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REVIEW ARTICLE

The effects of nicotinamide adenine dinucleotide in cardiovascular diseases: Molecular mechanisms, roles and therapeutic potential

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Abstract Recently, cardiovascular diseases (CVDs) were identified as the leading cause of mortality, imposing a heavy burden on health care systems and the social economy. Nicotinamide adenine dinucleotide (NAD^+), as a pivotal co-substrate for a range of different enzymes, is involved in many signal transduction pathways activated in CVDs. Emerging evidence has shown that NAD^+ can exert remediating effects on CVDs by regulating metabolism, maintaining redox homeostasis and modulating the immune response. In fact, NAD^+ might delay ageing through sirtuin and non-sirtuin pathways and thus contribute to interventions for age-related diseases such as CVDs. Considering that robust clinical studies of NAD^+ are ongoing, we discuss current challenges and the future translational potential of NAD^+ based on existing studies and our understanding. Despite some remaining gaps in its clinical application, NAD^+ has been shown to have broad prospects and pan-effects, making it a suitable prophylactic drug for CVDs.

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Introduction

Cardiovascular diseases (CVDs) are heart and circulatory system disorders that collectively are the leading cause of premature mortality and disability in humans worldwide.[1] The incidence of CVDs continues to increase worldwide, and it is predicted that by 2030 nearly 24 million individuals will die from a CVD each year.[2] CVDs are complex diseases, including coronary artery disease, arrhythmia, hypertension, cardiomyopathy and heart failure. The physiopathology of CVDs is also complex and mainly involves metabolic disorders, oxidative stress and inflammation.[3] For example, abnormal glucose and lipid metabolism can accelerate the formation of atherosclerosis.[4] Excessive reactive oxygen species (ROS) production can cause mitochondrial dysfunction and aggravate cardiomyocyte injury in ischemic cardiomyopathy.[5] Inflammatory signaling networks have also been indicated to contribute to ischaemic cardiovascular disease.[6]

Nicotinamide adenine dinucleotide (NAD^+) is a pyridine nucleotide that plays a crucial role in the catabolism of fuel substrates in a variety of cells.[7] In mammals, NAD^+ is synthesized from a variety of precursors and consumed in many pathways, which have been comprehensively reviewed,[8–11] and are thus only briefly described here. NAD^+ can be produced from tryptophan (Trp) in the de novo pathway or from nicotinic acid (NA), nicotinamide (NAM), nicotinamide riboside (NR), or nicotinamide mononucleotide (NMN) in the salvage pathway. NA, NAM and NR, also known as three different forms of vitamin B3, have shown potential as dietary supplements to increase intracellular NAD^+ levels.[8] The de novo pathway relies on tryptophan obtained from food for the synthesis of quinolinic acid (QA), which is then transformed by QAPRT (quinolinate phosphoribosyltransferase) into nicotinate mononucleotide (NAMN). Nicotinate mononucleotide (NAMN) can also be converted from NA by nicotinate phosphoribosyl transferase (NAPRT). Nicotinate mononucleotide (NAMN) is further catalysed by nicotinamide mononucleotide adenylyltransferase (NMNAT) to produce NAD^+. The reaction process in which NA is converted to NAD^+ is called the Preiss handler pathway.[12] In another reaction in the salvage pathway, NAM is first catalysed by nicotinamide phosphoribosyl transferase (NAMPT) to produce NMN, which is further transformed by nicotinamide mononucleotide adenylyltransferase (NMNAT) to produce NAD^+. NMN can also be converted from NR by nicotinamide riboside kinases (NRKs) (Fig. 1). Notably, the salvage pathway is the main source of NAD^+ in mammals.[11]

The homeostasis of NAD^+ in cells depends on the balance of NAD^+ biosynthesis and consumption. There are three major NAD^+-consuming enzymes: sirtuin family members (SIRTs), poly(ADP-ribose) polymerases (PARPs) and cyclic ADP-ribose synthases (cADPRs). The SIRT family consists of "longevity proteins" with conserved sequences that are ubiquitous in almost all organisms and NAD^+-dependent deacetylase activity, which is extensively involved in metabolic regulation, antioxidation and cell fate.[13] PARPs constitute a protease family consisting of 17 members. They transfer the ADP-ribose unit from NAD^+ to a target protein, which leads to the poly ADP-ribosylation of various proteases and itself, and these proteases are involved in nuclear DNA damage repair and induce

mitochondrial signalling.[14] cADPRs consist of a pair of extracellular enzymes, CD38 and CD157, called lymphocytic antigens that are involved in the calcium signalling pathway, cell life cycle and insulin secretion by hydrolysing NAD^+ to generate the second messenger cADP.[15] In this review, we explain our contention that NAD^+ is involved in the signalling pathways of CVDs and provide an overview of the benefits and underlying mechanisms of NAD^+ replenishment in the cardiovascular system. In addition, we discuss the therapeutic potential of NAD^+ in clinical practice (Fig. 2, 3).

Mechanism underlying the effects of NAD^+ in cardiovascular diseases

NAD^+ is an important component in metabolic pathways

Different subcellular NAD^+ pools are interconnected through a complex set of redox processes, thus modulating the activity of compartment-specific metabolic pathways such as glycolysis in the cytoplasm and the tricarboxylic acid (TCA) cycle in the mitochondria.[10] In the cytoplasm, the synthesis of pyruvate by glycolysis requires two NAD^+ molecules per molecule of glucose, following the synthesis of acetyl-CoA (coenzyme A) through pyruvate oxidation. Acetyl-CoA can also be produced by the β-oxidation of fatty acids, which reduces NAD^+ to nicotinamide adenine dinucleotide with one hydrogen atom (NADH). Acetyl-CoA then enters the tricarboxylic acid (TCA) cycle in the mitochondrial matrix, which reduces NAD^+ molecules to produce multiple NADH molecules. NADH molecules then undergo oxidative phosphorylation, which strips them of their electrons, leading to the oxidized form, NAD^+, in the electron transport chain (ETC) located at the mitochondrial membrane.

Metabolic disorders play crucial roles in CVDs, such as atherosclerosis,[4] cardiomyopathy[16] and heart failure.[17] Therefore, NAD^+ supplementation may improve these diseases through metabolic pathways. First, NAD^+ may influence the metabolic process of nutrients such as glucose and fatty acids. Studies have shown that NR supplementation can stimulate glycolysis and enhance citrate and acetyl-CoA metabolism in heart failure.[18] Fang et al observed a distinct metabolic profile (different levels of amino acids and metabolites in the TCA cycle) in ataxia-telangiectasia mutated (ATM)-deficient worms and mice. However, NAD^+ can restore most of these metabolites.[19] Recent evidence also indicates that NAD^+ can redirect glucose metabolic flux through the pentose phosphate pathway, which links glucose homeostasis to redox balance.[20] In addition, NAD^+ can regulate metabolism by modulating SIRT activity. Since SIRT1 has been reported to function as a low-energy sensor in concert with adenosine monophosphate-activated protein kinase (AMPK) and hypoxia inducible factors (HIFs) to enhance autophagy and maintain mitochondrial homeostasis,[21] NAD^+ may enhance the metabolic state in heart failure through SIRT1/AMPK/HIF pathways. NAD^+ can also regulate mitochondrial biogenesis and function through the SIRT1/PGC1α (peroxisome proliferator-activated receptor γ (PPARγ) coactivator 1α) pathway, which is essential for
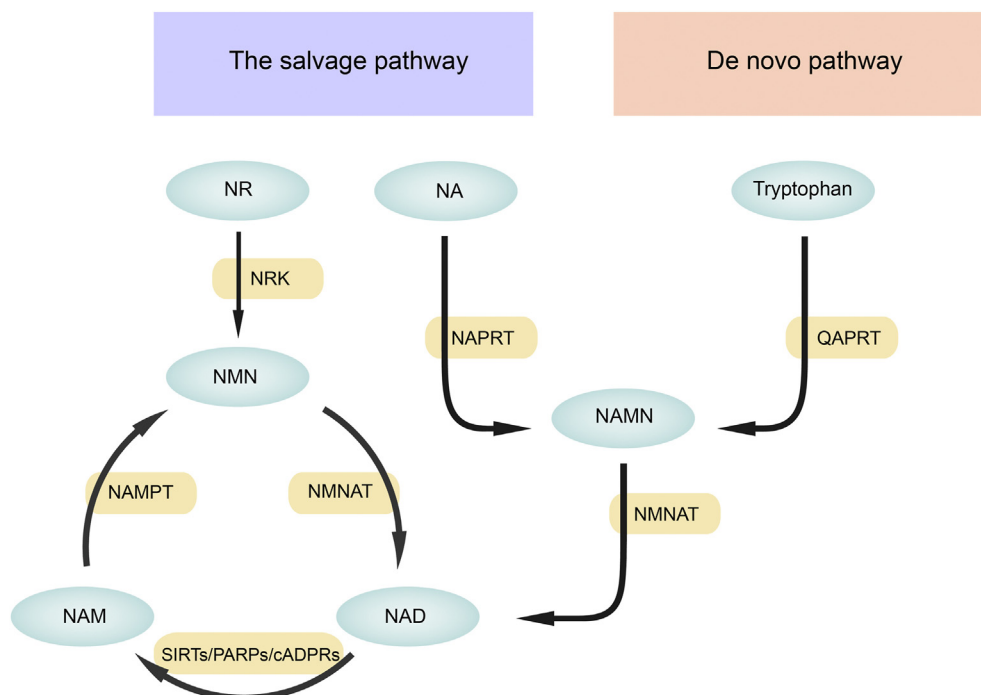
Figure 1 Nicotinamide adenine dinucleotide (NAD$^+$) biosynthetic pathways in mammals. There are two major pathways of NAD$^+$ synthesis: the de novo pathway from tryptophan and the salvage pathway from NA, NAM and NR with different catalysing enzymes. NAD$^+$ can also be converted to NAM by three major NAD$^+$-consuming enzymes, SIRTs, PARPs and cADPRs. cADPRs, cyclic ADP- ribose synthases; NA, nicotinic acid; NAD$^+$, nicotinamide adenine dinucleotide; NAM, nicotinamide; NAMN, nicotinic acid mononucleotide; NAMPT, nicotinamide phosphoribosyltransferase; NAPRT, nicotinic acid phosphoribosyltransferase; NMN, nicotinamide mononucleotide; NMNAT, nicotinamide mononucleotide adenylyltransferase; NR, nicotinamide riboside; NRK, nicotinamide ribose kinase; PARPs, poly-ADP-ribose polymerases; QAPRT, quinolinate phosphoribosyltransferase; SIRTs, sirtuin.

energy metabolism in cardiomyocytes.[22] Interestingly, as the beneficial effects of niacin on dyslipidaemia might be partly explained by increasing NAD$^+$ levels to promote SIRT1 activity,[23] NAD$^+$ supplementation may decrease serum triglyceride (TG) and free fatty acid (FFA) levels through a similar pathway. Although its specific role in metabolic control is still debated, NAD$^+$ has been shown to be a node in the metabolic network. As CVDs are closely associated with metabolic disorders and energy deficiency,[24] NAD$^+$ may be an attractive target to improve energy metabolism to ultimately improve cardiac performance.

NAD$^+$ is involved in sustaining redox homeostasis

Redox reactions involve the transfer of electrons between species in which atoms oxidation state have changed. Many diseases and disorders have been linked with oxidant-antioxidant imbalance within cells. Indeed, oxidative stress is an important factor involved in CVDs progression.[5] An increase in ROS production has been described in several CVDs, such as myocardial fibrosis,[25] cardiac hypertrophy,[26] myocardial infarction[27] and heart failure.[28] As a coenzyme, NAD$^+$ mediates redox reactions by carrying electrons from one reactant to another. The NAD$^+$/NADH ratio is a key component in the redox state of a cell.[29] An altered cytosolic redox state and energy deficiency have been observed in human heart failure, and their restoration can alleviate

heart failure by restoring the NAD$^+$ redox balance and thus suppress mitochondrial protein hyperacetylation, including that of the proteins in the malate aspartate shuttle and regulators of the mitochondrial permeability transition pore (mPTP),[30] which suggests a novel therapy for heart failure. More importantly, NAD$^+$ is a cofactor for aldehyde oxidation by aldehyde dehydrogenase 2 (ALDH2).[31] Studies have found that imbalanced NAD$^+$/NADH ratios in the heart during ischaemia can prevent the hydroxynonenal (HNE) oxidation catalysed by ALDH2,[32] thus increasing oxidative stress and mitochondrial injury. Therefore, increasing NAD$^+$ during ischaemia may be a pharmaceutical approach to confer cardioprotective effects by restoring the HNE reaction.

Additionally, the fact that the deacetylase activity of SIRTs is dependent upon NAD$^+$ lends credence to the idea that NAD$^+$ can regulate cellular antioxidant and redox signalling in a SIRT-dependent manner.[33,34] SIRTs have been found to act as critical regulators of the antioxidant response through multiple targets, such as the proteins in the forkhead box O (FOXO) family,[35] nuclear factor E2-related factor 2 (NRF2),[36] superoxide dismutase (SOD)[37] and glutathione (GSH),[38] which provides opportunities for NAD$^+$ supplementation to counter oxidative stress in CVDs. Moreover, exogenous NAD$^+$ administration has been reported to alleviate ischaemia/reperfusion-induced oxidative injury in rat hearts via the SIRT5-SDH-succinate pathway.[33] NMN, as an intermediate in NAD$^+$ synthesis, can
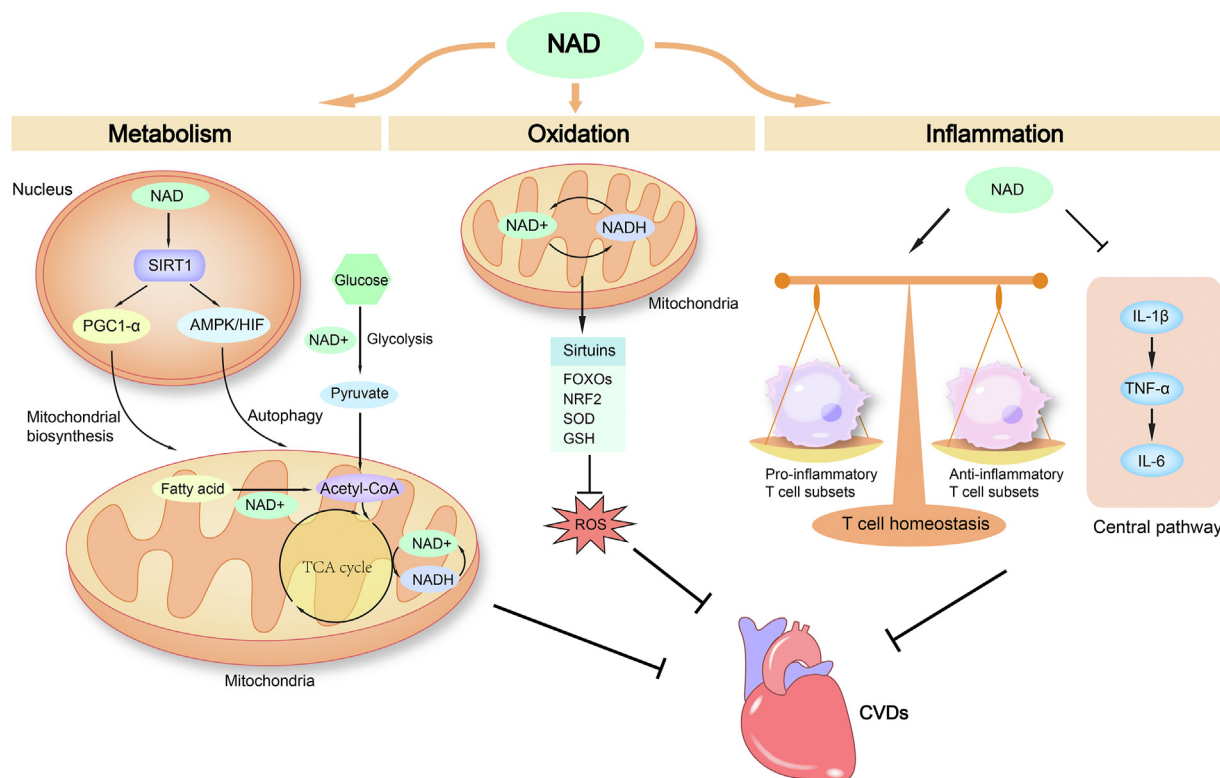
Figure 2 Nicotinamide adenine dinucleotide (NAD^+) exerts beneficial effects on CVDs by regulating metabolism, maintaining redox homeostasis and modulating immune responses. The NAD^+ coenzyme is reduced to NADH during glycolysis, fatty acid β-oxidation and the tricarboxylic acid (TCA) cycle. NAD^+ can regulate mitochondrial biogenesis through the SIRT1/PGC1α pathway and enhance autophagy through the SIRT1/AMPK/HIF pathway. These effects of NAD^+ in metabolism contribute to its invention potential for CVDs treatment. The balance of NAD^+ and NADH is a key component of the redox state of a cell. NAD^+ can exert antioxidant effects by activating SIRTs to regulate FOXOs, NRF2, SOD and GSH, thus countering oxidative stress in CVDs. NAD^+ can also suppress the central immune pathway and regulate T-cell homeostasis to exert immunomodulatory effects, which might be beneficial as a treatment of CVDs. AMPK, adenosine monophosphate-activated protein kinase; CVDs, cardiovascular diseases; FOXOs, class O of forkhead box family; GSH, glutathione; HIF, hypoxia inducible factors; NRF2, nuclear factor E2-related factor 2; PGC1α, peroxisome proliferator-activated receptor γ (PPARγ) coactivator 1α; ROS, reactive oxygen species; SOD, superoxide dismutase.

also protect the heart from ischaemia/reperfusion injury through the SIRT1/FOXO1 pathway.[39] Therefore, NAD^+ has shown great therapeutic potential in CVDs because it can be used to sustain redox homeostasis.

NAD^+ participates in anti-inflammation processes

Inflammation plays a crucial role in CVDs, and its involvement in the pathogenesis of heart failure[40,41] and ischaemic cardiovascular disease has been recognized.[6] However, the outcomes of anti-inflammatory strategies used in cardiovascular system therapy have been disappointing thus far,[42] which may explain the limited role of these regimens in the clinic. Interestingly, the administration of canakinumab, a therapeutic monoclonal antibody targeting interleukin-1β, demonstrates beneficial effects in those patients.[43] Although the underlying mechanisms are not fully understood, this antibody has shown promise for the treatment of CVDs. The following questions are raised at this stage: Will anti-inflammatory therapies eventually form the cornerstone of cardiovascular risk reduction? Which novel therapeutic

targets will be identified among the different pathological phenotypes of inflammation? NAD^+, a cofactor for numerous enzymes, may be involved in the modification of systemic immune responses. We hypothesize that NAD^+ can suppress the central immune pathway and regulate T-cell homeostasis to exert immunomodulatory effects. Supplementation with NAD^+ or its precursors might constitute a promising strategy in anti-inflammatory therapies. Recent studies have revealed that NAD^+ can depress the levels of inflammatory cytokines,[44,45] which may indicate its potential in targeting the central immune pathway, specifically by linking IL-1β, TNF-α, and IL-6, which are considered upstream inflammatory biomarkers.[46] NAD^+ has also been reported to regulate T cell homeostasis during inflammation by participating in the signalling pathway of $CD4^+$ T cell differentiation and apoptosis.[47,48] Since many acute and chronic inflammatory diseases are accompanied by an imbalance in T cell subsets, NAD^+ may be an ideal molecule to treat these diseases because of its homeostatic properties. Notably, NAD^+ exerts its effects through immunomodulation, not immunosuppression. It can promote the systemic production of anti-
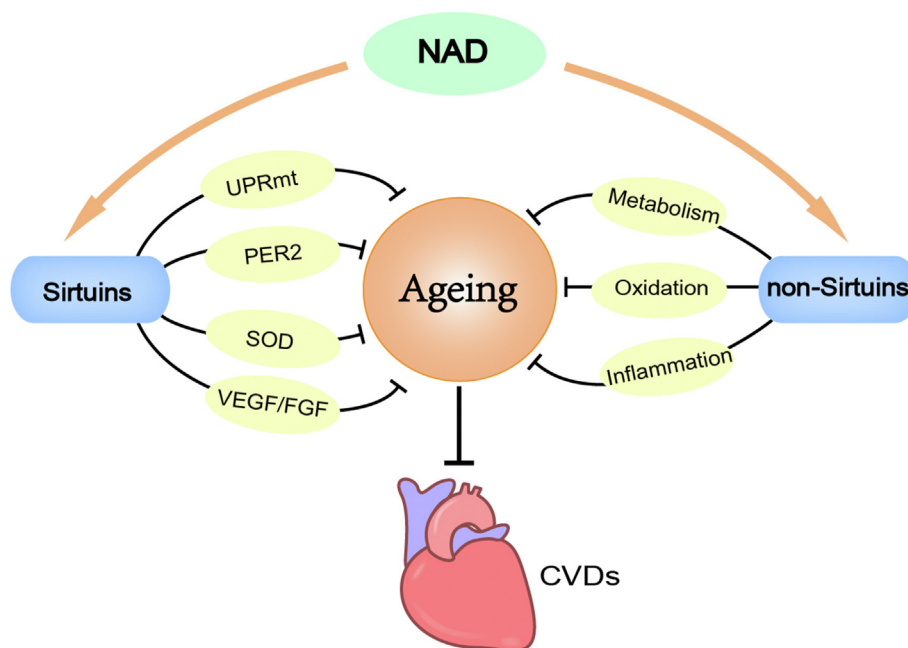
Figure 3 The role of NAD$^+$ in age-related CVDs. Nicotinamide adenine dinucleotide (NAD$^+$) can exert anti-ageing effects by modulating sirtuin activity to regulate UPRmt, PER2, SOD and VEGF/FGF. NAD$^+$ can also counter ageing in non-sirtuin-dependent ways through its beneficial effects on metabolism, oxidation and inflammation. Thus, NAD$^+$ can treat age-related CVDs by delaying ageing. CVDs, cardiovascular diseases; FGF, fibroblast growth factor; PER2, period 2; SOD, superoxide dismutase; UPRmt, mitochondrial unfolded protein response; VEGF, vascular endothelial growth factor.

inflammatory cytokines[49] and regulate CD4$^+$ T cell fate in the absence of antigen.[50] Despite its complicated role in immune responses, its anti-inflammatory potential may further support the clinical application of NAD$^+$ or its precursors for CVDs treatment.

NAD$^+$ exerts anti-ageing effects

Ageing is characterized by a progressive loss of physiological integrity leading to impaired function and increased vulnerability until death.[51] Slowing the ageing process is an enduring aim that has generated an exciting field of study. Ageing is also considered an important risk factor for CVDs because it is associated with excessive oxidative stress, chronic low-grade inflammation and the overactivation of the sympathetic nervous system.[52] Ageing contributes to many cellular and even structural changes in the cardiovascular system, including myocardial fibrosis,[53] amyloidosis in the myocardium,[54] cardiac hypertrophy,[55] endothelial dysfunction and artery stiffness.[56] These changes are associated with an increased risk of coronary artery disease, hypertension, atrial fibrillation, cardiomyopathy and heart failure.[57]

The coenzyme NAD$^+$ is critical in cellular bioenergetics and adaptive stress responses. Emerging evidence suggests that boosting cellular NAD$^+$ levels alleviates physical deterioration, chronic inflammation, metabolic dysregulation, ROS overproduction and DNA damage, which can delay ageing in four respects.[58] NAD$^+$ is widely involved in signal

transduction in the ageing process and has shown broad prospects for treating age-related CVDs. Both dietary and injected replenishment of NAD$^+$ have shown beneficial effects on age-associated phenotypes. A recent clinical trial reported that oral NR supplementation in 12 aged men was well tolerated and reduced the levels of circulating cytokines (IL-6, IL-5, IL-2, and TNF-α), with altered energy metabolism at the transcriptional level.[44] Other studies have provided evidence showing that NAD$^+$ can improve physical activity,[59] metabolic status[60] and memory function[19] in aged mice. Additionally, studies have found that NAD$^+$ replenishment significantly extends the lifespan of both worms and mice.[19,61,62] Notably, supplementation with NAD$^+$ precursors can prevent vascular ageing by enhancing endothelial relaxation, reducing artery stiffness[63] and increasing muscular capillary density.[59] As shown in Table 1, NAD$^+$ replenishment not only can extend the lifespan but can also improve healthspan. Table 1 also shows that NAD$^+$ can delay the processes implicated in age-related CVDs.

Potential NAD$^+$-mediated mechanisms have been explored. Because SIRTs are longevity-associated deacetylases, NAD$^+$ can be used to counter ageing by modulating SIRT activity. For example, NAD$^+$ can prevent age-associated metabolic decline and extend lifespan through the SIRT1/mitochondrial unfolded protein response (UPRmt)/superoxide dismutase (SOD) signalling pathway.[61,62] Additionally, NAD$^+$ has been demonstrated to counteract the age-related decline in circadian rhythms through SIRT1 to deacetylate

Table 1 Effects of NAD$^+$ on ageing in humans and animal models.

Animals & strain	Supplement method & dose	Benefits	Refs
12 men aged (median age of 75 years)	NR 1000mg/2 × /d 21 d	Downregulation of energy metabolism in skeletal muscle depressed circulating levels of IL-6, IL-5, IL-2, TNF-α	44
DBA/2J (D2) mice	NAM 550 mg/kg/d	Reduced vulnerability to glaucoma reduced the incidence of optic nerve degeneration	67
C57BL/6N mice	NMN 500 mg/kg NR 400 mg/kg/d	Enhanced nighttime locomotor activity rhythms.	64
C57BL/6 mice	NMN 300 mg/kg/d	Improved carotid artery endothelium-dependent dilation reduced aortic stiffness	63
C57BL/6J mice	NMN 400 mg/kg/d	Increased capillary density and blood flow Increased exercise endurance	59
C57BL/6N Mice	NMN 100 mg/kg/d 300 mg/kg/d	Decreased age-associated body weight gain, enhanced energy metabolism and physical activity, improved insulin sensitivity, plasma lipid profile, eye function and bone density.	60
C57BL/10SnJ mice	NR 400 mg/kg/d	Reduction in cardiac fibrosis, necrosis, and inflammatory cell infiltration	66
C57BL/6J mice	NR 400 mg/kg/d	Improved muscle function expanded lifespan	61
B6 Atm−/− Mice	NR 12 mM NMN 12 mM	Improved behavioral and memory function expanded lifespan	19
C57BL/6J mice	NAM 500 mg/kg 1000 mg/kg	Improved glucose metabolism enhanced physical performance reduced hepatic steatosis	20
N2(WT) C. elegans	NR 500uM	Improved locomotion and cognitive function expanded lifespan	19
N2(WT) C. elegans	NR 500uM NAM 200uM	Improved metabolic state expanded lifespan	62

NA, nicotinic acid; NAM, nicotinamide; NMN, nicotinamide mononucleotide; NR, nicotinamide riboside.

clock repressor period 2 (PER2).[64] Since evidence has indicated that metabolic homeostasis is intimately linked with the circadian clock,[65] NAD$^+$ may also have the potential to treat metabolic disorders associated with the ageing process. NMN supplementation has been reported to reverse age-related arterial dysfunction by decreasing oxidative stress in old mice, an outcome associated with the activation of SIRT1 and manganese superoxide dismutase (MnSOD).[63] NAD$^+$ can prevent vascular ageing by activating SIRT1 to mediate vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (FGF) secretion from myocytes to influence endothelial cells.[59] NR treatment reduced cardiac

fibrosis, necrosis, and inflammatory cell infiltration in aged *mdx* mice (a Duchene's muscular dystrophy model) with cardiomyopathy by activating SIRT1 and enhancing mitochondrial function.[66] Therefore, NAD$^+$ has shown promise for treating age-related CVDs in a SIRT-dependent manner.

The exact molecular mechanisms of the anti-ageing effect of NAD$^+$ are not yet understood. Therefore, our team has suggested that a non-sirtuin-dependent mechanism as an emerging new direction, especially for future anti-ageing studies of NAD$^+$. Although studies have shown that NAD$^+$ can delay ageing through its beneficial effects on metabolic regulation,[60] redox homeostasis[63] and anti-

inflammation,[44] the role of NAD$^+$ in these studies is still debated, and further investigations are needed to explore the underlying mechanisms.

Clinical evidence and future prospects for NAD$^+$ translation

Research and current problems

Given its multiple properties, including its ability to regulate metabolism, maintain the redox balance, exert anti-inflammatory effects and delay ageing, NAD$^+$ has gained attention for its therapeutic potential. The question

remains: Can increasing NAD$^+$ levels are clinically beneficial? As shown in Table 2, the administration of various NAD$^+$ precursors can lead to a dose-dependent increase in blood NAD$^+$ levels and does not appear to have significant adverse effects. Some studies indicate that NAD$^+$ may decrease the risk of certain CVDs, such as hypertension, arterial stiffness and hyperlipidaemia.[67–69] Several other studies also show that NAD$^+$ can improve metabolic and inflammatory profiles,[44,70,71] which suggests that NAD$^+$ is involved in related signalling pathways in humans.

However, these studies leave many questions unanswered. On the one hand, none of these studies show a direct benefit to the cardiovascular system, despite the benefits listed in Table 2 and 3, and many experiments had

Table 2 Studies that detected an increase in NAD$^+$ levels.

Pharmacological Agent	Subjects	Health Outcomes Observed	References
NR 1000 mg/d 6 weeks	13 men and women overweight (BMI 27–35 kg/m2)	NAD↑ body fat-free mass↑ sleeping metabolic rate↑	[70]
NR 1000mg/2 × /d 21d	12 men aged (median age of 75 years)	energy metabolism in skeletal muscle↑ IL-6, IL-5, IL-2, TNF-α↓	[44]
NR 100 mg/day, 300 mg/day, and 1000 mg/day 8weeks	140 men and women overweight (BMI 25–30 kg/m2)	NAD↑ No clinically meaningful changes in vital sign, hematological and biochemical indexes. No adverse events	[72]
NR 500 mg, 2 × /day 6 weeks	30 men and women lean and healthy (average BMI = 24 ± 4 kg/m2)	NAD↑ No effects on haematology, plasma glucose, insulin, TC and TG potential for reducing systolic blood pressure and arterial stiffness	[69]
NR 1000 mg/day 1week	A male Healthy; 52-year-old; 65 kg	NAD↑ No adverse events	[108]
NR 250–1000 mg/d 1 week	8 participants healthy	NAD↑ No adverse events	[74]
NRPT 1X; 250 mg NR + 50 mg PT NRPT 2X 500 mg NR + 100 mg PT 8weeks	120 participants healthy	NAD↑ No adverse events ALT↓ diastolic blood pressure ↓ mobility↑ lower body strength↑ total cholesterol ↑ LDL-cholesterol↑	[68]
NAM 1080 mg/day 12 weeks	65 patients hemodialysis	NAD↑ Serum phosphate↓, parathyroid hormone↓ HDL-C↑, LDL-C↓	[67]
Acipimox 250 mg, 3 × /day 2 weeks	21 patients T2DM	NAD↑ Mitochondrial unfolded- protein response↑, ATP↑, muscle lipid content↑, mitochondrial respiration↑	[71]

NAD, nicotinamide adenine dinucleotide; NAM, nicotinamide; NR, nicotinamide riboside; NRPT, nicotinamide riboside and pterostilbene; PT, pterostilbene; BMI, Body Mass Index; T2DM, type 2 diabetes; TG, triglyceride; TC, total cholesterol; ALT, Alanine aminotransferase; HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; ATP, Adenosine triphosphate.

Table 3 Studies in which NAD$^+$ levels were not determined.

Pharmacological Agent	Subjects	Health Outcomes Observed	References
NA 3000 mg/d 6 years	3908 patients previous MI	TG↓, TC↓ incidence of definite, non-fatal MI ↓	[78]
NA 2000 mg/d 7—14 months	315 Patients CHD	TG↓, TC↑, HDL-C↑, LDL-C↑ CIMT ↓	[85]
NA 375—1000 mg 4weeks + 1500 mg 6weeks	24 patients HDL-C < 0.9 mmol/L	TG↓, TC↓, LDL-C↓, HDL-C↑, Adiponectin↑, resistin↓	[84]
NA 1000 mg 4 × /d + colestipol 10000 mg 3 × /d 2.5 years	120 men high Apo B levels coronary artery disease a family history of vascular disease	TG↓, LDL-C↓, HDL-C↑ Atherosclerotic progression↓ incidence of cardiovascular events↓	[86]
NA + colestipol 3000—12000 mg/d 2 years	162 men previous coronary bypass surgery	TG↓, LDL-C↓, HDL-C↑ Atherosclerotic progression↓	[79]
NA + Simvastatin 1000—4000 mg/d 3 years	160 patients CHD low HDL-C levels	LDL-C↓, HDL-C↑ Coronary stenosis↓ occurrence of a first cardiovascular event↓	[81]
NA + statins 1000 mg/d 1 year	167 patients CHD low HDL-C levels	HDL-C↑ Atherosclerotic progression↓	[83]

NA 1500—2000 mg/
  d + Simvastatin 40—80 mg/
  d + ezetimibe 10 mg/d
3 years
	3414 patients
	cardiovascular disease
		TG↓, LDL-C↓, HDL-C↑	[80]

NA 2000 mg/d + ezetimibe/ simvastatin 10/20 mg/d 24weeks	1220 patients type IIa or IIb hyperlipidemic	TG↓, LDL-C↓, Apo B↓, lipid/ lipoprotein ratio↓, HDL-C↑, Apo A-I↑ well tolerated aside from N- associated flushing	[82]
NR 2000 mg/d 12 weeks	40 men healthy; obese (BMI > 30 kg/m2)	No effects on glucose and lipid homeostasis	[73]
NR 2000 mg/d 12 weeks	40 men obese (BMI 30 kg/m2)	No effects on fasting/ postglucose plasma glucose, insulin, C-peptide, glucagon and β-cell function	[109]
NAM 1500 mg 2 × /d 24 weeks	31 patients mild to moderate dementia	no significant effects on the cognitive function No adverse events	[76]
NMN (100 mg/d, 250 mg/d, and 500 mg/d)	10 men healthy	Bilirubin↑, creatinine↓, chloride↓, glucose ↓ No adverse events	[110]
Acipimox 1000 mg/d 1week	25 individuals overweight (BMI 22—30 kg/m2)	FFA↓, insulin sensitivity↑, C- peptide↓, HOMA index↓, systolic BP↓, cardiac function↓	[75]
Acipimox 750 mg/d 4—9 weeks	30 patients hyperlipoproteinaemia	TG↓, LDL-C↓, HDL-C↑	[111]
Acipimox 750—1200 mg/d 60 d—9months	17 patients hyperlipoproteinaemia	TG↓, plasma TC↓, HDL-C↑	[112]
Acipimox 1500 mg/d 3 d	8 patients NIDDM	FFA with rebound↓, TG↓, glucose↓, insulin↓	[113]
Acipimox 1000 mg/d	7 patients T2DM	FFA↓, insulin sensitivity↑, glucose↓, glucose tolerance↑	[114]

Table 3 (*continued*)

Pharmacological Agent	Subjects	Health Outcomes Observed	References
7 d			
Acipimox 1000 mg/d	8 men hypopituitary	Glucose↓, FFA↓, insulin sensitivity↑	[115]
Acipimox 150 mg/d	14 volunteers healthy	FFA↓, disposition index during 24-h fasting↑, insulin response↑, insulin sensitivity↑	[116]
Acipimox 750 mg/d	9 subjects, lean control 13 subjects, obese 10 subjects, obese, impaired glucose tolerance, 11 patients, T2DM	FFA↓, insulin↓, insulin-stimulated glucose uptake↑, glucose tolerance↑	[117]
Acipimox 750 mg/d	8 men overwerght (BMI: 30.06 0.7 kg/m2) 8 men, hypopituitary	FFA↓, GLP-1 ↑, glucose-infusion rate↑	[118]
Acipimox 250 mg/d	14 male patients T2DM	FFA during exercise↓, glucose and insulin during recovery from exercise↓, glycaemic control↑	[119]
EH301 1200 mg/d 4 months	32 people ALS	Fat mass↓, Pulmonary function↑, muscular strength↑	[120]

NA, nicotinic acid; NR, nicotinamide riboside; NAM, nicotinamide; NMN, nicotinamide mononucleotide; BMI, body mass index; T2DM, type 2 diabetes; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; CHD, coronary heart disease; CIMT, carotid intima-media thickness; ALS, amyotrophic lateral sclerosis; NIDDM, noninsulin-dependent diabetes mellitus; FFA, free fatty acid; GLP-1, glucagon-like peptide 1; HOMA index, homeostasis model assessment; BP, blood pressure; Apo A-I, apolipoprotein A-I; Apo B, apolipoprotein B.

only neutral outcomes.[72–76] On the other hand, in some studies, NAD$^+$ levels were not determined after supplementation with its precursors, including NA, NR, NAM and NMN, as shown in Table 3. Moreover, NA is not exclusively a NAD$^+$ precursor; it was shown a long time ago to also be a lipid-lowering agent.[77] Almost all studies indicate that the treatment of NA or its derivative acipimox can efficiently enhance lipid metabolism and thus exert beneficial effects on CVDs.[78–86] To some extent, it is still not clear whether these effects depend on NAD$^+$ replenishment. Some studies have attributed these beneficial actions of NA to the activation of the putative G protein-coupled receptor GPR109A.[87] However, emerging evidence suggests that some of the effects promoted by NA might be achieved through NAD$^+$-induced activation of SIRT1.[23,88] Arguing in favour of the latter hypothesis, many of the beneficial effects of NA take place at concentrations much higher than the EC50 for GPR109A (i.e., the concentration to activate 50% of the receptor).[89] NA also has some undesirable effects, such as flushing, pruritus and burning pain,[90] which largely limits its clinical application. Consequently, whether it is feasible to increase NAD$^+$ levels by supplementing endogenous NA continues to be debated.

Since these clinical trials have some disappointing outcomes, some points need to be reconsidered. First, the number of participants in some clinical trials was too small to enable the acquisition of significant results. Second, the time of these studies might be too short for NAD$^+$ to show its full beneficial effects. Moreover, there is a gap between the doses administered *in vitro* and the effective intracellular concentrations. Although NAD$^+$ may exert a long-term benefit on the cardiovascular system in the future, the side effects of NA indicate that we cannot neglect its risk. Further large, well-designed clinical studies are needed to clarify the therapeutic potential and to establish treatment protocols for the use of NAD$^+$ or its precursors.

Boosting NAD$^+$ levels and filling remaining gaps

As mentioned above, emerging evidence has shown that boosting NAD$^+$ levels may have favourable effects, and the best way to increase NAD$^+$ levels for clinical applications remains unclear. Pharmacologically modulating NAD$^+$ levels has emerged as a hot topic for drug development.[91] There are three main approaches that researchers and drug developers are exploring to increase NAD$^+$ levels, including supplementation with NAD$^+$ precursors or their biosynthetic intermediates, stimulating NAD$^+$ synthesis and inhibiting NAD$^+$ consumptions.[10] In addition, NAD$^+$ levels can be increased during physiological processes, such as caloric restriction[92] and exercise,[93] while they can be decreased upon intake of high-fat diets (HFDs).[94] Maintaining a low-energy lifestyle may be a simpler way to increase NAD$^+$ than taking medicine.

As the oral route is most commonly used for drug administration, we cannot neglect the interactions between the gut microbiome and administered drugs. A recent study has reported that *E. coli* can enhance the

chemotherapeutic toxicity of fluoropyrimidines by converting 5′-fluorodeoxyuridine (FUdR) into 5-fluorouridine monophosphate (FUMP).[95] The complexity of drug—microbe—host co-metabolism indicates that NAD^+ precursors may interact with the gut microbiome directly or indirectly,[96] thus modulating drug efficacy and toxicity.

Given the fact that NAD^+ is not evenly distributed in the cell[97–100] and its subcellular distribution is highly variable between tissues,[91] we cannot neglect the gap between the doses administered *in vitro* and the effective concentrations in different subcellular compartments. On the one hand, the permeability of subcellular organelles to NAD^+ is different. NAD^+ is considered to be easily translocated between the nucleus and cytoplasm, but the mitochondrial free NAD^+ level is thought to fluctuate differently than the nuclear and cytoplasmic levels.[97] However, a recent study was the first to identify a mammalian mitochondrial NAD^+ transporter, SLC25A51,[98] which shed light on the mechanism critical for the uptake of NAD^+ into mitochondria. Hence, whether it is feasible to replenish the mitochondrial NAD^+ pool by increasing the cytoplasmic NAD^+ level remains controversial. In addition, the subcellular distribution of NAD^+ biosynthetic enzymes differs. Most enzymes important for NAD^+ synthesis have been detected only in the cytosol, including nicotinamide riboside kinase 1 (NRK1), nicotinamide riboside kinase 2 (NRK2)[99] and nicotinate phosphoribosyl transferase (NAPRT).[101] However, nicotinamide mononucleotide adenylyltransferase (NMNAT) has been found in the cytosol, nucleus, mitochondria and even the Golgi apparatus, although as different isoforms.[100] Similarly, nicotinamide phosphoribosyl transferase (NAMPT) is ubiquitous in the cytosol, nucleus and mitochondria,[102] with NAM considered the common precursor of NAD^+. The manner of NAD^+ replenishment and the type of NAD precursors supplemented might affect its subcellular distribution.

Perspective

The SIRT family was first characterized in yeast as silent information regulator 2 (Sir2).[103] More homologues of Sir2 were found in *Caenorhabditis elegans* and *Drosophila melanogaster*.[104] In mammals, seven SIRTs (SIRT1-7) have different subcellular locations, enzymatic activities and targets.[13] As NAD^+ is an essential substrate of SIRTs, this compartmentalized localization of SIRTs might be regulated by the uneven distribution of NAD^+ pools and may be adjusted on the basis of the complex metabolic state of the cell. This evolutionary process might be partly due to the compartmentalized distribution of NAD^+ pools, which can help organisms adapt to externally changing environments and constant stress. We think the benefits of NAD^+ pool homeostasis may be related to this evolutionary process to some extent. Restoring the homeostasis of NAD^+ pools may contribute to the maintenance of SIRT activity, which has favourable effects on healthspan. This hypothesis can partly explain why a single SIRT agonist does not show more clinical benefits.[105,106] Therefore, we hypothesize that NAD^+ may be involved in the evolutionary process of SIRTs

and that the homeostasis of NAD^+ pools might be more important than the simple activation of SIRTs.

Importantly, the pan-effects of NAD^+ have shown broad application prospects for the treatment of CVDs, including atherosclerosis, coronary heart disease, hypertension, arrhythmias and heart failure.[107] In the future, applying NAD^+ for primary prevention may have profound significance for the management of CVDs. We confirmed that NAD^+ can be a prophylactic drug for CVDs, which can decrease health care costs and relieve socioeconomic burdens.

Author contributions

Xiaokai Zhang contributed to literature searching and wrote the manuscript. Yang Zhang initiated and designed the review. Junbo Ge and Aijun Sun supervised the study and revised the manuscript. All authors read and approved the final manuscript.

Conflict of interests

The authors declare that they have no competing interests.

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Abbreviations

cADPRs	cyclic ADP- ribose synthases
NA	nicotinic acid
NAD	nicotinamide adenine dinucleotide
NAM	nicotinamide
NAMN	nicotinic acid mononucleotide
NAMPT	nicotinamide phosphoribosyltransferase
NAPRT	nicotinic acid phosphoribosyltransferase
NMN	nicotinamide mononucleotide
NMNAT	nicotinamide mononucleotide adenylyltransferase
NR	nicotinamide riboside
NRK	nicotinamide ribose kinase
PARPs	poly-ADP-ribose polymerases
QAPRT	quinolinate phosphoribosyltransferase
SIRTs	sirtuins
AMPK	adenosine monophosphate-activated protein kinase
CVDs	cardiovascular diseases
TCA	tricarboxylic acid
FOXOs	class O of forkhead box family
GSH	glutathione
HIF	hypoxia inducible factors
NRF2	nuclear factor E2-related factor 2
PGC1α	peroxisome proliferator-activated receptor γ (PPARγ) coactivator 1α
ROS	reactive oxygen species
SOD	superoxide dismutase
FGF	fibroblast growth factor
PER2	period 2
UPRmt	mitochondrial unfolded protein response
VEGF	vascular endothelial growth factor

ATM   ataxia-telangiectasia mutated
TG   triglyceride
FFA   free fatty acid
ALDH2   aldehyde dehydrogenase 2
HNE   hydroxynonenal
FUdR   5′-fluorodeoxyuridine
FUMP   5-fluorouridine monophosphate

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