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Protective effects of sirtuins in cardiovascular diseases: from bench to bedside

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Sirtuins (Sirt1–Sirt7) comprise a family of nicotinamide adenine dinucleotide (NAD+)–dependent enzymes. While deacetylation reflects their main task, some of them have deacylase, adenosine diphosphate-ribosylase, demalonylase, glutarylase, and desuccinylase properties. Activated upon caloric restriction and exercise, they control critical cellular processes in the nucleus, cytoplasm, and mitochondria to maintain metabolic homeostasis, reduce cellular damage and dampen inflammation—all of which serve to protect against a variety of age-related diseases, including cardiovascular pathologies. This review focuses on the cardiovascular effects of Sirt1, Sirt3, Sirt6, and Sirt7. Most is known about Sirt1. This deacetylase protects from endothelial dysfunction, atherothrombosis, diet-induced obesity, type 2 diabetes, liver steatosis, and myocardial infarction. Sirt3 provides beneficial effects in the context of left ventricular hypertrophy, cardiomyopathy, oxidative stress, metabolic homeostasis, and dyslipidaemia. Sirt6 is implicated in ameliorating dyslipidaemia, cellular senescence, and left ventricular hypertrophy. Sirt7 plays a role in lipid metabolism and cardiomyopathies. Most of these data were derived from experimental findings in genetically modified mice, where NFκB, Pcsk9, low-density lipoprotein-receptor, PPARγ, superoxide dismutase 2, poly[adenosine diphosphate-ribose] polymerase 1, and endothelial nitric oxide synthase were identified among others as crucial molecular targets and/or partners of sirtuins. Of note, there is translational evidence for a role of sirtuins in patients with endothelial dysfunction, type 1 or type 2 diabetes and longevity. Given the availability of specific Sirt1 activators or pan-sirtuin activators that boost levels of the sirtuin cofactor NAD+, we anticipate that this field will move quickly from bench to bedside.

Keywords
Sirtuins • Cardiovascular • Translational • Metabolism • Aging

Sirtuins: mediators of caloric restriction

Silent Information Regulator 2 (SIR2) proteins—Sirtuins—are a family of histone deacetylases (HDACs) that catalyse deacetylation of both histone and non-histone lysine residues. Beyond deacetylase and deacylase activity, some sirtuins exert adenosine diphosphate (ADP)-ribosylase, demalonylase, desuccinylase, or glutarylase properties. Post-transcriptional modification of a wide range of protein targets of the seven mammalian orthologs (Sirt1–7) has been described in diverse settings. Their requirement for nicotinamide adenine dinucleotide (NAD+) distinguishes sirtuins from other HDAC classes and defines them as class III HDACs. This need of NAD+ for their enzymatic activity closely links sirtuins to the cellular energy status, increasing their activity at times of low energy availability such as caloric restriction and exercise (Figure 1).1–4

To date, caloric restriction is the most robust intervention that has been reproducibly shown to prolong life span and maintain health in both invertebrates and vertebrates, including mammals.5,6 The first association between sirtuins and longevity in budding yeast 16 years ago7 sparked efforts in numerous laboratories to unravel the mechanisms underlying sirtuin-mediated prolongation of life span and elucidate their potential to postpone the onset of age-related diseases. In this context, the seven mammalian sirtuin orthologs have been studied in diverse disease models, including insulin resistance and diabetes, inflammation, neurodegenerative disease, cancer, and more recently, in cardiovascular pathologies such as cardiac hypertrophy, heart failure, endothelial dysfunction, and atherosclerosis.
This review focuses on the recent advances of sirtuin research in experimental cardiovascular disease and discusses their potential clinical applications (Table 1).

**Sirtuins: emerging roles in cardiovascular and metabolic diseases**

**Atherosclerosis: a chronic immunometabolic disease**

Atherosclerosis with its sequelae myocardial infarction or stroke comprises the major cause for morbidity and mortality in the Western world. At the cellular and molecular level, atherosclerosis results from a complex interplay between modified low density lipoproteins (LDL), activated endothelial cells, monocyte-derived macrophages, T cells, and the vessel wall: Oxidative stress in endothelial cells, endothelial dysfunction, and subsequent recruitment of macrophages into the sub-endothelial space are major steps in early atherogenesis. In response to para- and autocrine inflammatory mediators such as tumour necrosis factor α and different interleukins, invading monocytes transdifferentiate into macrophages, proliferate, and ingest abundantly present modified LDL-cholesterol (LDL-C). Thereby, sub-endothelial macrophages turn into foam cells that promote plaque formation and subsequent plaque rupture. Plasma cholesterol and in particular its LDL subfraction are central players for both the initiation and progression of atherosclerosis.
It is intriguing that many risk factors for cardiovascular disease such as age, hypercholesterolaemia, obesity, and type 2 diabetes are conditions in which the activation of the sirtuins has been shown to exert protective effects in experimental models.

**Sirt1 in cardiovascular disease**

Sirt1 is found in the nucleus and cytoplasm (Figure 2). Among all seven sirtuin isoforms, most is known about Sirt1. First evidence of a connection between Sirt1 and endothelial cells was that Sirt1 activates endothelial nitric oxide synthase (eNOS).15 Later on, studies in genetically engineered mouse models have demonstrated that Sirt1 exerts atheroprotective effects by activating eNOS or by diminishing NFκB activity in endothelial cells and macrophages.12–14 Moreover, pharmacological Sirt1 activation protected endothelial cells from senescence induced by disturbed flow.15 Another report assigned Sirt1 in vascular smooth muscle cells a protective role against DNA damage, medial degeneration, and atherosclerosis.16

These reports place macrophages, endothelial, and vascular smooth muscle cells at centre stage for Sirt1-mediated atheroprotection.17 Sirt1 also has wide-ranging effects on metabolic homeostasis, mainly through its role as a master regulator of mitochondrial integrity.18,19 Sirt1 activation hence results in improved glucose tolerance and lipid homeostasis and reduced inflammatory tone, which all are also atheroprotective.1,2

Pharmacological Sirt1 inhibition has been reported to increase thrombosis by inhibiting tissue factor activation via NFκB.20 Similarly, cyclooxygenase-2-derived prostacyclin and PPARδ activation were found to decrease arterial thrombus formation by suppressing tissue factor activation.21 Thus, activation of Sirt1 protects from arterial thrombosis. In the context of atherosclerosis, pharmacological Sirt1 activation lowered plasma LDL-C levels by inhibiting proprotein convertase subtilisin/kexin 9 (Pcsk9) secretion, thereby increasing hepatic LDL-receptor (LDL-R) availability and consecutive LDL-C clearing.22 Conversely, absence of LDL-R...
abolished the atheroprotective effects of pharmacological Sirt1 activation. These experiments explained the controversial finding that genetic Sirt1 overexpression increased atherosclerosis in LDL-R knockout mice. Moreover, Sirt1 was reported to promote angiogenesis via inhibition of endothelial notch signaling.

Sirt1 also provides cardiac protection: Sirt1-deficient mice exhibit increased injury in response to ischaemia-reperfusion, whereas injury was decreased in Sirt1 transgenic mice. Moreover, Sirt1 was shown to protect from catecholamine-induced cardiomyopathy in mice.

**Sirt3 in cardiovascular disease**

Together with Sirt4 and Sirt5, Sirt3 is located in the mitochondria (Figure 3). Sirt3 regulates global mitochondrial lysine acetylation, boosting antioxidant defense, and preserving mitochondrial function. Mitochondrial dysfunction plays a central role in a number of cardiovascular diseases, ranging from hypertrophic and dilated cardiomyopathy, heart failure, and pulmonary hypertension to endothelial dysfunction in early atherogenesis.

The protective effects of caloric restriction on oxidative stress depend on the presence of Sirt3, which increased the ratio of glutathione to glutathione disulphide. Similarly, deletion of Sirt3 was associated with increased malondialdehyde levels in LDL-R knockout mice, yet without affecting atherogenesis. Concordantly, overexpression of Sirt3 decreased cellular levels of reactive oxygen species (ROS), an effect that was brought about by Sirt3-mediated deacetylation and activation of superoxide dismutase 2 (SOD2), the
mitochondrial isoform of this group of major ROS detoxifying enzymes. Importantly, excessive ROS, subsequent mitochondrial DNA damage, and progressive respiratory chain dysfunction activate several signalling pathways underlying endothelial dysfunction and vascular inflammation in atherogenesis. Sirt3-dependent transcriptional induction of SOD2 and catalase was shown to prevent cardiac hypertrophy by deacetylation of the transcription factor forkhead box O3a (Foxo3a). Moreover, Sirt3-deficient mice were more susceptible to age-dependent and trans aortic constriction-induced left ventricular hypertrophy via activation of the mitochondrial permeability transition pore. Sirt3 was also reported to prevent stress-induced mitochondrial apoptosis of mammalian cardiomyocytes and to protect endothelial mitochondria from oxidative damage.

Interestingly, Sirt3 knockout mice develop spontaneous pulmonary hypertension. Moreover, mitochondria isolated from pulmonary artery smooth muscle cells of Sirt3-deficient mice displayed a reduced oxygen consumption rate compared with controls. Concordantly, Sirt3 deficiency in both murine and human pulmonary artery smooth muscle cells was associated with an induction of the three transcription factors HIF1α, STAT3, and NFATc2, all known to be essential in the development of pulmonary arterial hypertension.

Sirt3 has been shown to orchestrate mitochondrial metabolism by driving not only oxidative phosphorylation but also the tricarboxylic acid cycle, and β-oxidation. In this context, Sirt3 deficiency accelerated the development of the metabolic syndrome in mice, a cluster of risk factors for cardiovascular diseases.

Sirt6 in cardiovascular disease

Sirt6 is a nuclear chromatin-associated deacylase (Figure 4). Recent reports provided mechanistic insight on the ADP-ribosyltransferase substrate-specific deacylase activity of both acetyl and long-chain fatty-acyl groups. Sirt6 plays a role in cardiovascular disease including cardiac hypertrophy, heart failure, myocardial hypoxic damage, and metabolism. Sirt6 has gained attention for its role in human telomere and genome stabilization, gene expression and DNA repair, glucose and fat homeostasis, and inflammation.

Sirt6 contributes to chromosomal stability by promoting double-strand break repair by homologous recombination (HR). DNA double-strand breaks are a hallmark of genomic instability in aging tissues. Notably, overexpression of Sirt6 in pre-senescent cells...
stimulated HR. This support in HR can be partially attributed to a physical interaction and consecutive stimulation of Sirt6 with poly[ADP ribose] polymerase 1, thereby preventing the premature aging phenotype of Sirt6 knockout mice. Concomitantly, male Sirt6 transgenic mice revealed an increased life span compared with wild-type controls. Interestingly, the heart is among the organs with the highest Sirt6 expression, suggesting a role for Sirt6-mediated protection from myocardial senescence. Indeed, Sirt6 was identified as a negative endogenous regulator of myocardial IGF-Akt signaling, a pathway that upon constitutive activation eventually leads to cardiac hypertrophy. Sirt6 deficiency enhanced H3K9 acetylation, facilitating binding of the stress-responsive transcription factor c-Jun. Subsequent boosting of myocardial IGF signalling resulted in cardiac hypertrophy and heart failure. Along this line, nicotinamide mononucleotide adenyllytransferase, a vital enzyme in NAD biogenesis, prevented angiotensin II-induced cardiac hypertrophy. Moreover, cardiomyocytes from Sirt6 transgenic mice were protected from prolonged hypoxia ex vivo, an effect that was in part attributed to increased Sirt6-mediated deacetylation and inactivation of RelA (p65), a subunit of NFkB. By the transcriptional repression of NFkB-dependent targets, Sirt6 plays an important role in stress response, thereby protecting against inflammatory and degenerative diseases. Importantly, this interaction with NFkB-signalling may contribute to premature senescence and early lethality upon Sirt6 deficiency. Vascular inflammation is a key regulatory process in atherogenesis. Thus, Sirt6 deficiency in cultured endothelial cells increased expression of pro-inflammatory cytokines such as interleukin 1β along with an increased transcriptional activity of NFkB. Intriguingly, hepatic Sirt6 was also shown to suppress transcription of Pcsk9, thereby preventing hepatic LDL-R degradation and consecutively reducing plasma LDL-C levels in mice. Increased levels of LDL-C in concert
with enhanced pro-inflammatory cytokines trigger endothelial activation and launch the vicious immunometabolic cycle of atherogenesis.

**Sirt7 in cardiovascular disease**

Data from two different laboratories, using two independently generated mouse lines have also linked Sirt7 with cardiomyopathy, the main cause of death of Sirt7-deficient mice (Figure 5). In fact, Sirt7 deficiency in mice induces multi-systemic mitochondrial dysfunction, which is reflected by increased blood lactate levels, reduced exercise performance due to cardiac dysfunction, hepatic micro-vesicular steatosis, and age-related hearing loss. This link between Sirt7 and mitochondrial function can be translated to humans, where Sirt7 overexpression rescues the mitochondrial functional defect in fibroblasts of patients with a mutation in NADH dehydrogenase [ubiquinone] iron-sulphur protein 1. These wide-ranging effects of Sirt7 on mitochondrial homeostasis are the consequence of the deacetylation of distinct lysine residues located in the hetero- and homodimerization domains of GA-binding protein (GABP)β1, a master regulator of nuclear-encoded mitochondrial genes. Along these lines, Sirt7 improves mitochondrial function in numerous tissues including cardiac and skeletal muscle where it protects from cardiomyopathy, lowers lactate levels, and improves exercise performance, respectively. Moreover, Sirt7 protects from hepatic micro-vesicular steatosis.

**Figure 5** Cardiovascular effects of Sirt7. Nuclear Sirt7 deacetylates distinct lysine residues located in the hetero- and homodimerization domains of GA-binding protein (GABP)β1, a master regulator of nuclear-encoded mitochondrial genes. Along these lines, Sirt7 improves mitochondrial function in numerous tissues including cardiac and skeletal muscle where it protects from cardiomyopathy, lowers lactate levels, and improves exercise performance, respectively. Moreover, Sirt7 protects from hepatic micro-vesicular steatosis.

**Sirtuins: a role in human cardiovascular disease**

Data on sirtuins in human cardiovascular diseases are scarce. Of note, reports about associations have to be differentiated from findings related to potential causal effects.

Most studies report lower sirtuin expression levels in contexts of cardiovascular disease compared with healthy controls. However, none of them tracked specific sirtuin activity. First, data were derived from patients with insulin resistance: Low Sirt1 expression in insulin-sensitive tissues correlated with impaired stimulation of
energy expenditure by insulin. Thus, these findings associated impaired regulation of mitochondrial function with insulin resistance in humans. Additional reports came from patients with atherosclerosis: Sirt1 expression levels were found to be lower in human atherosclerotic carotid arteries compared with non-diseased arteries. Sirt1 expression levels were also lower in monocyttes of patients with acute coronary syndromes compared with healthy controls. In parallel, Sirt6 expression and inflammatory activity in diabetic atherosclerotic plaques increased upon incretin treatment and was associated with a more stable plaque phenotype.

In contrast to associative reports, genetic studies imply a more stringent cause–effect relationship. A report in Finnish subjects demonstrated a direct involvement of Sirt1 in modulating energy expenditure and metabolic homeostasis: Three of five single-nucleotide polymorphisms (SNPs) were significantly associated with whole body energy expenditure as evaluated either during fasting or during a hyperinsulinemic clamp. Another study showed that a point mutation of Sirt1 was associated with human type 1 diabetes and ulcerative colitis; expression of this mutation in insulin-producing cells resulted in overproduction of nitric oxide and pro-inflammatory cytokines. Yet, no association was observed between five known SNPs of the Sirt1 gene with longevity using an extensive DNA data bank of 1573 long-lived individuals with matched younger controls. Of note, many other studies reporting SNPs confer limitations such as low patient numbers or insufficient adjustments in control groups.

Moreover, Sirt3 deficiency was associated with pulmonary arterial hypertension and the metabolic syndrome both in mice and humans; sirt6 levels were decreased in human failing hearts; in addition, genetic variants of Sirt6 and uncoupling protein 5 were associated with carotid atherosclerotic plaque burden. To date, there are no reports about genetic variants of Sirt7 and human disease.

**Pharmacological modulation of sirtuin activity**

Two reports that resveratrol protected mice against diet-induced obesity and insulin resistance with Sirt1 as a crucial mediator created considerable attention. These effects of resveratrol supplementation, mimicking caloric restriction, could later on be translated into humans. Subsequently, more specific Sirt1-activating compounds (STACs) applied in experimental diet-induced obesity mimicked the protective effects of caloric restriction, showing mitochondrial activation, and subsequent prevention of obesity along with an improvement of the diabetic phenotype. Moreover, STAC improved survival and healthspan in obese mice.

However, the specificity of these initial STAC was called into question: A non-physiological fluorescent substrate that was used for the Sirt1 activity assays was shown to lead to artefactual results. Nevertheless, the substrate specificity of STAC was consecutively underlined: Mutation of a single amino acid in Sirt1, Glu, located in a structured N-terminal domain was critical for Sirt1 activation. Given the conservation of Glu down to model organisms (drosophila), the presence of an endogenous activator appears logical, which however remains to be proven. Furthermore, the most recent pharmacological STAC have been used with beneficial effects in mice for atheroprotection and extension of lifespan, as well as in healthy smokers for improving endothelial dysfunction and lowering LDL-C.

While Sirt1-specific activators may ultimately prove operative, there is increasing evidence that pan-sirtuin activators might be more effective. The metabolite NAD+ is an essential co-substrate for the activity of all sirtuins. Its levels decline in response to high-fat diet, DNA damage, and aging. Reports on sirtuin-dependent beneficial effects of increasing NAD+ levels on metabolic homeostasis suggest that this strategy provides a novel and promising concept for cardiovascular protection.

NAD+ levels are maintained by balancing its biosynthesis/salvage and breakdown (Figure 1). NAD+ content can be boosted by exercise, inhibition of NAD+-consuming enzymes [sirtuins, poly-ADP-ribose-polymerases (PARPs), and cyclic ADP-ribose synthases], and administration of NAD+ precursors, such as nicotinic acid, nicotinamide, nicotinamide mononucleotide, and nicotinamide riboside. NAD+ precursor compounds as well as PARP and CD38 inhibitors have been used in several long-term mouse studies confirming wide-ranging health benefits on the metabolic, immune, and nervous systems. Raising NAD+ levels may improve cardiac function in the context of ischaemia-reperfusion, but may be damaging in the setting of acute myocardial infarction. Thus, the net effects of increasing NAD+ levels are likely to be dose- and time-dependent; such a biphasic response is well known from nitric oxide. The effects of raising NAD+ levels long term in the context of chronic cardiovascular disease are likely to be beneficial. Yet, this has to be formally tested.

**Sirtuins in cardiovascular diseases: conclusions and perspectives**

The beneficial effects of sirtuins on inflammation, lipid metabolism, and numerous areas of cardiovascular disease are well documented at the pre-clinical level. The prognostic value of deranged pathways of inflammation and lipids for human cardiovascular disease is established. Multi-omics approaches will enlarge our instrumentarium of diagnostic and prognostic biomarkers in cardiovascular diseases. Moreover, large databases for linking genotypes to cardiovascular phenotypes and prognosis are available. Yet, the tracking of specific sirtuin activity remains a challenge. Some genetic reports imply a cause–effect relationship of Sirt1 SNPs with metabolic homeostasis. Yet, conclusive genetic analyses from large databases (GWAS) about the role of Sirt1 SNPs are still pending. At the experimental level, both specific Sirt1 activators and pan-sirtuin-activating compounds are in development and hold great promise for future applications in treating and preventing cardiovascular and metabolic diseases.

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