Role of NAD+ and mitochondrial sirtuins in cardiac and renal diseases

Kathleen A. Hershberger[*], **Angelical S. Martin**[*], and **Matthew D. Hirschey**
Duke Molecular Physiology Institute and Sarah W. Stedman Nutrition and Metabolism Center, 300 N Duke Street, Durham, North Carolina 27701, USA

Abstract

The coenzyme nicotinamide adenine dinucleotide (NAD^+) has key roles in the regulation of redox status and energy metabolism. NAD^+ depletion is emerging as a major contributor to the pathogenesis of cardiac and renal diseases and NAD^+ repletion strategies have shown therapeutic potential as a means to restore healthy metabolism and physiological function. The pleotropic roles of NAD^+ enable several possible avenues by which repletion of this coenzyme could have therapeutic efficacy. In particular, NAD^+ functions as a co-substrate in deacylation reactions carried out by the sirtuin family of enzymes. These NAD^+-dependent deacylases control several aspects of metabolism and a wealth of data suggests that boosting sirtuin activity via NAD^+ supplementation might be a promising therapy for cardiac and renal pathologies. This Review summarizes the role of NAD^+ metabolism in the heart and kidney, and highlights the mitochondrial sirtuins as mediators of some of the beneficial effects of NAD^+-boosting therapies in preclinical animal models. We surmise that modulating the NAD^+–sirtuin axis is a clinically relevant approach to develop new therapies for cardiac and renal diseases.

The heart and kidneys have the highest numbers of mitochondria and are among the greatest oxygen consumers of all organs in the body[1]. Energy production is maintained in these organs through the oxidative metabolism of a range of fuels. Substrate metabolism is intricately tied to the pyridine dinucleotide, nicotinamide adenine dinucleotide (NAD^+). This essential metabolite serves as a key cofactor in a plethora of mitochondrial metabolic processes that facilitate energy production[2] and also has several other important cellular functions[3].

NAD^+ is a hydride acceptor that forms the reduced dinucleotide NADH. The NAD^+/NADH nucleotide pair is vital for driving reduction–oxidation (redox) reactions in energy production. Furthermore, NAD^+ is a precursor for the phosphorylated dinucleotide pair $NADP^+$/NADPH, which is required for several cellular biosynthetic pathways and to protect cells from reactive oxygen species (ROS). NAD^+ also acts as an enzyme substrate for

several non-redox reactions, such as those that occur in signalling pathways. In these reactions, the adenine diphosphate ribose (ADPR) moiety of NAD^+ is cleaved and used as a signalling molecule through the enzymatic activity of ADPR cyclases, used to post-translationally modify proteins via the activity of ADP ribosyltransferases, and used to remove post-translational acyl modifications via the activity of sirtuins. The mitochondrial sirtuins control metabolism by coupling NAD^+ consumption with deacylation on critical lysine residues of metabolic proteins, thereby regulating flux through cellular metabolism[4]. The multi-faceted functions of NAD^+ uniquely position this metabolite at the regulatory interface of energy metabolism and several cellular processes in metabolically active organs such as the heart and kidneys[5].

Consistent with these key roles of NAD^+, various cardiac and renal stresses have been associated with decreases in the levels of this metabolite, which contribute to disease pathogenesis[1,5]. Acute stresses, such as stroke, myocardial infarction, acute kidney injury (AKI) and ischaemia, deplete cellular NAD^+ levels and reduce NAD^+ synthesis[5,6]. In the setting of chronic stress, such as ageing, cardiac and renal levels of NAD^+ decline and correlate with a general reduction in mitochondrial function[7]. Thus, elevating intracellular NAD^+ levels is a potential therapeutic strategy for cardiac and renal diseases. Several therapeutic strategies to increase NAD^+ levels have been established, including exogenous supplementation of NAD^+ and its precursors or manipulation of key enzymes in its biosynthetic pathways. A number of studies in model organisms ranging from *Caenorhabditis elegans* to mice, as well as in humans, have established beneficial effects of boosting NAD^+ levels in a wide range of disease states[3]; however, the mechanisms by which such therapies are efficacious remain incompletely understood.

Here, we describe the primary mechanisms by which NAD^+ supplementation might influence cellular processes, and consider the evidence for the contribution of each mechanism to the potentially beneficial effects of NAD^+ in the heart and kidney. In particular, we highlight the mitochondrial sirtuins as key mediators of the effects of NAD^+ supplementation in preclinical animal models.

NAD^+ metabolism in the heart and kidneys

NAD^+ synthesis

The heart and kidneys are among the organs with the highest NAD^+ levels[8] and cellular control of this metabolite is crucial for cardiac and renal metabolic and bioenergetic homeostasis[5]. In mammals, NAD^+ is synthesized from four different biosynthetic precursors in two distinct pathways. *De novo* synthesis (the deamidated pathway) uses the amino acid tryptophan, which is metabolized to form biosynthetic intermediates. These intermediates ultimately generate nicotinamide (the pyridine moiety of NAD^+) and then form NAD^+. Dietary vitamin B_3 compounds, including nicotinic acid, nicotinamide and nicotinamide riboside, serve as NAD^+ biosynthetic precursors and are salvaged from the diet (the amidated pathway) to generate cellular NAD^+ (FIG. 1).

Comprehensive profiling of the physiological steady-state levels of components of the NAD^+ metabolome (NAD^+ precursors and intermediates) and the activities of enzymes

involved in NAD$^+$ biosynthesis and salvage in C57BL/6 mice showed that the amidated route accounts for 99.3% and 99.9% of the NAD$^+$ synthesized in the heart and kidneys, respectively[8]. These data indicate that nearly all cardiac and renal NAD$^+$ is generated from salvage of nicotinic acid, nicotinamide and nicotinamide riboside, although *de novo* synthesis can contribute to the NAD$^+$ pool under some conditions[8,9]. This conclusion is corroborated by earlier studies that found low expression and activity of enzymes involved in the deamidated route in these tissues[10]. Moreover, experiments in perfused rodent kidneys showed slow uptake and use of quinolinic acid, an intermediate in *de novo* NAD$^+$ synthesis[10,11]. The amidated salvage route is, therefore, crucial for providing the NAD$^+$ that is required to fulfil the high metabolic demands of the heart and kidneys, as well as for maintaining NAD$^+$ homeostasis in the setting of disease.

Uptake and use of NAD$^+$ and its precursors

Tissue-specific mechanisms of nicotinic acid, nicotinamide, and nicotinamide riboside transport are an emerging area of study. Renal uptake of NAD$^+$ and its salvage precursors occurs at the brush border membrane of the proximal tubules[12,13] (FIG. 1d). Nicotinic acid, lactate, glucose, and short-chain fatty acids are rapidly taken up into the kidneys via the sodium-dependent monocarboxylate transporter SLC5A8 (SMCT)[14], whereas uptake and transport pharmacokinetics of nicotinamide are much slower[9]. Uptake of nicotinamide does not occur through the SMCT, but is thought to occur through an unidentified carrier[14]. In the heart, nicotinic acid is believed to have a direct effect on lactate efflux through the lactate monocarboxylic acid transporter (MCT)[15]. Specific and/or overlapping mechanisms of nicotinic acid transport, such as active transport via a MCT, is an active area of investigation.

Once taken up by cardiac or renal cells, nicotinamide is converted into nicotinamide mononucleotide (NMN) via the activity of nicotinamide phosphoribosyltransferase (NAMPT) in the amidated pathway, and to nicotinic acid via multiple steps in the deamidated pathway (FIG. 1). The degree to which cardiac cells incorporate nicotinamide or nicotinic acid for NAD$^+$ synthesis seems to depend on precursor availability, but is overall similar for each metabolite[16]. In the kidneys, both nicotinic acid and nicotinamide are readily metabolized to NAD$^+$; nicotinamide metabolism is tightly regulated to maintain low levels of this precursor[9]. Radiolabelling studies in perfused rat kidneys showed rapid conversion of nicotinic acid to NAD$^+$ via the deamidated route, as well as rapid consumption of newly synthesized NAD$^+$ into NMN via the amidated route[9]. In these studies, radiolabelled nicotinamide was converted to NAD$^+$ so quickly that no trace of labelled intermediate NMN was detected. In murine saturation studies, excess nicotinic acid resulted in its decreased conversion to NAD$^+$, by both rate and overall amount incorporated into NAD$^+$ synthesis. The nicotinamide that was generated from the excess nicotinic acid was quickly converted into byproducts that were excreted in the urine[9,11,17,18] (FIG. 1d). This tight control of nicotinic acid and nicotinamide is thought to be crucial for regulating the activities of NAD-cleaving enzymes, such as sirtuins, ADP cyclases and ADPR-transferases[8]. These enzymes generate nicotinamide as a byproduct, which in turn provides feedback inhibition on their activities.

Extracellular NMN is converted to nicotinamide riboside, which is then taken up into cells via membrane transporters such as CD73 (REF. 19) or nicotinamide riboside kinases (NRKs)[20]. NRKs are required for the intracellular conversion of nicotinamide riboside into NMN, which is then used to synthesize NAD^+ by the activity of nicotinamide mononucleotide adenylytransferase (NMNAT). In liver cells, NRK1 activity also seems to be required for the uptake of exogenous nicotinamide riboside and NMN, but not for the uptake of the precursors nicotinamide or nicotinic acid[20]. In that study, mouse kidney expressed the highest levels of *Nmrk1*, whereas levels of cardiac *Nmrk1* were among the lowest of all the tissues analysed. Moreover, both nicotinamide riboside and NMN supplementation boosted renal NAD^+ levels by 150% in a wild-type mouse, but this increase was reduced by two-thirds in an NRK1-knockout mouse. These findings support a key role for NRK1 in nicotinamide riboside and NMN uptake and use in NAD^+ synthesis in the kidneys.

NRK1 was not required for increased NAD^+ levels in skeletal muscle resulting from either supplementation with nicotinamide riboside or NMN[20]. Expression of *Nrmk2* transcript and NRK2 protein levels were highest in heart and skeletal muscle, suggesting a role for NRK2 in mediating the uptake and metabolism of nicotinamide riboside and NMN in cardiomyocytes. In an NRK1/2-double knockout mouse, however, uptake of NR and NMN and conversation to NAD^+ in skeletal muscle was unchanged compared to that in wild-type mice, indicating a non-NRK route of NR and NMN uptake in muscle tissue[20]. In a parallel study, acute nicotinamide riboside supplementation was highly effective at elevating NAD^+ levels in the livers, but not in the hearts of wild-type mice[21]. Interestingly, intraperitoneal injection of nicotinamide riboside in mice greatly enhanced the cardiac NAD^+ metabolome, producing high levels of nicotinamide, which was quickly converted to the byproducts 1-methylnicotinamide and *N*-methyl-4-pyridone-5-carboxamide. These findings suggest that in the heart, exogenous nicotinamide riboside might have an alternative fate to NAD^+ synthesis. By contrast, a plethora of studies have demonstrated that intraperitoneal injection of NMN boosts NAD^+ levels in the heart[22–24]. Future studies are required to elucidate the disparate capacity of NMN and nicotinamide riboside to promote cardiac NAD^+ synthesis.

Intracellular fate of NAD⁺

Once in the cell, NAD^+ precursors might have different fates depending on the cellular compartment. In a tissue-wide NAD^+ metabolome study, NAD^+ biosynthetic rates were reconstructed, and NMNAT isoform expression and activity was found to be a key regulator of the rate of NAD^+ biosynthesis in the heart and kidney[8] (FIG. 1). NMNAT converts NMN to NAD^+ via the amidated route, and converts nicotinic acid mononucleotide to nicotinic acid dinucleotide via the deamidated route; the NMN substrate is an intermediate metabolite of nicotinamide and nicotinamide riboside (FIG. 1). NMNAT has three isoforms: the nuclear isoform, NMNAT1, is ubiquitously expressed and highly active in the heart and kidney[3]; the cytosolic isoform, NMNAT2, is highly expressed in the heart and poorly expressed in the kidney but has little activity in either tissue; and the mitochondrial isoform, NMNAT3, is robustly expressed in the kidney and weakly expressed in the heart[25], but highly active in both tissues[25]. Interestingly, *Nmnat3*-knockout mice show no change in steady-state NAD^+ levels in the heart or kidney compared with that of wild-type mice, but whether mitochondrial pools were affected by *Nmnat3* knockout was unclear[26]. Together these

findings support important functional roles of nuclear NMNAT1 and mitochondrial NMNAT3 in the heart and kidney. Furthermore, they show that the uptake and use of nicotinamide riboside, nicotinamide, and NMN are important for NAD^+ synthesis in a cellular compartment-specific manner.

A complex, tissue-specific, and compartment-specific system of NAD^+ uptake and homeostasis exists, but understanding the differential bioavailability and use of NAD^+ and its precursors requires further study. For example, future investigations will identify cardiac-specific mechanisms of uptake of NAD^+ and its precursors, and track cellular compartmental shifts in NAD^+ pools to establish flux in the NAD^+ metabolome with precursor supplementation. Such research will provide the information necessary to fully understand NAD^+ supplementation strategies in the setting of cardiac and renal diseases.

Potential benefits of NAD⁺ therapy

Homeostasis of the NAD^+ metabolome is key for responses to pathological stress in the heart and kidney. Cardiomyopathy studies have shown a critical role of NAMPT in protecting the heart against pathological stress[27,28], whereas in the kidneys, NAMPT has an important role in cell growth[29] and survival[30]. Several studies have demonstrated that NAD^+ supplementation has a beneficial effect on cardiac function. In a model of pressure-overload-induced left ventricle hypertrophy, supplementation with NMN or genetic manipulation of enzymes in the NAD^+ salvage pathways increased NAD^+ levels and improved cardiac function[23]. Similarly, in kidneys subjected to ischaemia, nicotinamide supplementation increased NAD^+ levels and decreased the levels of toxic fatty acids that have a critical role in the pathological progression of AKI[2].

Several studies have firmly established that NAD^+ therapies are efficacious in multiple models of cardiac and renal disease[22,23,31–34]. Given the re-emerging interest in NAD^+ biology and in supplementation strategies for a wide-range of diseases, additional studies will likely identify roles for NAD^+ supplementation in mitigating a wide range of cardiac and renal diseases. The challenge moving forward will be to develop a deeper understanding of the mechanisms that mediate the therapeutic benefits of NAD^+ supplementation.

NAD⁺ and sirtuins

A possible mechanism by which NAD^+ supplementation might improve outcomes in cardiac and renal diseases is by activating sirtuins (TABLE 1). This family of NAD^+-dependent enzymes regulates metabolism by removing acyl-lysine modifications from a variety of proteins[35]. Sirtuins were initially described as deacetylases that target acetyl-lysine for removal[36], but they have now been shown to also target several acyl-lysine modifications, including malonyl-lysine[37,38], succinyl-lysine[38], and glutaryl-lysine[39], as well as long-chain acyl-modifications[40].

In mammals, seven sirtuins (SIRT1-7) occupy different cellular compartments. SIRT1, SIRT6, and SIRT7 reside in the nucleus and nucleolus, whereas SIRT2 resides primarily in the cytoplasm. Notably, SIRT6 seems to have a role in mitigating cardiac stress[41,42]. SIRT3, SIRT4, and SIRT5 are localized in mitochondria and have roles in various metabolic

pathways. SIRT3 is a lysine deacetylase that controls several pathways, including lipid metabolism and oxidative stress[43]. SIRT4 has been described as an ADP-ribosyltransferase[44], a deacetylase[45], and a lipoadmidase[46], but its primary activity has not yet been established. This sirtuin has been shown to influence animo acid and lipid metabolism, as well as the tricarboxylic acid cycle[44,47]. SIRT5 is a lysine demalonylase, desuccinylase, and deglutarylase, which controls several metabolic pathways, including the urea cycle[39]. Lysine acetylation is thought to occur nonenzymatically[48–50] and to increase in response to elevated levels of acetyl-coenzyme A, such as those that occur in response to disease-associated shifts in metabolism[49]. Acylation of mitochondrial proteins is generally repressive[51], although lysine acylation has been described to activate enzyme activity in a few cases[52].

The dependence of sirtuins on NAD^+ links energy metabolism to sirtuin function[53]. Importantly, yeast sirtuins can be activated by manipulating NAD^+ biosynthetic pathways or by increasing concentrations of NAD^+. For example, increasing the levels of *NPT1,* which encodes nicotinate phosphoribosyltransferase (an enzyme with a role in NAD^+ biosynthesis), increased yeast sirtuin (Sir2, the homologue of mammalian SIRT1) activity and extended lifespan by up to 60% without changing NAD^+ levels[54]. Similar lifespan extension was observed with nicotinamide riboside supplementation, which increased NAD^+ levels[55]. Supplementation of nicotinic acid had no effect on Sir2 activity[56], however, demonstrating that different NAD^+ precursors elicit different biological effects, perhaps owing to differences in uptake, availability, or fate.

Investigations of NAD^+ supplementation and the effects on sirtuin activity in higher organisms have yielded similar results. Exogenous nicotinamide riboside or nicotinamide increased levels of NAD^+, improved mitochondrial function, and increased lifespan in *C. elegans*[57]. In a mouse model of type 2 diabetes mellitus induced by a high-fat diet, NMN supplementation restored NAD^+ levels, an effect that was partially attributed to SIRT1 activation[58]. Based on these studies and others, supplementation with NAD^+ precursors or boosting the activities of enzymes in the NAD^+ salvage pathway to activate sirtuins is being explored as a strategy to treat cardiac and renal diseases.

The influence of SIRT1 on ageing and lifespan and the role of this sirtuin in mediating the effects of NAD^+ supplementation has been extensively studied[59,60]. Many studies have linked NAD^+ supplementation to SIRT1 activation and shown that SIRT1 protects against cardiac and renal disease. The beneficial effects of SIRT1 in the heart include reduced inflammation, inhibition of cardiomyocyte apoptosis, defence against myocardial oxidative stress, and maintenance of energy metabolism[61,62]. In the kidney, SIRT1 promotes podocyte function and mitochondrial biogenesis, and inhibits fibrosis, inflammation, and apoptosis[63–65]. Although strong evidence supports a role for SIRT1 in mediating some of the beneficial effects of NAD^+ supplementation, here we focus on the role of mitochondrial sirtuins in cardiac and renal function and the potential effects of NAD^+ supplementation on these sirtuins.

Role in cardiac function

SIRT3—SIRT3 has a role in maintaining normal cardiac physiology and potentially in mitigating cardiac pathophysiology. Ablation of SIRT3 in the heart results in hyper-acetylation of metabolic enzymes, hypertrophy, and a marked reduction (>50%) in ATP levels, demonstrating an important role for SIRT3 in regulating cardiac bioenergetics[66,67]. SIRT3-knockout mice develop spontaneous cardiac hypertrophy with age[68] and have increased susceptibility to several pharmacological and non-pharmacological cardiac stressors[31,69]. In pathophysiological states, levels of NAD^+ are reduced, suggesting that SIRT3 activity is decreased[23,70]. Consistent with this conclusion, hyperacetylation of cardiac mitochondrial proteins has been reported in mouse models of heart failure and in failing human hearts[23,70].

Importantly, hyperacetylation of mitochondrial proteins is thought to be a driver of metabolic dysfunction in the heart[22,23,43,71]. In a model of obesity-induced cardiomyopathy and in a SIRT3-knockout mouse, hyperacetylation increased the activities of cardiac long chain acyl-CoA dehydrogenase and other enzymes involved in fatty acid oxidation (FAO)[71]. The researchers suggested that the resulting increase in rates of FAO likely reduced cardiac efficiency. By contrast, an earlier study showed that protein hyperacetylation was associated with reduced rates of FAO in the hearts of fasted animals[43]. This discrepancy suggests that the type of stress might be important in determining the effect of acetylation on protein activity. Future studies are required to elucidate this context-specific regulation of target proteins via acetylation and improve understanding the role of acetylation in various types of cardiac pathology.

SIRT3 mediates some of the beneficial effects of NAD^+ therapy in the heart. Early studies showed that treatment of mice or murine embryonic fibroblasts with nicotinamide riboside increased NAD^+ levels and activated SIRT1 and SIRT3 (REF. 72). Nicotinamide riboside supplementation also reduced the acetylation of specific targets of SIRT3, including superoxide dismutase 2 (SOD2) and NADH ubiquinone oxidoreductase subunit A9, suggesting that this treatment activates SIRT3. In another murine study, SIRT3 knockout increased the sensitivity of mice to isoproterenol-induced cardiac hypertrophy[31]. Treatment with exogenous NAD^+ blocked isoproterenol-induced hypertrophy in a SIRT3-dependent manner, highlighting an important role of SIRT3 in mediating the therapeutic efficacy of NAD^+ supplementation. Finally, NMN supplementation resulted in reduced acetylation of cardiac mitochondrial proteins in a mouse model of complex I deficiency[22,23], potentially via activation of SIRT3.

Together, these data show that NAD^+ has the capacity to activate SIRT3 in the heart and improve cardiac function. Supplementation with NMN has been shown to modulate cardiac acetylation[22] and exogenous NAD^+ can activate cardiac SIRT3 (REF. 31). The beneficial effects of NMN in the heart are consistent with efficient transport and conversion to NAD^+ in the cardiomyocyte. NMN supplementation seems to be an effective means to reduce mitochondrial protein acetylation and off-set functional cardiac decline during heart failure.

SIRT4—Similar to other mitochondrial sirtuins, SIRT4 is highly expressed in tissues with high metabolic demand, including the heart, kidney, liver and brain[44]. Little is known about

the role of SIRT4 in heart and kidney function. SIRT4 has, however, been shown to attenuate hypoxia-induced apoptosis of cardiomyoblast cells, suggesting a cardioprotective effect[73]. By contrast, SIRT4 knockout protected mice against angiotensin II-induced cardiac hypertrophy, fibrosis and oxidative stress, suggesting a cardioprotective effect of SIRT4 deficiency and implicating SIRT4 in the pathogenesis of disease[74]. Although the findings of these studies are conflicting, both highlight a role for SIRT4 in modulating oxidative stress. Clearly, additional studies are required to understand the role of SIRT4 in cardiac physiology and pathophysiology, particularly in the setting of NAD$^+$ precursor supplementation.

SIRT5—The role of SIRT5 in maintaining normal cardiac function is not well understood. An early study found that the heart weight, heart rate, and systolic blood pressure of SIRT5-knockout mice receiving a chronic high-fat diet were similar to those of wild-type controls, suggesting that this mitochondrial sirtuin has little influence over cardiac function[75]. Stress is often required, however, to elicit a phenotype in sirtuin-deficient animals, and cardiac stress may be needed to study the role of SIRT5 in the heart[76]. Although SIRT5 targets multiple protein modifications, protein succinylation is uniquely elevated in the hearts of SIRT5-knockout mice[77], suggesting that reversible succinylation of metabolic proteins might regulate cardiac metabolism. A large number of proteins involved in metabolic pathways are significantly succinylated in the absence of SIRT5, including those involved in fatty acid metabolism, branched-chain amino acid catabolism, the tricarboxylic acid cycle, oxidative phosphorylation (OXPHOS), stress pathways, ketogenesis, and pyruvate metabolism[76].

In mice subjected to cardiac ischaemia, SIRT5-knockout hearts had a greater infarct area post-reperfusion than those of wild-type controls, as well as slightly elevated ROS levels[76]. Succinylation activated succinate dehydrogenase (SDH) in the SIRT5-knockout hearts, and inhibition of SDH normalized infarct size to wild-type levels[76]. Together, these data suggest that SIRT5 influences the cardiac stress response by decreasing SDH activity via desuccinylation.

Ageing also elicits a phenotype in the hearts of SIRT5-knockout mice[78]. The hearts of 39-week-old SIRT5-knockout mice were significantly hypertrophied and had increased fibrosis compared to the hearts of similarly aged wild-type controls. In addition, echocardiograms showed that both the shortening fraction and ejection fraction of the SIRT5-knockout hearts were significantly decreased compared to wild-type levels at 8 weeks and 39 weeks of age. Succinylation and subsequent inhibition of the mitochondrial trifunctional protein α-subunit was a key mechanism that contributed to cardiomyopathy in the hearts of ageing SIRT5-knockout mice.

No studies have directly examined the effects of boosting NAD$^+$ levels on SIRT5, but phenotypic similarities between the hearts of SIRT3-knockout and SIRT5-knockout mice suggest that supplementation with NAD$^+$ precursors in the context of heart failure might activate SIRT5. In this setting, putative SIRT5 activation by NAD$^+$ precursors, such as NMN, would be expected to lead to wide-spread protein desuccinylation, and activation of metabolic pathways that might lead to a decreased pathological response to stresses such as

ischaemia–reperfusion injury or ageing. Future studies should be directed at testing the cardiac SIRT5–NAD$^+$ axis.

Role in renal function

SIRT3—In the kidney SIRT3-mediated deacetylation under basal and stress conditions is an emerging area of study. Early work correlated changes in *Sirt3* mRNA expression with various renal stresses[34,79,80]. In general, stress tends to reduce renal expression of *Sirt3*; however, few studies have measured corresponding changes in SIRT3 activity by monitoring changes in mitochondrial protein acetylation in response to stress. In a model of free fatty acid (FFA)-associated tubulointerstitial inflammation, *Sirt3* mRNA expression was significantly decreased, ROS accumulated, and markers of inflammation increased compared to levels in control animals[79]. Remarkably, retroviral overexpression of *Sirt3* alleviated these effects, demonstrating that SIRT3 is an important factor that maintains cellular function in response to FFA-associated tubulointerstitial inflammation. In addition, rats fed a high-fat diet had reduced renal *Sirt3* expression and increased oxidative stress, as measured by an increase in malondialdehyde and manganese superoxide dismutase acetylation[80].

Mitochondrial function, acetylation, and SIRT3 activity have also been studied in a model of cisplatin-induced AKI[34]. Cisplatin-treated renal tissue had reduced numbers of mitochondria with several hyperacetylated mitochondrial proteins and decreased *Sirt3* mRNA expression. Treatment with an 5′-AMP-activated protein kinase (AMPK) agonist (5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside) or an antioxidant (acetyl-L-carnitine) together with cisplatin resulted in an increase in *Nampt*, *Ppargc1a*, and *Sirt3* mRNA and a decrease in mitochondrial protein acetylation. Similarly, exposure of glomerular mesangial cells to high glucose levels resulted in decreases in the levels of *Sirt3* mRNA and protein expression[33], whereas supplementation with NAD$^+$ prevented the development of high-glucose-induced mesangial hypertrophy and increased SIRT3 mRNA and protein levels. This finding supports a potential protective role of renal SIRT3 in diabetic nephropathy.

Together, the available data support a role of SIRT3 as a therapeutic target for conditions of renal stress, particularly oxidative stress. In models of SIRT3 depletion or activation, however, deacetylase activity and subsequent pathway activation have not yet been directly linked to the observed phenotypes described above[34,79,80]. Despite this shortcoming, these early studies provide a strong rationale for NAD$^+$ supplementation to activate SIRT3 and improve kidney function in renal disease.

SIRT4—SIRT4 has a potential role in maintaining kidney function after AKI[81]. In rats, cisplatin-induced nephrotoxicity resulted in decreased expression of NAMPT, SIRT1, SIRT3, and SIRT4. Upon cotreatment with cisplatin and the anti-inflammatory agent curcumin, the reduction in levels of these proteins was partially abrogated and kidney function was improved compared with that of cisplatin-treated controls. The data showed a positive correlation between kidney function, NAD$^+$ metabolism, and SIRT4 levels. The improvement in kidney function cannot be directly attributed to SIRT4, however, because the levels of NAMPT, SIRT1 and SIRT3 also increased in response to cisplatin and circumin

treatment. Much work remains to determine the role of SIRT4 in kidney metabolism and physiology.

SIRT5—Ablation of SIRT5 leads to hypersuccinylation of mitochondrial proteins in the kidney and other tissues[39,77]. The levels of other post-translational modifications that are regulated by SIRT5, namely mal-onylation[77] and glutarylation[39], also increase in kidneys in the absence of SIRT5. These observations suggest a role for SIRT5 in renal metabolism, but this hypothesis remains largely unexplored.

Some studies have reported a role for SIRT5 in the regulation of circulating ammonia levels. Liver carbamoyl-phosphate synthase 1 (CPS1) is an integral protein in the urea cycle and a target of SIRT5 (REFS 38,39,82). During fasting, SIRT5 deacylates liver CPS1, enabling CPS1 activation and lowering of blood ammonia levels. Following a 48 h fast, blood ammonia levels were increased in mice with global knockout of SIRT5 compared with levels in wild-type controls[75]. Consistent with a role for SIRT5 in regulation of ammonia handling, activation of glutaminase via SIRT5-mediated desuccinylation of lysine residues resulted in elevated renal ammonia excretion[83]. Although the effect of AKI or other renal stresses on protein succinylation and SIRT5 expression is not yet known, these studies highlight a key role of SIRT5 in the control of ammonia metabolism, and identify an emerging area for investigation.

Activation by NAD⁺ supplementation

A wealth of literature demonstrates that pathways affected by NAD^+ supplementation converge on pathways affected by sirtuins, which together can be activated by supplementation with NAD^+ or NAD^+ precursors or by manipulation of NAD^+ biosynthesis. In mitochondria, levels of NAD^+ are thought to reach a magnitude higher than the Michaelis constants (Km) for SIRT3 and SIRT5, suggesting that sirtuin activity could be rate-limited by mitochondrial NAD^+ levels[3], and therefore poised for activation.

Given the important role of the mitochondrial sirtuins in cardiac function, these proteins could partly mediate the beneficial effects of NAD^+ supplementation. Currently, the strongest evidence exists for a role of SIRT3 in mediating the beneficial effects of NAD^+ therapy. Although the effect of NAD^+ supplementation on the function of SIRT4 or SIRT5 has not been studied directly, these mitochondrial sirtuins could also protect against cardiac or renal stress. Determining whether the activity of SIRT4 or SIRT5 changes in the setting of NAD^+ therapy, for example by measuring changes in sirtuin-specific protein modifications, will be important in future studies.

Several models of renal or cardiac stresses are associated with increases in mitochondrial protein acetylation, which might be due to, or indicative of, depleted intracellular NAD^+ and reduced sirtuin activity. Protein acetylation is also observed in human failing hearts[23,70], suggesting that these findings have clinical relevance. NAD^+ supplementation could activate multiple sirtuins and improve mitochondrial function, thereby improving cardiac and renal function under diseased conditions.

NAD+ and cellular redox

NAD$^+$ supplementation also influences cardiac and renal function by directly influencing the cellular redox state. The heart and kidneys maintain a high degree of metabolic flexibility by consuming various fuel substrates to generate ATP, which is crucial for tissue function. Both organs prioritize fats as the preferred substrate for energy production (fats are thought to provide the greatest output of energy per carbon), but can also use other substrates such as glucose (heart), lactate (heart and kidney), ketones (heart), and amino acids (heart and kidney)[5,84]. Metabolism of these substrates to generate ATP is achieved predominantly via mitochondrial OXPHOS. This process involves a series of redox reactions whereby free energy released from the catabolism of carbon substrates is captured by exchanges between electron donors and acceptors through the electron transport chain (ETC). Catabolism of mitochondrial substrates generates electrons that are accepted by electron acceptors such as flavin adenine dinucleotide and NAD$^+$. Reduction of NAD$^+$ generates NADH, which is ultimately oxidized by complex I of the ETC to regenerate NAD$^+$ (REF. 3). Ultimately, the exchanges within these redox reactions result in the efficient conversion of chemical energy to ATP, which is a more stable and universal energy-bearing metabolite. The ratio of NAD$^+$ to NADH is a key regulator of several enzymes, including a wide range of oxidoreductases. For example, elevated levels of NADH inhibit several oxidative NAD$^+$-dependent enzymes. NAD$^+$ is an obligatory cofactor of metabolism and its availability controls the overall production of cardiac and renal ATP (REF. 3).

The importance of NAD$^+$/NADH redox exchange as a means to generate energy is underscored by the many cardiac and renal pathologies that are associated with defective OXPHOS resulting from inborn errors in metabolism. Impairment of the OXPHOS machinery contributes to reduced substrate oxidation, decreased ATP production, compromised intracellular Ca^{2+} flux, generation of ROS, and an altered NAD$^+$ to NADH ratio[5,84,85]. Various cardiac stressors lead to a switch from oxidative to anaerobic metabolism to reduce ROS damage and salvage ATP. This switch halts OXPHOS and exhausts mitochondrial NAD$^+$ (REF. 24).

In a model of cardiac-specific complex I deficiency (NDUSF4KO), mice had reduced NAD$^+$ to NADH ratio, hyperacetylated mitochondrial proteins, OXPHOS dysfunction, and a sensitivity to cardiac stress. The increased susceptibility to cardiac stress of these mice was mediated by increased acetylation of both the mitochondrial permeability transition pore (mPTP), which sensitized the pore to premature opening, and the malate aspartate shuttle, which inhibited shuttle activity[86]. Treatment with NMN partially normalized NAD$^+$ levels, reversed hyperacetylation of the mPTP and the malate aspartate shuttle, and attenuated the development of heart failure in NDUFS4KO mice undergoing chronic stress; activation of SIRT3 upon NMN supplementation could have an important role in mediating these beneficial effects. Another provocative mechanistic explanation is that NAD$^+$ supplementation directly shifts the NAD$^+$ to NADH ratio, thereby reactivating cellular oxidoreductases. NDUFS4KO mice had a reduced NAD$^+$ to NADH ratio and possible inhibited SIRT3 (REF. 22). Sirtuins are direct sensors of NAD$^+$, but they do not seem to sense NADH at physiologically relevant levels[87]; therefore, they are unlikely to sense shifts in the NAD$^+$ to NADH ratio in the same way that several cellular oxidoreductases sense the

ratio. If NDUFS4KO mice have NAD$^+$ deficiency and elevated levels of NADH, adding NAD$^+$ might have a direct, sirtuin-independent effect on oxidoreductase activity, which could contribute to the beneficial effects of NMN treatment. Regardless of this alternative hypothesis, SIRT3 and its regulation of acetylation are key mediators of the beneficial effects of NMN in the pathological cardiac remodelling observed in the NDUFS4KO mouse.

In the kidney, the dependence on the NAD$^+$ to NADH ratio and susceptibility to OXPHOS impairment is underscored by the relationship between oxidative metabolism and renal protection against AKI-induced tissue damage[2]. Peroxisome proliferator-activated receptor γ co-activator 1α (PGC1α) is a key transcriptional regulator of NAD$^+$ biosynthesis that protects against AKI. In PGC1α-deficient mice, supplementation with nicotinamide enhanced fatty acid oxidation and resulting NAD$^+$/NADH redox-driven ATP production, reversed AKI damage, and protected against AKI[2]. Nicotinamide supplementation seemed to directly influence the NAD$^+$/NADH redox state to enable substrate oxidation, thereby restoring metabolic and OXPHOS activity, and overall ATP production in this model of renal disease.

In summary, under cardiac and renal conditions of impaired OXPHOS or other states associated with a deficiency in electron acceptors (NAD$^+$), supplementation with NAD$^+$ and its precursors might directly relieve inhibition of some OXPHOS enzymes and improve cellular redox. As cellular redox is an important contributor to cardiac and renal metabolism and the response to stress, manipulating redox using NAD$^+$ precursors might have therapeutic efficacy. Future studies are required to test this hypothesis.

NAD$^+$ and cell signalling

In the heart and kidney, NAD$^+$ is also a substrate for enzymes involved in DNA damage repair and calcium signalling pathways, the ADP-ribosyltransferases (ARTDs; also known as PARPs) and the ADPR cyclases, respectively. Substantial efforts have been directed at determining if these enzymes can be manipulated as a strategy to alter intracellular NAD$^+$ levels or, conversely, if they are influenced by NAD$^+$ supplementation therapies.

ARTDs and ADPR cyclases have emerged as major nodes that influence NAD$^+$ levels[5,88]. Both consume NAD$^+$ and use the ADPR group to relay cellular signals (FIG. 2). Specifically, ARTDs transfer ADPR to accepting molecules to signal DNA damage and induce cell death[89]. Likewise, ADPR cyclases hydrolyse NAD$^+$ to form ADPR and other signalling metabolites that function as second messengers in calcium signalling[90]. These enzymatic activities generate nicotinamide as a byproduct, which can feedback to inhibit their activities. Several studies have shown that these enzymes deplete intracellular NAD$^+$ levels under conditions of cardiac and renal pathological stress[5]. For this reason, manipulating NAD$^+$ by inhibiting ARTDs and ADPR cyclases is currently being explored as a therapy to alter cell viability and function, respectively, in cardiac and renal diseases.

ARTDs and NAD$^+$

The ARTD family of enzymes transfer the ADP-ribose group of NAD$^+$ to histones, DNA and proteins as a signal to repair damaged DNA (REF. 89). ARTDs consume NAD$^+$ to

synthesize poly(ADP)ribose polymers (PARylation) that covalently attach to accepting molecules (FIG. 2). ARTD1 is the most abundant isoform and serves as a protector of genome stability upon activation by ROS-induced DNA lesions. Activated ARTD1 increases PARylation of enzymes, which is a signal for DNA repair or induction of cell death (parthanatos)[3]. In the heart and kidneys, persistent oxidative stress triggers over-activation of ARTD1, which contributes to myocardial infarction, ischaemic kidney injury, heart and renal failure, and other cardiovascular and renal pathologies[91]. Inhibition of ARTD1 protects against several pathological insults in the heart and kidney, such as ischaemia–reperfusion injury, which results in a high level of ROS-induced tissue damage[92,93].

Over-activation of ARTD1 can deplete NAD^+, resulting in reduced levels of cellular ATP and induction of parthanatos[89]. Research in skeletal muscle (ARTD1)[94], liver (ARTD7), and fat (ARTD5)[3] support a role for ARTD-mediated NAD^+ depletion as a mediator of cell death. The contribution of NAD^+ depletion to cellular death in the heart and kidneys in the setting of various pathological stresses remains to be determined.

By contrast, evidence is mounting that ARTD PARylation rather than NAD^+ decline is a direct cause of cellular death in response to pathological stress. For example, direct inhibition of the glycolytic enzyme hexokinase by ARTD1 PARylation reduced glycolysis before NAD^+ depletion, increased mitochondrial dysfunction, and led to cortical neuronal death[92]. Similarly, in the kidney, direct PARylation of the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was a key driver of cell death in renal tubules upon ischaemic injury[93]. ARTD1-mediated PARylation of GAPDH reduced anaerobic respiration, ultimately exacerbating ATP depletion and inducing cell death. These data suggest that the therapeutic benefit of ARTD1 inhibition might be due to altered metabolism at key nodes, independent of altered NAD^+ levels under pathological stress. Further research is required to disentangle the role of NAD^+ depletion upon ARTD over-activation in renal and cardiomyocyte cell death.

ADPR cyclases and NAD^+

Similar to ATRDs during cardiac and renal stress, ADPR cyclases are considered to be important regulators of intracellular NAD^+ levels in normal physiology[95]. These cyclases, primarily CD38 and CD157, serve as important effector molecules for generating Ca^{2+}-mobilizing second messengers that regulate cardiac and renal cell function. In cardiomyocytes, exogenous signals stimulate an initial release of Ca^{2+}, which activates CD38 (REF. 90). Subsequently, CD38 catalyses the conversion of NAD^+ to cyclic ADP-ribose (cADPR), which then triggers Ca^{2+} release from stores in the cardiac sarcoplasmic reticulum[96]. This Ca^{2+}-induced calcium release serves as the basis of rhythmic contraction of cardiac muscle[5]. In the kidneys, cADPR signalling is mediated by glomerular mesangial cells[97]. cADPR stimulates Ca^{2+} release from the endoplasmic reticulum[98], which contributes to renal vasoconstriction in response to various G-protein-coupled receptor agonists[99]. Mesangial cells and the capillary vasculature form a biomechanical meshwork, whereby Ca^{2+} signalling regulates mesangial cell contraction and capillary cell surface, ultimately controlling filtration rate and blood volume within the glomerulus[99].

Prolonged CD38 activation in response to cardiac stress leads to sustained Ca^{2+} release and signalling that results in hypertrophy and arrhythmia[5,100]. Consistent with these findings, male *Cd38*-knockout mice had enhanced cardiac function, including improved contractility[101], and ADPR cyclase inhibitors had anti-arrhythmic effects in models of cellular or cardiac Ca^{2+} overload[100]. Several studies support a role of ADPR cyclases other than CD38 in kidney function. For example, inhibition of CD73 has been shown to protect against renal stress such as AKI, and the enzymatic activity of CD39 has been shown to protect against acute renal ischaemic injury[102]. Kidney-specific compounds that inhibit these ADPR cyclases have been generated, but their influence on kidney function is still under investigation[97].

CD38 also alters intracellular NAD^+ levels in the heart and kidneys. One study showed that *Cd38*-knockout mice had ~25-fold higher NAD^+ levels in the heart and ~two-fold higher levels in the kidney than did wild-type controls[88,95]. By contrast, another study showed no changes in NAD^+ levels in these tissues in *Cd38* knockout mice compared with wild-type controls[103]. Thus, the extent to which CD38 inhibition or ablation enhances NAD^+ levels in the heart and kidney requires further investigation. Despite this gap in knowledge, inhibitors that target ADPR cyclases are currently in development for the treatment of cardiac and renal disease. Thorough characterization of the cellular impact of these inhibitors could identify a role for these enzymes in mediating the therapeutic efficacy of NAD^+ supplementation.

Remaining challenges

NAD^+ supplementation therapies are efficacious in several animal models of cardiac and renal diseases but two key challenges remain for their continued development and clinical translation. First, elucidating the mechanisms of the therapeutic effects of NAD^+ is important to enable their potential to be fully realized in the settings of cardiac and renal pathologies. Disentangling the individual and/or concerted contributions of sirtuin activation, redox, and cellular signalling will be challenging (FIG. 2). Activation of sirtuins, especially SIRT3, via exogenous NAD^+ improves cellular bioenergetics, mitochondrial function, and stress resistance. Additionally, NAD^+ supplementation seems to correct redox imbalances in the setting of disrupted bioenergetics, which could be mediated partly by directly increasing enzyme redox activity and partly via manipulation of mitochondrial protein acetylation. Finally, increasing NAD^+ levels might mitigate the damage induced by overactive ARTDs and ADPR cyclases. Which of these pathways confers the greatest effects on overall cardiac and renal function remains unknown. Specific activators of each pathway, independent of NAD^+, might shed light on this question. Alternatively, continued investigation into NAD^+ precursor supplementation in animal models lacking relevant pathways (for example NAD^+ supplementation in the absence of CD38 (REF. 104) or a sirtuin[31]) would directly test the role of each pathway in mediating NAD^+ therapeutic efficacy.

A second major challenge will be to identify the best pharmacological strategies to alter cellular NAD^+ levels (for example precursor supplementation versus inhibition of NAD^+ consumers) in patients with various diseases. These strategies have not yet been tested in

patients with cardiac or renal diseases. Ongoing clinical trials for other indications are primarily focused on NAD^{+} precursor supplementation because preclinical studies have demonstrated efficacy of this approach for increasing NAD^{+} levels and improving a variety of cellular functions. Several clinical trials are underway to determine the pharmacokinetics[105–107], bioavailability[108], and effect of nicotinamide riboside supplementation in metabolic diseases, with a particular focus on obesity and insulin sensitivity[109,110]; no adverse effects have been reported with 100–1,000 mg per day. The efficacy of nicotinimide riboside supplementation to improve mitochondrial function, energy metabolism, and parameters of cardiovascular risk is also currently being tested[110,111]. Interestingly, supplementation with nicotinamide riboside has been reported to increase the NAD^{+} metabolome in human blood in a dose-dependent manner[21]. In this study the intermediate nicotinic acid adenine dinucleotide was formed from the recycling of newly synthesized NAD^{+} and served as a biomarker of enhanced NAD^{+} levels.

Conclusions

The potential of NAD^{+} as a therapeutic target has renewed interest in this metabolite, which was discovered over 100 years ago[112]. NAD^{+} is a versatile metabolic and redox substrate that is essential for all living cells and has a central role as a co-substrate for sirtuin deacylation reactions, as a coenzyme in OXPHOS reactions, as a substrate in ARTD and ADPR cyclase signalling pathways, and likely also has a role in many as yet undiscovered biological pathways. NAD^{+} depletion is emerging as a central feature of cardiac and renal disorders that has an important role in the pathogenesis of these diseases[2,5]. As such, therapeutic approaches to treat these diseases by inhibiting NAD^{+}-consuming enzymes and/or augmenting intracellular NAD^{+} levels via stimulation of NAD^{+} biosynthesis or supplementation with NAD^{+} precursors is an area ripe for discovery.

Acknowledgments

Glossary

Nicotinamide adenine dinucleotide
(NAD^{+}). A pyridine dinucleotide and important metabolic cofactor

ADPR cyclases
Effector molecules that generate calcium-mobilizing second messengers

ADP ribosyltransferases
Enzymes that transfer the ADPR group of NAD^{+} as a signal to repair damaged DNA

Sirtuins

NAD^+-dependent protein deacylases that consume NAD^+ to remove post-translational acyl modifications from proteins

Biosynthetic precursors

The biosynthetic precursors of NAD^+ are dietary vitamin B_3 compounds, including nicotinic acid, nicotinamide, and nicotinamide riboside. These precursors are recycled from the diet and used by tissues to generate NAD^+

Nicotinamide phosphoribosyltransferase

(NAMPT). An enzyme that converts nicotinamide into NMN in the NAD^+ salvage pathway

Oxidative phosphorylation

(OXPHOS). The electron transport pathway that is used by cells to generate ATP

Parthanatos

A form of programmed cell death that is induced by accumulation of poly(ADP) ribose and the nuclear translocation of apoptosis-inducing factor from mitochondria.

References

1. Forbes JM. Mitochondria-power players in kidney function? Trends Endocrinol Metab. 2016; 27:441–442. [PubMed: 27215468]

2. Tran MT, et al. PGC1α drives NAD biosynthesis linking oxidative metabolism to renal protection. Nature. 2016; 531:528–532. This study demonstrates that NAM treatment improves renal function in a mouse model of AKI. [PubMed: 26982719]

3. Cantó C, Menzies KJ, Auwerx J. NAD^+ metabolism and the control of energy homeostasis: a balancing act between mitochondria and the nucleus. Cell Metab. 2015; 22:31–53. [PubMed: 26118927]

4. He W, Newman JC, Wang MZ, Ho L, Verdin E. Mitochondrial sirtuins: regulators of protein acylation and metabolism. Trends Endocrinol Metab. 2012; 23:467–476. [PubMed: 22902903]

5. Mericskay M. Nicotinamide adenine dinucleotide homeostasis and signalling in heart disease: pathophysiological implications and therapeutic potential. Arch Cardiovasc Dis. 2015; 109:207–215. [PubMed: 26707577]

6. Ebrahimkhani MR, et al. Aag-initiated base excision repair promotes ischemia reperfusion injury in liver, brain, and kidney. Proc Natl Acad Sci USA. 2014; 111:E4878–E4886. [PubMed: 25349415]

7. Braidy N, et al. Age related changes in NAD+ metabolism oxidative stress and Sirt1 activity in wistar rats. PLoS ONE. 2011; 6:e19194. [PubMed: 21541336]

8. Mori V, et al. Metabolic profiling of alternative NAD biosynthetic routes in mouse tissues. PLoS ONE. 2014; 9:e113939. [PubMed: 25423279]

9. Lin LF, Henderson LM. Pyridinium precursors of pyridine nucleotides in perfused rat kidney and in the testis. J Biol Chem. 1972; 247:8023–8030. [PubMed: 4344988]

10. Ikeda M, et al. Studies on the biosynthesis of nicotinamide adenine dinucleotide. II A role of picolinic carboxylase in the biosynthesis of nicotinamide adenine dinucleotide from tryptophan in mammals. J Biol Chem. 1965; 240:1395–1401. [PubMed: 14284754]

11. Shibata K, Morita N, Shibata Y, Fukuwatari T. Enzymes that control the conversion of L-tryptophan-nicotinamide and the urinary excretion ratio (N^1-methyl-2-pyridone-5-carboxamide + N^1-methyl-4-pyridone-3-carboxamide)/N^1-methylnicotinamide in mice. Biosci Biotechnol Biochem. 2013; 77:2105–2111. [PubMed: 24096677]

12. Eto N, Miyata Y, Ohno H, Yamashita T. Nicotinamide prevents the development of hyperphosphataemia by suppressing intestinal sodium-dependent phosphate transporter in rats

with adenine-induced renal failure. Nephrol Dial Transplant. 2005; 20:1378–1384. [PubMed: 15870221]

13. Kempson SA, Colon-Otero G, Ou SY, Turner ST, Dousa TP. Possible role of nicotinamide adenine dinucleotide as an intracellular regulator of renal transport of phosphate in the rat. J Clin Invest. 1981; 67:1347–1360. [PubMed: 6453134]

14. Gopal E, et al. Sodium-coupled and electrogenic transport of B-complex vitamin nicotinic acid by slc5a8, a member of the Na/glucose co-transporter gene family. Biochem J. 2005; 388:309–316. [PubMed: 15651982]

15. Trueblood NA, Ramasamy R, Wang LF, Schaefer S. Niacin protects the isolated heart from ischemia-reperfusion injury. Am J Physiol Heart Circ Physiol. 2000; 279:H764–H771. [PubMed: 10924076]

16. Collins PB, Chaykin S. The management of nicotinamide and nicotinic acid in the mouse. J Biol Chem. 1972; 247:778–783. [PubMed: 4333514]

17. Corr PB, May DG. Renal mechanisms for the excretion of nicotinic acid. J Pharmacol Exp Ther. 1975; 192:195–200. [PubMed: 235633]

18. Nomura K, et al. Hepatectomy-related hypophosphatemia: a novel phosphaturic factor in the liver–kidney axis. J Am Soc Nephrol. 2014; 25:761–772. [PubMed: 24262791]

19. Grozio A, et al. CD73 protein as a source of extracellular precursors for sustained NAD+ biosynthesis in FK866-treated tumor cells. J Biol Chem. 2013; 288:25938–25949. [PubMed: 23880765]

20. Ratajczak J, et al. NRK1 controls nicotinamide mononucleotide and nicotinamide riboside metabolism in mammalian cells. Nat Commun. 2016; 7:13103. [PubMed: 27725675]

21. Trammell SAJ, et al. Nicotinamide riboside is uniquely and orally bioavailable in mice and humans. Nat Commun. 2016; 7:12948. The first clinical study of nicotinamide riboside supplementation; shows a dose-dependent increase in NAD$^+$ with nicotinamide riboside, demonstrating the potential of NAD$^+$ boosting therapies in a clinical setting. [PubMed: 27721479]

22. Karamanlidis G, et al. Mitochondrial complex I deficiency increases protein acetylation and accelerates heart failure. Cell Metab. 2013; 18:239–250. [PubMed: 23931755]

23. Lee CF, et al. Normalization of NAD+ redox balance as a therapy for heart failure. Circulation. 2016; 134:883–894. This study demonstrates that NMN and cardiac NAMPT increase NAD$^+$, reduce protein hyperacetylation, and improve cardiac function in a mouse model of heart failure. [PubMed: 27489254]

24. Yamamoto T, et al. Nicotinamide mononucleotide, an intermediate of NAD+ synthesis, protects the heart from ischemia and reperfusion. PLoS ONE. 2014; 9:e98972. [PubMed: 24905194]

25. Berger F, Lau C, Dahlmann M, Ziegler M. Subcellular compartmentation and differential catalytic properties of the three human nicotinamide mononucleotide adenylyltransferase isoforms. J Biol Chem. 2005; 280:36334–36341. [PubMed: 16118205]

26. Yamamoto M, et al. Nmnat3 is dispensable in mitochondrial NAD level maintenance *in vivo*. PLoS ONE. 2016; 11:e0147037. [PubMed: 26756334]

27. Hsu CP, Yamamoto T, Oka S, Sadoshima J. The function of nicotinamide phosphoribosyltransferase in the heart. DNA Repair (Amst). 2014; 23:64–68. [PubMed: 25277684]

28. Wang P, Miao CY. NAMPT as a therapeutic target against stroke. Trends Pharmacol Sci. 2015; 36:891–905. [PubMed: 26538317]

29. Aboud OA, et al. Dual and specific inhibition of NAMPT and PAK4 by KPT-9274 decreases kidney cancer growth. Mol Cancer Ther. 2016; 15:2119–2129. [PubMed: 27390344]

30. Benito-Martin A, et al. Endogenous NAMPT dampens chemokine expression and apoptotic responses in stressed tubular cells. Biochim Biophys Acta. 2014; 1842:293–303. [PubMed: 24287278]

31. Pillai VB, et al. Exogenous NAD blocks cardiac hypertrophic response via activation of the SIRT3–LKB1–AMP-activated kinase pathway. J Biol Chem. 2010; 285:3133–3144. Study showing that exogenous NAD$^+$ supplementation blocked agonist-induced hypertrophic responses in cardiomyocytes and mouse models; implicates SIRT3 deacetylase activity in the mechanism of this cardioprotection. [PubMed: 19940131]

32. Hsu CP, Oka S, Shao D, Hariharan N, Sadoshima J. Nicotinamide phosphoribosyltransferase regulates cell survival through NAD+ synthesis in cardiac myocytes. Circ Res. 2009; 105:481–491. [PubMed: 19661458]

33. Zhuo L, et al. NAD blocks high glucose induced mesangial hypertrophy via activation of the sirtuins–AMPK–mTOR pathway. Cell Physiol Biochem. 2011; 27:681–690. This study shows that exogenous NAD$^+$ maintains SIRT1 and SIRT3 activity in the setting of high-glucose-induced mesangial hypertrophy and provides a mechanism for how sirtuin activity protects against mesangial hypertrophy. [PubMed: 21691086]

34. Morigi M, et al. Sirtuin 3-dependent mitochondrial dynamic improvements protect against acute kidney injury. J Clin Invest. 2015; 125:715–726. [PubMed: 25607838]

35. Anderson KA, Green MF, Huynh FK, Wagner GR, Hirschey MD. SnapShot: mammalian sirtuins. Cell. 2014; 159:956–956.e1. [PubMed: 25417168]

36. Denu JM. The Sir2 family of protein deacetylases. Curr Opin Chem Biol. 2005; 9:431–440. [PubMed: 16122969]

37. Peng C, et al. The first identification of lysine malonylation substrates and its regulatory enzyme. Mol Cell Proteomics. 2011; 10:M111.012658.

38. Du J, et al. Sirt5 is a NAD-dependent protein lysine demalonylase and desuccinylase. Science. 2011; 334:806–809. [PubMed: 22076378]

39. Tan M, et al. Lysine glutarylation is a protein posttranslational modification regulated by SIRT5. Cell Metab. 2014; 19:605–617. [PubMed: 24703693]

40. Feldman JL, Baeza J, Denu JM. Activation of the protein deacetylase SIRT6 by long-chain fatty acids and widespread deacylation by mammalian sirtuins. J Biol Chem. 2013; 288:31350–31356. [PubMed: 24052263]

41. Sundaresan NR, et al. The sirtuin SIRT6 blocks IGF–Akt signaling and development of cardiac hypertrophy by targeting c-Jun. Nat Med. 2012; 18:1643–1650. [PubMed: 23086477]

42. Gupta M, Samant S, Bao R, Pillai V. The sirtuin SIRT6 represses expression of cachexia-associated cytokine myostatin by blocking its NF-kB-dependent gene transcription. FASEB J. 2016; 30:1009.11.

43. Hirschey MD, et al. SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation. Nature. 2010; 464:121–125. [PubMed: 20203611]

44. Haigis MC, et al. SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic beta cells. Cell. 2006; 126:941–954. [PubMed: 16959573]

45. Michishita E, Park JY, Burneskis JM. Evolutionarily conserved and nonconserved cellular localizations and functions of human SIRT proteins. Mol Biol Cell. 2005; 16:4623–4635. [PubMed: 16079181]

46. Mathias RA, et al. Sirtuin 4 is a lipoamidase regulating pyruvate dehydrogenase complex activity. Cell. 2014; 159:1615–1625. [PubMed: 25525879]

47. Jeong SM, et al. SIRT4 has tumor-suppressive activity and regulates the cellular metabolic response to DNA damage by inhibiting mitochondrial glutamine metabolism. Cancer Cell. 2013; 23:450–463. [PubMed: 23562301]

48. Wagner GR, Payne RM. Mitochondrial acetylation and diseases of aging. J Aging Res. 2011; 2011:1–13.

49. Wagner GR, Hirschey MD. Nonenzymatic protein acylation as a carbon stress regulated by sirtuin deacylases. Mol Cell. 2014; 54:5–16. [PubMed: 24725594]

50. Ghanta S, Grossmann RE, Brenner C. Mitochondrial protein acetylation as a cell-intrinsic, evolutionary driver of fat storage: biocehmical and metabolic logic of acetyl-lysine modifications. Crit Rev Biochem Mol Biol. 2013; 48:1–42. [PubMed: 23173916]

51. Chhoy, P., et al. Sirtuins. Houtkooper, R., editor. Vol. 10. Sirtuins; 2016. p. 105-138.

52. Park J, et al. SIRT5-mediated lysine desuccinylation impacts diverse metabolic pathways. Mol Cell. 2013; 50:919–930. [PubMed: 23806337]

53. Imai S, Armstrong CM, Kaeberlein M, Guarente L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature. 2000; 403:795–800. [PubMed: 10693811]

54. Anderson RM, et al. Manipulation of a nuclear NAD+ salvage pathway delays aging without altering steady-state NAD+ levels. J Biol Chem. 2002; 277:18881–18890. [PubMed: 11884393]

55. Belenky P, et al. Nicotinamide riboside promotes Sir2 silencing and extends lifespan via Nrk and Urh1/Pnp1/ Meu1 pathways to NAD+ Cell. 2007; 129:473–484. [PubMed: 17482543]

56. Bitterman KJ, Anderson RM, Cohen HY, Latorre-Esteves M, Sinclair DA. Inhibition of silencing and accelerated aging by nicotinamide, a putative negative regulator of yeast sir2 and human SIRT1. J Biol Chem. 2002; 277:45099–45107. [PubMed: 12297502]

57. Mouchiroud L, et al. The NAD$^+$/sirtuin pathway modulates longevity through activation of mitochondrial UPR and FOXO signaling. Cell. 2013; 154:430–441. [PubMed: 23870130]

58. Yoshino J, Mills KF, Yoon MJ, Imai SI. Nicotinamide mononucleotide, a key NAD+ intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice. Cell. 2011; 14:528–536.

59. Imai S, Guarente L. NAD+ and sirtuins in aging and disease. Trends Cell Biol. 2014; 24:464–471. [PubMed: 24786309]

60. Giblin W, Skinner ME, Lombard DB. Sirtuins: guardians of mammalian healthspan. Trends Genet. 2014; 30:271–286. [PubMed: 24877878]

61. Ma L, Li Y. SIRT1: role in cardiovascular biology. Clin Chim Acta. 2015; 440:8–15. [PubMed: 25444742]

62. Winnik S, Auwerx J, Sinclair DA, Matter CM. Protective effects of sirtuins in cardiovascular diseases: from bench to bedside. Eur Heart J. 2015; 36:3404–3412. [PubMed: 26112889]

63. Kong L, et al. Sirtuin 1: a target for kidney diseases. Mol Med. 2015; 21:87–97. [PubMed: 25587857]

64. Dong YJ, et al. Renal protective effect of sirtuin 1. J Diabetes Res. 2014; 2014:843786. [PubMed: 25386563]

65. Kitada M, Kume S, Koya D. Role of sirtuins in kidney disease. Curr Opin Nephrol Hypertens. 2014; 23:75–79. [PubMed: 24247822]

66. Ahn BH, et al. A role for the mitochondrial deacetylase Sirt3 in regulating energy homeostasis. Proc Natl Acad Sci USA. 2008; 105:14447–14452. [PubMed: 18794531]

67. Sundaresan NR, et al. Sirt3 blocks the cardiac hypertrophic response by augmenting Foxo3a-dependent antioxidant defense mechanisms in mice. J Clin Invest. 2009; 119:2758–2771. [PubMed: 19652361]

68. Hafner AV, et al. Regulation of the mPTP by SIRT3-mediated deacetylation of CypD at lysine 166 suppresses age-related cardiac hypertrophy. Aging (Albany NY). 2010; 2:914–923. [PubMed: 21212461]

69. Porter G, Urciuoli WR, Brookes PS, Nadtochiy SM. SIRT3 deficiency exacerbates ischemia-reperfusion injury: implication for aged hearts. Am J Physiol Heart Circ Physiol. 2014; 306:H1602–H1609. [PubMed: 24748594]

70. Horton JL, et al. Mitochondrial protein hyperacetylation in the failing heart. JCI Insight. 2016; 1:1–14.

71. Alrob OA, et al. Obesity-induced lysine acetylation increases cardiac fatty acid oxidation and impairs insulin signalling. Cardiovasc Res. 2014; 103:485–497. [PubMed: 24966184]

72. Cantó C, et al. The NAD$^+$ precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet-induced obesity. Cell Metab. 2012; 15:838–847. [PubMed: 22682224]

73. Liu B, et al. SIRT4 prevents hypoxia-induced apoptosis in H9c2 cardiomyoblast cells. Cell Physiol Biochem. 2013; 32:655–662. [PubMed: 24029877]

74. Luo, Y-X., et al. Sirt4 accelerates Ang II-induced pathological cardiac hypertrophy by inhibiting manganese superoxide dismutase activity. Eur Heart J. 2016. http://dx.doi.org/10.1093/eurheartj/ehw138

75. Yu J, et al. Metabolic characterization of a Sirt5 deficient mouse model. Sci Rep. 2013; 3:2806. [PubMed: 24076663]

76. Boylston JA, et al. Characterization of the cardiac succinylome and its role in ischemia-reperfusion injury. J Mol Cell Cardiol. 2015; 88:73–81. The first publication of the cardiac succinylome; the data suggest a role of SIRT5 in the response to ischaemia–reperfusion injury. [PubMed: 26388266]

77. Nishida Y, et al. SIRT5 regulates both cytosolic and mitochondrial protein malonylation with glycolysis as a major target. Mol Cell. 2015; 59:321–332. [PubMed: 26073543]

78. Sadhukhan S, et al. Metabolomics-assisted proteomics identifies succinylation and SIRT5 as important regulators of cardiac function. Proc Natl Acad Sci USA. 2016; 113:4320–4325. This study shows that SIRT5-knockout hearts have impaired fatty acid oxidation that contributes to cardiac hypertrophy with ageing. [PubMed: 27051063]

79. Koyama T, et al. SIRT3 attenuates palmitate-induced ROS production and inflammation in proximal tubular cells. Free Radic Biol Med. 2011; 51:1258–1267. [PubMed: 21664458]

80. Yang H, et al. Green tea polyphenols attenuate high-fat diet-induced renal oxidative stress through SIRT3-dependent deacetylation. Biomed Environ Sci. 2015; 28:455–459. [PubMed: 26177907]

81. Ugur S, et al. The renoprotective effect of curcumin in cisplatin-induced nephrotoxicity. Renal Fail. 2014; 37:332–336.

82. Nakagawa T, Lomb DJ, Haigis MC, Guarente L. SIRT5 deacetylates carbamoyl phosphate synthetase 1 and regulates the urea cycle. Mol Cell. 2009; 137:560–570.

83. Polletta L, et al. SIRT5 regulation of ammonia-induced autophagy and mitophagy. Autophagy. 2015; 11:253–270. [PubMed: 25700560]

84. Hall AM, Unwin RJ. The not so 'mighty chondrion': emergence of renal diseases due to mitochondrial dysfunction. Nephron Physiol. 2007; 105:1–10.

85. Fosslien E. Mitochondrial medicine — molecular pathology of defective oxidative phosphorylation. Ann Clin Lab Sci. 2001; 31:25–67. [PubMed: 11314862]

86. Fung Lee C, Garcia-Menendez L, Karamanlidis G, Tian R. Restoration of NAD redox balance ameliorates pressure overload-induced cardiac hypertrophy and dysfunction via regulation of mitochondrial protein acetylation and permeability transition. Free Radic Biol Med. 2013; 65:S75.

87. Madsen AS, et al. Investigating the sensitivity of NAD+-dependent sirtuin deacylation activities to NADH. J Biol Chem. 2016; 291:7128–7141. [PubMed: 26861872]

88. Aksoy P, White TA, Thompson M, Chini EN. Regulation of intracellular levels of NAD: a novel role for CD38. Biochem Biophys Res Commun. 2006; 345:1386–1392. [PubMed: 16730329]

89. Rouleau M, Patel A, Hendzel MJ, Kaufmann SH, Poirier GG. PARP inhibition: PARP1 and beyond. Nat Rev Cancer. 2010; 10:293–301. [PubMed: 20200537]

90. Malavasi F, et al. Evolution and function of the ADP ribosyl cyclase/CD38 gene family in physiology and pathology. Physiol Rev. 2008; 88:841–886. [PubMed: 18626062]

91. Xiao CY, et al. Poly(ADP-ribose) polymerase promotes cardiac remodeling, contractile failure, and translocation of apoptosis-inducing factor in a murine experimental model of aortic banding and heart failure. J Pharmacol Exp Ther. 2005; 312:891–898. [PubMed: 15523000]

92. Andrabi SA, et al. Poly(ADP-ribose) polymerase-dependent energy depletion occurs through inhibition of glycolysis. Proc Natl Acad Sci USA. 2014; 111:10209–10214. [PubMed: 24987120]

93. Devalaraja-Narashimha K, Padanilam BJ. PARP-1 inhibits glycolysis in ischemic kidneys. J Am Soc Nephrol. 2009; 20:95–103. [PubMed: 19056868]

94. Pirinen E, et al. Pharmacological Inhibition of poly(ADP-ribose) polymerases improves fitness and mitochondrial function in skeletal muscle. Cell Metab. 2014; 19:1034–1041. [PubMed: 24814482]

95. Ying W. NAD+/NADH and NADP+/NADPH in cellular functions and cell death: regulation and biological consequences. Antioxid Redox Signal. 2008; 10:179–206. [PubMed: 18020963]

96. Rizzuto R, De Stefani D, Raffaello A, Mammucari C. Mitochondria as sensors and regulators of calcium signalling. Nat Rev Mol Cell Biol. 2012; 13:566–578. [PubMed: 22850819]

97. Kim SY, Park KH, Gul R, Jang KY, Kim UH. Role of kidney ADP-ribosyl cyclase in diabetic nephropathy. Am J Physiol Renal Physiol. 2009; 296:F291–F297. [PubMed: 19073639]

98. Stockand JD, Sansom SC. Glomerular mesangial cells: electrophysiology and regulation of contraction. Physiol Rev. 1998; 78:723–744. [PubMed: 9674692]

99. Thai TL, Arendshorst WJ. Mice lacking the ADP ribosyl cyclase CD38 exhibit attenuated renal vasoconstriction to angiotensin II, endothelin-1, and norepinephrine. Am J Physiol Renal Physiol. 2009; 297:F169–F176. [PubMed: 19403649]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

100. Kannt A, Sicka K, Kroll K, Kadereit D, Gögelein H. Selective inhibitors of cardiac ADPR cyclase as novel anti-arrhythmic compounds. Naunyn Schmiedebergs Arch Pharmacol. 2012; 385:717–727. [PubMed: 22526470]

101. Gan L, et al. Disruption of CD38 gene enhances cardiac functions by elevating serum testosterone in the male null mice. Life Sci. 2011; 89:491–497. [PubMed: 21840325]

102. Rajakumar SV, et al. Deficiency or inhibition of CD73 protects in mild kidney ischemia-reperfusion injury. Transplantation. 2010; 90:1260–1264. [PubMed: 21063242]

103. Young GS, Choleris E, Lund FE, Kirkland JB. Decreased cADPR and increased NAD + in the Cd38$^{-/-}$ mouse. Biochem Biophys Res Commun. 2006; 346:188–192. [PubMed: 16750163]

104. Guan XH, et al. CD38 deficiency protects the heart from ischemia/reperfusion injury through activating SIRT1/FOXOs-mediated antioxidative stress pathway. Oxid Med Cell Longev. 2016; 2016:7410257. [PubMed: 27547294]

105. US National Library of Medicine. ClinicalTrials.gov. 2015. https://clinicaltrials.gov/ct2/show/NCT02300740

106. US National Library of Medicine. ClinicalTrials.gov. 2014. https://clinicaltrials.gov/ct2/show/NCT02191462

107. US National Library of Medicine. ClinicalTrials.gov. 2016. https://clinicaltrials.gov/ct2/show/NCT02689882

108. US National Library of Medicine. ClinicalTrials.gov. 2017. https://clinicaltrials.gov/ct2/show/NCT02712593

109. US National Library of Medicine. ClinicalTrials.gov. 2016. https://clinicaltrials.gov/ct2/show/NCT02303483

110. US National Library of Medicine. ClinicalTrials.gov. 2016. https://clinicaltrials.gov/ct2/show/NCT02835664

111. US National Library of Medicine. ClinicalTrials.gov. 2016. https://clinicaltrials.gov/ct2/show/NCT02812238

112. Harden A, Young WJ. The alcoholic ferment of yeast-juice. Part II. — the conferment of yeast-juice. Proc R Soc Lond B. 1906; 78:369–375.

Key points

- Nicotinamide adenine dinucleotide (NAD^+) is a coenzyme with roles in several cardiac and renal metabolic processes

- NAD^+ depletion is emerging as a major contributor to the pathogenesis of cardiac and renal disease

- Preclinical data suggest that NAD^+ repletion strategies have the potential to restore healthy renal and cardiac metabolism and physiology

- The mitochondrial sirtuins mediate some of the beneficial effects of NAD^+ supplementation

- NAD^+ supplementation can directly enhance metabolism and improve cellular redox reactions in the setting of cardiac and renal disease

- NAD^+ is also a substrate for enzymes involved in DNA damage repair and calcium signalling pathways; NAD^+ supplementation could alter these pathways to influence cell viability, organ function and disease outcomes
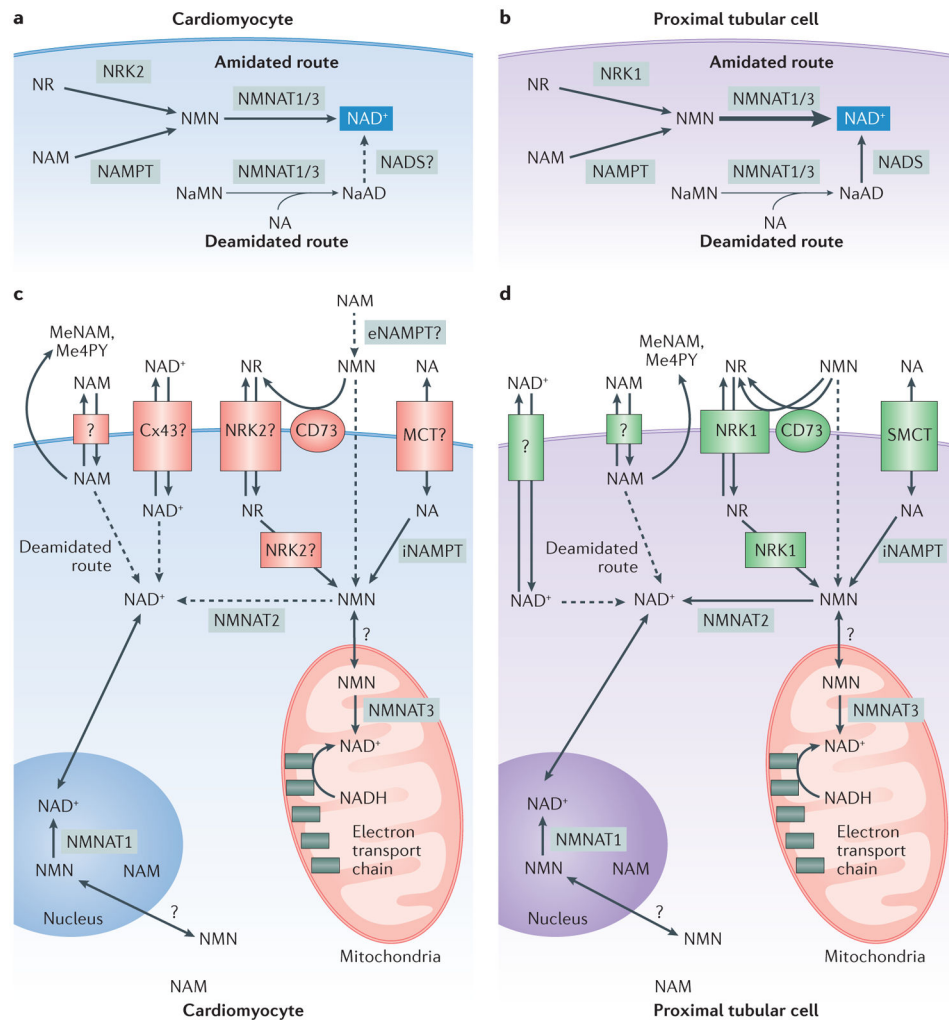
Figure 1. Cardiac and renal biosynthesis and the metabolome of nicotinamide adenine dinucleotide (NAD$^+$)

Two routes of NAD$^+$ synthesis — deamidated (*de novo* synthesis) and amidated (precursor salvage) — occur in the heart (parts **a,c**) and the kidney (parts **b,d**) (REF. 8). In the amidated route, which dominates in both tissues, the activities of nicotinamide riboside kinase (NRK) isoforms (NRK2 in the heart and NRK1 in the kidney) enable generation of nicotinamide mononucleotide (NMN) from the salvage of nicotinamide riboside (NR), whereas nicotinamide phosphoribosyltransferase (NAMPT) activity converts salvaged nicotinamide (NAM) to NMN. Nicotinamide mononucleotide adenyltransferase (NMNAT) isoforms 1 (nuclear) and 3 (mitochondrial) convert NMN to NAD$^+$ in the amidated route and nicotinic acid mononucleotide (NaMN) to nicotinic acid dinucleotide (NaAD) in the deamidated route. The activities of NMNAT1/3 in the amidated route are much greater in the kidney (part **b**) than in the heart (part **a**), as indicated by the arrow thicknesses. Nicotinic acid (NA) can also be converted to an intermediate in the deamidated route, and the activity of NAD synthase (NADS) enables conversion of NaAD to NAD$^+$, although the role of NADS in the heart is speculative. Uptake of NAD$^+$ and its precursors occurs through many means in the heart (part **c**) and kidney (part d). NAD$^+$ can be directly transported into the heart by the

connexion 43 hemichannel (Cx43). Renal uptake of NA occurs through the sodium monocarboxylate transporter (SMCT) (REF. 14). NR and NMN require NRK1 for optimal uptake and conversion to NAD^+ in the kidney[20]. NRK2 might serve this role for these NAD^+ precursors in the heart. Additionally, extracellular NMN might be converted into NR by membrane bound cluster of differentiation 73 (CD73) (REF. 19) and subsequently taken up by renal NRK1 or cardiac NRK2. Renal NAM uptake is thought to occur through an unidentified transporter[14]. NAM — generated from exogenous sources or other NAD^+ precursors such as NA — is maintained at low levels in the heart and kidney[9] through its conversion into the byproducts 1-methylnicotinamide (MeNAM) and *N*-methyl-4-pyridone-5-carboxamide (Me4PY), which are excreted by the cell. 5′-ectonucleotidase; MCT, monocarboxylate transporter; NADH, reduced NAD.
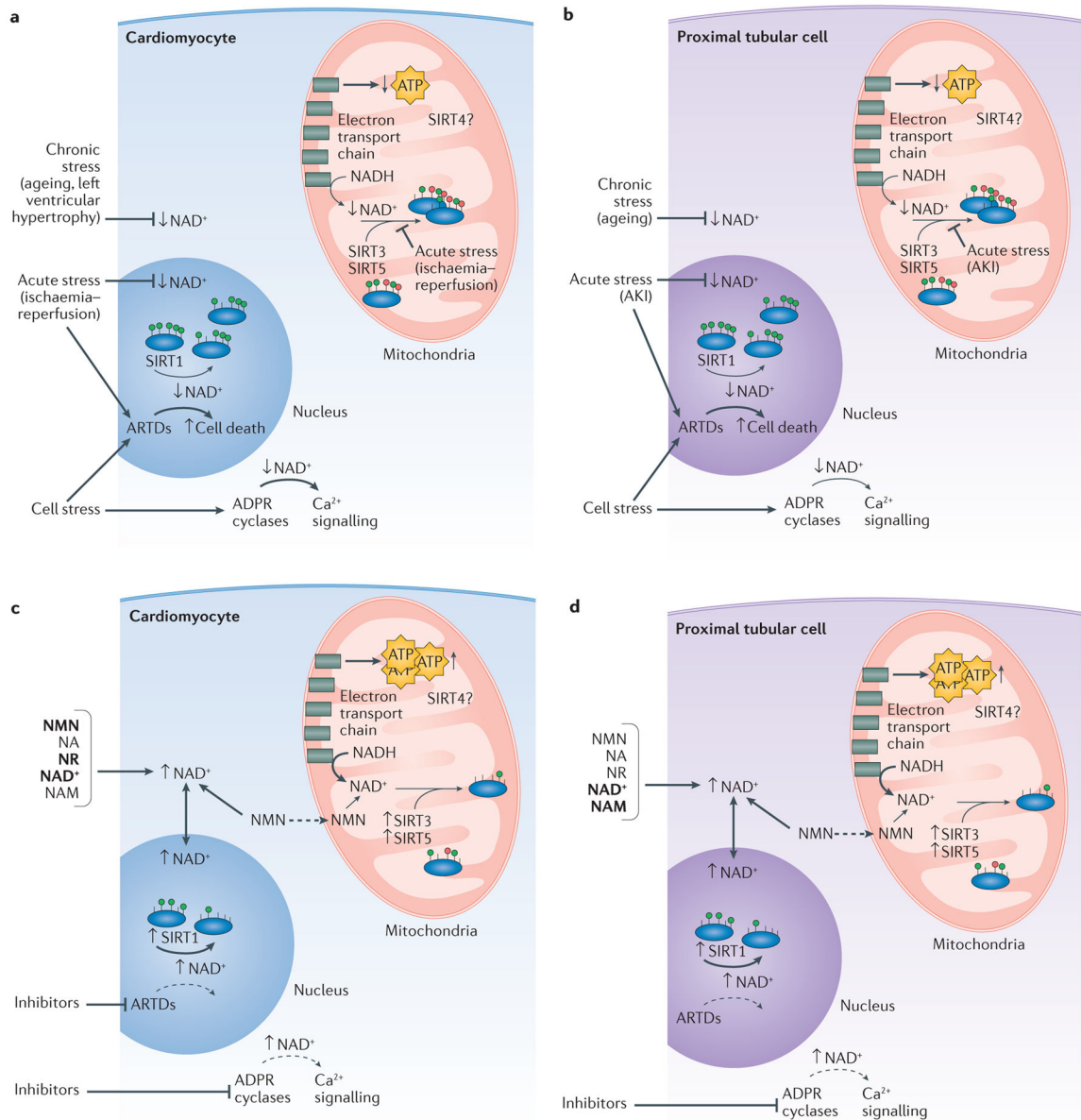
Figure 2. The potential effects of nicotinamide adenine dinucleotide (NAD⁺) therapy on cardiac and renal pathophysiology

Chronic cardiac (part **a**) and renal (part **b**) stresses such as ageing and left ventricular hypertrophy result in depleted cellular NAD$^+$ levels. Mitochondrial protein hyperacylation accompanies general mitochondrial dysfunction and might contribute to impaired reduction–oxidation (redox) reactions and ATP depletion. Cellular stress causes overactivation of ADP-ribosyltransferases (ARTDs) and adenine diphosphate ribose (ADPR) cyclases, potentially contributing to overall NAD$^+$ depletion. Acute stresses including ischaemia and acute kidney injury (AKI) deplete cellular NAD$^+$ levels by lowering NAD$^+$ synthesis (primarily through the amidated route). Supplementation with NAD$^+$ or NAD$^+$ precursors might boost cellular NAD$^+$ levels and improve cardiac (part **c**) and renal (part **d**) function in disease states. The primary mechanism might be activation of mitochondrial sirtuins (SIRTs), which activate

protein lysine deacylation and improve mitochondrial function, leading to improved cellular health. Other NAD$^+$ boosting therapies include inhibiting ARTDs and ADPR cyclases to maintain nuclear and cytoplasmic NAD$^+$ levels. In the heart, ARTD inhibition has beneficial effects but the mechanism is unclear. Inhibition of ADPR cyclases substantially increases NAD$^+$ levels in the heart, but only minimally increases NAD$^+$ levels in the kidney. Such inhibition might improve cardiac and renal function in disease states by normalizing calcium signalling. NA, nicotinic acid; NADH, reduced NAD; NAM, nicotinamide; NMN, nicotinamide mononucleotide; NR, nicotinamide riboside.

Table 1

Effects of manipulating NAD$^+$ on sirtuins in the heart and kidney

NAD therapy	Murine model and tissue	Physiological effect	Effect on NAD metabolome	Effect on sirtuins	Ref
Exogenous NAD$^+$	Cardiac hypertrophy Model	NAD$^+$ blocked cardiac hypertrophic response	NAD$^+$ maintained intracellular NAD	↑ SIRT3 deacetylase activity	31
	High-glucose-induced mesangial hypertrophy	NAD$^+$ blocked prohypertrophic signaling	NAD$^+$ restored intracellular NAD$^+$:NADH ratio	• ↑ SIRT3 activity • ↑ SIRT1 activity	33
Genetic manipulation of NAD$^+$:NADH ratio	Cardiac-specific mitochondrial complex I deficiency	• ↑ Sensitivity of the mPTP • ↑ Mitochondrial protein acetylation	↓ NAD$^+$:NADH ratio	SIRT3 overexpression in cardiomyocytes resulted in: • ↓ Mitochondrial protein acetylation • ↓ Sensitivity of the mPTP	22
NMN supplementation	Pressure-overload-induced hypertrophy in cardiac-specific mitochondrial complex I deficiency	NMN improved fractional shortening, left ventricular dilation and hypertrophy via: • ↓ Sensitivity of the mPTP • ↓ Mitochondrial protein hyperacetylation	NMN normalized cardiac NAD$^+$:NADH ratio	NMN reduced hyperacetylation of two pathological protein targets, which corrected the phenotype: malate aspartate shuttle and regulators of the mPTP	23
Pharmacological manipulation of NAMPT via AICAR	Cisplatin-induced acute kidney injury	Improved renal function, decreased tubular injury	↑ NAMPT expression	• ↑ *Sirt3* mRNA • ↓ Mitochondrial protein hyperacetylation	34

AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide; mPTP, mitochondrial permeability transition pore; NAD$^+$, nicotinamide adenine dinucleotide; NADH, reduced NAD; NAMPT, nicotinamide phosphoribosyltransferase; NMN, nicotinamide mononucleotide; SIRT, sirtuin.