

Sirtuins and NAD⁺ in the Development and Treatment of Metabolic and Cardiovascular Diseases

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Abstract: The sirtuin family of nicotinamide adenine dinucleotide–dependent deacylases (SIRT1–7) are thought to be responsible, in large part, for the cardiometabolic benefits of lean diets and exercise and when upregulated can delay key aspects of aging. SIRT1, for example, protects against a decline in vascular endothelial function, metabolic syndrome, ischemia-reperfusion injury, obesity, and cardiomyopathy, and SIRT3 is protective against dyslipidemia and ischemia-reperfusion injury. With increasing age, however, nicotinamide adenine dinucleotide levels and sirtuin activity steadily decrease, and the decline is further exacerbated by obesity and sedentary lifestyles. Activation of sirtuins or nicotinamide adenine dinucleotide repletion induces angiogenesis, insulin sensitivity, and other health benefits in a wide range of age-related cardiovascular and metabolic disease models. Human clinical trials testing agents that activate SIRT1 or boost nicotinamide adenine dinucleotide levels are in progress and show promise in their ability to improve the health of cardiovascular and metabolic disease patients. (*Circ Res.* 2018;123:868-885. DOI: 10.1161/CIRCRESAHA.118.312498.)

Key Words: aging ■ atherosclerosis ■ cardiomyopathies ■ dyslipidemias ■ insulin resistance
■ metabolic syndrome ■ obesity

The lifespan extending abilities of the sirtuins were first discovered in yeast in the 1990s^{1,2} when the silent information regulator (SIR2) gene was shown to increase the replicative lifespan of yeast when upregulated.² Sir2 is a yeast gene silencing protein, which silences transcription at the HM mating-type loci in young yeast but relocalizes to the ribosomal DNA as cells age to prevent DNA damage that contributes to yeast aging.³ Upregulating the expression of Sir2 reduces DNA damage and increases the lifespan of yeast.² Subsequently, Sir2 was shown to have histone deacetylase activity that requires nicotinamide adenine dinucleotide (NAD),^{4,5} and sirtuins in mammals (SIRT1–7) were identified. Mammalian sirtuins were also shown to have important beneficial roles in aging, longevity, and stress responses.^{6–8} Increasing the activity of the sirtuins is associated with the delay of age-related diseases^{9–13} and, in some cases, increased longevity.^{14–17}

Mammalian sirtuins are NAD⁺-dependent deacylases with a huge range of roles in transcription regulation, energy metabolism modulation, cell survival, DNA repair, inflammation, and circadian rhythm regulation.^{8,18} SIRT1 which is the most extensively studied sirtuin is found in the nucleus and cytosol and along with histone deacetylation also modulates transcription factors, such as p53,¹⁹ nuclear factor κ -light-chain-enhancer of activated B cells (NF κ B),²⁰ forkhead box (FOXO)²¹ and peroxisome proliferator-activated receptor γ coactivator 1- α (PGC1 α),²² and DNA repair proteins, such as poly ADP-ribose polymerase 1 (PARP1).²³ SIRT2 is a cytosolic sirtuin while SIRT3, 4, and 5 are located in the mitochondria

and have roles in oxidative stress and lipid metabolism.^{24–26} SIRT6 and 7 are nuclear sirtuins with roles in gene expression and DNA repair.^{27,28} There is growing evidence that sirtuins, along with deacetylation, also have roles in catalyzing other reactions, such as demalonylation, desuccinylation, and mono-ADP-ribosylation,^{29,30} collectively termed deacylase reactions.

The activity of SIRT1 can be increased with pharmaceuticals known as sirtuin-activating compounds (STACs). One of the first STACs, resveratrol, was discovered in a screen for molecules that increased the activity of human SIRT1 and extended the lifespan of yeast.³¹ Since then, there have been several generations of STACs with increasing potency and specificity, including SRT1720 and SRT2104.^{6,9,32} STACs act as allosteric activators of SIRT1, binding to the STAC-binding domain in the N terminus and via a bend-at-the-elbow model increasing the binding affinity of a substrate for SIRT1.^{33,34} Whether STACs activate sirtuins other than SIRT1 and the mechanism of this activation is still not completely understood.³⁵ In addition to SIRT1, resveratrol has been reported to activate SIRT3³⁶ and SIRT5,³⁷ as well as nonsirtuin targets.^{38,39} Honokiol is believed to be primarily a SIRT3 activator^{40,41} although it may also activate SIRT1.^{42,43} Sirtuin transgenic mice should be used in combination with STACs to confirm the roles of individual sirtuins in mechanistic studies.

For almost a century, NAD⁺ has been known as a fundamental housekeeping molecule that catalyzes electron transfer in metabolic reduction-oxidation reactions. In the past 10 years, NAD⁺ has also emerged as a key modulator of

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Nonstandard Abbreviations and Acronyms

ApoE	apolipoprotein E
HFD	high-fat diet
IR	ischemia-reperfusion
LDL	low-density lipoprotein
NAD⁺	nicotinamide adenine dinucleotide
NAMPT	nicotinamide phosphoribosyltransferase
NMN	nicotinamide mononucleotide
NO	nitric oxide
NR	nicotinamide riboside
PARP1	poly ADP-ribose polymerase 1
SIR2	silent information regulator 2
STACs	sirtuin-activating compounds
TAC	transverse aortic constriction
TG	triglyceride

cell signaling and survival pathways.^{7,44} The discovery that NAD⁺ is an essential substrate of sirtuin deacetylation created renewed interest in NAD⁺ as a potential modulator of longevity and health. The sirtuin deacetylation reaction involves the removal of an acetyl group from target substrates via the conversion of NAD⁺ to nicotinamide and O-acetyl-ADP-ribose^{4,30} (Figure 1).

The first evidence that NAD⁺ boosting was beneficial to health came from yeast studies. Overexpression of yeast *PNC1*, which encodes a nicotinamidase that catalyzes the first step in the NAD⁺ salvage pathway, increased stress resistance and lifespan of yeast cells by mimicking caloric restriction.⁴⁶

Increasing age is associated with significantly reduced levels of NAD⁺,⁴⁷ which is coupled with an increase in NADH,^{48,49} suggesting that reduction-oxidation imbalance may contribute to decreased NAD⁺ with aging. Increasing NAD⁺ levels, especially in old age, is associated with a range of beneficial health effects in a variety of models.^{7,45}

NAD⁺ supplies in the cells are replenished by either de novo synthesis from dietary tryptophan or via salvage pathways from precursors including nicotinamide, nicotinamide riboside (NR), and nicotinic acid⁵⁰ (Figure 1). In the heart, >99% of NAD⁺ is synthesized via the salvage pathway.⁵¹ Levels of NAD⁺ can be raised by providing precursors to NAD⁺ such as NR, nicotinamide, or nicotinamide mononucleotide (NMN) or by increasing the levels of enzymes involved in the NAD⁺ synthesis pathways, such as nicotinamide phosphoribosyltransferase (NAMPT).⁵² Another potential approach is to inhibit enzymes that consume NAD⁺ including cluster of differentiation 38 (CD38),^{53–55} SARM1 (sterile α and TIR motif-containing protein 1),^{56,57} and PARP1.⁵⁸

As described below, the sirtuins and NAD⁺ have major roles in maintaining homeostasis of metabolic processes and cardiovascular function, which are disturbed in aging and in cardiovascular and metabolic diseases. The manipulation of sirtuins and NAD⁺ could lead to radical new medicines to prevent and treat these and other age-related diseases.

Sirtuins and NAD⁺ in Age-Related Cardiovascular and Metabolic Diseases

Loss of sirtuin activity and NAD⁺ levels with age are implicated in the pathogenesis of a wide variety of cardiovascular

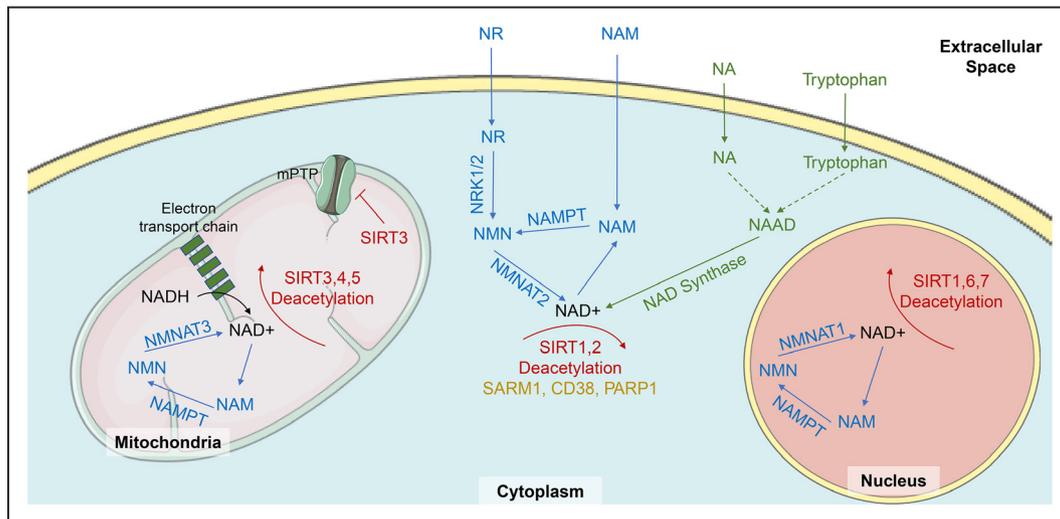


Figure 1. Synthesis, salvage, and metabolism of nicotinamide adenine dinucleotide (NAD⁺). NAD⁺ supplies in the body come from either de novo synthesis or via salvage pathways. NAD⁺ is synthesized de novo from dietary tryptophan via a series of enzymatic reactions ultimately resulting in the production of nicotinic acid adenine dinucleotide (NAAD), which is converted to NAD⁺ by NAD synthase. However, in the heart, the vast majority of NAD⁺ is synthesized via salvage routes from nicotinamide (NAM), nicotinamide riboside (NR), and nicotinic acid (NA). NAM and NR are converted to nicotinamide mononucleotide (NMN) by nicotinamide phosphoribosyltransferase (NAMPT) and nicotinamide riboside kinases (NRKs), respectively. NMN is then converted to NAD⁺ by nicotinamide mononucleotide adenylyltransferase (NMNAT) 2 in the cytoplasm, NMNAT3 in the mitochondria, and NMNAT1 in the nucleus. NA is converted, via a series of reactions into NAAD and finally to NAD⁺ by NAD synthase. NAD⁺ is a necessary substrate of sirtuin deacetylation reactions, by which it is converted into NAM. NAD⁺ is broken down by sterile α and TIR motif-containing protein 1 (SARM1), cluster of differentiation 38 (CD38), poly ADP-ribose polymerase 1 (PARP1), and SIRT1–7. SIRT1, 4, and 5 are located in the mitochondria, SIRT 2 is located in the cytoplasm, SIRT6 and 7 are found in the nucleus, and SIRT1 is found in both the cytoplasm and nucleus. Sirtuins and NAD⁺ have a variety of roles in cardiac and vascular cells that can effect cardiovascular function. For example, SIRT3 acts to block opening of the mitochondrial permeability transition pore (mPTP) to reduce cell swelling and hypertrophy. Figure made using adapted images from <http://smart.servier.com>.

and metabolic diseases including atherosclerosis, endothelial dysfunction, acute cardiac syndromes, cardiomyopathy, hypertrophy and heart failure, arrhythmias, hypertension, metabolic syndrome, obesity and fatty liver, diabetes mellitus, and dyslipidemia. The majority of these studies have been completed in animal models, but human studies are increasing. Preclinical studies were completed in either genetically manipulated mice (summarized in Table 1) or using STACs and NAD⁺ boosters to modulate sirtuin activity (summarized in Table 2).

Table 1. Effect of Over or Under Expression of SIRT1–7 on Cardiac and Metabolic Function in Mice

	Key Cardiac and Metabolic Effects	
	Gain of Function	Loss of Function
SIRT1	↑/↓ atherosclerosis	↑/↓ atherosclerosis
	↓ endothelial inflammation	↑ endothelial dysfunction, ↓ angiogenesis
	↓ IR injury susceptibility	↑ IR injury susceptibility
	↑/↓ hypertrophy and cardiomyopathy	↑/↓ hypertrophy
	↓ hepatic steatosis	↑/↓ hepatic steatosis
	↓ obesity	↑ obesity
	↑/↔ glucose homeostasis	↑/↓ glucose homeostasis
	↑/↓ serum lipid levels	↑/↓ serum lipid levels
	↓ hypertension	↑ arrhythmias
SIRT2	↓ hypertrophy	↑ hypertrophy and fibrosis
SIRT3	↓ hypertrophy and fibrosis	↑ hypertrophy and fibrosis
		↑ IR injury susceptibility
		↑ obesity
		↑/↔ insulin resistance
		↑ serum lipid levels
SIRT4	↑ hypertrophy and cardiac dysfunction	↓ hypertrophy and fibrosis
		↓ obesity
		↑ insulin resistance
SIRT5	...	↑ IR injury susceptibility
		↑ hypertrophy and fibrosis
SIRT6	↓ hypertrophy	↑ hypertrophy
	↓ obesity	↑ obesity
	↑ glucose homeostasis, ↑ insulin sensitivity	↓ glucose homeostasis
	↓ serum lipid levels	↑ serum lipid levels
SIRT7	...	↑ hypertrophy and fibrosis
		↓ systolic function
		↓ hepatic steatosis
		↓ glucose intolerance

IR indicates ischemia-reperfusion; and SIRT, sirtuin.

Atherosclerosis

Atherosclerosis is the major risk factor for myocardial infarction, coronary artery disease, and stroke in the older population. It is the number one cause of death in the developed world, and the incidence of atherosclerosis increases with age.⁸⁹ Atherosclerosis results from increased LDL (low-density lipoprotein) cholesterol in the plasma, dysfunctional endothelial cells, endothelial oxidative stress, an inflammatory response, and ultimately plaque formation^{90,91} (Figure 2). Risk factors for atherosclerosis, along with age, include hypercholesterolemia, metabolic syndrome, hypertension, obesity, and type 2 diabetes mellitus.⁸⁹ Sirtuins and NAD⁺ play a role in the modulation of many of these risk factors, and there is increasing evidence that sirtuins may also modulate atherosclerosis itself (Figure 2). SIRT1 levels are decreased in atherosclerotic regions of patients.^{92,93} Monocytes isolated from patients with coronary artery disease also have lower SIRT1 levels than control patients,⁹⁴ suggesting a systemic decrease in SIRT1 in this disease.

Preclinical studies have shown a protective role for SIRT1 in atherosclerosis, at least in vascular endothelial and smooth muscle cells. Apolipoprotein E (ApoE) knockout mice have increased serum cholesterol levels and can be used as a model of atherosclerosis, especially when fed a high-fat diet (HFD). ApoE knockout mice have reduced SIRT1 expression,⁹⁵ particularly in atherosclerotic plaques.⁹³ Endothelial cell-specific overexpression of SIRT1 in ApoE knockout mice protects against atherosclerotic plaques.⁹⁶ In addition, ApoE knockout mice crossed with smooth muscle cell-specific SIRT1 knockout mice showed increased atherosclerosis.⁹³ Treatment of ApoE knockout mice with the STAC SRT3025 reduced atherosclerosis in these mice by increasing LDL uptake.⁸⁵ However, whole-body SIRT1 overexpressors that were placed on an atherogenic diet to induce plaque formation had worse lipid profiles and more severe atherosclerosis than wild-type mice on the same diet.⁹⁷ The proposed mechanism was increased lipid accumulation and secretion in the liver as a result of Creb modulation which resulted in overall proatherogenic effects despite potential benefits of SIRT1 overexpression in the vasculature.⁹⁷ More research is needed to understand the complex role of SIRT1 in lipid metabolism in the liver, especially in the context of HFD or high-cholesterol diet. This is discussed further in sections Diabetes, Glucose/Insulin Dysregulation and Metabolic Syndrome and Dyslipidemia.

Other factors associated with atherosclerosis are also modulated by SIRT1. Age-related dedifferentiation of vascular smooth muscle cells is implicated in the disease, and the induction of SIRT1 with resveratrol can promote differentiation of these cells and may contribute to protection against atherosclerosis.⁹⁸ Thrombosis contributes to an increased risk of ruptured plaques and clots in atherosclerosis. In mice, inhibition of SIRT1 with sirtinol results in increased tissue factor expression and thrombi promotion, indicating SIRT1 may protect against thrombosis.^{99,100} Interestingly, treatment with resveratrol to increase sirtuin activity reversed the prothrombotic status of cyclooxygenase-2 knockout mice.⁹⁹

Overall, the majority of studies show a protective role of SIRT1 in modulating atherosclerosis and many of the factors that contribute to its development. Increasing SIRT1 activity

Table 2. Preclinical Animal Studies of STACs and NAD⁺ Boosters in Cardiovascular and Metabolic Diseases

Drug	Disease	Model	Effects	References
NR	Metabolic syndrome	HFD in mice	↓ fat mass ↑ insulin sensitivity ↓ LDL	59
NMN	Endothelial dysfunction	Aging mice	↑ capillary density ↑ endothelial function	60
		Aging mice	↑ capillary density ↑ endurance	61
	Hypertrophy	Cardiomyopathy mouse	↑ cardiac function	62
		TAC in mice	↓ hypertrophy	63
	Cardiomyopathy	Ndufs4 KO mice	↓ sensitivity to pressure overload	64
	IR injury	Ischemia in mice	↓ ischemic damage	65
NR	Cardiomyopathy	Aged MDX mice	↓ inflammation and fibrosis	66
Resveratrol	Hypertrophy	Rats+doxorubicin	↓ fibrosis ↓ diastolic dysfunction	67
		TAC in rats	↑ cardiac function	68
		Dahl salt-sensitive rats	↑ cardiac function ↓ hypertrophy	69
		Spontaneously hypertensive rats	↑ cardiac function ↓ hypertrophy	70
		TAC in mice	↑ cardiac function ↓ hypertrophy	36
	Cardiomyopathy	TO-2 hamster	↓ fibrosis ↑ cardiac function	71
		Type 1 diabetes mellitus model	↑ cardiac function	72
		Autoimmune myocarditis	↑ cardiac function ↓ hypertrophy ↓ fibrosis	73
	Endothelial dysfunction	Rats+cigarette smoke	↓ ROS ↑ vascular relaxation	74
		Rhesus monkeys+HFD	↓ arterial stiffening ↓ inflammation	75
		Rat MI model	↑ angiogenesis ↑ cardiac function	76
	I/R injury	IR in mice	↓ ischemic damage	77
		IR in rats	↓ ischemic damage	78
	Insulin/glucose regulation	Rhesus monkeys+HFD	↑ insulin sensitivity	79
		Mice+HFD	↑ lifespan ↑ insulin sensitivity	80
		Mice+HFD	↓ obesity ↑ insulin sensitivity	81
SRT2104	Insulin/glucose regulation	Mice	↑ lifespan ↑ insulin sensitivity	17
SRT1720	Metabolic syndrome	Mice	↑ lifespan ↓ metabolic syndrome ↓ cholesterol	16
		Genetically obese mice	↑ lifespan ↓ serum glucose	32
		Mice+HFD	↓ obesity ↑ endurance ↓ insulin resistance	82
		Mice+HFD	↑ lifespan ↓ insulin resistance ↓ hepatic steatosis	83
	Endothelial dysfunction	Aging mice	↑ vascular relaxation ↓ superoxide ↓ inflammation	84
SRT3025	Atherosclerosis	ApoE KO+HFD	↓ serum LDL and cholesterol ↓ atherosclerosis	85
Curcumin	IR injury	IR in rats+curcumin	↑ cardiac function ↓ ischemic damage	86
Honokiol	Hypertrophy	TAC in mice	↓ hypertrophy ↓ fibrosis	40
		Mice+doxorubicin	↓ oxidative stress	41
4-aminobenzamide	Endothelial dysfunction	Diabetes mellitus rat model	↓ endothelial dysfunction ↓ inflammation	87
Luteolinidin	IR injury	Isolated rat hearts	↓ endothelial dysfunction ↓ contractile dysfunction	88
Apigenin	Metabolic syndrome	Mice+HFD	↓ glucose dysregulation	53

ApoE indicates apolipoprotein E; HFD, high-fat diet; I/R, ischemia/reperfusion; KO, knockout; LDL, low-density lipoprotein; MI, myocardial infarction; NAD⁺, nicotinamide adenine dinucleotide; NMN, nicotinamide mononucleotide; NR, nicotinamide riboside; ROS, reactive oxidative species; STAC, sirtuin-activating compound; and TAC, transverse aortic constriction.

should be further investigated as a promising treatment option for atherosclerosis and its risk factors. More research is needed, however, to confirm the role of SIRT1 in lipid modulation in the liver in the context of different diets and the effect of this on atherosclerosis.

There are limited studies exploring the effect of the other sirtuins and NAD⁺ in atherosclerosis. Knockout of SIRT3 in mice lacking the LDL receptor had no effect¹⁰¹ while *SIRT6* gene variants have been associated with the development of plaques in patients.¹⁰² Inhibition of NAMPT with the drug

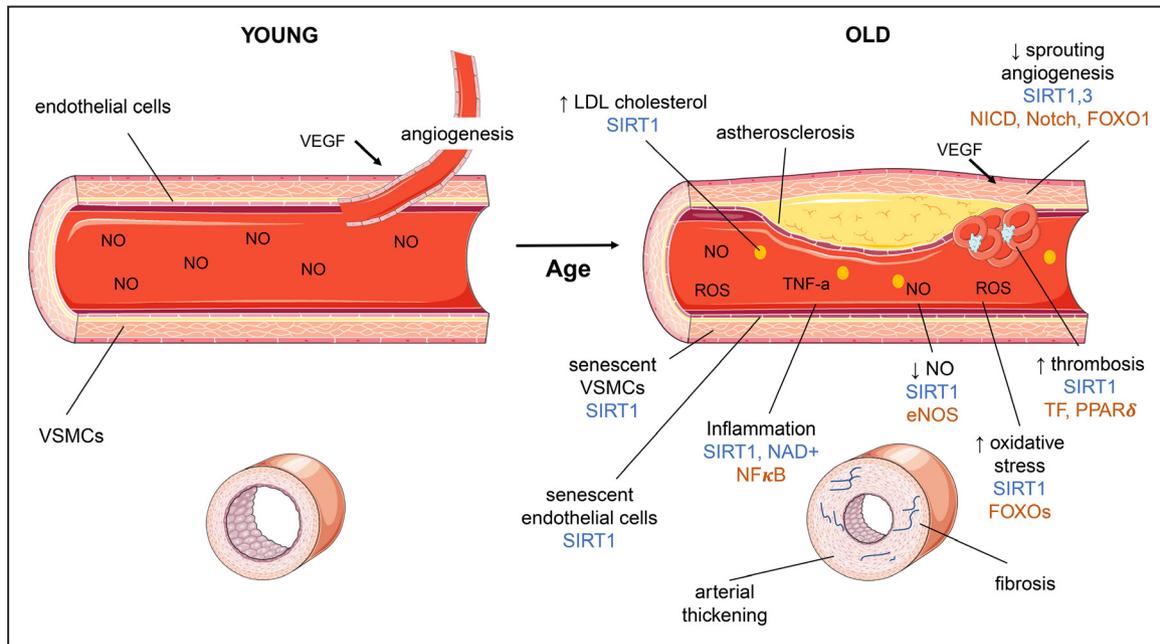


Figure 2. The role of sirtuins and nicotinamide adenine dinucleotide (NAD⁺) in the aging vasculature. A, A young blood vessel shows healthy vascular smooth muscle cells (VSMCs), lined with healthy endothelial cells. Stimulation with nitric oxide (NO) results in vessel contraction. Angiogenesis, the growth of new blood vessels, is induced when vascular endothelial growth factor (VEGF) acts on SIRT1 to relieve notch intracellular domain (NICD) inhibition of Notch. **B,** Aging results in changes to the structure and function of the vasculature. With increasing age, there are increased atherosclerotic plaques, increased low-density lipoprotein (LDL) cholesterol in the blood stream, decreased sprouting angiogenesis, increased thrombosis, increased oxidative stress, decreased nitric oxide, increased inflammation, senescence of endothelial cells and VSMCs, arterial thickening, and fibrosis. These changes contribute to reduced blood flow and reduced reactivity of the blood vessels in aging. Sirtuins and NAD⁺ can improve or reverse some aspects of vascular aging (marked in blue on the figure) through modulation of targets (marked in brown). In particular, SIRT1 decreases LDL cholesterol levels, reduces vascular cell senescence, increases NO through endothelial nitric oxide synthase (eNOS), reduces inflammation through nuclear factor κ -light-chain-enhancer of activated B cells (NF κ B) modulation, decreases oxidative stress by modulating forkhead box (FOXOs) and thrombosis via tissue factor (TF) and peroxisome proliferator-activated receptor delta (PPAR δ), and increases angiogenesis through modulation of FOXO1 and NICD-Notch. SIRT3 also has a protective role in angiogenesis, and NAD⁺ reduces vascular inflammation. Figure made using adapted images from <http://smart.servier.com>.

FK866 in ApoE knockout mice resulted in reduced C-X-C motif chemokine 1 (CXCL1) chemokine levels and thus reduced neutrophil infiltration in atherosclerotic plaques,¹⁰³ indicating a potential role of NAD⁺ in increasing inflammation in atherosclerosis.

Endothelial Dysfunction and Artery Stiffness

A hallmark of vascular aging is increased dysfunction of the vascular endothelium, characterized by a reduced nitric oxide (NO)-mediated vasodilatory response to stimuli. In addition, with aging, there are changes to the vascular smooth muscle cells, which result in increased arterial stiffness and reduced elasticity.^{104,105} These changes reduce the ability of the vasculature to relax and dilate in old age and are associated with an increased risk of a range of cardiovascular diseases, including hypertension, heart failure, and coronary artery disease.¹⁰⁴ The role of SIRT1 has been explored in the context of endothelial dysfunction and seems to contribute to normal endothelial activity and protect against dysfunction in aging and other models of damage (Figure 2).

Inhibition of SIRT1 is associated with poor vascular function and increased artery stiffness. Mice with knockout of both ApoE and SIRT1 show increased endothelial superoxide production and increased NF κ B compared with the ApoE knockout alone, indicating a role of SIRT1 in reducing endothelial dysfunction, at least in the context of atherosclerosis.¹⁰⁶ SIRT1 inhibition with sirtinol in young wild-type mice resulted in

artery stiffness to a similar level to that seen in aging wild-type mice.¹⁰⁷ Specific inhibition of SIRT1 in the arterial endothelium of mice resulted in a reduction in NO and an inhibition of endothelium-dependent vasodilation.¹⁰⁸ Importantly, the same study showed in vitro that SIRT1 increased endothelial NO by directly deacetylating and thus stimulating the activity of endothelial NO synthase.¹⁰⁸

Several studies have explored the protective effects of increasing SIRT1 activity on the vascular endothelium. A cigarette smoke model of vascular aging in rats resulted in impaired artery relaxation, increased reactive oxidative species, and increased inflammatory markers. Activation of SIRT1 either genetically or with resveratrol prevented these effects on the vasculature.⁷⁴ SIRT1-overexpressing mice fed an HFD had better endothelium-dependent vasorelaxation, and increased endothelial NO synthase expression, compared with wild-type mice fed an HFD.⁹⁶ SIRT1 activation with SIRT1720 in old mice restored endothelial function by increasing cyclooxygenase-2-mediated dilation and also reduced superoxide production and decreased inflammation.⁸⁴ SIRT1 also acts to prevent senescence of endothelial cells, which may contribute to vascular endothelial dysfunction in aging.^{109,110} Importantly for clinical translation in rhesus monkeys, increasing SIRT1 activity with resveratrol treatment prevented arterial stiffening and inflammation,⁷⁵ and a human study found that in older patients with stiffer vessels there was reduced SIRT1 expression in artery endothelial cells.¹⁰⁷

The role of the other sirtuins in endothelial dysfunction has not been well characterized. SIRT6 seems to have a role in protecting against endothelial cell senescence,^{111,112} which may contribute to protection against age-related endothelial dysfunction. NAD⁺ may also have a protective role in the aging vasculature as NMN treatment of old mice improved endothelial relaxation in arteries and reduced artery stiffness.⁶⁰ Treatment with a PARP1 inhibitor in a diabetes mellitus-induced vascular dysfunction rat model was shown to prevent endothelial dysfunction and inflammation although NAD⁺ levels were not explicitly investigated.⁸⁷ Targeting detrimental changes to the endothelium in aging with SIRT1 activators or NAD⁺ boosters shows promise as an area of focus for treating age-related vascular dysfunction and arterial stiffening.

Angiogenesis

Angiogenesis, which involves endothelial cell proliferation and migration in response to vascular endothelial growth factor, is also decreased in aging.¹¹³ Angiogenesis may have a protective role in cardiovascular disease by increasing blood flow and myocardial function, especially in ischemic heart disease.^{114,115}

SIRT1 and SIRT3 have both been shown to have roles in inducing angiogenesis. SIRT1 acts as a negative regulator of Notch and FOXO1, key regulators of blood vessel growth.¹¹⁶ In vitro studies show that SIRT1 knockout results in reduced angiogenesis of endothelial cells.¹¹⁷ In mice and zebrafish, a reduction in SIRT1 reduces sprouting blood vessel formation.¹¹⁷ A more recent study showed that in aged endothelial cell-specific SIRT1 knockout mice, there is reduced capillary density which was exacerbated

by transverse aortic constriction (TAC), an intervention to model hypertrophy, and heart failure in mice.¹¹⁸ In vitro studies showed that SIRT1 knockout resulted in reduced angiogenic response to vascular endothelial growth factor, possibly because of reduced expression of the vascular endothelial growth factor receptors FLT1 (Fms-related tyrosine kinase 1) and FLK2 (fetal liver kinase-2).¹¹⁸ Resveratrol pretreatment to increase SIRT1 activity in a rat myocardial infarction model upregulated modulators of angiogenesis and increased capillary density which ultimately resulted in improved cardiac function.⁷⁶ Bone marrow cell treatment increased angiogenesis and improved cardiac function in a mouse model of myocardial infarction.¹¹⁹ Interestingly, it seems that SIRT3 is required for this protection as treatment with bone marrow cells isolated from SIRT3 knockout mice did not result in angiogenesis or the associated cardiac benefits.¹¹⁹

In addition, recent findings showed that NMN treatment in aging mice resulted in a reversal of the age-related decline in capillary density and blood flow in muscles, which resulted in increased mobility and endurance.⁶¹ Importantly, the authors demonstrated that the mechanism of reduced blood flow with aging is related to reduced NAD⁺-dependent SIRT1 activity and that increasing the NAD⁺-SIRT1 axis with NMN resulted in SIRT1-dependent inhibition of Notch1 intracellular domain and promoted vascular endothelial growth factor-stimulated sprouting angiogenesis⁶¹ (Figure 3). Hydrogen sulfide was also shown to improve angiogenesis via NAD⁺- and SIRT1-dependent mechanisms⁶¹ although the exact mechanism of the hydrogen sulfide-SIRT1 interaction remains to be

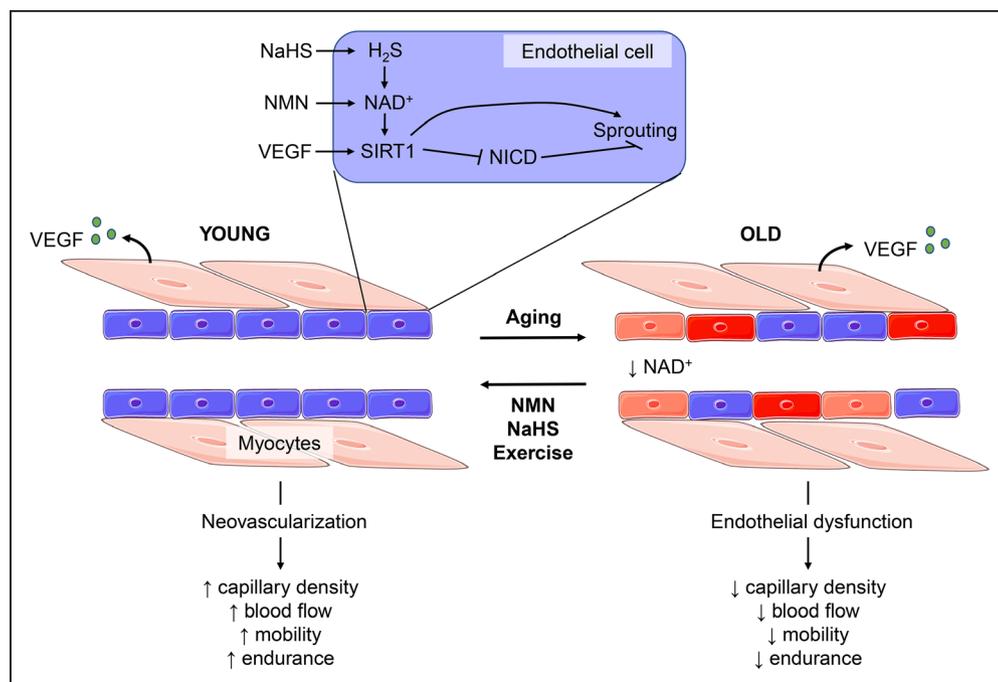


Figure 3. The role of SIRT1 and nicotinamide adenine dinucleotide (NAD⁺) in age-related endothelial dysfunction. In young healthy capillaries, angiogenesis occurs after stimulation of endothelial cells with vascular endothelial growth factor (VEGF) released from myocytes. Characteristic changes of the vasculature with old age include dysfunctional endothelial cells and reduced VEGF-induced angiogenesis. These age-related changes result in reduced capillary density, reduced blood flow, and ultimately reduced mobility and endurance. A mechanism of this reduced angiogenesis in old age is reduced endothelial SIRT1 and NAD⁺, which ostensibly reduces the sensitivity of endothelial cells to VEGF. Increasing the hydrogen sulfide (H₂S)-NAD⁺-SIRT1 axis in endothelial cells, either with NaHS to boost H₂S, NMN to boost NAD⁺, or with exercise, causes SIRT1-dependent inhibition of Notch intracellular domain (NICD), which results in increased sprouting angiogenesis. These interventions can reverse endothelial dysfunction and increase angiogenesis in old age to return capillary density and endurance to the levels of that seen in young mice. Figure derived from Das et al⁶¹ using adapted images from <http://smart.servier.com>.

elucidated.¹²⁰ Furthermore, Das et al⁶¹ found that NMN treatment also increased sprouting of aortic rings from aging wild-type, but not SIRT1 knockout mice, indicating that NMN may also be beneficial in inducing SIRT1-dependent angiogenesis in tissues other than skeletal muscle.

These promising results indicate that NAD⁺ boosters and sirtuin activators can be used to increase angiogenesis in cardiovascular diseases and age-related blood flow dysfunction.

Ischemia-Reperfusion Injury

Many studies over the past decade have investigated the role of sirtuins and NAD⁺ in the context of ischemia-reperfusion (IR) injury. IR injury occurs clinically in the context of myocardial infarction or during cardiovascular surgery when blood supply to the heart, or part of the heart, is stopped. The period of ischemia can result in tissue injury and cell death as a result of rapidly reduced ATP levels. This damage is exacerbated by rapid reperfusion, which results in the induction of oxidative stress and apoptosis. IR injury increases with age,¹²¹ and periods of ischemia or hypoxia in cardiomyocytes are associated with a reduction in endogenous NAMPT,^{65,122} as well as downregulation of SIRT1,¹²³ SIRT3,¹²⁴ and SIRT4.¹²⁵

Increasing SIRT1 has a protective role in IR injury (Figure 4). Cardiac-specific SIRT1 knockout mice display increased cardiac damage after exposure to IR while mice overexpressing SIRT1 in the heart are protected from IR damage.¹²³ Overexpression of SIRT1 resulted in upregulation of antioxidant pathways mediated by FOXO1 and manganese

superoxide dismutase and downregulation of proapoptotic pathways mediated by caspase 3 and Bax.¹²³ A similar role of SIRT1 was observed in rats exposed to IR injury,⁸⁶ and resveratrol pretreatment protected both mice and rats from IR injury.^{77,78} Other in vitro studies have shown the same anti-apoptotic role of SIRT1 by mediating FOXO1 expression, in the context of hypoxia.¹²⁶

The mitochondrial sirtuins SIRT3, 4, and 5 also have protective roles in IR injury. In vitro studies of SIRT3 overexpression in cardiomyocytes exposed to hypoxia showed protection against cell damage via a downregulation of apoptosis involving Ku70 and Bax.¹²⁷ The same role is seen in vivo, where SIRT3 knockout mice are more susceptible to IR injury and have increased damage and reduced mitochondrial function postinjury when compared with wild-type mice.¹²¹ Overexpression of SIRT4 in cardiomyoblast cells increased their viability posthypoxia by decreasing caspase activity and apoptosis, while knockdown of SIRT4 in the same cells results in reduced viability and an induction of apoptosis.¹²⁵ SIRT5 knockout mice have increased infarct size after IR injury compared with wild-type mice and an inhibitor of succinate dehydrogenase protected against this injury, implying a role of SIRT5 in modulating protein succinylation in the heart in the context of IR injury.¹²⁸ In vitro studies also support a role of SIRT5 in protecting against IR injury, at least in part by reducing apoptosis.¹²⁹

Little is known about the role of the nuclear sirtuins SIRT6 and SIRT7 and the other cytoplasmic sirtuin SIRT2 in IR injury. One study showed that cardiomyocytes isolated from mice

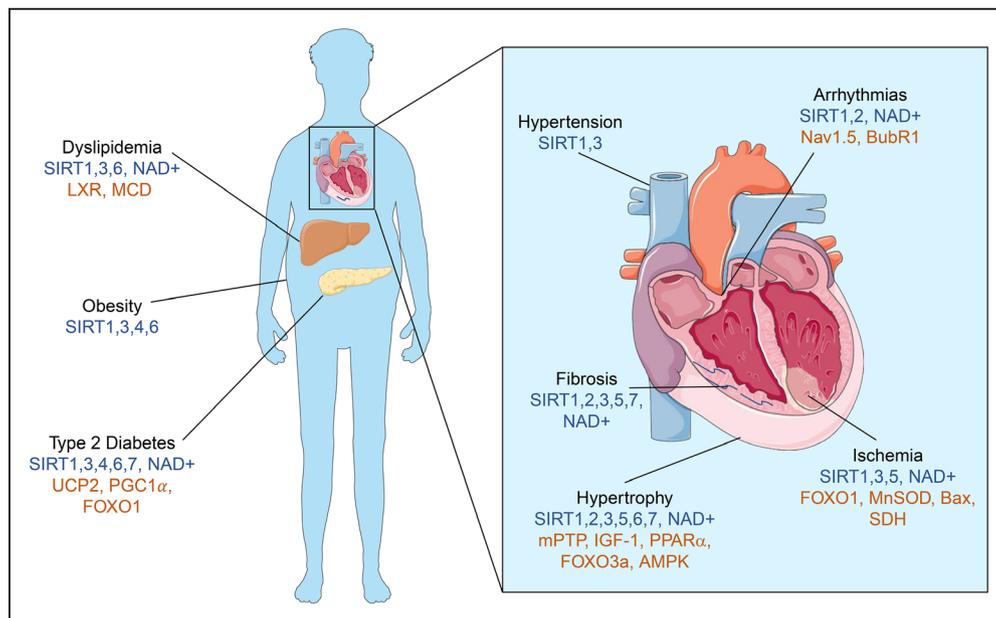


Figure 4. The role of sirtuins and nicotinamide adenine dinucleotide (NAD⁺) in age-related metabolic and heart diseases. In old age, there is increased risk of cardiovascular and metabolic diseases, including fatty liver, dyslipidemia, obesity, type 2 diabetes mellitus, hypertension, arrhythmias, fibrosis, hypertrophy, and ischemia/reperfusion injury. Sirtuins and NAD⁺ have roles in protecting against or preventing the development of these diseases (shown in blue on figure) through modulation of a variety of proteins (shown in brown on figure). SIRT1, 3, 6 and NAD⁺ have roles in modulating dyslipidemia, with the liver X receptor (LXR) and malonyl-CoA decarboxylase (MCD) identified as targets, and SIRT1, 3, 4, and 6 have roles in preventing obesity. SIRT1, 3, 4, 6, 7 and NAD⁺ may have protective roles in type 2 diabetes mellitus and identified targets include mitochondrial uncoupling protein 2 (UCP2), peroxisome proliferator-activated receptor γ coactivator 1- α (PGC1 α), and forkhead box 1 (FOXO1). SIRT1 and 3 have protective roles in hypertension, and SIRT1, 2, 3, 5, 7 and NAD⁺ have been associated with reduced cardiac fibrosis. SIRT1, 2, 3, 4, 6, 7 and NAD⁺ protect against cardiac hypertrophy with identified targets, including the mitochondrial permeability transition pore (mPTP), insulin-like growth factor (IGF)-1, peroxisome proliferator-activated receptor α (PPAR α), eukaryotic initiation factor 2 (eIF2 α), FOXO3a, and AMP-activated protein kinase (AMPK). SIRT1, 3, 5 and NAD⁺ protect against ischemia/reperfusion injury via FOXO1, manganese superoxide dismutase (MnSOD), B-cell lymphoma 2 (Bcl-2)-associated X protein (Bax), and succinate dehydrogenase (SDH). SIRT1, 2 and NAD⁺ improve arrhythmias via sodium channel Nav1.5 and BubR1. Figure made using adapted images from <http://smart.servier.com>.

overexpressing SIRT6 had less damage when exposed to hypoxia than those isolated from wild-type mice, via a downregulation of apoptosis and oxidative stress through modulation of B-cell lymphoma 2, NF κ B, phosphorylated Akt, and phosphorylated-5' adenosine monophosphate-activated protein kinase (AMPK).¹³⁰ An *in vitro* study suggested that SIRT2 may play a contributing role in IR injury with downregulation of SIRT2 protecting against hypoxia in H9c2 cells.¹³¹ This supports other evidence suggesting that SIRT2 acts as a negative regulator of biological stress.¹³²

Cardiac-specific overexpression of NAMPT in mice results in an expected increase in cardiac NAD⁺ levels and protection against IR injury.¹²² In addition, ischemic preconditioning, which is known to reduce damage to subsequent IR injury, seems to induce protection at least in part through NAMPT upregulation.⁶⁵ NAMPT knockout mice show no protection against IR injury with preconditioning.⁶⁵ Increasing NAD⁺ levels with the precursor NMN also protects against IR injury.⁶⁵ PARP inhibitors also protect against IR injury in a variety of animal models,¹³³ possibly through increased NAD⁺ levels. The CD38 inhibitor luteolinidin also prevented left ventricular contractile and endothelial dysfunction after IR injury in isolated rat hearts.⁸⁸

Overall, sirtuins and NAD⁺ seem to have a strongly protective role in IR injury. There is the potential for the development of STACs or NAD boosting therapeutics for use during cardiac surgery or to attenuate damage postmyocardial infarction in older patients.

Hypertrophy, Fibrosis, and Heart Failure

Aging is associated with distinct structural changes in the heart, including increased fibrosis, left ventricular hypertrophy, and dilation.^{134–136} Hypertrophy and fibrosis in the aging heart cause systolic and diastolic dysfunctions and can ultimately result in heart failure.^{134–136} Age-related hypertension can contribute to the development of hypertrophy and heart failure in humans. Mice do not naturally develop age-related hypertension, so this is modeled in rodents with interventions including TAC that causes pressure overload, angiotensin II exposure, doxorubicin exposure, transgenic cardiomyopathy models, diabetic cardiomyopathy, and other stress-induced cardiomyopathy models.

SIRT1 has apparently opposing roles in age-related structural changes of the heart. Several mouse studies have observed the development of cardiac hypertrophy in mice with SIRT1 overexpression,¹³⁷ with one study showing the degree of impairment was dependent on the degree of overexpression.¹³⁸ High levels of SIRT1 overexpression (20-fold) resulted in dilated cardiomyopathy while moderate overexpression (6.8-fold) caused diastolic dysfunction, and low overexpression (3.2-fold) resulted in normal function at baseline but the development of cardiac dysfunction with pressure overload.¹³⁸ Knockout of either SIRT1 or peroxisome proliferator-activated receptor α (PPAR α) reduced hypertrophy in a TAC mouse study while the overexpression of both proteins exacerbated it,¹³⁹ ostensibly because of upregulation of estrogen-related receptors and resulting mitochondrial dysfunction.¹³⁹

Conversely, SIRT1 can also have a protective function (Figure 4). Another study exploring levels of cardiac-specific

SIRT1 overexpression on age-related remodeling in 18-month-old mice found that low to moderate overexpression (2.5–7.5 fold) protected against age-related hypertrophy, fibrosis, and cardiac dysfunction while only a high level of overexpression (12.5-fold) resulted in cardiomyopathy and hypertrophy.¹⁴⁰ The authors suggest that the detrimental effect of a high level of SIRT1 overexpression is because of induction of mitochondrial dysfunction,¹⁴⁰ which may also contribute to hypertrophic damage in the context of TAC pressure overload and increased SIRT activity.¹³⁸ Cardiomyocytes with SIRT1 activation either genetically or with resveratrol were protected against phenylephrine-induced hypertrophy and related inflammation. Interestingly, in direct opposition to that seen above, the inhibition of PPAR α stopped this protective effect.¹⁴¹ The same study also showed that SIRT1 overexpression in mice protected against isoproterenol-induced hypertrophy.¹⁴¹ Mouse studies using either stress-induced or diabetes mellitus models of cardiomyopathy observed that SIRT1 knockout increased hypertrophy and decreased cardiac function, and that treatment with either the sirtuin activator STAC-3 or resveratrol was protective against this hypertrophy.^{72,142} Consistent with this, resveratrol treatment in rat or hamster models of heart failure and cardiomyopathy also prevented hypertrophy and a decline in cardiac function although the exact target of resveratrol is difficult to prove without the use of SIRT1 knockouts.^{67–71,73}

The conflicting roles of SIRT1 in these studies imply a potentially different effect of SIRT1 on cardiac remodeling in different contexts and models. Investigation of the role of sirtuins in more clinically relevant models of cardiac aging, or studies in aging humans themselves, will be essential. Importantly, the one study discussed above that did use naturally aging mice found a protective effect of low to moderate SIRT1 overexpression on hypertrophy.¹⁴⁰ More studies are required to fully understand the complex effects of SIRT1 in cardiomyopathy and hypertrophy in animal models and humans.

SIRT3 has a clear protective role in cardiac hypertrophy. Doxorubicin-induced cardiomyopathy, TAC-induced hypertrophy, and a Friedreich ataxia model of cardiomyopathy all resulted in reduced SIRT3 levels, which were associated with hyperacetylation of mitochondrial proteins.^{62,143,144} SIRT3 knockout mice develop hypertrophy and fibrosis as they age as a result of increased mitochondrial permeability transition pore opening (Figure 1) and transforming growth factor- β 1 signaling.^{145,146} SIRT3 knockout mice are also more sensitive to the hypertrophic effects of TAC, angiotensin II,^{144,145,147} doxorubicin,^{143,148} diabetes mellitus,¹⁴⁹ and isoproterenol¹⁵⁰ than wild-type mice. SIRT3 overexpression results in resistance to hypertrophy-inducing stimuli, as a result of increased FOXO3a, manganese superoxide dismutase, oxoguanine glycosylase 1, parkin, and catalase activation.^{143,147,149} HFD is also associated with hypertrophy and cardiac dysfunction which is accompanied by a decrease in cardiac SIRT3. Moreover, SIRT3 knockout mice with HFD had greater cardiac dysfunction than HFD-fed wild-type mice.¹⁵¹ The known protective effects of resveratrol on hypertrophy and fibrosis may be as a result of both SIRT1 and SIRT3 activation as the protective effects of resveratrol were not observed in SIRT3 knockout mice in one study.³⁶ Honokiol is a

polyphenolic compound that is thought to activate SIRT3. Honokiol treatment in mice exposed to pressure overload and drug-induced heart failure prevented hypertrophy and fibrosis,⁴⁰ as well as the associated mitochondrial damage and cell death.⁴¹

The role of the other sirtuins in cardiac hypertrophy and fibrosis has not been as extensively studied. SIRT2 knockout mice have increased hypertrophy and fibrosis and decreased systolic function compared to wild-type mice.¹⁵² Moreover, cardiac-specific SIRT2 overexpression protected against angiotensin II-induced hypertrophy by activating liver kinase B1, which ultimately resulted in maintenance of AMPK levels in hypertrophy.¹⁵² SIRT4 seems to contribute to remodeling, with SIRT4 knockout mice having reduced fibrosis and hypertrophy in response to angiotensin II, and cardiac-specific SIRT4 overexpression increasing hypertrophy and reducing cardiac function.¹⁵³ SIRT5 prevents age-related hypertrophy with aging SIRT5 knockout mice displaying more fibrosis, hypertrophy, and systolic dysfunction than age-matched wild types.¹⁵⁴ SIRT6 knockout mice have increased hypertrophy while overexpression of SIRT6 was associated with protection against hypertrophic stimuli in mice.¹⁵⁵ Overexpression of nicotinamide mononucleotide adenylyltransferase 2 in rat cardiomyocytes to increase NAD⁺ protected against hypertrophy in a SIRT6- but not SIRT1-dependent manner, further suggesting a protective role of SIRT6.¹⁵⁶ Finally, SIRT7 knockout in mice results in hypertrophy, fibrosis, inflammation, systolic dysfunction, and increased apoptosis,^{157,158} indicating a protective role of SIRT7 against cardiomyopathy and hypertrophy as well.

Several studies suggest that **increasing NAD⁺ may protect against hypertrophy**. Increasing NAD⁺ in mice by either dosing with NMN or overexpressing NAMPT prevented hypertrophy induced by TAC and improved cardiac function⁶³ by increasing energy production in the mitochondria and reducing mitochondrial permeability transition pore opening.⁶³ The addition of NAD⁺ prevented isoproterenol-induced hypertrophy in mice, and interestingly, this was mediated by SIRT3, but not SIRT1, through activation of liver kinase B1-AMPK signaling.¹⁵⁰ NMN treatment protected against cardiac dysfunction in a Friedreich ataxia cardiomyopathy mouse model in a SIRT3-dependent manner⁶² and increased cardiac function in *Ndufs4* knockout mice exposed to pressure overload.⁶⁴ NR treatment also reduced inflammation and fibrosis in aged MDX mice, a muscular dystrophy model that develops cardiomyopathy.⁶⁶ Finally, PARP inhibitors protected against hypertrophy and improved cardiac function in several rodent models,¹³³ possibly in part by increasing NAD⁺ levels.

The role of sirtuins and NAD⁺ in heart failure, rather than remodeling, has not been as extensively studied. Levels of SIRT1 increase in failing hamster,⁷¹ rat,⁶⁸ mouse,¹⁴⁰ and dog hearts,¹⁵⁹ but whether this increase is cardioprotective or contributes to the development of heart failure is not clear. The association between heart failure and the other sirtuins is not well understood although one study found reduced SIRT6 expression in failing human hearts.¹⁵⁵ Failing hearts also have extensive mitochondrial hyperacetylation that may contribute

to dysfunction in heart failure,¹⁶⁰ and NAD⁺ levels are reduced in failing mouse and human hearts.^{63,160}

Overall, moderately increasing SIRT1 activity, increasing SIRT3 activity, or boosting NAD⁺ levels seem to be promising approaches for protection against age-related hypertrophy and fibrosis. More research in clinically relevant models of heart aging is needed, however, to understand the potentially detrimental effects of high overexpression of SIRT1.

Arrhythmias

The risk of **atrial fibrillation and other types of arrhythmias increases significantly with age**.¹³⁵ The role of sirtuins and NAD⁺ in arrhythmias has been minimally investigated. A recent study showed that SIRT1 deacetylates the voltage-gated Na⁺ channel (Nav1.5), which is necessary for normal cardiac electrophysiology, to increase its activity. Cardiac-specific **SIRT1 knockout mice had decreased Nav1.5 expression, which resulted in abnormal cardiac conduction**.¹⁶¹ One study in *BubR1* knockout mice, which are short-lived in part because of **defective cardiac electrophysiology, showed a potential role of NAD⁺ and SIRT2 in treating arrhythmias**. They found that either overexpressing SIRT2 or **increasing NAD⁺ levels** with NMN increased *BubR1* and partly reversed the repolarization defects observed.¹⁶² **Given the association between reduced NAD⁺ and sirtuin activity in the heart with age and the increased risk of arrhythmias, this is an area that warrants further research.**

Hypertension

Hypertension is a risk factor for other cardiovascular diseases and the risk of hypertension increases with age, especially in women.^{163,164} Few studies have explored the effects of the sirtuins on hypertension. Angiotensin II exposure, which is a commonly used model of hypertrophy in mice, also causes hypertension although most studies of angiotensin II-induced hypertrophy discussed above did not investigate blood pressure changes. One study of SIRT1 overexpression in the context of angiotensin II found that SIRT1 protected against the systolic blood pressure increase seen in wild-type mice.¹⁶⁵ In addition, a study in rats with spontaneous pulmonary artery hypertension found that they had reduced SIRT3 expression and that SIRT3 overexpression prevented the development of hypertension.¹⁶⁶ Polymorphisms in SIRT3 were also associated with pulmonary artery hypertension in patients.¹⁶⁶ More research in this area will identify whether boosting sirtuins or NAD⁺ may protect against hypertension in aging.

Diabetes Mellitus, Glucose/Insulin Dysregulation, and Metabolic Syndrome

The metabolic syndrome is a set of risk factors for cardiovascular diseases including obesity, diabetes mellitus, increased LDL levels, increased triglyceride (TG) levels, and hypertension.^{167,168} Type 2 diabetes mellitus is characterized by high blood glucose levels, low levels of insulin, and insulin resistance. The risk of diabetes mellitus is increased by obesity and a lack of exercise, and diabetes mellitus is associated with an increased risk of cardiovascular disease, stroke, and kidney failure.¹⁶⁹ Increasing age is a significant risk factor for insulin resistance and impaired glucose tolerance.^{170–172} The role of sirtuins in glucose and insulin control, obesity,

and the development and prevention of diabetes mellitus has been well explored, at least for SIRT1, 3, 4, and 6. A variety of animal models are used to study diabetes mellitus and insulin resistance including aging mice, transgenic mouse models of diabetes mellitus, such as db/db, and the induction of obesity and diabetes mellitus with an HFD. Human studies are limited, but a specific SIRT1 mutation was associated with type 1 diabetes mellitus in one patient study,¹⁷³ and low SIRT1 has been associated with insulin resistance in the offspring of those with type 2 diabetes mellitus.¹⁷⁴ Two human studies found associations between SIRT3 polymorphisms and the metabolic syndrome, suggesting a role of SIRT3 in this syndrome in humans.^{166,175}

SIRT1 transgenic overexpressors fed a diet high in fat and cholesterol have better glucose homeostasis than wild-type mice fed the same diet but with no difference in body weight.^{12,13,97} SIRT1-overexpressing mice fed a standard diet also show increased glucose tolerance, which is accompanied by reduced cholesterol, reduced fat mass, and lower body weight.¹⁷⁶ SIRT1 overexpression in the db/db diabetic mouse model also improved glucose tolerance and reduced hepatic glucose production.¹³ Pancreatic β -cell-specific overexpression of SIRT1 was associated with increased insulin secretion and improved glucose tolerance, even as the mice aged.¹⁷⁷ Both mice and rhesus monkeys with insulin resistance induced by an HFD had increased insulin sensitivity when SIRT1 activity was increased with resveratrol or SRT1720 treatment.^{79–83} Mice fed a standard diet and dosed with the SIRT1 activators SRT2104 or SRT1720 also had increased insulin sensitivity.^{16,17} The role of reduced SIRT1 function in mice is less clear. One study found that SIRT1 knockout mice displayed reduced basal and glucose-stimulated insulin levels as a result of upregulation of UCP (uncoupling protein) 2 expression.¹⁷⁸ Another study, however, showed that on a standard diet, liver-specific SIRT1 knockout mice had no change in insulin or glucose modulation and that on an HFD, they maintained insulin sensitivity, implying a role of SIRT1 in contributing to insulin resistance in this model.¹⁷⁹

Although overexpression of SIRT1 in high caloric exposure generally acts to decrease glucose and increase insulin response, increased SIRT1 in fasting seems to increase glucose production. Acute SIRT1 knockout in the liver of fasted mice actually resulted in increased insulin sensitivity, decreased serum glucose, and decreased glucose production, and overexpression reversed these effects.¹⁸⁰ In vitro studies show that SIRT1 negatively regulates glycolysis through phosphoglycerate mutase 1 and that in fasted cells there is increased SIRT1 activity and thus reduced glucose breakdown.¹⁸¹ Fasting in mice also resulted in increased SIRT1 modulation of the glycolytic and gluconeogenesis pathways via FOXO1 and PGC1 α to potentially increase glucose levels.²² Thus, there is clearly a complex and potentially tissue-specific relationship between nutritional state, SIRT1, and insulin and glucose regulation that requires further research.

Unlike SIRT1, SIRT3 knockout mice fed a high-cholesterol diet gained more weight but had the same glucose tolerance as wild-type mice.¹⁰¹ One study of HFD-fed SIRT3 knockout mice found increased fatty acid oxidation, implying that SIRT3 may act to protect against insulin resistance in high

calorie environments by decreasing fatty acid oxidation.¹⁸² Interestingly, SIRT3 seems to have the opposite effect in low calorie environments as SIRT3 knockout mice have decreased levels of fatty acid oxidation in fasting.²⁴ SIRT4 knockout mice have upregulation of fatty acid oxidation and metabolism,^{25,183,184} which results in protection against diet-induced obesity.¹⁸⁵ SIRT4 knockout mice also have increased insulin secretion in response to amino acids,²⁵ which results in insulin resistance and glucose intolerance.¹⁸⁶ Interestingly, SIRT4 knockout mice also have increased SIRT1 activity, so there are likely several sirtuin pathways involved in the regulation of glucose and insulin homeostasis.¹⁸³

Whole-body SIRT6 knockout mice die prematurely from hypoglycemia. The mechanism seems to be increased AKT activation and reduced insulin-like growth factor-1 levels, which results in upregulation of glucose transporters and increased glucose uptake in skeletal muscle and brown adipose tissue.^{187–189} SIRT6 knockout mice also have increased glucose production as normally SIRT6 downregulates hepatic glucose production via PGC1 α ¹⁹⁰ as well as increased glycolysis, possibly through increased activity of hypoxia-inducible factor-1 α .^{189,191} Interestingly, overexpression of SIRT6 improved glucose homeostasis in aging mice¹⁵ and increased glucose tolerance and insulin sensitivity in the context of an HFD, along with a reduction in visceral fat.¹¹ The authors suggest that the HFD caused defective insulin secretion as a result of increased fat and inflammatory cells in the pancreas and that SIRT6 overexpression improved insulin sensitivity by reducing this inflammatory pancreatic infiltration.¹¹ Neural-specific SIRT6 knockout mice develop obesity overtime implying that SIRT6 may act as a central regulator of growth and obesity.¹⁹² As such, SIRT6 may be a promising target for therapeutics to modulate obesity, increase insulin sensitivity, and reduce glucose levels in patients. SIRT7 may also have a role in regulating glucose levels as SIRT7 knockout mice were resistant to glucose intolerance.¹⁹³ Supplementation of mice on an HFD with NR increased insulin sensitivity and reduced fat mass compared with HFD alone. Interestingly, NR also increased the activity of SIRT2 and SIRT3.⁵⁹ Similarly, supplementation with NMN also prevented the insulin resistance and glucose intolerance associated with an HFD in mice and improved glucose tolerance in aging mice.¹⁹⁴ Finally, treatment with the CD38 inhibitor apigenin in mice with diet-induced obesity resulted in increased NAD⁺ levels, as well as protection against glucose dysregulation.⁵³

Overall sirtuins seem to have a complex but generally protective effect on obesity, but whether this effect is related to or independent from effects on insulin and glucose regulation is still not known. Boosting NAD⁺ levels or increasing SIRT1 and SIRT6 activity in the context of the metabolic syndrome, type 2 diabetes mellitus, and insulin resistance could be an important therapeutic approach for preventing and treating these diseases in aging.

Dyslipidemia

Dyslipidemia is characterized by abnormal levels of lipids, including TGs and cholesterol, in the blood. The most common of these abnormalities in the elderly is hypercholesterolemia that is an increased level of LDL cholesterol.^{195,196} The role of sirtuins and NAD⁺ in dyslipidemia has not been well

characterized. SIRT1 has a role in cholesterol homeostasis as a positive regulator of the cholesterol-sensing proteins, liver X receptors.¹⁹⁷ SIRT1 knockout results in reduced liver X receptor expression and reduced high-density lipoprotein and TG lipid levels, implying a protective role of SIRT1 in increasing the levels of beneficial high-density lipoprotein cholesterol.¹⁹⁷ The role of SIRT1 in lipid homeostasis also seems to depend on the nutritional state of the mice. Acute SIRT1 knockout in the liver of fasted mice results in decreased serum cholesterol levels and increased liver cholesterol.¹⁸⁰ The opposite role for SIRT1 was seen on standard or HFD, however, where SIRT1 overexpression resulted in decreased serum cholesterol levels.^{176,198}

SIRT3 knockout mice with HFD exposure develop hyperlipidemia, implying a role of SIRT3 in maintaining lipid homeostasis.²⁴ SIRT4 also has a role in lipid metabolism by regulating mast cell degranulating peptide activity.¹⁸⁵ SIRT6 plays a role in negatively regulating glycolysis and TG synthesis¹⁹¹ and may also have a role in protecting against increased LDL cholesterol levels.¹⁹⁹ SIRT6 knockout mice have increased LDL while SIRT6 overexpressing mice fed an HFD have lower LDL and TG levels than wild-type mice.^{11,199} NR in HFD-fed mice was associated with reduced LDL cholesterol levels compared with HFD alone.⁵⁹ Niacin, which is a collective term for nicotinamide and nicotinic acid, has for many years been used as a treatment for hypercholesterolemia,²⁰⁰ but the mechanism of this protection is still not known.²⁰¹ NAD⁺-dependent activation of sirtuins is a possible mechanism,²⁰² but the effect may also be driven by NAD⁺-independent activation of the G-coupled receptor GPR109A.²⁰¹

NAD⁺ and sirtuin boosters may have beneficial roles in treating dyslipidemia, especially in the context of Western diets, but more research is required to fully understand the mechanisms behind this protection and the role of each of the sirtuins in lipid homeostasis.

Clinical Trials of Molecules to Modulate Sirtuins and NAD⁺ in Cardiovascular and Metabolic Diseases

The use of both STACs and NAD⁺ boosters in cardiovascular and metabolic diseases has shown beneficial effects in a variety of animal models (Table 2). Given the preclinical evidence for benefits of sirtuins and NAD⁺ in preventing and treating cardiovascular and metabolic diseases, many researchers recognize the potential of these pathways as medicines. As a result, there are a growing number of human trials of sirtuin and NAD⁺-boosting drugs, some of which are showing protective effects in cardiovascular and metabolic diseases (Table 3).

Sirtuin-Activating Compounds

There have been >20 clinical trials of resveratrol, including at least 6 investigating cardiovascular outcomes and 8 with primary metabolic outcomes, including diabetes mellitus.²²⁰ Clinical trials of resveratrol have been recently and thoroughly reviewed^{6,220,221} with most trials showing beneficial cardiovascular and metabolic effects of resveratrol.^{6,220,221} For example, administration of resveratrol to healthy obese men for 30 days improved insulin sensitivity and decreased systolic blood pressure²⁰⁵ while no effect was seen in healthy nonobese men.²⁰⁶

Resveratrol treatment in patients with metabolic syndrome resulted in reduced body weight, reduced fat mass, and decreased total insulin secretion,²⁰⁷ and patients with type 2 diabetes mellitus treated with resveratrol for 45 days had lower fasting glucose and decreased insulin resistance than controls.²⁰⁸ Resveratrol has also shown improvements in patients with coronary artery disease²⁰⁹ and nonalcoholic fatty liver disease.^{36,211} Not all studies, however, have seen improvements with resveratrol treatment.^{203,220,221} Resveratrol treatment in glucose intolerant patients for 6 weeks resulted in improved vascular function with no effect on glucose tolerance or insulin sensitivity,²⁰⁴ and resveratrol treatment in patients with peripheral artery diseases did not improve the primary outcome of walking performance.²⁰³ Potential differences in clinical trial design, such as drug formulation, dose, and timing, may explain these varied results.⁶

Clinical trials of the STAC SRT2104 have shown beneficial serum lipid-lowering effects in patients. A trial in healthy older volunteers found that SRT2104 decreased serum total cholesterol, LDL, and TG levels with no change in glucose tolerance.²¹³ SRT2104 also reduced LDL and TG levels in healthy smokers but had no effect on the vasculature.²¹⁴ A small crossover trial of SRT2104 in patients with type 2 diabetes mellitus found that drug treatment was associated with weight loss, but there was little effect on endothelial function or serum lipid profiles, and there was a potentially adverse effect on glucose control.²¹² The authors suggest that this negative effect may be a result of acute rather than chronic exposure to SRT2104 and that long-term exposure may mimic what is seen in preclinical studies.²¹²

NAD⁺ Boosters

There have been a few clinical trials of NAD⁺ boosters in treating cardiovascular or metabolic diseases with more currently underway.^{6,44} Niacin has been used for many years for the treatment of hypercholesterolemia and acts to decrease very-low-density lipoprotein TGs by suppressing lipolysis²⁰⁰ although this effect may be NAD⁺ independent.²⁰¹ The first study of NR pharmacokinetics in healthy volunteers demonstrated safety and efficacy of single doses of NR in boosting NAD⁺ levels.²¹⁶ A second pharmacokinetic study investigated the effect of 8 days of increased dosing of NR in healthy volunteers and also found that NR was well-tolerated and increased circulating NAD⁺ levels.²¹⁷ Recently, these safety and pharmacokinetic results were confirmed in a 6-week clinical trial of NR in healthy older adults, and secondary analyses suggest it also reduced blood pressure and arterial stiffness.²¹⁸ A combination of NR and the polyphenol compound pterostilbene was also found to be tolerated without side effects in a recent phase I trial.²¹⁵ There are many more trials investigating NR in cardiovascular outcomes, including heart failure and atherosclerosis, currently underway.^{6,44} The first human trials of NMN are also ongoing.²²² Safety and pharmacokinetics of the PARP inhibitor INO-1001 were investigated in a small study of patients with myocardial infarction, and it was found to be effective and well tolerated.²¹⁹

The overall promising results of STACs and NAD⁺ boosters in cardiovascular and metabolic diseases will result in further clinical trials of these compounds over the next decade and beyond.

Table 3. Clinical Trials of STACs and NAD⁺ Boosters in Cardiovascular and Metabolic Diseases

Drug	Effect	Study Population	References	
Resveratrol	No change in walking performance	Patients with peripheral artery disease	203	
	↑ Vascular function, No change in glucose metabolism	Glucose intolerant adults	204	
	↓ Systolic blood pressure ↑ Insulin sensitivity	Healthy obese men	205	
	No effect	Healthy nonobese men	206	
	↓ Body weight ↓ Fat mass ↓ Total insulin secretion	Metabolic syndrome patients	207	
	↓ Insulin resistance ↓ Fasting glucose	Type 2 diabetes mellitus patients	208	
	↑ Endothelial function ↑ Diastolic function	Patients with coronary artery disease	209	
	↓ Serum LDL ↓ Insulin resistance	NAFLD patients	210	
	↓ Hepatic steatosis ↓ Inflammation	NAFLD patients	211	
	SRT2104	↓ Body weight Changes to glucose control	Type 2 diabetes mellitus patients	212
		↓ Serum LDL ↓ Serum TGs	Healthy older volunteers	213
↓ Serum LDL ↓ Serum TGs		Healthy smokers	214	
Niacin	↓ Dyslipidemia	Varied	200	
NRPT	Safely tolerated	Healthy volunteers	215	
NR	Safely tolerated	Healthy volunteers	216,217	
	↓ Blood pressure ↓ Arterial stiffening	Healthy volunteers	218	
	Underway, aim to improve atherosclerosis and heart failure	Varied	No. NCT02812238 No. NCT03423342 (Clinicaltrials.gov)	
NMN	Underway, aim to improve cardiometabolic outcomes	Women 55+ with dyslipidemia	No. NCT03151239 (Clinicaltrials.gov)	
INO-1001	Safely tolerated	Patients with myocardial infarction	219	

LDL indicates low-density lipoprotein; NAD⁺, nicotinamide adenine dinucleotide; NAFLD, nonalcoholic fatty liver disease; NMN, nicotinamide mononucleotide; NR, nicotinamide riboside; NRPT, nicotinamide riboside and pterostilbene; STAC, sirtuin-activating compound; and TG, triglyceride.

Conclusions

This review has shown the clear role of sirtuins and NAD⁺ in a range of cardiovascular and metabolic diseases and identified exciting opportunities to boost NAD⁺ and sirtuin activity to treat or prevent these diseases. In particular, there is extensive preclinical, and recently clinical, evidence for increasing SIRT1 activity to target type 2 diabetes mellitus and dyslipidemia. In addition, there is promising preclinical evidence for increasing SIRT6 activity in insulin resistance, increasing SIRT3 activity to protect against age-related cardiac hypertrophy, and boosting NAD⁺ levels to promote angiogenesis and increase blood flow, which may translate into clinical studies in the near future.

There are still many questions that remain unanswered, however, and more research to be done. There is little known about sex differences in the roles of NAD⁺ and sirtuins in cardiovascular and metabolic diseases, and most preclinical studies have only been completed in males. Given the important sex differences in the cardiovascular system with aging^{223,224} and sex differences in response to longevity interventions,²²⁵ this area warrants further research. In addition, more research is needed on unraveling the role of each of the sirtuins in these age-related diseases, especially SIRT2, 4, 5, and 7, where little

is currently known. The potentially divergent roles of different sirtuins in these diseases are not well understood, especially the possible detrimental roles of SIRT2 in IR injury and SIRT4 in hypertrophy. More research could improve understanding of how STACs may act differently on different sirtuins. Moreover, future studies should provide a better understanding of the effect of nutritional status on the role of sirtuins in glucose and insulin regulation and in dyslipidemia to optimize the use of sirtuin-modulating therapies in these conditions. In addition, there is still much to learn about the basic physiology and pharmacology of the NAD⁺-boosting drugs, such as their cellular transport, metabolism, and dosing before they can be widely used clinically. Although not discussed in this review, there is also the potential that diets that improve cardiovascular or metabolic outcomes^{226,227} may modulate NAD⁺ levels and increase sirtuin activity. For example, it has been suggested that increased NAD⁺ may be a significant mechanism contributing to the benefits of a high ketone diet.²²⁸

In conclusion, there is clear preclinical evidence for NAD⁺-boosting and sirtuin-activating therapies in treating and preventing age-related cardiovascular and metabolic diseases, which are resulting in growing translation of these promising interventions into clinical trials.

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Disclosures

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