	December 2023	NCT03821623
	1 g/d	Peripheral artery disease	Randomized, placebo-controlled, double-blind trial	3	90	6 mo	Effects on walking performance, physical activity, quality of life, and skeletal muscle phenotype	Skeletal muscle	April 2022	NICE NCT03743636
	Dose escalation up to 2 g/d (or maximum tolerated dose if <2 g)	HFrEF	Randomized, placebo-controlled, double-blind trial	1	30	12 wk	Safety and tolerability, cardiac function as well as mitochondrial function in PBMCs	Whole blood	June 2019	NCT03423342
	0.5 g/d	Young compared with old volunteers with normal or prehypertensive blood pressures	Randomized, placebo-controlled, double-blind trial with crossover design	N/A	16	7 d	Effects on blood lipids and vascular function after dietary high-fat intake	Whole blood and PBMCs	December 2019	NCT03501433
	1 g/d	Healthy elderly females	Randomized, placebo-controlled, double-blind trial	N/A	48	6 mo	Cardiopulmonary functional capacity, physical performance, as well as skeletal muscle phenotyping	…	December 2022	NCT03818802

(*Continued*)

Table 2. Continued

Precursor	Regimen/ dosage	Disease/target population	Study design	Trial phase	Estimated enrollment	Follow-up	Study outcome(s)	NAD+ measurement	Estimated completion	Trial title or identifier
NMN	300 mg/d	Overweight and obese subjects with prediabetes	Randomized, placebo-controlled, double-blind trial	N/A	56	16 wk	Cardiovascular risk factors, including glucose tolerance and insulin sensitivity	…	September 2025	VAN NCT04571008
	300 mg/d	Middle-aged and old healthy volunteers	Randomized, placebo-controlled, double-blind trial	N/A	66	2 mo	Safety and efficacy in reducing systolic and diastolic blood pressures	Whole blood	March 2021	NCT04228640
	250 mg/d or 500 mg/d	Healthy volunteers with moderate physical activity	Randomized, placebo-controlled, double-blind trial	N/A	150	38 d	Muscle recovery, physical activity, and cardiopulmonary capacity	Whole blood	September 2022	NCT04664361
	400 mg/d	Healthy volunteers	Exploratory, open-label, single-arm trial	N/A	20	28 d	Tolerability, pharmacodynamics, and cardiovascular effects, including arterial blood pressure, heart rate, blood lipids	Whole blood	October 2021	NCT04862338

We searched the US clinical trial registry (https://www.clinicaltrials.gov/) using terms "nicotinamide" and "cardiovascular disease" for pending or ongoing clinical trials of NAD+ supplementation that have yet to publish results (from database inception to May 2021). HFrEF indicates heart failure with reduced ejection fraction; LVAD, left ventricular assist device; N/A, not applicable; NAD+, nicotinamide adenine dinucleotide, oxidized form; NAM, nicotinamide; NMN, nicotinamide mononucleotide; NR, nicotinamide riboside; PBMCs, peripheral blood mononuclear cells; and SBP, systolic blood pressure.

content, for instance by inhibiting NAD+ consumption, have yielded promising preclinical results. However, future trials must establish their long-term safety profile, especially on the risk of infections and immunosuppression.[172,173] Another important issue is the standardization of reliable biomarkers of NAD+ metabolism, including quantitation of NAD+ precursors and metabolites in body fluids and tissues as well as that of proxies of their bioactivity that may include specific protein acetylation patterns, autophagy and mitophagy. Resolving these issues will be instrumental for the design of future NAD+-centered therapeutic interventions on cardiovascular diseases and other age-related diseases.

PERSPECTIVES AND CONCLUDING REMARKS

A recent wave of intense research has transformed our understanding of NAD+ biology and led to new and evolving concepts on the biosynthesis, transport, catabolism, and functions of NAD+ in health and disease.[1] Although this knowledge may have yielded novel targets to prevent or treat cardiovascular diseases with a remarkable number of patent applications (8778 as of September 11, 2021, according to the European Patent Office's worldwide database), several obstacles need to be overcome to translate these findings into the clinical arena. Thus, future clinical trials need to be of much longer duration, include a follow-up beyond treatment discontinuation, involve larger numbers of patients, and consider adapting drug doses from rodent studies to human studies on a per-weight rather than on a per-surface basis.[174,175] In this respect, quantification of potential long-term adverse effects (eg, hepatotoxicity and bleeding) will be critical to ensure that the administration NAD+ precursor administration at higher doses is clinically safe. Reported differences in bioavailability and stability of NAD+ precursors have fueled the debate on the choice of the optimal NAD+ enhancer. Available evidence indicates that none among the natural NAD+ precursors is optimal for all indications. However, the lack of systematic direct comparisons among NAD+ precursors (NA, nicotinamide, NR, and NMN) at the preclinical and clinical levels precludes firm therapeutic recommendations. Given that NAD+ and its close metabolites can be subjected to continuous interconversion with a half-life in the order of minutes,[22] future studies should measure NAD+ flux to explore the dynamics and kinetics of NAD+ biosynthesis, degradation, and metabolism in a tissue-specific manner.[22,59] This approach combined with the advent of NAD+ biosensors will allow for accurate NAD+ quantification in intact tissues, cells, and even defined subcellular compartments.[176,177] Dynamic monitoring of the entire NAD+ metabolome also will advance our understanding of inherited or acquired alterations in NAD+ levels and its related intermediates, help to optimize methods for raising NAD+ levels, explore the pathogenesis of defined cardiovascular diseases, and allow comparisons with the current standard of care for these conditions.

In conclusion, we emphasize that NAD+ and its precursors are pleotropic molecules involved in multiple processes that cannot entirely depend on the activity of 1 single target (Figure 4). For instance, although sirtuins are key downstream targets of NAD+, global

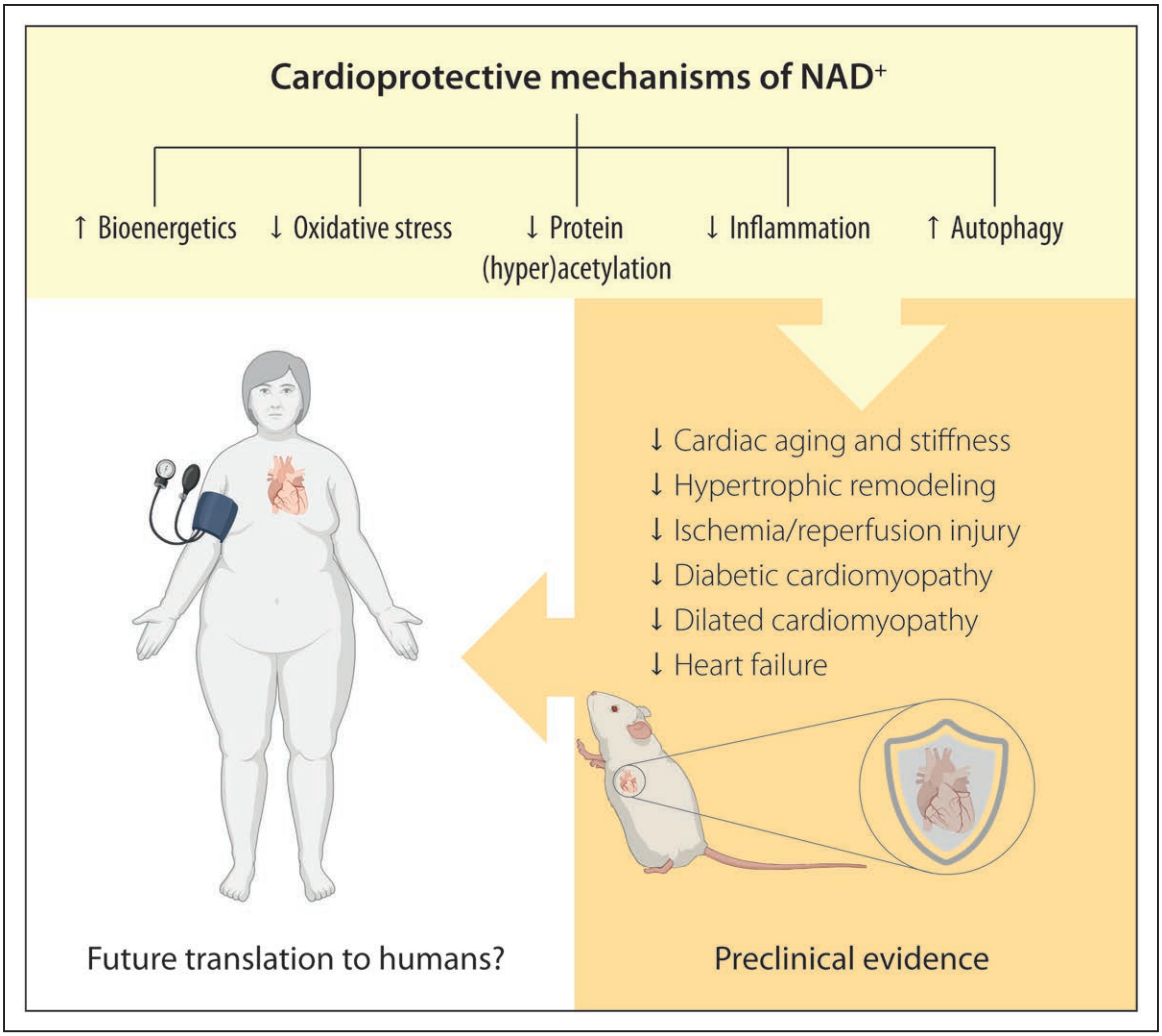

Figure 4. Cardioprotective mechanisms of NAD+.
NAD+ is a pleotropic molecule involved in multiple processes that do not entirely depend on the activity of a single downstream effector. Indeed, various animal models of cardiovascular diseases treated with NAD+ precursors reveal that mutually nonexclusive mechanisms might contribute to the benefits of NAD+ depending on the precursor used and the underlying condition. These include, but are not limited to, improved myocardial bioenergetics, reduced oxidative stress, and attenuated hyperacetylation of mitochondrial or sarcomeric proteins, as well as reduced inflammation and increased autophagy activation. Thus far, overwhelming preclinical evidence indicates that NAD+-based therapeutics might be effective against cardiac aging and several cardiomyopathies, including ischemic, hypertrophic, dilated, and diabetic cardiac disease, as well as heart failure. However, these experimentally attested cardiovascular benefits await translation to humans. NAD+ indicates nicotinamide adenine dinucleotide, oxidized form. The clip art included in this figure was created with BioRender.com.

analyses of cardiac protein acetylation profiles in response to NAD+-replacement interventions, with the exception of NAMPT overexpression,[101] failed to show clear-cut effects.[15,16,25] Hence, future acetylation-centric studies should rely on more refined analyses of specific protein targets. Furthermore, other NAD+-modulated processes, like inflammation and autophagy, which are dampened or induced by NAD+, respectively, might be involved in the broad physiological effects of NAD+ precursors in vivo. Recently, a dynamic model has been proposed in which NAD+ influences its flux through mitochondrial pathways, improving oxidative phosphorylation without any involvement of sirtuins.[178] Deciphering the mechanisms underlying the mode of action of NAD+ in a cell type- and precursor-specific manner will be decisive for the future implementation of NAD+ targeting interventions.

ARTICLE INFORMATION

Affiliations

Department of Cardiology, Medical University of Graz, Austria (M.A., S.S.). Metabolomics and Cell Biology Platforms, Institut Gustave Roussy, Villejuif, France (M.A., G.K.). Centre de Recherche des Cordeliers, Equipe labellisée par la Ligue contre le cancer, Université de Paris, Sorbonne Université, Institut national de la santé et de la recherche médicale (INSERM) U1138, Institut Universitaire de France (M.A., G.K.). BioTechMed Graz, Austria (S.S.). Institute of Physiology, Faculty of Medicine, University of Maribor, Slovenia (S.S.). Pôle de Biologie, Hôpital Européen Georges Pompidou, Assistance Publique – Hôpitaux de Paris (AP-HP), Paris 7015, France (G.K.).

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Disclosures

Drs Abdellatif and Sedej are involved in a patent application related to the cardiometabolic effects of caloric restriction mimetics, including nicotinamide. The other author reports no conflicts.

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