

Review



NAD⁺ and Vascular Dysfunction: From Mechanisms to Therapeutic Opportunities

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ABSTRACT

Nicotinamide adenine dinucleotide (NAD⁺) is an essential and pleiotropic coenzyme involved not only in cellular energy metabolism, but also in cell signaling, epigenetic regulation, and post-translational protein modifications. Vascular disease risk factors are associated with aberrant NAD⁺ metabolism. Conversely, the therapeutic increase of NAD⁺ levels through the administration of NAD⁺ precursors or inhibitors of NAD⁺-consuming enzymes reduces chronic low-grade inflammation, reactivates autophagy and mitochondrial biogenesis, and enhances oxidative metabolism in vascular cells of humans and rodents with vascular pathologies. As such, NAD⁺ has emerged as a potential target for combatting age-related cardiovascular and cerebrovascular disorders. This review discusses NAD⁺-regulated mechanisms critical for vascular health and summarizes new advances in NAD⁺ research directly related to vascular aging and disease, including hypertension, atherosclerosis, coronary artery disease, and aortic aneurysms. Finally, we enumerate challenges and opportunities for NAD⁺ repletion therapy while anticipating the future of this exciting research field, which will have a major impact on vascular medicine.

Keywords: Vascular disease; Hypertension; Nicotinamide adenine dinucleotide; Inflammation; Mitochondria; Autophagy; Aging

INTRODUCTION

Cardiovascular and cerebrovascular diseases are the leading causes of morbidity in the elderly and are responsible for at least one in every 3 deaths globally.^{1,2} Hence, identifying the pathophysiological mechanisms contributing to age-related vascular decline is key to the prevention and treatment of these disorders and has the potential to exert a major impact on human health.³ In this regard, emerging experimental and epidemiological evidence indicates that aging is associated with a systemic decline in nicotinamide adenine dinucleotide (NAD⁺), which is an essential coenzyme in cellular metabolism.⁴⁻⁶ Accordingly, dysregulated NAD⁺ metabolism has been implicated in the age-related functional decline of

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Conflict of Interest

Drs. Abdellatif and Sedej are involved in a patent application related to the cardiometabolic effects of caloric restriction mimetics, including nicotinamide. G.K. has held research contracts with Daiichi Sankyo, Eleor, Kaleido, Lytix Pharma, PharmaMar, Samsara, Sanofi, Sotio, Vascage, and Vasculox/Tioma. G.K. is on the Board of Directors of the Bristol Myers Squibb Foundation France. G.K. is a scientific co-founder of everImmune, Samsara Therapeutics, and Therafast Bio. G.K. is the inventor of patents covering therapeutic targeting of aging, cancer, cystic fibrosis and metabolic disorders. H.B. reports no conflicts. H.B. is an editor of Journal of Lipid and Atherosclerosis; however, he was not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

Author Contributions

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various tissues and organs, including those composing the circulatory system.⁷⁻⁹ Restoring NAD⁺ homeostasis through supplementation of its precursors (also known as vitamin B3 derivatives), including nicotinamide riboside (NR), nicotinamide (NAM, also named niacinamide) and nicotinic acid (NA), in addition to the NAD⁺ intermediate nicotinamide mononucleotide (NMN), mitigates age-associated diseases in many clinically relevant animal models.^{4,10,11} These findings have transformed views on NAD⁺ metabolism and shaped further research activities with the aim to gain a deeper understanding of why NAD⁺ levels decline during aging, and how that decline affects body functions in health and disease.

In the context of vascular disease, NAD⁺ metabolism is increasingly recognized as an attractive actionable target. Replenishment of NAD⁺ in vascular cells—either by the stimulation of NAD⁺ synthesis or the inhibition of its degradation—protects against age-related arterial stiffening and endothelial dysfunction⁸ and improves conditions characterized by abnormal blood flow, such as ischemia/reperfusion injury.¹² This is particularly important as these vascular pathologies often co-exist. Indeed, hypertension is a risk factor for atherosclerosis, and coronary artery disease often occurs via thrombotic complications of atherosclerotic plaques. Although intense research in recent years has revolutionized our view on NAD⁺ biology and generated new and evolving concepts related to the biosynthesis, transport, catabolism, and bioactivity of NAD⁺ in health and disease,¹³ we are only beginning to understand the pathophysiological implications of dysregulated vascular NAD⁺ metabolism. In this review, we summarize how disruption of NAD⁺ metabolism affects vascular function, which vasoprotective mechanisms are regulated by NAD⁺, and how the restoration of NAD⁺ homeostasis mitigates common vascular diseases, including hypertension, atherosclerosis, coronary artery disease, and aortic aneurysm. For a comprehensive overview of NAD⁺ biochemistry and metabolism, as well as of its role in other organs, including the heart, we refer readers to other relevant in-depth reviews.^{5,14,16}

VASOPROTECTIVE MECHANISMS OF NAD⁺

1. NAD⁺ suppresses vascular inflammation

Epidemiological and experimental studies suggest that old age and chronic systemic low-grade inflammation (i.e., inflammaging) are the principal drivers of cardiovascular and cerebrovascular diseases.^{17,18} Very recently, Covarrubias and colleagues demonstrated a causal link between age-dependent decrease in NAD⁺ and persistent low-grade inflammation.¹⁹ The authors found that senescent cells promote the proliferation of M1-like mouse macrophages expressing high levels of CD38, which is a major NAD⁺-consuming enzyme in mammals.²⁰ Accordingly, high CD38 levels contribute to the age-dependent decline of NAD⁺, at least in metabolically active tissues, such as the liver and adipose tissue. Of note, CD38 is strongly expressed in endothelial cells,²¹ as well as in human macrophages and monocytes in inflammatory conditions²² and in blood samples from aged individuals.²³ Therefore, reduced NAD⁺ consumption, increased NAD⁺ synthesis, or a combination of both have been proposed as plausible strategies to attenuate age-induced inflammatory processes (**Fig. 1**). In support of this idea, chronic supplementation of the NAD⁺ precursor NAM reduced inflammation and improved many aspects of healthspan in aged mice fed a high-fat diet,¹⁰ likely by promoting the differentiation of monocytes to macrophages with reduced pro-inflammatory phenotype.²⁴ Interestingly, similar anti-inflammatory actions have also been reported for niacin, which stimulated M2 polarization of peripheral monocytes *in vitro*, both in mice and humans.²⁵ In another study, NAM reduced renal mRNA levels of inflammatory markers, which was

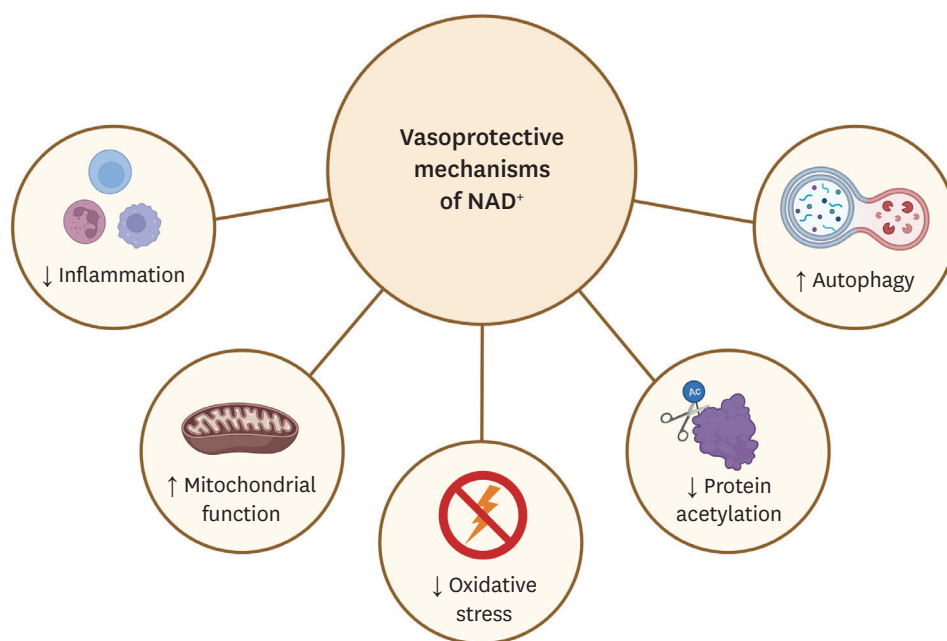


Fig. 1. Elevating cellular NAD⁺ activates various vasoprotective mechanisms. Pharmacological modulation of NAD⁺ levels via NAD⁺ precursors or inhibitors of NAD⁺-consuming enzymes reduces chronic low-grade inflammation and protein acetylation, reactivates autophagy and mitochondrial biogenesis, and enhances oxidative metabolism in vascular cells. Up-arrows indicate increases, down-arrows indicate decreases. The clip art included in this figure was created with BioRender.com.
NAD⁺, nicotinamide adenine dinucleotide.

associated with lowered arterial blood pressure in hypertensive mice with genetically or pharmacologically induced dysfunction of endothelial nitric oxide synthase (eNOS).²⁶ Since NAM administration is safe in humans,²⁷ this observation merits further evaluation in the subgroup of patients with hypertension who have an impaired eNOS system, in whom the inhibition of inflammation might be particularly efficient.

Similar anti-inflammatory effects were reported for alternative NAD⁺ precursors, such as NR and NMN, which inhibit interleukin-1 β and tumor necrosis factor- α (TNF- α)-induced inflammation in cultured endothelial cells and improve endothelial dysfunction in aortic rings *ex vivo*.²⁸ Interestingly, NMN reversed endothelial dysfunction and inflammation by extracellular conversion to NR via CD73, an ecto-5'-nucleotidase localized on the luminal surface of endothelial cells, whereas the NR-induced vasoprotective effects were CD73-independent.²⁸ Although the precise mechanisms underlying the vasoprotective effects of NR remain elusive, endothelial SIRT1 (an NAD⁺-dependent lysine deacetylase) appears to be involved,²⁸ likely through the modulation of eNOS activity.²⁹ In another study, the activation of SIRT1 with SRT1720 was shown to ameliorate vascular endothelial dysfunction in aged mice by reducing arterial inflammation and oxidative stress, but these effects were linked to elevated COX-2 signaling rather than increased nitric oxide (NO) production.³⁰ Regardless, NR also inhibits TNF- α signaling, thereby lowering systolic blood pressure and, at least in part, reducing multimorbidity and premature aging in mice with dysfunctional mitochondria owing to mitochondrial transcription factor A (TFAM) deficiency in T cells.³¹ In sum, considering that inflammation renders the vasculature prone to dysfunction, pharmacological strategies to increase vascular NAD⁺ concentrations might constitute a promising approach to prevent inflammatory-mediated endothelial dysfunction and consequent vascular disease.

2. NAD⁺ attenuates vascular oxidative stress and mitochondrial dysfunction

The depletion of intracellular NAD⁺ levels impedes mitochondrial fatty acid β -oxidation and oxidative phosphorylation, underscoring the critical role of NAD⁺ in maintaining mitochondrial function in the vasculature and beyond. Preclinical studies have demonstrated that supplementation of different NAD⁺ precursors reduces mitochondrial oxidative stress and reverses related vascular dysfunction (**Fig. 1**).^{32,33} For example, chronic NMN supplementation improved NO-related endothelial dysfunction and decreased aortic pulse wave propagating velocity (a proxy of arterial stiffness) by attenuating collagen accumulation and increasing elastin content in aged mouse arteries.³² These NMN-induced vasoprotective effects correlated with reduced oxidative stress and increased SIRT1 activity. Similarly, NMN administration normalized mitochondrial production of reactive oxygen species (ROS) and improved mitochondrial bioenergetics in primary cerebrovascular endothelial cells of old mice.³⁴ Additionally, the neurovascular protective effects of NMN were accompanied by the transactivation of genes involved in mitochondrial rejuvenation, anti-inflammatory, and anti-apoptotic pathways.³⁵ NMN, especially in combination with exogenous hydrogen sulfide, also improved skeletal muscle blood flow by attenuating the age-associated reduction in capillary density (i.e., microvascular rarefaction) through the activation of vascular endothelial growth factor signaling in a SIRT1-dependent manner.⁸ In the same vein, many health benefits of SIRT1 activation are related to improved mitochondrial function. Indeed, similar to NAD⁺ precursors, SIRT1-activating compounds such as resveratrol and SRT1720 induced mitochondrial biogenesis,³⁶ attenuated mitochondrial oxidative stress,^{37,38} activated the antioxidant defense response³⁹ and inhibited apoptosis in endothelial and vascular smooth muscle cells from old mice and rats.⁴⁰ NMN also activates SIRT3,⁴¹ which deacetylates numerous mitochondrial proteins (e.g., superoxide dismutase 2, SOD2), thereby reducing vascular oxidative stress.^{42,43} In the aged mouse aorta, NMN reverted changes in the microRNA expression profile, which correlated with enhanced mitochondrial biogenesis.⁴⁴ However, future studies are required to explain the relationship between microRNAs and age-related vascular diseases, and to delineate the mechanistic role of microRNA gene expression regulatory networks in the vasoprotective effects of NMN.

Another strategy for raising intracellular NAD⁺ levels is to inhibit its degradation by blocking NAD⁺-consuming enzymes,⁵ such as the cyclic ADP-ribose synthase CD38, which is considered the principal NADase in mammalian tissues.²⁰ CD38 is highly expressed in the endothelium,²¹ where it is strongly activated by hypoxia-reoxygenation, leading to loss of eNOS-mediated NO generation and exaggerated eNOS uncoupling. Of note, CD38 is inhibited by the naturally occurring flavonoid apigenin, resulting in elevated NAD⁺ and decreased global acetylation in cell cultures.⁴⁵ In old mice, apigenin rescued endothelial dysfunction, which was associated with increased NO bioavailability, normalized arterial ROS, and reduced oxidative stress.⁴⁶ Additionally, *in vitro*, apigenin prevented the formation and accumulation of foam cells, which are known to propagate the development of atherosclerotic lesions, and alleviated age-associated aortic stiffening, reducing adverse remodeling of the extracellular matrix and suppressing vascular inflammation. Considering that apigenin is a Food and Drug Administration-approved dietary supplement, these preclinical findings provide an experimental basis for future translational studies testing the potential of this CD38 inhibitor to improve arterial dysfunction and reduce vascular disease risk in the elderly.

In sum, considering the key role of mitochondrial homeostasis in maintaining vascular health,⁴⁷ age- and disease-related depletion of NAD⁺ might have severe consequences on mitochondrial redox balance with implications for vascular disease risk.

3. NAD⁺ activates vascular autophagy

In recent years, substantial progress has been made towards better understanding the connection between cardiovascular dysfunction and autophagy.^{48,49} As is also true for several other tissues, autophagic flux is reduced in the vasculature of aged mice and humans.⁵⁰ Consistent with this finding, genetic manipulation studies have demonstrated that reducing or completely blocking autophagy by inactivating essential autophagy genes in vascular endothelial or smooth muscle cells markedly deteriorates vascular physiology.⁵¹ For instance, mice harboring a vascular smooth muscle cell-specific *Atg7* deficiency showed premature defects in calcium homeostasis, as well as abnormal vascular reactivity and smooth muscle cell contractility.⁵² Along similar lines, mice with endothelial cell-specific deletion of *Prkaa*—an α catalytic subunit of AMP-activated protein kinase that regulates mitochondrial biogenesis, function, and turnover—displayed reduced autophagy, which was sufficient to cause aortic endothelial dysfunction and mitochondrial fragmentation.⁵³ These findings indicate that manipulation of autophagy may impair vascular functions and that intact autophagic responses are required for vascular homeostasis. Increasing evidence suggests that enhancing NAD⁺ availability stimulates autophagic flux to protect from ischemic vascular diseases, including in the heart and brain.^{54,55} For example, NAD⁺ treatment preserved coronary microvascular density, reduced infarct size, and improved postischemic vascular repair by rescuing coronary microvascular endothelial cells upon ischemia/reperfusion damage in the rat heart.¹² This microvascular protection was mediated, at least in part, through TFEB-induced lysosomal autophagy, which was also reported to stimulate postischemic angiogenesis in a mouse hindlimb ischemia model.⁵⁶

Mechanistically, recent studies have identified that sirtuins play an important role in mediating NAD⁺-induced autophagy.⁵⁷ In fact, SIRT1 can induce autophagy by epigenetic mechanisms, namely through histone modifications that influence autophagy-related gene expression and by post-translational mechanisms through the action of forkhead box transcription factors.⁵⁸ Moreover, SIRT1 directly deacetylates several essential proteins of the autophagy machinery, including the products of the autophagy genes *Atg5*, *Atg7*, and *Atg8*.^{59,60} Since NAD⁺ might affect multiple other downstream targets relevant for autophagy, further research is warranted to obtain a full understanding of the mechanisms underlying NAD⁺-dependent autophagy activation. Of note, SIRT6 has been recently implicated in autophagy activation and reduced macrophage foam cell formation, suggesting that yet another NAD⁺-responsive sirtuin might protect against atherosclerosis progression.⁶¹

Taken together, emerging evidence underscores that autophagic flux plays a major role in the NAD⁺-induced maintenance of vascular homeostasis (**Fig. 1**). Conversely, defective autophagy appears to be a common cause of vascular aging and the development of associated pathologies. However, further studies testing causality and dose–response relationships are required to confirm whether and to which extent autophagy underlies NAD⁺-induced vasoprotection. Future research is required to delineate the role of autophagy subroutines, such as mitophagy (i.e., mitochondrial autophagy). This is particularly important because age-dependent impairment of mitophagy might cause endothelial dysfunction, metabolic imbalance, inflammation, and senescence, which may collectively contribute to atherosclerosis.⁶² Hence, future studies focusing on the mitochondrial axis are needed to elucidate the role of NAD⁺ in decelerating the manifestation of age-related diseases.⁶³

TARGETING NAD⁺ METABOLISM IN VASCULAR DISEASE

A decrease in NAD⁺ is centrally involved in cardiovascular diseases, such as cardiac ischemia in the context of coronary artery disease.⁶⁴⁻⁶⁶ Disrupted NAD⁺ homeostasis also accompanies other human vascular pathologies, including hypertension,⁶⁷ atherosclerosis,⁶⁸ and aortic aneurysm,⁶⁹ and restoration of NAD⁺ content via different NAD⁺ precursors has yielded promising results in animal models (**Fig. 2** and **Table 1**).

1. Hypertension

Currently, one in 3 adults worldwide has hypertension,⁷⁰ and the global epidemic of hypertension is expected to rise owing to the demographic shift towards ever more aged populations. Although hypertension is a modifiable risk factor for cardiovascular disorders, including ischemic and hemorrhagic stroke, coronary and valvular heart diseases, as well as heart or renal failure,⁷¹ hypertension management has remained a major public health challenge. This has been largely attributed to the multifactorial nature of hypertension and its complex pathogenesis, which remains incompletely understood.

In this respect, NAD⁺ metabolism has emerged as a potential therapeutic target for hypertension and associated vascular dysfunction.⁷² The expression of the rate-limiting enzyme in NAD⁺ biosynthesis, nicotinamide phosphoribosyltransferase (NAMPT), has been recently found downregulated both in mice and humans with hypertension,⁶⁷ implying that restoring NAD⁺ homeostasis might have an anti-hypertensive effect. In support of this possibility, systemic overexpression of NAMPT was found to protect against angiotensin II-induced hypertension in a SIRT1-dependent manner by reducing ROS production in aortic endothelial cells and vascular smooth muscle cells.⁶⁷ By contrast, mice with systemic *Nampt* haploinsufficiency displayed elevated blood pressure and ROS levels in response to angiotensin II infusion, and the administration of recombinant human NAMPT reversed this effect.⁶⁷ Along the same lines, increased NAMPT-mediated NAD⁺ biosynthesis upon NAM supplementation prevented the increase in systolic blood pressure induced by the non-selective NOS inhibitor, L-NAME (N[ω]-nitro-L-arginine methyl ester). Consistently,

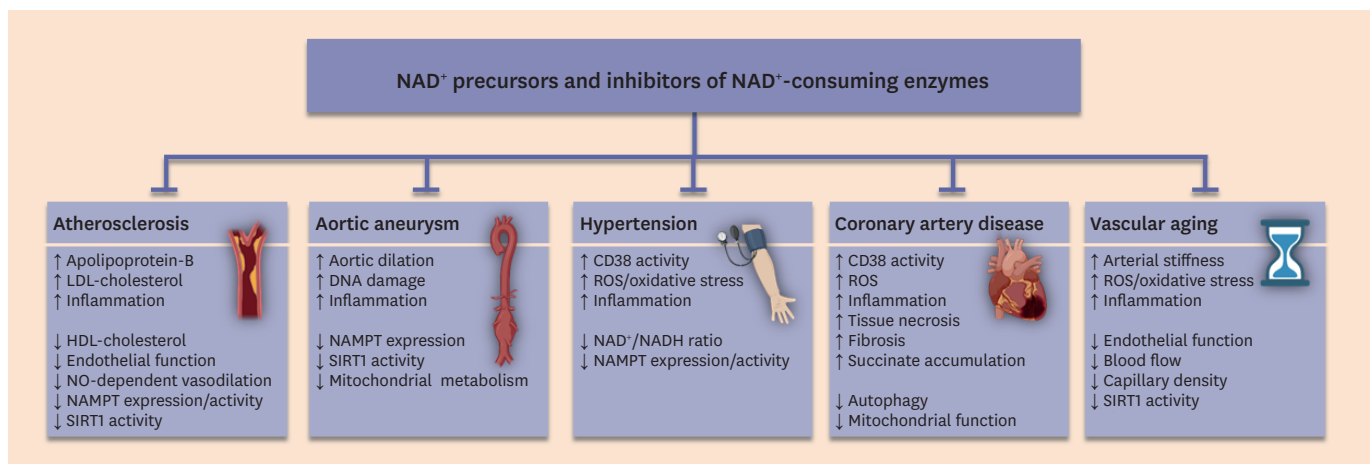


Fig. 2. Targeting NAD⁺ metabolism to treat vascular diseases. Restoration of NAD⁺ content through different NAD⁺ precursors and inhibitors of NAD⁺-depleting enzymes is an emerging therapeutic strategy to improve hallmarks of various vascular disorders. Up-arrows indicate increases, down-arrows indicate decreases. The clip art included in this figure was created with BioRender.com.

NAD⁺, nicotinamide adenine dinucleotide; LDL, low-density lipoprotein; HDL, high-density lipoprotein; NO, nitric oxide; NAMPT, nicotinamide phosphoribosyltransferase; ROS, reactive oxygen species.

Table 1. Vascular disorders, against which NAD⁺ precursors show beneficial effects

Vascular disease	NAD ⁺ precursor	Experimental setting	Effects	Ref.		
Hypertension	NAM	<i>Dahl</i> salt-sensitive rats	Reduced high blood pressure	26,73		
		<i>eNOS</i> ^{-/-} mice	Improved endothelial function			
	L-NAME-treated mice	Reduced inflammation				
Atherosclerosis	NA	Nephrectomized rats	Reduced high blood pressure	76		
	NR	Middle-aged and old humans	Reduced inflammation			
			Reduced oxidative stress			
NA	Humans and (APOE) mice with atherosclerosis	A trend towards reduced blood pressure and aortic stiffness	72			
Coronary artery disease	NAM	A swine model of myocardial ischemia-reperfusion	Decreased LDL cholesterol	83,132		
			Increased HDL cholesterol			
	NAM	APOE mice	Improved protection against ApoB-containing lipoprotein oxidation		79	
Aortic aneurysm	NA and NAM	Calcium chloride- and angiotensin II-treated mice	Reduced inflammation and atherogenesis	112		
			Decreased necrosis, fibrosis and stiffness			
			Improved recovery of cardiac function			
			Reduced inflammation			
Vascular aging	NMN	Naturally aged mice	Decreased myocardial infarction size	104,133		
			Reduced oxidative stress			
			NR	Ischemia-reperfusion injury in mice	Improved ejection fraction and reduced infarct size	105
			NMN	Ischemia-reperfusion injury in mice and aged rats	Smaller infarct size	106,107
Aortic aneurysm	NA and NAM	Calcium chloride- and angiotensin II-treated mice	Ameliorated cardiac function	112		
			Improved ROS and mitochondrial membrane potential			
			Decreased formation of abdominal aortic aneurysms			
Vascular aging	NMN	Naturally aged mice	Reduced inflammation and immune cell infiltration	32,34		
			Lower matrix degradation			
			Reduced arterial stiffness			
			Cerebromicrovascular protection			
Vascular aging	NMN	Naturally aged mice	Improved neurovascular coupling	32,34		
			Improved endothelial function			
			Lower oxidative stress			

NAD⁺, nicotinamide adenine dinucleotide; NAM, nicotinamide; eNOS, endothelial nitric oxide synthase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; L-NAME, N[ω]-nitro-L-arginine methyl ester; ROS, reactive oxygen species; NA, nicotinic acid; NR, nicotinamide riboside; NMN, nicotinamide mononucleotide.

NAM lowered the elevated systolic blood pressure in *Dahl* salt-sensitive rats as well as in *eNOS*^{-/-} mice, likely through reduced inflammation.^{26,73} In pregnant mice with pre-eclampsia, NAM lowered arterial blood pressure through the reduction of cADPR,⁷⁴ a product of CD38-mediated NAD⁺ consumption that regulates calcium signaling.⁷⁵ Similarly, NA attenuated high blood pressure, inflammation, and oxidative stress in rats with chronic kidney disease,⁷⁶ whereas NR treatment lowered systolic blood pressure in mice with T cell-specific TFAM deficiency while restoring the NAD⁺/NADH ratio.³¹ These findings were corroborated in a recent human phase I study showing that NR supplementation led to a mild reduction in blood pressure and aortic stiffness in middle-aged and old, otherwise healthy, individuals.⁷²

Pharmacological and genetic CD38 inhibition, which increases cellular NAD⁺, significantly attenuated angiotensin II-induced hypertension and vascular remodeling in mice.⁷⁷ *CD38*^{-/-} mice and WT mice treated with NMN or the CD38-specific inhibitor 78c displayed lower blood pressures, reduced vascular media thickness, media-to-lumen ratio, and collagen deposition, as well as normalized elastin expression. Moreover, NMN supplementation and CD38 inhibition alleviated the senescence of vascular smooth muscle cells.⁷⁷

In aggregate, restoring NAD⁺ levels by supplementation of NAD⁺ precursors or CD38 inhibitors is being explored as an adjuvant therapy for hypertension. However, large clinical trials are warranted to provide conclusive evidence as to whether incrementation of NAD⁺ levels provides tangible benefits.

2. Atherosclerosis

Atherosclerosis is associated with endothelial dysfunction, the recruitment of pro-inflammatory M1-like macrophages, and the degeneration of smooth muscle cells in the vasculature. During early atherosclerosis, macrophages differentiate into foam cells by ingesting modified low-density lipoprotein cholesterol, which in turn promotes the formation of atherosclerotic plaques. NAD⁺-dependent activation of SIRT1 has been shown to have beneficial effects on all these cell types and to protect against atherosclerosis.⁷⁸ For instance, dietary NAM supplementation in *ApoE*-deficient mice prevented atherogenesis and improved protection against ApoB-containing lipoprotein oxidation and aortic inflammation.⁷⁹ Of note, the protective effects of NAM might also be achieved by increasing the plasma concentration of N-methyl-nicotinamide (methyl-NAM, a metabolic product of NAM). In fact, epidemiological studies have demonstrated that methyl-NAM may exert anti-thrombotic and anti-inflammatory effects on the endothelium by promoting NO-dependent vasodilation, thereby improving endothelial function.⁸⁰ In the same vein, methyl-NAM was found to be atheroprotective in *ApoE*^{-/-}/*Ldlr*^{-/-} mice, which displayed improved endothelial dysfunction associated with reduced atherosclerotic plaque area, plaque inflammation, and cholesterol content in the brachiocephalic artery.⁸¹ Similarly, the aortas of *ApoE*^{-/-} mice fed methyl-NAM and a high-fat, high-cholesterol diet exhibited improved endothelium-dependent vasorelaxation.⁸² Mechanistically, this effect was attributed, at least in part, to decreased asymmetric dimethylarginine concentrations due to the induction of dimethylarginine dimethylaminohydrolase 2.⁸²

Niacin (nicotinic acid, NA) is a well-known lipid-lowering compound that reduces apolipoprotein-B-containing lipoproteins while raising the levels of atheroprotective high-density lipoproteins.⁸³ Notably, the anti-dyslipidemia effects of niacin were known long before the discovery of statins and the link between NAD⁺ and sirtuins.⁸⁴ Although niacin has a potent anti-atherogenic effect, it failed to reduce the residual cardiovascular risk in patients receiving statins.⁸⁵⁻⁸⁷ Furthermore, a recent meta-analysis revealed that the combinatory administration of niacin and standard lipid-lowering therapy using statins might be associated with adverse effects on survival.^{88,89} It is important to note, however, that niacin monotherapy was previously shown to reduce mortality.⁹⁰ Regardless, niacin-treated patients exhibit poor compliance due to an unpleasant flushing side effect; thus, niacin is no longer recommended or only prescribed to statin-intolerant patients.^{91,92} Although more tolerable formulations of NA have been developed,⁹³ available preclinical evidence on the role of NAD⁺, and especially NAMPT—the rate-limiting enzyme of NAD⁺ salvage biosynthesis⁹⁴—in atherosclerosis is rather scarce and contradictory. On the one hand, leukocyte-specific overexpression of NAMPT attenuated atherosclerotic plaques in low-density lipoprotein receptor-deficient (*Ldlr*^{-/-}) mice.⁹⁵ Additionally, a reduced number of atherosclerotic plaques in *Ldlr*^{-/-} mice coincided with increased macrophage resistance to apoptosis and skewed polarization towards a more anti-inflammatory M2 phenotype.⁹⁵ On the other hand, systemic NAMPT inhibition has been reported to exert an atheroprotective effect,⁹⁶ while global NAMPT overexpression aggravated atherosclerosis in *ApoE*^{-/-} mice.⁹⁷

In light of the inconsistent findings regarding the impact of NAD⁺ on atherosclerosis, more studies geared towards targeted and cell type-specific interventions must examine the general importance of maintaining cellular NAD⁺ levels in atherosclerosis and delineate the specific role of NAMPT in atherogenesis. Furthermore, it is important to mention that, despite the initial discouraging clinical effects of niacin on patients with cardiometabolic risk, current research is shifting towards other NAD⁺ precursors, which do not necessarily reduce

lipid levels, but arguably possess higher NAD⁺ repletion capacity than niacin.⁵ In view of the pleiotropic actions of NAD⁺, it would not be surprising if the possible (cardio)vascular benefits of NAD⁺ precursors might be uncoupled from the correction of hyperlipidemia. In fact, as we discuss in the next sections, various studies have consistently shown that, both in the presence or absence of adiposity, NAD⁺ repletion counteracts life-threatening vascular disorders.

3. Coronary artery disease

Experimental models of coronary artery disease induced by transient coronary artery ligation have clearly demonstrated that myocardial ischemia is associated with NAD⁺ depletion.⁶⁴⁻⁶⁶ One possible explanation for the NAD⁺ decline in postischemic hearts is the over 50-fold higher CD38 activity in endothelial cells than in cardiomyocytes.²¹ This CD38 overactivation appears to be an important cause of postischemic endothelial dysfunction, suggesting that CD38 is an actionable target to prevent this dysfunction in unstable coronary syndrome.⁹⁸ In this regard, both genetic deletion and pharmacological inhibition of CD38 by luteolinidin and the thiazoloquin(az)olin(on)e 78c protected against ischemia/reperfusion injury, preserved contractile function, enhanced coronary flow, and decreased infarct size.⁹⁹⁻¹⁰¹ Similarly, NAD⁺ administration (10 mg/kg body weight [BW] per day intraperitoneally) lowered the ischemic accumulation of succinate and ROS, which were both associated with reduced cardiac injury in isolated rat hearts.¹⁰² Furthermore, intravenous NAD⁺ administration (20 mg/kg BW before reperfusion) attenuated ischemic cardiac tissue necrosis, fibrosis, and inflammation upon reperfusion of the transiently occluded left anterior descending coronary artery in pigs.¹⁰³ NAD⁺ precursors, including NAM, NR, and NMN exert similar protective effects. For instance, dietary administration of NAM (0.5 g/kg diet) reduced infarction size in an *ex vivo* model of myocardial ischemia-reperfusion.¹⁰⁴ Mice treated with the alternative precursor NR (100 mg/kg BW) also exhibited improved cardiac function and smaller infarcts.¹⁰⁵ The NAD⁺ intermediate NMN consistently normalized alterations in the mitochondrial membrane potential and ROS levels associated with ischemic myocardial injury in aged rats.¹⁰⁶ NMN not only protected against ischemic injury, but also had beneficial effects against coronary reperfusion injuries.¹⁰⁷ Of note, the NAD⁺-induced protective effects in coronary artery disease models coincide with the reactivation of autophagy flux.^{12,54} However, more studies are required to elucidate whether autophagy is protective or detrimental in this setting.¹⁰⁸

Accumulating evidence implicates NAD⁺ deficiency in coronary artery disease and associated cardiac events. Like several CD38 inhibitors, various NAD⁺ precursors have been shown to improve postischemic endothelial dysfunction and, thus, protect against experimental ischemia/reperfusion injury of the myocardium. Therefore, future clinical trials should examine whether treatment with NAD⁺ precursors may exert beneficial effects in patients with acute coronary syndrome. Furthermore, the harmful effects of CD38 overactivation in the postischemic heart highlight the need for further research to delineate the mechanisms involved.⁹⁸ In this respect, future studies should focus on the mechanisms of CD38 activation in response to hypoxia-reoxygenation in endothelial cells, which display the highest CD38 expression among all major cardiac cell types.¹⁹

4. Aortic aneurysm

Apart from lowering lipid levels, NA mediates potent anti-inflammatory effects on human endothelial and immune cells.^{109,110} In this regard, persistent adventitial and medial infiltration of immune cells contributes to the pathogenesis of abdominal aortic aneurysms. Consistently, NA (0.3% w/v in the drinking water)¹¹¹ reduced immune cell infiltration and matrix degradation, thereby protecting against abdominal aortic aneurysm formation in

mice subjected to calcium chloride or angiotensin II infusion.¹¹² Interestingly, NAM (0.4% w/v), which ostensibly does not exert significant lipid-lowering effects,¹¹³ also protected against abdominal aortic aneurysms.¹¹² Notably, NAM-treated mice exhibited increased SIRT1 activity, and co-administration of the SIRT1 inhibitor EX-527 effectively abolished the vasoprotective effects of NAM.¹¹² Similarly, the alternative NAD⁺ precursor NR has been recently shown to improve mitochondrial metabolism, aortic function, and aortic diameter, thereby reversing Marfan syndrome-associated aortic aneurysms in a relevant mouse model.¹¹⁴ In support of a causal role of NAD⁺ in the development of aortic dilation and aneurysms, mice with smooth muscle cell-specific knockout of *Nampt* exhibited increased susceptibility to angiotensin II-induced aortic aneurysms, as denoted by exaggerated medial hemorrhage and dissection.⁶⁹ In human subjects with thoracic aortic aneurysms, unrepaired DNA strand breakages were detected in smooth muscle cells, and this damage was particularly enriched in smooth muscle cells with the lowest NAMPT expression.⁶⁹

In sum, supplementation of NAD⁺ precursors improves aortic wall structure and function as it protects or even reverses aortic aneurysms in mice. Various mechanisms, including reduced pro-inflammatory signals, enhanced mitochondrial metabolism, and sirtuin activation may mediate these vasoprotective effects. Taking into account that the aortic diameter in patients with aortic aneurysm inversely correlates with NAMPT expression in smooth muscle cells,⁶⁹ future clinical studies should explore whether NAD⁺ precursors may improve the course of this disease in humans.

5. Aging and related vascular decline

The integrity of most organs and tissues relies on an ample and functional microcapillary network that provides transport routes for the circulation of cells, oxygen, nutrients, and metabolic waste products.¹¹⁵ Recent observations suggest that the organ-specific loss of vascular abundance is an important characteristic of aging tissues in mice and humans.¹¹⁶ On the one hand, impaired vascular function and structure comprises stiffening of the large elastic arteries, intimal thickening, and media calcification, which are mediated by increases in oxidative stress, inflammation, and vascular smooth muscle tone. On the other hand, vascular dysfunction also encompasses a decrease in the number and function of endothelial cells at the interface between circulating blood and tissues. Endothelial dysfunction, which is characterized by reduced NO production and bioavailability, as well as an imbalance between the vasoconstrictors and vasodilators derived from the endothelium, leads to local dysregulation of the vascular tone and, thus, to reduced blood flow to tissues, culminating in end-organ damage.^{117,118}

A decline in the cellular NAD⁺ pool is closely related to cellular aging, whereas an increase in NAD⁺ synthesis or a decrease in its degradation delays aging in various organ systems.¹⁵ Similar geroprotective effects have been reported for the vascular system. For example, aged mice treated with NMN, although not suffering from hyperlipidemia or hypertension, exhibited several vascular benefits, including restored endothelial-dependent vascular relaxation, reduced arterial stiffness, and reduced oxidative stress.³² In another study, NMN administration to naturally aged mice conferred cerebrovascular protective effects, which led to improved neurovascular coupling and cognitive function.³⁴ In addition, NMN supplementation improved blood flow and increased treadmill endurance in old mice by promoting a SIRT1-dependent increase in capillary density in the skeletal muscle.⁸ Since adequate blood flow is vital to every tissue and organ, not only skeletal muscle, it will be important to test whether increased endothelial NAD⁺ availability stimulates angiogenesis and improves blood flow in the aging brain and heart.

NAD⁺ METABOLISM AS A TARGET TO IMPROVE VASCULAR HEALTH IN HUMANS

Despite extensive preclinical evidence on the benefits of NAD⁺, clinical studies still lag behind. In fact, only a handful of trials have been concluded, and these trials mainly focused on safety, as well as on the ability of NAD⁺ precursors to increase NAD⁺ bioavailability. Besides NA, which has been historically tested for its lipid-lowering impact, NR is the most common precursor evaluated in ongoing trials with vascular endpoints (**Table 2**). NR supplementation appears to be safe, well-tolerated, has no apparent side effects, and is effective in increasing whole-blood NAD⁺ levels.⁷² Importantly, in healthy middle-aged and older adults, NR tended to lower blood pressure and reduce aortic stiffness.⁷² In addition, NR improved mitochondrial fitness and dampened activation of the NLRP3 inflammasome in circulating leukocytes isolated from healthy volunteers.¹¹⁹ However, not all studies support the therapeutic potential of NR supplementation. For example, NR administration failed to ameliorate endothelial dysfunction, as determined by brachial artery flow-mediated dilation in middle-aged and older adults.⁷² Similarly, oral NR failed to improve blood flow, mitochondrial bioenergetics, and metabolism of skeletal muscle, although it did succeed in reducing the levels of circulating inflammatory cytokines in 70- to 80-year-old men.¹²⁰ Future large-scale trials are needed to provide conclusive evidence on the putative health benefits provided by NR.

Table 2. Ongoing NAD⁺ clinical trials with vascular endpoints

NAD ⁺ precursor	Dose	Condition (demographics)	Trial design and phase	No. of recruited participants	Vascular endpoint(s)	Expected completion	Identifier
NAM	2,500 mg/day	Early-onset pre-eclampsia (age: 18–55 years; gender: women)	Single group, open-label (phase 2)	25	Changes in mean blood pressure	July 2020	NCT03419364
NA	Up to 2,000 mg/day	Healthy volunteers (age: 18–99 years; gender: men and women)	Single group, open-label (phase 2)	24	Changes in lipoprotein composition and function as well as vascular compliance	July 2020	NCT02322203
NR	1,000 mg/day	Hypertension (SBP >130 mmHg; age: 65–105 years; gender: men and women)	Randomized, placebo-controlled, double-blind (phase 1)	74	Changes in systolic blood pressure and arterial stiffness	May 2021	NCT04112043
	1,000 mg/day	Moderate to severe chronic kidney disease (age: 35–80 years; gender: men and women)	Randomized, placebo-controlled, double-blind (phase 2)	118	Changes in aortic stiffness and arterial blood pressure	September 2024	NCT04040959
	1,000 mg/day	(Pre)hypertension (SBP: 120–139 mmHg; age: 50–79 years; gender: men and women)	Randomized, placebo-controlled, double-blind (phase 2)	118	Changes in systolic blood pressure and arterial stiffness	December 2023	NCT03821623
	1,000 mg/day	Peripheral artery disease (age: >18 years; gender: men and women)	Randomized, placebo-controlled, double-blind (phase 3)	90	Effects on walking performance, physical activity, quality of life, and skeletal muscle phenotype	April 2022	NCT03743636
NMN	300 mg/day	Middle-aged and old healthy volunteers (age: 40–65 years; gender: men and women)	Randomized, placebo-controlled, double-blind (phase: N/A)	66	Safety and efficacy in reducing systolic and diastolic blood pressures	March 2021	NCT04228640
	400 mg/day	Healthy volunteers (age: 30–60 years; gender: men and women)	Single group, open-label (phase: N/A)	20	Tolerability, pharmacodynamics and cardiovascular effects, including arterial blood pressure; heart rate, blood lipids	October 2021	NCT04862338
	800 mg/day	Hypertension (SBP: 140–159 mmHg and DBP: 90–99 mmHg; age: 18–65 years; gender: men and women)	Randomized, single (assessor)-blind (phase 4)	20	Changes in flow-mediated dilation, pulse wave velocity, as well as systolic and diastolic blood pressures	July 2022	NCT04903210

We searched the US clinical trial registry (<https://www.clinicaltrials.gov/>) using terms “nicotinamide” and “vascular disease” for recently completed or ongoing clinical trials of NAD⁺ supplementation that have yet to publish results (from database inception to January 2022).

NAD⁺, nicotinamide adenine dinucleotide; NAM, nicotinamide; NA, nicotinic acid; NR, nicotinamide riboside; NMN, nicotinamide mononucleotide; DBP, diastolic blood pressure; SBP, systolic blood pressure; N/A, not available.

Observational findings indicate that a diet rich in NAM (and NA) is linked to lower blood pressure and a reduced risk of cardiac mortality in humans.⁷³ In view of the good tolerability of NAM in relatively high doses over months or even years,¹²¹ it is conceivable to examine the therapeutic utility of NAM as an adjuvant therapy for hypertension. Although 2 recent clinical studies have shown that NMN supplementation is safe and can increase NAD⁺ bioavailability in blood,^{122,123} the impact of NMN supplementation on vascular health has not yet been reported. Results from ongoing studies examining the effects of NMN on vascular function and arterial blood pressure in older adults and individuals with hypertension (**Table 2**) are awaited to determine whether NMN has the potential to improve vascular health.

Despite the promising vasoprotective effects of CD38 inhibitors, which have vasorelaxant and antioxidant properties in experimental models of cardiac ischemia/reperfusion injury,⁹⁹ only a few clinical studies have so far been completed. The flavonoids epicatechin and quercetin (both abundant in tea) were tested in middle-aged and old men and women with increased systolic blood pressure.¹²⁴ Supplementation with both CD38 inhibitors failed to improve flow-mediated dilation, arterial stiffness, NO bioavailability, or blood lipid profiles. Similarly, the polyphenol and CD38 inhibitor quercetin failed to increase endothelial function in healthy men with the APOE genotype.¹²⁵ However, quercetin lowered postprandial systolic blood pressure, which was associated with decreased postprandial triacylglycerol concentrations in parallel with increased high-density lipoprotein cholesterol concentrations. In light of the recent finding that CD38 hyperactivation might drive NAD⁺ depletion in aged mice,¹⁹ further research efforts are necessary to determine whether specific CD38 inhibition may alleviate age-related vascular remodeling. In support of this idea, new preclinical evidence suggests that suppressing vascular smooth muscle cell senescence by means of CD38 inhibitors delays vascular aging.⁷⁷

In aggregate, the available human studies suggest that oral administration of various NAD⁺ precursors is safe and modestly increases levels of NAD⁺ or its metabolites, albeit to varying degrees and in a tissue-specific fashion.¹²⁶ Hence, well-powered and carefully designed clinical trials should determine whether chronic supplementation of NAD⁺ precursors, especially those with high NAD⁺-increasing capacity, may improve vascular health, perhaps independently from lipid-lowering effects.¹²⁶

CONCLUSION AND FUTURE PERSPECTIVES

Although NAD⁺ studies focusing on the vasculature have been largely overshadowed by cardiac-centric studies, the significant benefits of restoring NAD⁺ homeostasis in animal models of vascular disease have spurred interest in the therapeutic potential of NAD⁺ at the clinical level. However, many difficulties and challenges related to the administration of NAD⁺-regenerative therapeutics must be resolved to translate the experimental findings to medical practice. For this, future large-scale clinical studies with long-term follow-up that extends beyond treatment discontinuation are needed. These trials should consider adapting drug doses from rodent studies to human studies while considering major differences in metabolic rate and body surface area between mice and humans, but rather similar cellular NAD⁺ turnover rates in both species.¹²⁷ Another important question is how NAD⁺ precursors are best administered (i.e., at which dose, formulation, and route of administration, and at what time of day, considering chronobiological variations in NAD⁺ levels).¹²⁸ On the one hand, utilizing the human-equivalent dosage of NAD⁺ precursors could have a more favorable

and consistent effect on vascular-related endpoints. On the other hand, high doses of NAD⁺ precursors can cause hepatotoxicity and other adverse effects in patients,^{16,129} emphasizing the necessity to rigorously measure therapeutic and toxicological endpoints in healthy and diseased states. Another important consideration is the standardization and development of reliable biomarkers of NAD⁺ metabolism, including the quantitation of NAD⁺ precursors and metabolites in the circulatory system as well as the proxies of their bioactivity, which can encompass specific patterns of protein acetylation, autophagy, and mitophagy. Solving these current limitations will be critical for designing future NAD⁺-centered therapeutic interventions in patients.

Based on current evidence, both NR and NMN seem promising candidates for boosting NAD⁺ levels in vascular cells. In addition to bypassing the rate-limiting step in NAD⁺ synthesis, another advantage of administering NR or the NAD⁺ intermediate NMN might reside in the fact that both precursors avoid the negative feedback exerted by NAM on sirtuin deacetylases (which typically produce NAM as an end product). However, recent *in vivo* data challenge this long-held view, as all 3 precursors (i.e., NAM, NMN, and NR) exerted ambiguous effects on global protein acetylation in various tissues including the heart^{41,73,130,131} and liver.¹⁰ Furthermore, a recent study has demonstrated that almost all NAD⁺ precursors are metabolized to NAM before reaching peripheral tissues,¹²⁷ implying that the inhibitory feedback exerted by NAM might occur irrespective of the chemical nature of the NAD⁺ precursor that has been administered. Regardless, this is an emerging area of investigation, and future head-to-head comparisons must elucidate the exact (and perhaps subtle) effects of different NAD⁺-increasing therapeutics on protein acetylation, which might depend on the precise (vascular) cell types and subcellular compartments where target proteins reside. Other open questions involve the cell type-specific mechanisms underlying the vasoprotective benefits of NAD⁺ repletion. Given the central role of NAD⁺ in mitochondrial metabolism and bioenergetics, future studies should examine mitochondrion-initiated stress pathways, with a particular focus on the mitochondrial unfolded protein response in mammalian models *in vivo* to identify key signaling molecules involved in mitochondrioprotection. We anticipate that this type of knowledge will advance our understanding of vascular diseases associated with mitochondrial dysfunction, and will accelerate the discovery of novel targets to modulate this proteotoxic stress-sensing pathway.

In summary, targeting vascular NAD⁺ metabolism holds significant therapeutic potential for the clinical management of age-related cardiovascular and cerebrovascular disorders. Although much remains to be done, based on ever-accumulating evidence, the pharmacological modulation of NAD⁺ levels via NAD⁺ precursors or inhibitors of NAD⁺-consuming enzymes appears to be an attractive strategy for reducing chronic low-grade inflammation, reactivating autophagy and mitochondrial biogenesis, and enhancing oxidative metabolism in vascular cells (**Fig. 3**). These approaches represent an exciting avenue to improve vascular health.

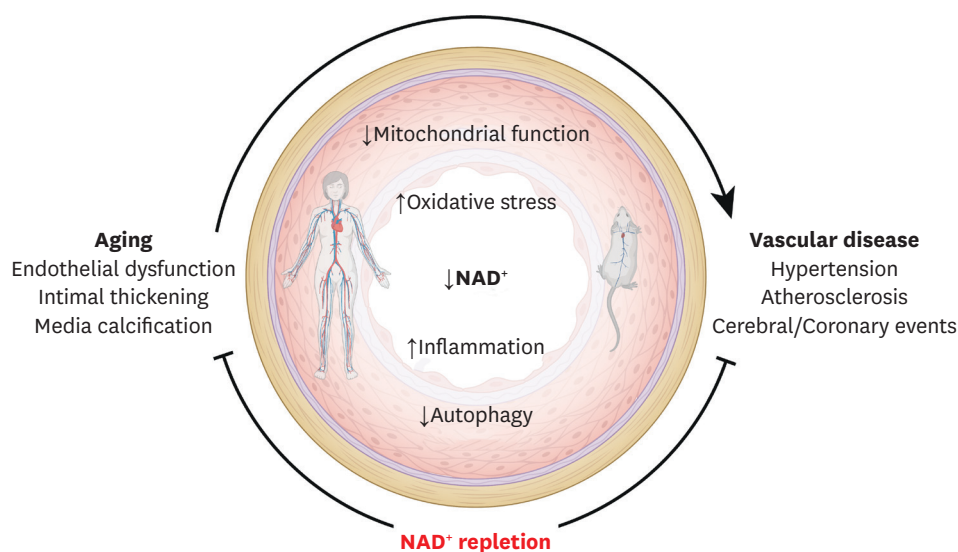


Fig. 3. NAD⁺ repletion therapy delays vascular aging and improves vascular health. Aging and related vascular disorders are associated with a decline in cellular NAD⁺ content, which coincides with reduced mitochondrial function and autophagy, as well as increased oxidative stress and pro-inflammatory signaling. Collectively, these contribute significantly to vascular pathologies, including hypertension, atherosclerosis, coronary artery disease, and aortic aneurysm. Hence, emerging NAD⁺-regenerative therapies are increasingly recognized as a potential strategy to protect vascular health during aging and disease both in animals and humans. Up-arrows indicate increases, down-arrows indicate decreases. The clip art included in this figure was created with BioRender.com. NAD⁺, nicotinamide adenine dinucleotide.

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