Nicotinamide adenine dinucleotide homeostasis and signalling in heart disease: Pathophysiological implications and therapeutic potential

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Nicotinamide adenine dinucleotide homeostasis and signaling in heart disease: pathophysiological meaning and therapeutic potential

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Homéostasie et signalisation du nicotinamide adénine dinucléotide dans les pathologies cardiaques: implications physiopathologiques et potentiel thérapeutique

Titre abrégé: Homéostasie et signalisation du NAD dans les pathologies cardiaques

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Abstract (147 words)

Heart failure is a highly morbid syndrome generating enormous socio-economic costs. The failing heart is characterized by a state of deficient bioenergetics that is not currently addressed by classical clinical approaches. Nicotinamide adenine dinucleotide (NAD^+/NADH) is a major coenzyme for oxidoreduction reactions of the energy metabolism that has recently emerged as a signaling molecule with broad range of activities ranging from Ca^{2+} signaling (CD38) to epigenetic regulation of gene expression involved in oxidative stress response, catabolic metabolism and mitochondrial biogenesis (Sirtuins, PARP). Here we review the current knowledge on alterations of myocardial NAD homeostasis that has been observed in various models of heart failure and its impact on mitochondrial functions, Ca^{2+}, Sirtuin and PARP signaling. We highlight the therapeutic approaches that are currently in use or in development that inhibit or stimulate NAD^+ consuming enzymes and emerging approaches aiming at stimulating NAD biosynthesis in the failing heart.

Abbreviations: FAO, fatty acid β-oxidation; HF, heart failure; NA, nicotinic acid; NAD, nicotinamide adenine dinucleotide; NAM, nicotinamide; Nampt, nicotinamide phopshoribosyl transferase; Nmrk, nicotinamide riboside kinase; Nmnat, nicotinamide mononucleotide adenylyl transferase; NR, nicotinamide riboside; PARP, poly(ADPribose) polymerase; Pgc, PPAR gamma co-activator; PPAR, peroxisome proliferator-activated receptor; Sirt, sirtuin;T2DM, type 2 diabetes mellitus.

Resumé (178 mots)

L'insuffisance cardiaque est un syndrome hautement morbide qui génère un coût socio-économique considérable. Le cœur insuffisant est caractérisé par un état de déficit bioénergétique qui n'est pas directement adressé par les thérapies les plus communément utilisées en clinique à l'heure actuelle. Le nicotinamide adénine dinucléotide (NAD^+/NADH) est un coenzyme majeur des réactions d'oxydo-réduction du métabolisme énergétique qui a récemment émergé comme une molécule de signalisation avec un large spectre d'action allant de la signalisation Ca^{2+} (CD38) à la régulation épigénétique de l'expression des gènes de la biogénèse mitochondriale et du métabolisme catabolique et de la résistance au stress oxydant (Sirtuines, PARP). Dans cette revue, nous reprenons l'état des connaissances actuelles sur les altérations de l'homéostasie du NAD qui ont été observées dans différents modèles d'insuffisance cardiaque et leur impact sur les fonctions mitochondriales et les fonctions de signalisation Ca^{2+}, Sirtuines et PARP. Nous mettons en avant les molécules thérapeutiques déjà utilisées ou en cours de développement qui inhibent ou stimulent les enzymes hydrolysant le NAD^+ et les approches nouvelles visant à stimuler les voies de biosynthèse du NAD^+.

Energy failure in heart failure

Population is ageing worldwide leading to a higher prevalence of age-related cardiovascular and metabolic diseases such as hypertension, coronary heart diseases, and type 2 diabetes mellitus (T2DM), all rising the risk of developing heart failure (HF). Despite improvement in HF therapy in the last two decades, patients still suffer poor quality of life, repeated hospitalization and have a reduced life expectancy. The last decade of research showed that mitochondrial dysfunctions leading to bioenergetics defects and increased reactive oxygen species (ROS) production, are key players in the process of cardiac ageing and development of HF [1]. Current therapies for HF are mainly based on the reduction of heart rate (β-blockers) and cardiac workload and remodeling (angiotensin converting enzyme inhibitors, mineralocorticoid receptor antagonists, vasodilatators) that contributes to spare energy consumption but do not address the issue of deficient energy production in HF. The decline in energy production and usage capacity observed in the failing heart is due to a global alteration in bioenergetics systems including deficient creatine kinase-mediated energy transfer systems, decreased fatty acid β-oxidation (FAO) and decreased mitochondrial oxidative phosphorylation capacities (OXPHOS) [2]. The exact causes of this decline in energy production in the failing heart are far from being completely understood but are thought to be associated with the repression of important transcriptional regulators of metabolic pathways and mitochondrial biogenesis including PGC1α and β (Peroxisome-proliferator-

activated receptor gamma co-activator) and ERRα and γ (estrogen related receptor) [3]. Alteration of Ca^{2+} handling systems in failing cardiomyocytes may also affect mitochondrial Ca^{2+} load and oxidative phosphorylation capacities. A common end-point to these metabolic alterations is that the myocardium becomes energy-starved, which is manifests by the alteration in the homeostasis of high-energy metabolites: first the major energy reserve compound phosphocreatine (PCr) declines (reduced PCr/ATP ratio), then ATP/ADP ratio and finally total ATP levels are progressively reduced [2].

NAD at the crossroad between energy metabolism, cell signaling and epigenetics

NAD coenzyme functions - While the alteration in ATP nucleotide homeostasis has been extensively studied in the context of cardiac ageing and HF, much less is known on the homeostatic regulation of the nicotinamide adenine dinucleotide (NAD) despite its major role as a coenzyme of oxidoreduction reactions in the energy metabolism. Oxidation of glucose and fatty acids lead to the reduction of NAD^+ into NADH. Glycolysis occurring in the cytosol produces 2 NADH. This cytosolic NADH is converted back to NAD^+ by the malate dehydrogenase that initiates the transfer of reducing equivalents to the mitochondria though the malate-aspartate shuttle system. On the other hand, FAO takes place inside the mitochondria and generates 1 FADH2, 1 NADH and 1 acetyl-CoA for each cycle of 2 carbons cleavage. For instance, a 16-carbon long palmitate molecule generates 7 NADH and FADH2 molecules and 8 acetyl-CoA. Since the Krebs cycle produces 3 NADH and only 1 FADH2, for

each cycle, NADH is the major electron donor to the electron transport chain in the mitochondria. In addition to this major function in energy metabolism, NAD is also the precursor of NADP by the mean of NAD kinase-mediated phosphorylation or the mitochondrial nicotinamide nucleotide transhydrogenase (Nnt). NADP(H) is the essential coenzyme of the enzymatic pathways dedicated to the detoxification of reactive species of oxygen (ROS) such as the glutathione and thioredoxin reductase systems. In all these functions of coenzyme for oxidoreductases of the energy metabolism, the recycling of NAD^+ and NAD does not modify the total pool of NAD. However, several cellular pathways exist that are net consumers of NAD^+.

NAD consumption by signaling pathways

Indeed, NAD^+ has emerged in the recent years as an important signaling molecule that is used by different pathways involved in the regulation of energy metabolism (Sirtuins), response to oxidative stress (PARP, Sirtuins) and Ca^{2+} signaling (CD38), setting NAD^+ as a major regulatory hub directly interfacing energy metabolism and cellular functions (**Figure 1**).

NAD^+-dependent Sirtuin deacetylases

Sirtuins function as metabolic regulators in response to energy stress through the stimulation of mitochondrial biogenesis and OXPHOS genes [4]. Sirtuins are enzymes (1 to 7) cleave NAD into nicotinamide (NAM) and ADP-ribose (ADPR) moieties (**Figure 1A**) to perform different type of post-translational modifications (PTMs) on cellular proteins. In one type of reaction, Sirtuins remove acetyl (Sirt1, -2, -3, -6, -7), succinyl (Sirt5) or lipids (Sirt6, -7) groups that have been

covalently linked to Lysine residues on cellular proteins modifying their charge and activity [4]. In that case, ADPR is used as the acceptor of the removed group. Alternatively, in the mono-ADP-ribosylation reaction performed by somes sirtuins (Sirt4, -6, -7), the ADPR moiety is transferred to arginine residues on target proteins modifying their charges and activities. Sirt1, -6, and -7 are predominantly nuclear and exert their functions through a direct effect on transcription factors involved in the regulation of metabolism, autophagy and cell survival. Sirt3 an -5 reside in the mitochondrial matrix and enhance the activity of enzymes involved in the Krebs cycle and OXPHOS metabolism [4]. One of the most extensively studied Sirtuin is Sirt1. Sirt1 plays a role in chromatin remodelling and gene expression by deacetylating histones as well as transcription factors including FOXO factors, p53 and PGC1α, which regulate autophagy, survival, and metabolic pathways. Sirt1-mediated deacetylation of the PGC-1α transcription factor stimulates its activity and increases mitochondrial biogenesis [5]. Resveratrol, a Sirt1 activator improves mitochondrial function in cardiac and skeletal muscles [5,6]. Moderate level of Sirt1 overexpression in the myocardium protects the heart against aging and oxidative stress [7] as well as against ischemia/reperfusion injury [8]. Other Sirtuins including Sirt3, Sirt4, Sirt6 and Sirt7 have also been shown to play a protective role in the heart by targeting mitochondrial or nuclear proteins [4]. Notably, Sirt3 plays an essential role to counteract inhibitory non-enzymatic acetylation of mitochondrial proteins caused by the excess of free acetyl-CoA, observed in situation of high-fat diet [9]. Sirt3 overexpression in transgenic mice protects the heart from pressure overload stress [10]. Angiotensin 2 or

phenylephrin agonists were shown to lower NAD^+ levels in the heart and NAD^+ administration was able to blunt the LV hypertrophy in a Sirt3 dependent manner [11].

NAD^+-dependent ADP-ribosylases

NAD^+ is also used as a ADP-ribose donor by a large family of enzymes collectively known as the ADP-ribosyl transferases ARTD1 to 18 (previously known as the poly(ADPribose) polymerases PARP) and ARTC1 to 5 [12]. PARP1 (ARTD1 in the new nomenclature) is one major NAD consuming enzyme in the cell that recognizes DNA lesions induced by an excess of ROS. When activated, PARP1 catalyzes the formation of long polymers of ADPR (Parylation) on itself and partner proteins to recruit the machinery of DNA repair enzymes. Mild activation of PARP1 is protective but overactivation can deplete the cellular pool of NAD and alter Sirt1 activity leading to cardiomyocytes death in mouse model of pressure overload hypertrophy [13,14]. Interestingly Sirt1 was shown to deacetylate and repress PARP1 [13]. Since both enzymes use NAD^+ for their catalytic activity, they appear to be involved in competitive pathways to decipher cell fate in situation of stress. PARP1 is also involved in the regulation energy metabolic pathways since it was recently shown to bind to PPARγ nuclear receptor enhancing ligand binding and co-factor exchange in adipocytes [15]. Other ART enzymes are less well characterized but the ectoenzymes ARTC1, for instance, has been shown to ADP-ribosylate a number of membrane proteins including the integrin α7 in skeletal muscle [16].. Interestingly, this modification was shown to enhance the adhesion of the

integrin α7 to the laminin protein in the extracellular matrix [16], which may be of importance in the highly active cardiac muscle.

NAD^+-dependent Ca^{2+} signaling

A major enzyme involved in NAD hydrolysis is the CD38 ectoenzyme that generates NAM, ADPR and cyclic ADPR (cADPR), the two latters acting as a second messengers in calcium signaling [17]. CD38 can also generate the NAADP derivative of NADP involved in lysosomal Ca^{2+} mobilization [17]. The activity of the ryanodine receptor is stimulated by cADPR in cardiac myocytes [18] and cADPR increases the frequency of Ca^{2+} sparks, that are essential for the Ca^{2+}-induced Ca^{2+} release (CICR) mechanism at the basis of cardiac rhythmic contractility [19].The cADPR second messenger is also required for the Angiotensin II -induced sustained Ca^{2+} rise that is observed after the initial rapid transient Ca^{2+} elevation triggered via the inositol trisphosphate (IP3) receptor [20]. Some authors however found that Connexin 43 hemichannels could be an alternative route of entry for cADPR [21] and NAD^+ may also directly enter by these channels [22].

CD38 hydrolysis of NAD^+ also generates ADPR, which together with cADPR is a known activator of the TRPM2 channel, a member of the M-family of transient receptor potential channels that are permeable to Ca^{2+} [23,24]. Interestingly, Ca^{2+} entry via Trpm2 is essential for cardiac myocyte bioenergetics maintenance in the context of ischemy-reperfusion injury or doxorubicin cardiomyopathy [25] suggesting that Trpm2 is a potential link between NAD^+ and Ca^{2+} signaling for cardiomyocyte survival.

The CD38 was once thought to essentially hydrolyze extracellular NAD^+ but recent data show that the enzyme exist in 2 conformations, one with the catalytic side on the outer side of the membrane and one with the catalytic side facing the cytosol, hence able to hydrolyze the intracellular pool of NAD^+ [17]. The CD38 KO mice display a general increase in NAD^+ tissue level though discrepancy in results were notable for the heart between the study of Aksoy et al. reporting a 30 fold increased [26] versus no change in the study of Young et al [27]. Myocardial contractility, contraction and relaxation velocities are significantly enhanced in male CD38 KO mice [28]. Altogether, these studies suggest that CD38 is an important regulator of the balance of extra and intracellular NAD homeostasis. It also establishes a potential link between NAD^+ and Ca^{2+} signaling though further research is needed to fully understand this connection.

Alteration of NAD^+/NADH ratio and NAD loss in ageing and heart failure

Tissue levels of NAD^+ decline in different organs including heart, liver, kidney and lungs during ageing in the rat [29]. The decline was most pronounced in the heart with a loss of 70% of NAD^+ between 3 and 24 month, compensated by a 50% increase in NADH levels, so that the change was mainly at the level of NAD^+/NADH ratio that was strongly reduced from 0.7 to 0.1.

Alterations in NAD levels and NAD^+/NADH ratio has been involved in multiples pathogenic mechanisms leading to HF notably in links with the impact of Ca^{2+} signaling in mitochondria. An original report by the group of Bernardi showed

that Ca^{2+} overload of isolated rat heart mitochondria resulted in a profound decrease in their NAD^+ content [30]. In this study, 30 min ischemia in isolated hearts led to a 30% decrease in NAD^+ both in mitochondria and at the tissue level. This loss was due to an increased hydrolysis of the mitochondrial-released NAD^+ by an unidentified glucohydrolase, and was worsened by reperfusion. Interestingly, the permeability transition pore (PTP) inhibitor Cyclosporin A (CsA) preserved NAD^+ levels in the mitochondria and protected the heart from reperfusion damage suggesting that NAD^+ transit through this complex.

In a guinea pig model of non ischemic HF obtained by ascending aorta constriction, the elevated cytosolic level of Na^+ that is characteristics of failing cardiomyocytes reduced mitochondrial Ca^{2+} level by accelerating Ca^{2+} efflux via the mitochondrial Na^+/Ca^{2+} exchange [31]. In turn, because Ca^{2+} normally stimulates enzymes of the Krebs cycle involved in NADH production, low mitochondrial Ca^{2+} levels decreased the mitochondrial NADH content and bioenergetic capacities in failing cardiomyocytes.

Conversely, the same group showed that the $NADH/NAD^+$ redox state can modulate Na^+ current (I_{Na}) in the heart. A mutation in the gene encoding the glycerol-3-phosphate dehydrogenase 1-like (GPD1-L) protein leads to abnormally elevated NADH levels and was shown to cause Brugada syndrome. Elevated NADH levels were shown to inhibit I_{Na} through increased ROS signaling mediated by the NADPH oxidase and PKC mediated inhibition of the $Na_v1.5$ channel encoded by the SCN5A gene [32]. In this study, NAD^+ perfusion restored $NADH/NAD^+$ redox state and had antiarrhythmic property on isolated

SCN5A deficient mouse hearts, the more classical model of Brugada. The same authors showed in a mouse model of hypertensive HF obtained by unilateral nephrectomy and deoxycorticosterone acetate (DOCA) pellet implantation that NADH levels measured by metabolite extractions and colorimetric assay were increased [33] on the contrary to what was observed in the guinea pig model cited above [31]. Differences in the method of NADH quantifications and the stage of HF in different animal models may account for this discrepancy. In the study of Liu M et al. [33], I_{Na} was decreased in failing cardiomyocytes and treating the cells with NAD^+ or mitoTEMPO, a mitochondria-targeted antioxidant, restored I_{Na} levels. The NAD^+ effect was dependent on CD38 activity to allow entry of NAD^+ in the cell. Since CD38 is a major NAD^+ hydrolase, we hypothesize that an intermediate step of intracellular NAD^+ regeneration from NAM would be required. The latter hypothesis has not been tested yet. Correlating with their mouse studies, these authors also showed that NAD^+ perfusion of isolated human failing heart improved conduction velocity consistent with a positive effect on Nav1.5 activity [33].

Recently, a model of cardiomyopathy induced by deletion of a complex I subunit of the mitochondrial electron transport chain was also shown to decrease NAD^+/NADH ratio in mitochondrial matrix by accumulation of NADH leading to diminished Sirt3 activity and hyperacetylation of mitochondrial proteins [34]. Nicotinamide mononucleotide (NMN) treatment of these mice as a precursor of NAD^+, helped to restore normal mPTP sensibility, ROS levels and deacetylation of mitochondrial proteins. Finally, several type of mouse models of chronic HF, including pressure overload hypertrophy induced by transverse aorta

constriction and ischemia-reperfusion injury were shown to display globally reduced myocardial NAD^+ levels in the heart [13,35,36]. However, it should be noted that in most of these studies, the NAD^+ and NADH were quantified based on metabolite extraction in acidic buffer for NAD^+ and basic buffer for NADH. This gives indications on the raw steady-state levels of each metabolite but it should not be considered as directly reflecting the redox state of the cell. When NaOH basic buffer is used to extract NADH, it is known to liberate a high amount of protein bound NADH, which is all right to estimate total level of NADH but do not correspond to the free NADH level in the cytosol. The latter defines the redox state and is about 2 orders of magnitude inferior to free NAD^+ levels. Recently, new magnetic resonance (MR) -based in vivo NAD assay was designed that is capable of noninvasively assessing NAD^+ and NADH contents [37]. This technology was applied to the analysis of NAD^+ and NADH levels in the human brain and showed a significant decline of total NAD as well as of NAD^+/NADH ratio between 20 and 75 year-old individuals. No doubts that this technology applied to cardiac tissue will be extremely powerful to better define the alterations in NAD homeostasis considering how ^{31}P MRS has proved so efficient to characterize energy failure in HF.

Stimulation of the NAD biosynthetic pathways in HF

Altogether, these studies showing that NAD^+ is lost in pathological conditions raised the interest in the pathways linked to NAD biosynthesis [38]. NAD^+ can be derived from deamidated precursors such as tryptophane (TRP) through the kyurenine pathway, and nicotinic acid (NA) a Vitamin B3 (niacin) precursor of

NAD^+. In the mouse heart, this “deamidated precursors” pathway provides a limited contribution [39].. The main source of NAD^+ precursors seems to be the amidated vitamins B3 nicotinamide (NAM) and nicotinamide riboside (NR) (**Figure 1B,** green pathway). NAD^+ contained in the food is essentially hydrolyzed into these 2 precursors in the intestinal lumen before to reach the circulation and being distributed to the body [40].

NAM is converted into NMN by the nicotinamide phosphoribosyl transferase (Nampt) that transfers an alpha-d-5-phosphoribosyl-1-pyrophosphate (PRPP) to the NAM ring and consumes 1 ATP in the process through a transient autophosphorylation of its histine 247 residue [41]. NMN is then condensed with the ADP moiety of an ATP molecule by the Nmnat enzymes (Nmnat 1 to 3) to form NAD^+. Because NAM is the by-product of all enzymatic activities hydrolyzing NAD (Sirtuins, PARP, CD38), Nampt is a key enzyme for the regeneration of the NAD^+ pool in the cell. Nampt was found to be repressed in different models of HF including pressure overload and ischemy-reperfusion [13,36]. Nampt plays a crucial role for the maintenance of the myocardial NAD^+ pool and Sirt1 activity in the heart. The group of Sadoshima reported that transgenic overexpression of Nampt in the mouse heart or NAD^+ supplementation to the mice had a protective role against ischemia-reperfusion, notably through the restoration of the autophagic flux, with no deleterious impact on cardiac functions at baseline [36]. However the group of Gupta showed that overexpression of Nampt, possibly at higher levels than in the study of Hsu et al., can trigger cardiac hypertrophy whereas mice with a half-dose of Nampt (Nampt +/-) were protected against agonist (isoproterenol and

angiotensin II)-induced hypertrophy showing that NAD^+ production is an important intermediate in this process [42]. Interestingly, the Nampt cDNA open reading frame was found to be identical to a cytokine named pre-B-cell colony-enhancing factor (PBEF) and was recently re-identified as a hormone named visfatin, reported to exert insulin-mimetic effects but also proinflammatory roles in different context [43]. Hence these studies revealed that Nampt can exist in 2 forms, intracellular iNampt and excreted eNampt (Visfatin). The existence of these two forms of Nampt led to the hypothesis that Nampt is centrally involved in a systemic regulatory network that regulates NAD^+ levels in the different organs, a model for which the term “NAD world was coined by Shin-ichiro Imai [44]. However it is still not clear at this stage whether eNampt can effectively synthesize NMN in the extracellular compartment and in fact recent evidences suggest that it not the case at least in human plasma [45]. So eNampt/PBEF/Visfatin may mainly function as a cytokine binding to yet unknown receptor(s) [43]. The study of Pillai et al. [42] showed that Nampt can also bee excreted by the cardiomyocytes and in vitro, eNAMPT added to the culture medium triggered hypertrophy of cultured cardiomyocytes.

Inhibitors of Nampt catalytic activity such as FK866 have been evaluated in clinics in the context of cancer therapy because Nampt is found to be overexpressed in different tumor cells [43]. However, its central role in NAD biosynthesis and its impact on mitochondrial functions should raise concerns about the potential cardiotoxicity of these compounds as for other chemiotherapies.

Nicotinamide riboside (NR) is a more recently characterized NAD^+ precursor that can be found in milk and beer [38]. The group of Brenner showed that NR promotes yeast replicative longevity through a Sir2 (Sirt1 homolog) pathway [46]. This group cloned the 2 mammalian homologs of the nicotinamide riboside kinase (Nmrk1 and 2) that phosphorylate NR to form NMN. The role of Nmrk enzymes has not been addressed in mammals so far. The interrogation of Gene Expression Omnibus (GEO) dataset profiles reveal that Nmrk1 is ubiquitously expressed while Nmrk2 appears to be specific to striated muscle tissue (skeletal and cardiac). Interestingly, the Nmrk2 ORF corresponds to the sequence of a muscle integrin binding protein (MIBP) previously shown to bind α7β1 integrin heterodimers in C2C12 myoblast cell line and to inhibit the deposition of laminin in the extracellular matrix (ECM) [47]. This suggest a potential link between the function of integrin binding and the function of NAD biosynthesis for Nmrk2/MIBP. One link could be signaling mechanisms related to the ADP-ribosylation of integrin α7 by ARTC1 [16] that require local NAD^+ hydrolysis at the membrane though this hypothesis remains to be tested.

NR enters in yeast cells through a nucleoside transporter Nrt1 without clearly identified homolog in humans [48] or through the Fun26, a homolog of human ENT (equilibrative nucleoside transporter) [49]. In addition, when NMN is given to the cells to stimulate NAD synthesis, it may be in fact first transformed into NR by the CD73 ecto-5'-nucleotidase to allow the entry into the cell [50].

NA supplementation was one of the first niacin to be used to show protection of the heart in stress conditions [51]. NA derivatives such as acipimox, were used efficiently in clinics for the treatment of hyperlipidemia in T2DM patients though

some rebound effect in free fatty acid levels in blood occurred after few days of treatment and a “flushing” effect is induced by acipimox that is difficult to bear for the patient, lowering the prospects of clinical use [52]. Yet acipimox was shown to improve oxidative metabolism in skeletal muscle of T2DM patients [53]. NR supplementation in mice was shown to have similar impact on oxidative metabolism without some of the limitations of NA and allowed the mice to partially resist to high-fat diet induced obesity [54]. More recently NR was shown to stimulate mitochondrial biogenesis in the skeletal muscle of mouse models of mitochondrial diseases [55,56]. So far the impact of NR supplementation on cardiac functions is unknown.

Conclusion

NAD^+ has emerged as a central regulator of energy metabolism, both through its direct role as a coenzyme in oxidoreduction reactions of glycolysis, FAO and oxidative phosphorylation and through its multiples facets as a signaling molecule connecting Ca^{2+} signaling to mitochondrial functions and transcription of genes involved in metabolic and oxidative stress resistance. Hence, we propose that acting on NAD bioavailability and usage in the failing heart may have a strong impact on the evolution of the disease (**Figure 2**). Several drugs already available in clinics or in development are in fact dealing with the rate of NAD consumption such as the PARP inhibitors that could be useful as inhibitors of cell death and inflammation in cardiovascular diseases [57]. Alternatively available inhibitors or therapeutic antibodies targeting CD38 could be tested for

their ability to raise the level of NAD^+ [58,59], considering that CD38 KO male present improved cardiac contractility [28]

Since the rate of NAD^+ consumption is likely to be increased in the failing heart in the face of PARP activation by oxidative stress and Sirt1 activation by energetic stress, strategies aiming at pushing the rate of NAD^+ biosynthesis by niacin supplementation could be an interesting alternative to restore bioenergetics capacities in the heart.

Finally the fact that NAD^+ is also regulated at the systemic level and can be found in serum raises the possibility to use circulating levels of NAD^+ or NAD^+ metabolites as biomarkers of energetic defect in cardiovascular diseases. In line with this hypothesis, NAD^+ levels were found to be decreased in multiple sclerosis patients in correlation with the severity of the disease [60].

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Conflict of interest: none

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Figure legends

Figure 1. Multiple roles of NAD^+ in energy metabolism and cell signaling. (**A**). Skeletal formula of the Nicotinamide Adenine Dinucleotide NAD^+ showing the site of reduction that gives rise to NADH in oxidoreduction reactions. The boxes indicate the nicotinamide and ADP-ribose moieties that are released after cleavage by NAD^+ consuming enzymes. (**B**) NAD^+ and its vitamins B3 precursors NAM and NR can be found in the extracellular compartment. NAD^+ synthetic pathways are highlighted in **green** and consuming pathways are highlighted in **red**. NAD^+ present in food is broke down in NMN, NAM or NR components. NMN is converted to NR by the CD73 5'-ectonucleotidase. NR can enter the cells through nucleoside transporters. The NAD^+ biosynthetic pathways are initiated by the Nampt and Nmrk enzymes forming NMN followed by the Nmnat enzymes fusing an NMN to an ADP moiety to form NAD^+. NAD^+ coenzyme is reduced in NADH during glycolysis, fatty acid β-oxidation and mitochondrial oxidative phosphorylation and is the precursor of $NADP^+$ / NADPH in the cytosol and mitochondria.. NAD^+ is cleaved by enzymes like the Sirtuin and the PARP involved in gene regulation for oxidative stress resistance and mitochondrial biogenesis. NAD is also used by ADPribosylases like ARTC1 located at the membrane. The CD38 cleaves NAD^+ to generate cADPR and ADPR second messengers or nicotinic acid adenine dinucleotide phosphate (NAADP) from NADP. The second messengers are involved in Ca^{2+} mobilization from extracellular compartment (Trp2) and intracellular stores notably, the sarcoplasmic reticulum through the activation of the Ryanodin receptor (RyR) or the lysosomal stores.

Figure 2. Therapeutic potential of compounds modulating NAD^+ homeostasis and signaling in heart failure.

Vitamins B3 (nicotinic acid (NA) and NA derivatives such as Acipimox, NAM, NR) and nicotinamide mononucleotide (NMN) can be used to stimulate NAD^+ synthesis and stimulate oxidative metabolism. NAM is not only a precursor of NAD^+ but also an inhibitor of Sirtuins. So its use maybe counterproductive.

PARP inhibitors (e.g. olaparib, veliparib, niraparib, L-2286, AG-690/11026014) can limit the high NAD^+ consumption of by PARP1 and were shown to be beneficial in preclinical models. Alternatively inhibitors of the other major NAD^+ hydrolase CD38 (e.g. 4-Amino-8-quinoline carboxamides compounds, Daratumumab (HuMax-CD38, Genmab), a human IgG1κ monoclonal antibody) could help to maintain NAD levels in the myocardium hough they have not been tested in preclinical models of heart failure yet. Sirtuins consume NAD^+ but at moderate level and, overall their action is thought to be protective in the context of pathological cardiac remodelling. This is supported by the beneficial action of sirtuin activators on cardiovascular health (e..g. resveratrol, SRT1460, SRT1720, SRT2183, STAC-5, STAC-9, STAC-10). Importantly, beneficial side-effect of Sirt1 activators could also be a repression of deleterious PARP1 activity.

Figure

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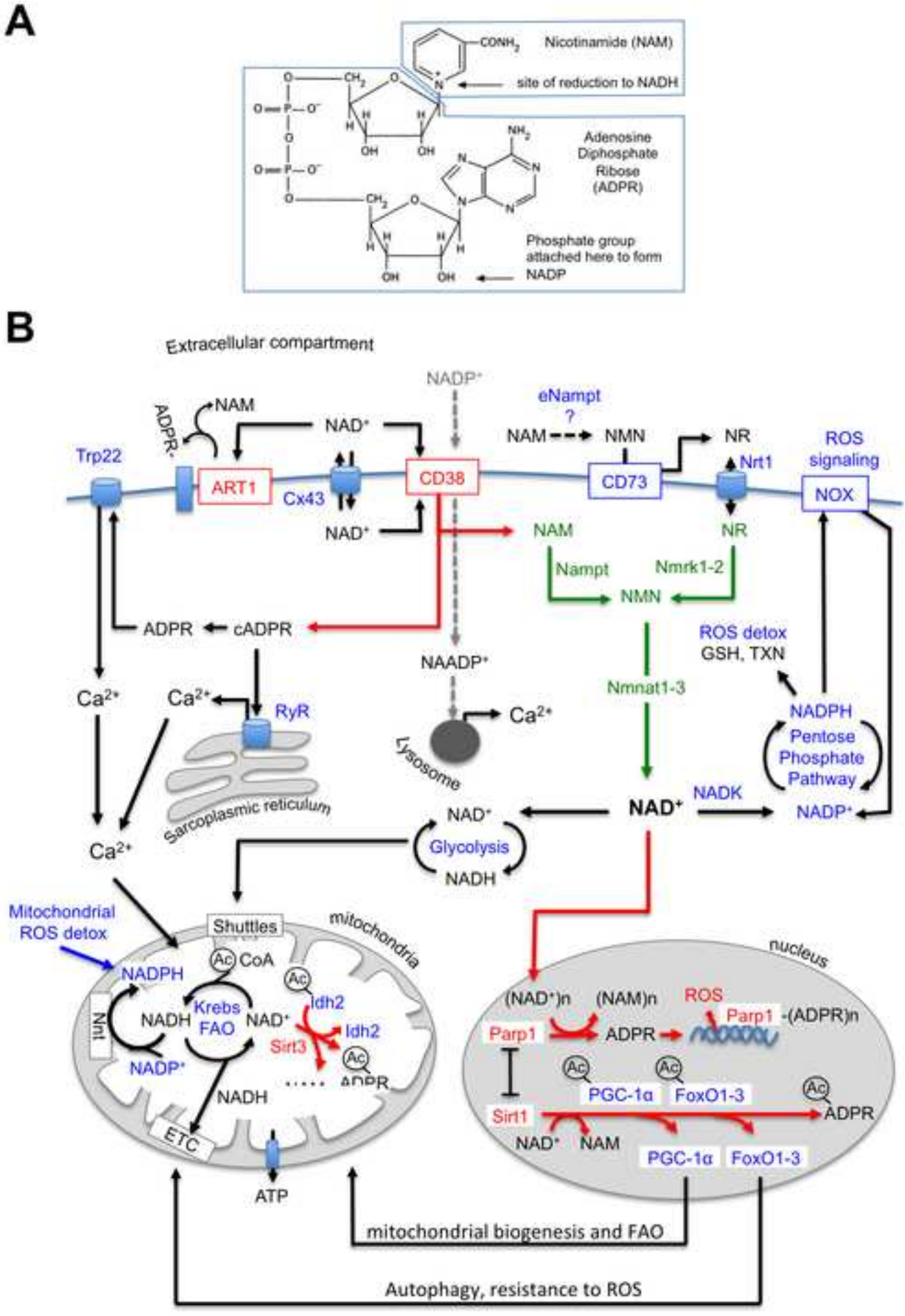

Figure
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