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# Sirtuins in atherosclerosis: guardians of healthspan and therapeutic targets

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Abstract | Sirtuins are $NAD^+$-dependent deacetylase and deacylase enzymes that control important cellular processes, including DNA damage repair, cellular metabolism, mitochondrial function and inflammation. Consequently, mammalian sirtuins are regarded as crucial regulators of cellular function and organism healthspan. Sirtuin activity and $NAD^+$ levels decrease with age in many tissues, and reduced sirtuin expression is associated with several cardiovascular diseases. By contrast, increased sirtuin expression and activity slows disease progression and improves cardiovascular function in preclinical models and delays various features of cellular ageing. The potential cardiometabolic benefits of sirtuins have resulted in clinical trials with sirtuin-modulating agents; although expectations are high, these drugs have not yet been proven to improve healthspan. In this Review, we examine the role of sirtuins in atherosclerosis, summarize advances in the development of compounds that activate or inhibit sirtuin activity and critically evaluate the therapeutic potential of these agents.

Sirtuins are a family of $NAD^+$-dependent deacetylase and deacylase enzymes that are widely recognized for their role in lifespan regulation and ageing. The *sir2* gene was first identified in yeast as an important suppressor of DNA damage and a positive regulator of cell lifespan through histone deacetylation[1]. Subsequently, seven members of the mammalian sirtuin family have been identified (SIRT1 to SIRT7), which share a highly conserved $NAD^+$-binding and catalytic core domain, but with varying subcellular localization and distinct enzymatic and biological functions and substrate specificity[2] (TABLE 1). Although originally classified as class III histone deacetylase enzymes, sirtuins have multiple deacylase and mono-ADP-ribosyltransferase activities and many non-histone and non-nuclear targets. The need for the energy metabolite $NAD^+$ as a crucial cofactor identifies sirtuins as key sensors of cellular metabolism[3], and a positive feedback loop between the $NAD^+$ pathway and sirtuins is also implicated in the control of mammalian ageing[4]. Indeed, both sirtuin and $NAD^+$ availability decrease with age, which can be exacerbated by cardiometabolic complications. In addition, nicotinamide phosphoribosyltransferase (NAMPT), the rate-limiting enzyme in $NAD^+$ biosynthesis and salvage, positively regulates sirtuin activity[5], and sirtuins seem at least partially to account for the effects of calorie restriction on extending organismal lifespan[6].

Sirtuins have received much attention in the past two decades, partly because they regulate crucial cellular processes in preclinical models and in human cells — including DNA damage, cellular senescence, inflammation, mitochondrial function and cell metabolism — that are disturbed in ageing and cardiovascular disease. Activation of sirtuins or boosting $NAD^+$ levels slows important processes of ageing and elicits lipid-lowering and anti-inflammatory effects in many cardiovascular diseases, including atherosclerosis[7]. For example, SIRT1 and SIRT6 protect against atherosclerosis by preventing endothelial cell dysfunction, vascular smooth muscle cell (VSMC) senescence and macrophage foam cell formation through regulation of DNA damage repair and anti-apoptotic and anti-inflammatory pathways. Mitochondrial sirtuins protect against vascular cell death mostly by promoting antioxidant defence and mitochondrial homeostasis, although their full role in atherosclerosis remains largely unexplored.

Although SIRT1 was previously considered to be the most promising drug target of the sirtuin family, focus has now shifted to SIRT6 with the development of SIRT6-specific activators[8–11], and new insights have been gained into the role of SIRT6 in endothelial cells[12], VSMCs[13] and macrophages[14] in atherosclerosis. After rather disappointing trial outcomes with SIRT1 activators, the field is now waiting for clinical trials with these SIRT6-activating drugs. In this Review, we discuss the functions of the various sirtuins, discoveries made in the past decade on their role in vascular cells in the development and progression of atherosclerosis, and the therapeutic potential of sirtuin-modulating agents in atherosclerosis.

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**Key points**

- Sirtuins protect against many processes associated with ageing and atherosclerosis, but only SIRT6 has been shown to extend lifespan in mice.
- Natural compounds with sirtuin-modulating activity can delay or prevent the development of atherosclerosis in preclinical models, but have low bioavailability and off-target effects.
- Small-molecule activators of sirtuins and $NAD^+$ boosters have shown promising cardiometabolic effects in animal models and are well tolerated in healthy volunteers, but clinical trials showing prevention of atherosclerosis or clinical events are lacking.
- Drug discovery has shifted from SIRT1 to SIRT6, because the lifespan-extending and chromatin-remodelling effects of SIRT6 make it an attractive therapeutic target.
- Unravelling the multiple enzymatic activities of the sirtuins and their targets will facilitate drug discovery of specific sirtuin-activating compounds to prevent or treat atherosclerosis.

## Sirtuin functions and targets

To date, seven mammalian sirtuins have been identified, which are localized in different cellular compartments and have different enzymatic functions (FIG. 1; TABLE 1). SIRT3, SIRT4 and SIRT5 are predominantly localized in mitochondria, SIRT6 in the nucleus and SIRT7 in the nucleolus, whereas SIRT1 and SIRT2 can shuttle between the nucleus and the cytosol. Each sirtuin has different amino-terminal and carboxy-terminal extensions carrying cellular localization sequences that flank the conserved catalytic domain (FIG. 1). In addition, 23 human sirtuin isoforms and 15 mouse sirtuin isoforms have been identified so far[15], and alternative splicing could result in their relocalization, with potential structural and functional changes. Alternative splicing is not fully conserved between humans and mice and can be tissue-specific or disease-specific, which complicates the testing of sirtuin-modulating agents in various disease models across species and translation to human disease.

Sirtuins regulate various important cellular processes through their $NAD^+$-dependent deacylase functions — including DNA damage repair, apoptosis, autophagy, cell senescence, inflammation, cell metabolism, antioxidant defence and mitochondrial homeostasis (FIG. 2) — all of which are dysregulated in vascular disease. However, each sirtuin has different targets and exerts different effects.

### *Nuclear sirtuins*

***SIRT1.*** SIRT1 is largely found in the nucleus, but can relocate to the cytoplasm in response to certain cellular stimuli (such as oxidative stress)[16]. SIRT1 has a crucial role in DNA damage repair, telomere maintenance, apoptosis mediated by reactive oxygen species (ROS), cellular senescence, cellular metabolism and inflammation (FIG. 2). In addition, SIRT1 is upregulated during calorie restriction and might partly mediate its lifespan-extending effect[6]. *Sirt1* knockout in mice is embryonically lethal[17], emphasizing the importance of this sirtuin in regulating a wide range of cellular processes.

SIRT1 directly deacetylates histones, resulting in chromatin structural remodelling and transcriptional silencing, and catalyses deacetylation of many non-histone targets (including transcription factors and coregulators), altering their activity, cellular distribution or both. For example, histone deacetylation or direct deacetylation and activation of DNA damage response (DDR) proteins (such as Werner syndrome ATP-dependent helicase (WRN) and nibrin) facilitates DNA damage repair by promoting the recruitment of DNA damage proteins[18]. SIRT1 is directly recruited to the damage site and regulates many DDR proteins, such as X-ray repair cross-complementing protein 6 (XRCC6; also known as Ku70), breast cancer type 1 susceptibility protein (BRCA1), DNA repair protein complementing XP-A cells (XPA), DNA-(apurinic or apyrimidinic site) endonuclease (APEX1) and transcription factors (including cellular tumour antigen p53 and forkhead box protein O3a (FOXO3a)) (FIG. 2).

SIRT1 also controls key regulators of antioxidant defence, energy metabolism and inflammation via deacetylation. For example, SIRT1 deacetylates FOXO3a, which regulates the transcription of mitochondrial superoxide dismutase (SOD2)[19], and activates peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α) through deacetylation, thereby stimulating liver gluconeogenesis and fatty acid metabolism[20]. Moreover, SIRT1 inhibits nuclear factor-κB (NF-κB)-dependent inflammatory signalling via direct deacetylation of the p65 subunit[21] (FIG. 2).

***SIRT6.*** SIRT6 is a nuclear protein that primarily regulates cellular processes via chromatin remodelling and has few known non-histone targets. SIRT6 has a crucial role in telomere maintenance by deacetylating histone H3 lysine 9 (H3K9), thereby preventing telomeric DNA damage and cellular senescence[22]. SIRT6 is also recruited and binds to double-strand break sites following DNA damage[23] and initiates a DDR via multiple mechanisms, including chromatin relaxation by recruitment of chromatin remodellers[24,25], recruitment of DDR proteins and ROS-dependent activation of poly(ADP-ribose) polymerase 1 (PARP1) via mono-ADP-ribosylation[18] (FIG. 2).

SIRT6 is the only sirtuin that has been shown to increase lifespan in mice[26,27], and its capacity to repair double-strand breaks in DNA has been directly linked to longevity[28]. Interestingly, SIRT6 is also a deacetylation target of SIRT1, suggesting that SIRT6 and SIRT1 work synergistically to potentiate DNA damage repair[29]. The importance of SIRT6 in genomic maintenance has been further demonstrated in *Sirt6*$^{-/-}$ mice, which show premature ageing and death at 4 weeks of age[30]. In addition to its role in DNA repair, SIRT6 is upregulated during calorie restriction and epigenetically controls the expression of many genes encoding proteins involved in energy metabolism and inflammation through deacetylation. For example, SIRT6 deacetylates H3K9 at the promoter region of glycolytic and NF-κB target genes resulting in transcriptional repression[31]. However, unlike SIRT1, NF-κB is not a direct deacetylation target of SIRT6. Interestingly, NAMPT is a direct deacetylation target of SIRT6, leading to increased NAMPT activity and NAD(P)(H) pools[32], thereby creating a positive feedback loop. Moreover, SIRT6 has a role in antioxidant defence by coactivating nuclear factor erythroid 2-related factor 2 (NRF2)[33] and promoting transcription of NRF2 target genes by mono-ADP-ribosylation of SWI–SNF complex

**Apoptosis**
A distinctive and important mode of 'programmed' cell death, characterized by cell shrinkage, chromatin condensation, DNA fragmentation and cell death.

**Autophagy**
A natural process in which unnecessary or damaged cellular components are removed by intrinsic mechanisms, thereby balancing energy sources at crucial times in development and in response to cellular stress.

**Cell senescence**
A stable cell cycle arrest that can be triggered in normal cells in response to intrinsic or extrinsic stimuli, particularly after DNA damage.

**DNA damage response**
(DDR). A complex network of genes and intracellular signalling pathways responsible for sensing and responding to DNA damage, regulating DNA repair, cell cycle regulation, replication stress responses and apoptosis.

**Chromatin remodelling**
Dynamic modification of the chromatin architecture to allow condensed genomic DNA to access the regulatory transcription machinery proteins, thereby controlling gene expression.

subunit SMARCC2 (also known as BRG1-associated factor 170; a subunit of the chromatin remodelling complex)[34]. Studies also suggest a defatty-acylase function for SIRT6, which could be more potent than its deacetylase activity[31]. SIRT6 promotes the secretion of tumour necrosis factor (TNF) by removing long-chain fatty acyl groups (for example, through myristoylation)[35], suggesting that SIRT6 is not restricted to the nucleus and might exert pro-inflammatory effects.

***SIRT7.*** SIRT7 is a nuclear protein found preferentially in nucleoli that has important roles in ribosomal gene biogenesis[36] and DNA damage repair. SIRT7 is recruited to double-strand break sites in DNA in a PARP1-dependent manner, facilitating chromatin condensation and DNA repair[37] through histone deacetylation or desuccinylation[38]. p53 is also an important SIRT7 deacetylation target, and loss of SIRT7 leads to impaired DNA repair and increased cell death[39] (FIG. 2). Although *Sirt7* knockout is not lethal in mice, reduced survival and cardiomyopathy are likely, due to increased p53 activity and cardiomyocyte apoptosis[40].

### *Cytoplasmic sirtuin*

***SIRT2.*** SIRT2 is predominantly localized to the cytosol, where it deacetylates cytoplasmic proteins (for example, tubulin-α) to maintain cell structure[41]. SIRT2 also transiently shuttles to the nucleus during mitosis, where

Table 1 | **Enzymatic functions and cellular targets of sirtuins in biological processes relevant to vascular disease**

| Sirtuin | Localization | Enzymatic functions | Targets | Biological functions | Phenotype of whole-body knockout mice |
|---|---|---|---|---|---|
| SIRT1 | Nuclear, cytosolic | Deacetylase | APEX1, FOXO3a[a], H3K9, H4K16, nibrin, p53[a], SIRT6, WRN, XPA, XRCC6 | DNA damage repair | Embryonically lethal |
| | | | PGC1α | Energy homeostasis | |
| | | | NF-κB | Inflammation | |
| SIRT2 | Cytosolic, nuclear (during mitosis only) | Deacetylase | α-Tubulin | Cell structure | Develop normally, but with metabolic abnormalities |
| | | | FOXO3a[a] | Transcriptional control | |
| | | | H4K16 | Cell cycle regulation | |
| | Mitochondrial | | Several mitochondrial proteins | ATP production, mitophagy, ROS homeostasis | |
| SIRT3 | Mitochondrial | Deacetylase | FOXO3a[a], IDH2, SOD2 | ROS homeostasis | Develop normally, but with metabolic abnormalities and cardiac hypertrophy with age |
| | | | ACS2, SDH | ATP production | |
| | | | VLCAD | Fatty acid oxidation | |
| | | | Many other mitochondrial proteins | Not reported | |
| SIRT4 | Mitochondrial | Mono-ADP-ribosylase | GDH | Glutamine metabolism | Develop normally, but with metabolic abnormalities |
| | | Lipoamidase | PDH | Tricarboxylic acid cycle | |
| SIRT5 | Mitochondrial | Deacetylase | CPS1 | Urea cycle | Develop metabolic abnormalities and hypertrophic cardiomyopathy |
| | | Deglutarylase | G6PD | ROS homeostasis | |
| | | Demalonylase | Malonyl-CoA | Fatty acid metabolism | |
| | | Desuccinylase | PDH, SDH | Glycolysis | |
| | | | IDH2 | ROS homeostasis | |
| | | | Many other targets | Not reported | |
| SIRT6 | Nuclear | Deacetylase | H3K9, H3K18, H3K27, H3K56, TRF2 | (Telomeric) chromatin remodelling | Die at 4 weeks of age |
| | | | KAT2A | Gluconeogenesis | |
| | | Deacylase | TNF | Cytokine secretion | |
| | | Mono-ADP-ribosylase | DDB2, PARP1 | DNA damage repair | |
| | | | SMARCC2 | Antioxidant defence | |
| SIRT7 | Nuclear (nucleolar) | Deacetylase | (Indirect) RNA polymerase I and II | Ribosomal biogenesis | Develop hypertrophic cardiomyopathy |
| | | | ATM, H3K18, p53[a] | DNA damage repair | |
| | | Desuccinylase | H3K122 | | |

ACS2, acetyl-CoA synthetase 2; APEX1, DNA-(apurinic or apyrimidinic site) endonuclease 1; ATM, serine-protein kinase ATM; CPS1, mitochondrial carbamoyl phosphate synthetase (ammonia); DDB2, DNA damage-binding protein 2; FOXO3a, forkhead box protein O3a; G6PD, glucose-6-phospate 1-dehydrogenase; GDH, glutamate dehydrogenase; H3K9, histone H3 lysine 9; H3K18, histone H3 lysine 18; H3K27, histone H3 lysine 27; H3K56, histone H3 lysine 56; H3K122, histone H3 lysine 122; H4K16, histone H4 lysine 16; IDH2, mitochondrial isocitrate dehydrogenase (NADP); KAT2A, histone acetyltransferase KAT2A; NF-κB, nuclear factor-κB; PARP1, poly(ADP-ribose) polymerase 1; PDH, pyruvate dehydrogenase; PGC1α, proliferator-activated receptor-γ coactivator 1α; ROS, reactive oxygen species; SDH, succinate dehydrogenase; SMARCC2, SWI–SNF complex subunit SMARCC2; SOD2, mitochondrial superoxide dismutase; TNF, tumour necrosis factor; TRF2, telomere repeat-binding factor 2; VLCAD, mitochondrial very long-chain specific acyl-CoA dehydrogenase; WRN, Werner syndrome ATP-dependent helicase; XPA, DNA repair protein complementing XP-A cells; XRCC6, X-ray repair cross-complementing protein 6. [a]Transcription factors.

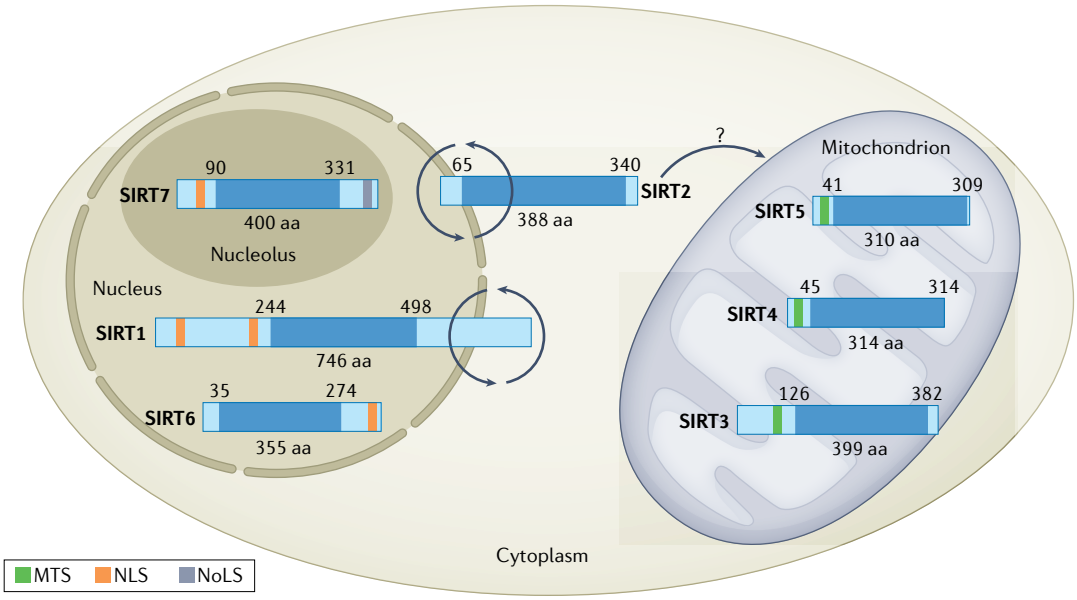


Fig. 1 | **Structure and localization of human sirtuins.** SIRT3, SIRT4 and SIRT5 are localized to the mitochondria and are characterized by a mitochondria-targeting sequence (MTS). The nuclear sirtuins (SIRT1, SIRT6 and SIRT7) carry a nuclear localization sequence (NLS), and SIRT7 is specifically found in the nucleolus and carries a nucleolar localization sequence (NoLS) in the carboxy terminus. SIRT1 can shuttle to the cytoplasm in response to specific cellular stimuli. SIRT2 is largely found in the cytoplasm, but can shuttle to the nucleus during mitosis via a process regulated by the C terminus and does not carry a clearly defined NLS signal. SIRT2 can also be found in the mitochondria, although no MTS has yet been identified in the primary sequence. Catalytic domains (dark blue boxes) and N-terminal and C-terminal extensions are shown in relative proportions. The numbers above the sirtuins relate to amino acid (aa) positions, and those below the sirtuins indicate the total protein length.

it epigenetically regulates the cell cycle by deacetylating histone H4 lysine 16 (REF.[42]) and controls a network of DDR proteins through deacetylation to maintain genomic stability[43]. SIRT2 also has an important role in mitochondrial function and redox homeostasis. For example, SIRT2 promotes SOD2 expression by deacetylating FOXO3a in response to oxidative stress[44] and might also be present in mitochondria, where it regulates protein acetylation and autophagic degradation (mitophagy)[45] (FIG. 2). Consistent with this function, mice with whole-body *Sirt2* knockout have metabolic abnormalities[46], although a mitochondria-targeting sequence has not yet been identified in SIRT2[15].

### *Mitochondrial sirtuins*

Mitochondrial quality control is vital for cell survival, energy production and metabolic homeostasis and for controlling inflammation. Mitochondrial dysfunction can lead to excess ROS production and chronic activation of inflammatory pathways (for example, NF-κB), which are important drivers of cardiovascular disease. Whereas nuclear sirtuins exert most effects via chromatin remodelling, mitochondrial sirtuins regulate cellular metabolism by direct deacetylation of mitochondrial proteins (FIG. 2).

***SIRT3.*** SIRT3 directly deacetylates and activates SOD2 (REF.[47]) and also promotes transcription of SOD2 and catalase via activation of FOXO3a[48]. SIRT3 is upregulated in response to calorie restriction, during which it increases mitochondrial glutathione antioxidant defence mechanisms by deacetylating and activating mitochondrial isocitrate dehydrogenase (NADP) (IDH2)[49]. SIRT3 also controls oxidative phosphorylation through deacetylation of several components of the tricarboxylic acid cycle and electron transport chain[50] and regulates fatty acid oxidation by deacetylating mitochondrial very long-chain specific acyl-CoA dehydrogenase (VLCAD)[51]. SIRT3 also has a crucial role in mitochondrial quality control by modulating mitochondrial mass, ATP production, mitophagy and protection against oxidative damage. These functions are at least partially the result of FOXO3a deacetylation[52], which controls the transcription of various mitochondrial proteins involved in mitochondrial biogenesis, mitochondrial fusion and fission, and mitophagy (FIG. 2).

***SIRT4.*** SIRT4 regulates cellular metabolism by post-translational modification of various mitochondrial proteins, including through its ADP-ribosyltransferase activity (FIG. 2). For example, ADP-ribosylation of glutamate dehydrogenase (GDH) by SIRT4 represses GDH activity and suppresses glutamine metabolism[53]. SIRT4 also has deacetylase[54], deacylase[55] and lipoamidase[56] functions. For example, the pyruvate dehydrogenase complex is a target of SIRT4 lipoamidase, preventing pyruvate from entering the tricarboxylic acid cycle, and this activity could be more important than the deacetylation function of SIRT4 (REF.[56]). Therefore, SIRT4 seems to oppose SIRT1 and SIRT3 and is downregulated in response to calorie restriction. This finding emphasizes the importance of SIRT4 as a control switch in

cellular metabolism, providing the cell with energy during reduced calorie intake. Blocking glutamine metabolism is also a crucial step in DDR pathways and requires SIRT4, such that loss of SIRT4 leads to increased glutamine-dependent proliferation, accumulation of DNA damage and tumorigenesis[57]. SIRT4 has been found in a few studies to be an endogenous inhibitor of certain sirtuins. For example, SIRT4 can compete with other sirtuins, including SIRT1, for $NAD^+$ as part of a mitochondrial retrograde signalling pathway, leading to decreased SIRT1 activity[58]. SIRT4 also competes with SIRT3 for SOD2 binding[59], and SIRT4 binding to SOD2 prevents its deacetylation by SIRT3, thereby increasing ROS accumulation and pathological cardiac remodelling[59]. However, the role of SIRT4, including its capacity to act as an endogenous inhibitor of other sirtuins, has never been studied in vascular cells specifically, nor in atherosclerosis.

***SIRT5.*** SIRT5 is upregulated in response to calorie restriction, has weak lysine deacetylation function[60], but has strong deacetylase, deglutarylase, demalonylase and desuccinylase activities. SIRT5 has hundreds of mitochondrial substrates with vital roles in energy

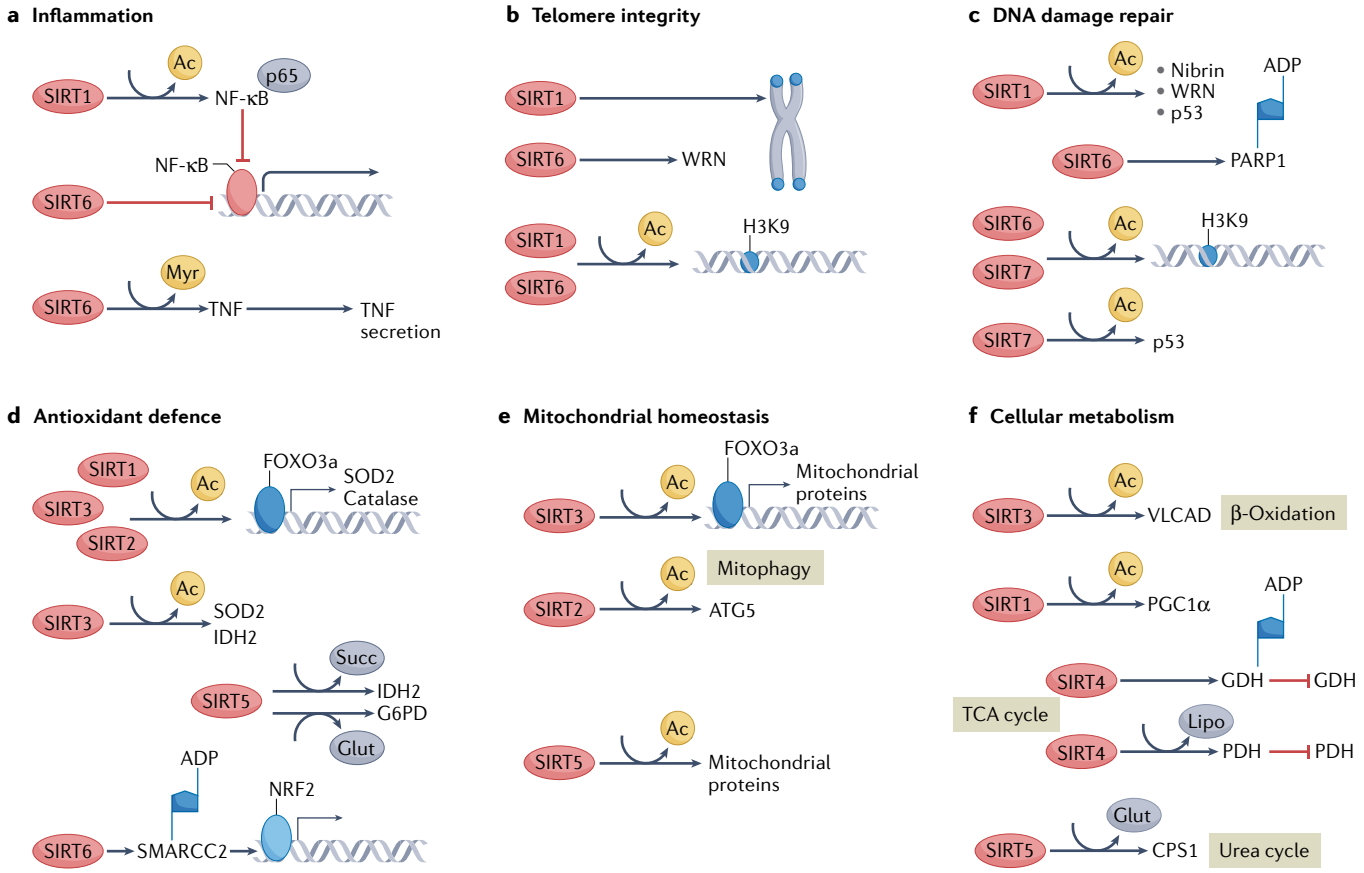


Fig. 2 | **Biological targets and cellular processes regulated by sirtuins.** **a** | Inflammation. SIRT1 inhibits inflammation by direct deacetylation of the nuclear factor-κB (NF-κB) p65 subunit, whereas SIRT6 binds to NF-κB, thereby repressing transcription of NF-κB target genes. SIRT6 can also promote secretion of tumour necrosis factor (TNF) through demyristoylation of TNF. **b** | Telomere integrity. SIRT1 binds to telomeric repeats and preserves telomere length. SIRT6 stabilizes Werner syndrome ATP-dependent helicase (WRN) during telomere DNA unwinding, thereby promoting telomeric DNA replication. Both SIRT1 and SIRT6 preserve telomere integrity by deacetylation of histone H3 lysine 9 (H3K9) in telomeric chromatin. **c** | DNA damage repair. SIRT1 facilitates DNA damage repair by deacetylating nibrin, WRN and p53. In response to oxidative stress, SIRT6 activates poly(ADP-ribose) polymerase 1 (PARP1) through mono-ADP ribosylation, thereby promoting PARP-mediated DNA repair. Both SIRT6 and SIRT7 promote DNA damage repair through H3K9 deacetylation. p53 is also a deacetylation target of SIRT7. **d** | Antioxidant defence. Both mitochondrial and non-mitochondrial sirtuins regulate antioxidant defence, either by promoting the expression of mitochondrial superoxide dismutase (SOD2) and catalase by activating forkhead box protein O3a (FOXO3a) through deacetylation, or by direct deacetylation of SOD2 or mitochondrial isocitrate dehydrogenase (NADP) (IDH2). IDH2 is also a target of SIRT5 for desuccinylation. SIRT5 promotes antioxidant defence through deglutarylation of glucose-6-phospate 1-dehydrogenase (G6PD). SIRT6 activates nuclear factor-erythroid 2-related factor 2 (NRF2) target genes via mono-ADP-ribosylation of SWI/SNF complex subunit SMARCC2 (SMARCC2). **e** | Mitochondrial homeostasis. SIRT3 regulates mitochondrial function by deacetylation and activation of FOXO3a, thereby promoting the expression of many proteins involved in mitochondrial dynamics and mitophagy. SIRT2 can also be found in mitochondria, where it positively regulates mitophagy via deacetylation of autophagy protein 5 (ATG5). SIRT5 has many targets for deacetylation that control mitochondrial homeostasis. **f** | Cellular metabolism. SIRT1 and SIRT3 control β-oxidation through deacetylation of proliferator-activated receptor-γ coactivator 1α (PGC1α) and mitochondrial very long-chain specific acyl-CoA dehydrogenase (VLCAD), respectively. SIRT4 inhibits the tricarboxylic acid (TCA) cycle by ADP ribosylation and lipomidation of glutamate dehydrogenase (GDH) and pyruvate dehydrogenase (PDH), respectively. SIRT5 also regulates the urea cycle by deglutarylation of mitochondrial carbamoyl phosphate synthetase (ammonia) (CPS1). Ac, acetyl; Glut, glutaryl; Lipo, lipoyl; Myr, myristoyl; Succ, succinyl.

metabolism[50]. However, the specific functions of SIRT5-induced post-translational modifications and their biological effects are unclear. Similar to SIRT3, SIRT5 has an important role in antioxidant defences by promoting the activity of the NADPH-producing enzymes IDH2 and glucose-6-phosphate 1-dehydrogenase (G6PD) through desuccinylation or deglutarylation, respectively[61] (FIG. 2). Surprisingly, mice lacking SIRT3, SIRT4 or SIRT5 develop normally, but show metabolic abnormalities under extreme metabolic stress, and can develop cardiomyopathy[50].

## Role of sirtuins in atherosclerosis

Atherosclerosis is a chronic inflammatory disease that results in arterial atherosclerotic plaques and leads to clinical complications such as myocardial infarction and stroke. Plaque formation is initiated by a series of events, including endothelial dysfunction, uptake of LDL cholesterol into the arterial wall, inflammation with infiltration of immune cells, and proliferation and migration of VSMCs into the lesion. The main risk factors for atherosclerosis include age, hypercholesterolaemia, smoking, hypertension and diabetes mellitus. Sirtuins modulate many of these risk factors, but also directly affect atherogenesis and plaque stability by regulating key pathological processes, including cell proliferation, cell senescence, DNA damage, oxidative stress, cellular metabolism and inflammation in lesions.

Studies have shown that sirtuins are downregulated in human atherosclerotic plaques (compared with normal arteries), in plaques from patients with diabetes (compared with plaques from individuals without diabetes)[62], in arteries from individuals (aged 64 ± 1 years) with increased vessel stiffness[63], in aortas of mice with hypertension[12,64] and in plaques of mice fed a high-fat diet (HFD)[13,65]. Although these observations do not differentiate cause and effect, they do indicate that downregulation of sirtuins could be pro-atherogenic. Indeed, sirtuins have major, and largely beneficial, effects on endothelial cells, VSMCs, macrophages and other immune cells, such as dendritic cells and T cells (FIGS 3,4), but whether all sirtuin isoforms have atheroprotective effects remains to be clarified.

### *Endothelial cells*

Endothelial cells are the first line of defence against lipid accumulation in the arterial wall. However, SIRT1 and SIRT6 are downregulated in endothelial cells by multiple disease-related stimuli, including hypertension[12], hyperglycaemia[66], hyperlipidaemia[13,65] and vascular ageing[63]. SIRT1 promotes endothelium-dependent vasorelaxation by deacetylating and activating endothelial nitric oxide synthase (eNOS), leading to increased NO production and vasodilatation in aortas from healthy rats[67] and HFD-fed mice[68]. Conversely, expression of a catalytically inactive SIRT1 mutant decreases NO bioavailability, confirming that the deacetylase activity of SIRT1 is crucial for its vasomotor effect (FIGS 3a,4). Interestingly, NO regulates SIRT1, creating a positive feedback loop between SIRT1 and eNOS to maintain endothelial cell function, although the NO-dependent protective effect of SIRT1 overexpression is lost in mice aged 45 weeks[69]. Instead, endothelial overexpression of SIRT1 prevents age-induced impairment of vasodilatory responses by upregulating soluble guanylyl cyclase signalling in VSMCs[69].

SIRT1 also inhibits endothelial cell apoptosis[68,69], senescence[66,70] and inflammation[71] (FIGS 3a,4). Cellular senescence is prevented via various mechanisms, including deacetylation of p53 (facilitating its degradation)[66,70] and deacetylation of the tumour suppressor serine/threonine protein kinase STK11 (also known as liver kinase B1), thereby antagonizing STK11-dependent 5′-AMP-activated protein kinase hyperactivation[72]. These actions suggest an atheroprotective role for SIRT1 and, indeed, endothelium-specific overexpression of human SIRT1 in HFD-fed, *Apoe*$^{-/-}$ mice reduces atherosclerosis[68], whereas *Sirt1* haploinsufficiency in mice is associated with increased endothelial cell activation in atherosclerotic plaques[71]. SIRT1 could also protect against atherothrombosis by downregulating tissue factor expression by reducing NF-κB activity in endothelial cells[73].

Like SIRT1, SIRT6 protects against endothelial dysfunction through pleiotropic effects on oxidative stress, DNA damage and inflammation (FIGS 3a,4). SIRT6 promotes endothelium-dependent vasodilatation[74,75] and regulates blood pressure by increasing NO bioavailability and inducing the expression of transcription factor GATA5 (REF.[12]) by inhibiting the transcriptional repressor homeobox protein Nkx-3.2 (REF.[12]). Loss of SIRT6 in endothelial cells results in growth arrest and cellular senescence, associated with upregulation of the cyclin-dependent kinase inhibitor p21 (REF.[76]) and accumulation of DNA damage, including to telomeres[77]. SIRT6 also inhibits endothelial cell apoptosis, promotes autophagy[12] and suppresses inflammation through various mechanisms, including decreased endothelial cell–monocyte adhesion and reduced expression of pro-atherogenic genes, such as *TNFLSF4* (REF.[74]). In vivo, SIRT6 depletion in endothelial cells augments hyperlipidaemia-induced vascular inflammation[78] and exacerbates atherosclerosis in HFD-fed, *Apoe*$^{-/-}$ mice[79]. Nevertheless, the role of SIRT6 in atherosclerosis could be complex, given that virus-based overexpression of SIRT6 increases endothelial cell growth, angiogenesis and intraplaque haemorrhage in an unstable carotid plaque tandem stenosis model in mice[80]. SIRT6 increases ROS in endothelial cells by sustained inhibition of catalase, and increases expression of hypoxia-inducible factor 1α (HIF1α)-dependent angiogenic factors by preventing HIF1α degradation[80]. These observations conflict with previous reports describing SIRT6 as an antioxidant defence mechanism and a corepressor of HIF1α transcriptional activity, albeit in different cells[33,34,81].

Despite the crucial role of mitochondrial sirtuins in regulating cellular metabolism and ROS generation, the few published studies found rather limited effects. For example, SIRT3 alters endothelium-dependent relaxation in HFD-fed mice[82], but does not affect atherosclerosis development[83]. Nevertheless, SIRT3 protects endothelial cells from oxidative damage by increasing SOD2 activity[82,84] (FIGS 3a,4), and reducing endothelial cell

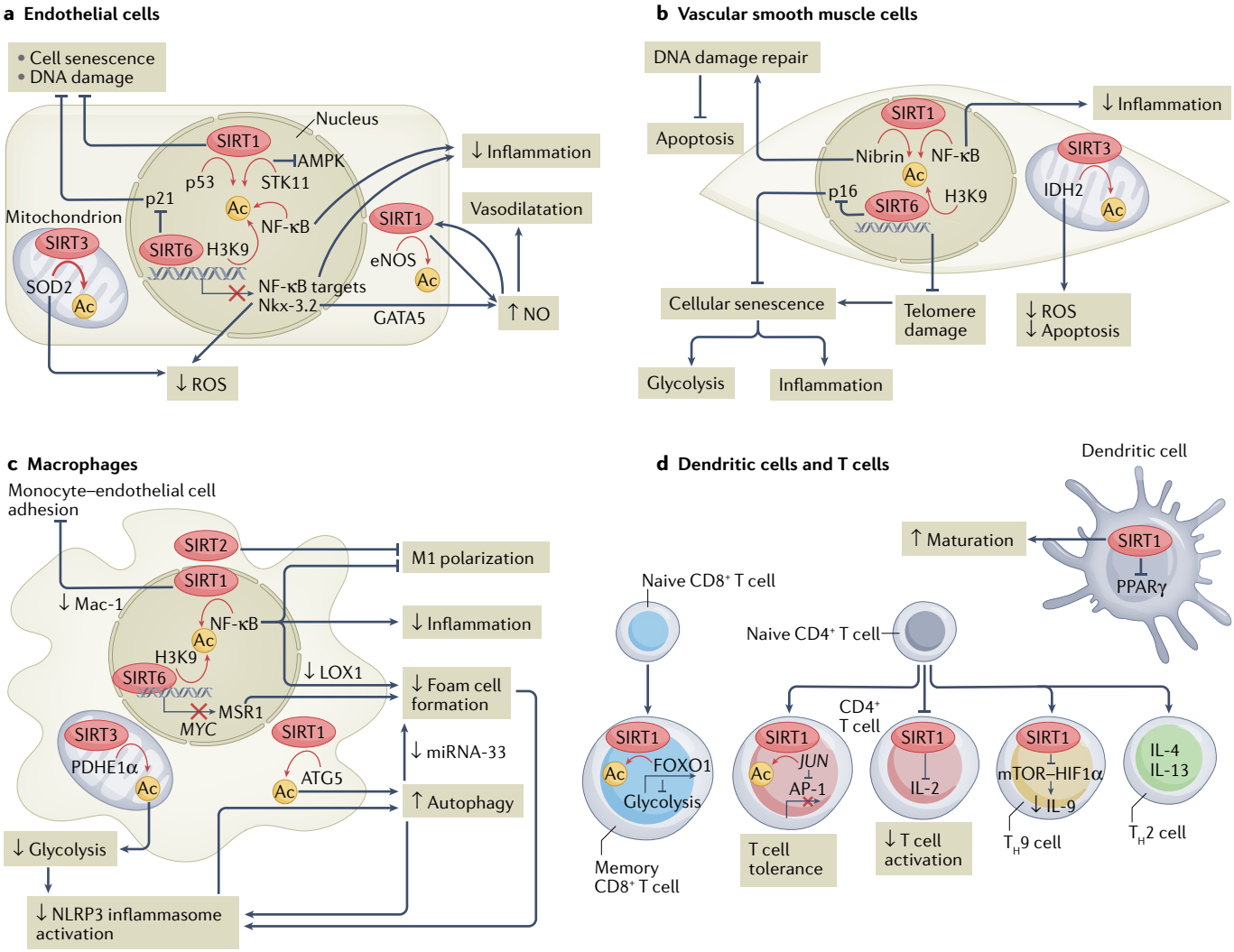


Fig. 3 | **The cellular roles of sirtuins. a** | In endothelial cells, SIRT1 and SIRT6 are important endogenous inhibitors of cellular senescence. SIRT1 prevents senescence by facilitating p53 degradation through deacetylation, or inhibiting 5′-AMP-activated protein kinase (AMPK) hyperactivation by serine/threonine protein kinase STK11 deacetylation. SIRT6 protects against DNA damage and p21-mediated cell cycle arrest. SIRT1 promotes nitric oxide (NO) production and vasodilatation by deacetylation and activation of endothelial NO synthase (eNOS); NO also regulates SIRT1, creating a positive feedback loop. SIRT1 reduces inflammation by direct deacetylation of the nuclear factor-κB (NF-κB) p65 subunit, whereas SIRT6 reduces inflammation by deacetylating histone H3 lysine 9 (H3K9) at NF-κB target gene promoters. SIRT6 promotes vasodilatation through H3K9 deacetylation and inhibition of homeobox protein Nkx-3.2, which is a transcriptional repressor of transcription factor GATA5, thereby increasing GATA5 expression and GATA5-mediated regulation of endothelial cell function. SIRT3 prevents the accumulation of mitochondrial reactive oxygen species (ROS) by deacetylating mitochondrial superoxide dismutase (SOD2). **b** | In vascular smooth muscle cells, SIRT1 promotes DNA damage repair via nibrin deacetylation, protecting against apoptosis. SIRT6 protects against telomere-specific DNA damage by deacetylation of H3K9 at telomeric chromatin, preventing p16-mediated cellular senescence associated with a metabolic shift to glycolysis and inflammation. SIRT1 reduces inflammation directly by deacetylation of NF-κB p65. SIRT3 reduces ROS levels and oxidative stress-induced apoptosis by increasing mitochondrial isocitrate dehydrogenase (NADP) (IDH2) activity via deacetylation. **c** | In macrophages, both SIRT1 and SIRT6 reduce NF-κB-dependent inflammation and foam cell formation via distinct mechanisms. SIRT1 reduces LDL uptake by reducing expression of oxidized LDL receptor 1 (LOX1), whereas SIRT6 represses transcription of macrophage scavenger receptor 1 (MSR1) via *MYC*. Foam cell formation can also be inhibited by activation of autophagy through SIRT1-mediated deacetylation and activation of autophagy protein 5 (ATG5) or by SIRT6-mediated cholesterol efflux in a microRNA-33-dependent manner. SIRT1 also limits monocyte–endothelial cell adhesion via reduced expression of macrophage-1 antigen (Mac-1), and both SIRT1 and SIRT2 can reduce macrophage polarization to a pro-inflammatory M1 phenotype. SIRT3 inhibits glycolysis by deacetylation of mitochondrial pyruvate dehydrogenase E1 component subunit-α, somatic form (PDHE1α), thereby preventing NLRP3 inflammasome activation. The latter could also be facilitated by SIRT3-dependent inhibition of foam cell formation. **d** | Sirtuin activity is important for dendritic cell maturation and stimulation of antigen-specific T cells. SIRT1 signalling in dendritic cells promotes a T helper 2 ($T_H2$) cell inflammatory response by repressing peroxisome proliferator-activated receptor-γ (PPARγ). In T cells, SIRT1 intrinsically inhibits CD4⁺ T cell activation, which is associated with reduced IL-2 production and represses cell differentiation of $T_H9$ cells by blocking mechanistic target of rapamycin (mTOR)–hypoxia-inducible factor 1α (HIF1α)-dependent glycolysis and IL-9 production. SIRT1 promotes induction of T cell tolerance by deacetylating the proto-oncogene *JUN*, thereby repressing activator protein 1 (AP-1) transcriptional activity. In memory CD8⁺ T cells, SIRT1 transcriptionally regulates glycolysis through deacetylation and activation of forkhead box protein O1 (FOXO1). Notably, none of these findings has been established in infiltrating dendritic cells or T cells in atherosclerotic plaques. Ac, acetyl.

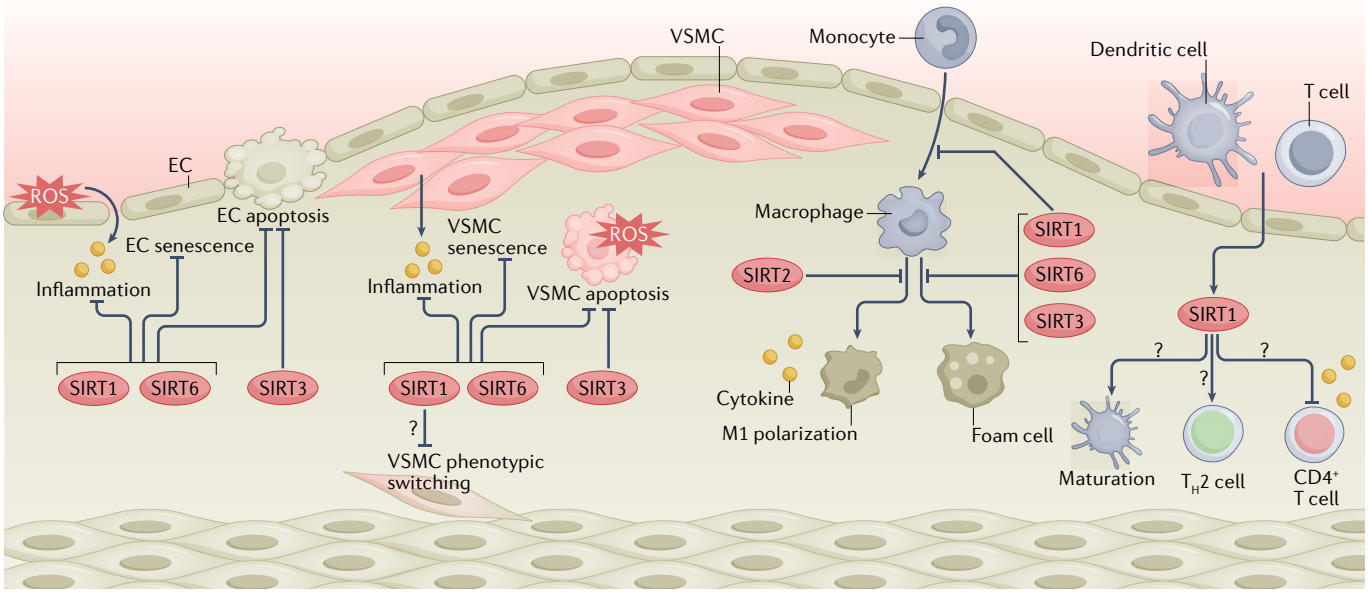


Fig. 4 | **The protective role of sirtuins in atherosclerosis.** SIRT1, SIRT3 and SIRT6 protect endothelial cells (ECs) from stressors, including oxidative stress and inflammation, thereby preventing apoptosis or cellular senescence. Sirtuins in vascular smooth muscle cells (VSMCs) prevent apoptosis and inflammatory signalling and inhibit cellular senescence. Whether sirtuins also control VSMC phenotypic switching is uncertain. In macrophages, SIRT1, SIRT3 and SIRT6 prevent foam cell formation via different mechanisms. SIRT1 also inhibits monocyte adhesion to ECs, and SIRT2 prevents polarization into a pro-inflammatory M1 macrophage phenotype. The role of sirtuins in dendritic cells and T cells that infiltrate the atherosclerotic plaque is unclear, but SIRT1 could positively regulate dendritic cell maturation and T helper 2 ($T_H2$) cell differentiation, and inhibit $CD4^+$ T cell activation. ROS, reactive oxygen species.

dysfunction could reduce age-dependent hypertension and arterial ageing[84].

### *Vascular smooth muscle cells*

During atherogenesis, arterial VSMCs switch from a contractile to a synthetic phenotype, proliferate, migrate into the lesion and form a fibrous cap. However, VSMCs in advanced atherosclerotic plaques show markers of DNA damage, telomere shortening and cellular senescence, all of which contribute to plaque instability[85]. The expression of SIRT1 and SIRT6 is reduced in human plaque VSMCs[13,65], in aortic VSMCs exposed to hyperlipidaemic agents (such as oxidized LDL or fatty acids) or hypertensive conditions[64], in atherosclerotic plaques of HFD-fed mice and in VSMCs undergoing (replicative) senescence[13,65]. SIRT1 regulates multiple processes in VSMCs, but with variable potency (FIGS 3b,4). For example, overexpression of SIRT1 increases the culture lifespan of human VSMCs only modestly, unless the $NAD^+$ salvage pathway is concomitantly boosted[86]. However, overexpression of NAMPT delays VSMC senescence through a mechanism mediated by SIRT1 (REF.[87]), suggesting that SIRT1 and $NAD^+$ biogenesis work together to control VSMC senescence. SIRT1 in VSMCs also protects against ROS-induced apoptosis and facilitates DNA damage repair by deacetylating and activating nibrin[65]; although surprisingly, p53 does not seem to be a major target of SIRT1 in VSMCs[65].

In vivo, VSMC-specific overexpression of an inactive human SIRT1 protein worsens atherosclerosis and some features of plaque instability in HFD-fed, *Apoe*$^{-/-}$ mice[65]. This finding was associated with increased DNA damage and apoptosis of VSMCs in the atherosclerotic plaque[65]. Conversely, VSMC-specific overexpression of SIRT1 reduces neointima formation after carotid artery ligation injury in mice[88], attenuates angiotensin II-induced vascular remodelling and hypertension associated with reduced ROS generation and inflammation in the arterial wall[64], and reduces aortic stiffness[89]. However, although SIRT1 can deacetylate NF-κB, the full contribution of SIRT1 in VSMCs to inflammation is not yet known.

Whereas SIRT1 regulates global DNA damage repair in VSMCs, SIRT6 has a more specific action through histone acetylation of telomeric chromatin[13] (FIGS 3b,4). Consequently, SIRT6 overexpression in VSMCs does not inhibit ROS-induced apoptosis, but prevents cellular senescence, increases lifespan and reduces telomere DNA damage. All these functions are lost in VSMCs overexpressing a catalytically inactive SIRT6 protein[13], emphasizing the importance of its deacetylase activity. SIRT6 also partially suppresses inflammatory cytokine expression and prevents a metabolic switch to glycolysis, although this function was seen only at senescence, suggesting that SIRT6 does not directly control energy metabolism and NF-κB-dependent inflammation in VSMCs[13]. In vivo, VSMC-specific SIRT6 overexpression reduces plaque size and cell senescence in HFD-fed, *Apoe*$^{-/-}$ mice, whereas expression of a SIRT6 inactive mutant increases several features of plaque instability[13].

Phenotypic switching of VSMCs occurs in atherogenesis[90], and SIRT1 activators can preserve the contractile phenotype of VSMCs[91,92], but the mechanisms involved and whether they require sirtuin deacetylase

activity at chromatin or other (unidentified) targets is not known. SIRT1 activation might also protect against DNA damage-induced VSMC calcification by activation of the DDR pathway[93], and VSMCs can transform into foam cells when exposed to lipids[90]. Whether SIRT1 or SIRT6 also reduce foam cell formation in VSMCs, as they do in macrophages, is not known (FIG. 4).

SIRT3 overexpression in human VSMCs protects against angiotensin II-induced ROS production and inflammation, which is associated with reduced activation of NF-κB and increased acetylation of the mitochondrial antioxidant defence protein IDH2 (REF.[94]). Reduced apoptosis in VSMCs overexpressing SIRT3 is likely to be the result of the (de)activation of a combination of pathways but, again, the precise mechanisms are unclear.

### *Macrophages*

Macrophage infiltration is one of the earliest events in atherosclerotic plaque formation, promoted by dyslipidaemia, endothelial cell upregulation of adhesion molecules, and release of inflammatory cytokines and chemokines by vascular cells. Although exposure of macrophages to oxidized LDL reduces SIRT1 and SIRT6 expression[95,96], some of these processes can be inhibited by sirtuins (FIGS 3c,4). For example, SIRT1 overexpression in monocytes inhibits adhesion to endothelial cells by reducing macrophage 1 antigen (Mac-1) expression[97]. In addition, SIRT1 overexpression prevents macrophage foam cell formation by reducing oxidized LDL receptor 1 (also known as lectin-like oxidized LDL receptor 1; LOX1) expression through suppression of NF-κB[98] or by promoting autophagy via the deacetylation of the autophagy protein 5 (ATG5)[95]. SIRT1 also represses the expression of many pro-inflammatory NF-κB target genes in macrophages through deacetylation of p65 (REF.[99]) (FIGS 3c,4).

Like SIRT1, SIRT6 reduces oxidized LDL uptake and foam cell formation by reducing the transcription of macrophage scavenger receptor type 1 (REF.[14]) and increasing autophagy and cholesterol efflux, which is at least partly mediated through a reduction in microRNA-33 levels[96]. SIRT6 depletion in bone marrow-derived cells increases atherosclerotic plaque formation, lipid content and macrophage accumulation in HFD-fed, *Apoe*$^{-/-}$ mice[14], confirming an anti-atherogenic role for myeloid SIRT6. Interestingly, myeloid-specific depletion of SIRT6 also promotes macrophage polarization towards a pro-inflammatory M1 phenotype by promoting NF-κB activation and IL-6 production[100].

Some mitochondrial sirtuins also regulate macrophage function through modulation of cellular metabolism, inflammation and foam cell formation. For example, SIRT2 overexpression reduces M1 macrophage polarization and plaque formation in HFD-fed, *Ldlr*$^{-/-}$ mice[101]. In addition, depletion of SIRT3 in macrophages induces activation of the NLRP3 inflammasome by shifting metabolism towards glycolysis through reduced deacetylation and deactivation of pyruvate dehydrogenase E1 component subunit-α, somatic form, mitochondrial[102]. SIRT3 depletion also increases foam cell formation and NLRP3 inflammasome activation in oxidized LDL-stimulated macrophages[103], although the latter could also be caused by impaired autophagy in response to reduced SIRT3 expression[104].

### *Dendritic cells and T cells*

A large and dynamic pool of both innate and adaptive immune cells infiltrate the atherosclerotic plaque at different stages of the disease, which can either promote or inhibit inflammation. The sirtuin–NAD$^+$ axis has a key regulatory role in metabolic rewiring of T cells and is important for the maturation of dendritic cells and their stimulation of antigen-specific T cells[105] (FIGS 3d,4). For example, SIRT1 promotes a T helper 2 ($T_H2$) cell inflammatory response by repressing peroxisome proliferator-activated receptor-γ (PPARγ) activity in dendritic cells, inhibits CD4$^+$ T cell activation, promotes T cell tolerance, and regulates the transcriptional metabolic reprogramming of CD8$^+$ memory T cells through FOXO1 (REF.[106]). Whether other sirtuins regulate T cell phenotype and response and any role for these processes in atherosclerosis are unclear.

## Sirtuin-modulating agents

In response to the predominantly beneficial effects of sirtuins in preclinical studies, considerable efforts have been made to discover and develop sirtuin-modulating compounds as potential therapeutics in cardiovascular and other diseases. However, human trials of both natural and synthetic sirtuin modulators have had mixed results (TABLE 2).

### *Preclinical studies*

***Sirtuin-activating compounds.*** Owing to the beneficial effects of SIRT1 on the cardiovascular system in animal studies, this sirtuin is a promising drug target[107]. Small molecules that activate sirtuins (sirtuin-activating compounds; STACs) were first described in 2003 (REF.[108]) and include resveratrol, a polyphenol present in red wine and grapes. Resveratrol is the most widely studied natural STAC and is a potent SIRT1 activator that can mimic calorie restriction and extend lifespan in yeast[108]. However, resveratrol can activate multiple sirtuins and, therefore, can have many downstream targets[108]. Similar concerns apply to other polyphenolic STACs, such as quercetin and curcumin[109]. The limited target specificity and bioavailability of these natural STACs has led to the development of synthetic SIRT1-activating compounds with an improved toxicity, bioavailability, selectivity and potency profile[110] (TABLE 2). For example, SIRT1 activators (such as SRT2104)[111] and new-generation small molecules (such as STAC-5, STAC-9 and STAC-10)[112] are 1,000 times more potent than resveratrol in inhibiting the deacetylase function of SIRT1 (REF.[111]). All STACs seem to rely on a shared mechanism of allosteric activation of SIRT1 at the N-terminal domain[113], but the binding mechanisms and differences between chemical moieties of the various STACs are beyond the scope of this Review and have been described previously[113–115].

The most convincing evidence for the health benefits of STACs comes from studies on hyperlipidaemic mice. Resveratrol extends the lifespan of HFD-fed mice[116], but not mice fed a normal diet, despite improving various

Table 2 | **Sirtuin-activating compounds and NAD$^+$ boosters tested in atherosclerosis**

| Compound | Sirtuin target | Effect on atherosclerosis | Additional information | Refs |
|---|---|---|---|---|
| ***Natural compounds: preclinical studies*** | | | | |
| Resveratrol | Activates SIRT1; might also activate SIRT5 and inhibit SIRT3 (plus non-specific targets) | ↓ Atherosclerosis, lipid levels, endothelial dysfunction and inflammation | Low oral bioavailability; low specificity | 120–124 |
| Curcumin | Activates SIRT1; many other targets | ↓ Atherosclerosis, inflammation, oxidative stress, foam cell formation, and VSMC migration and proliferation | Low aqueous solubility; low oral bioavailability | 109,166, 167 |
| Quercetin | Activates SIRT1 and SIRT6; might inhibit SIRT6 at low concentrations | ↓ Atherosclerosis, lipid levels, endothelial dysfunction, oxidative stress and inflammation<br>↑ Cholesterol efflux | Several quercetin derivatives act as sirtuin inhibitors; isoquercetin activates SIRT6 with improved specificity | 9,109, 168,169 |
| ***Natural compounds: clinical trials*** | | | | |
| Resveratrol | Activates SIRT1; might also activate SIRT5 and inhibit SIRT3 (plus non-specific targets) | ↓ LDL levels and platelet aggregation in patients with stable coronary artery disease | NA | 149 |
| | | ↓ Inflammatory biomarkers in healthy volunteers | NCT01244360 | 150 |
| | | ↓ Inflammatory and fibrinolytic status in patients at high risk of cardiovascular disease | NA | 151 |
| Curcumin | Activates SIRT1; many other targets | ↓ Atherogenic risk in patients with T2DM | NCT01052597 | 170 |
| | | ↓ Serum free fatty acid and triglyceride levels | NA | 171 |
| | | ↑ Blood antioxidant capacity | NA | 172 |
| Quercetin | Activates SIRT1 and SIRT6; might inhibit SIRT6 at low concentrations | ↓ Systolic blood pressure; no change in inflammatory biomarkers in women with T2DM | NA | 173 |
| ***Synthetic small molecules: preclinical studies*** | | | | |
| SRT1720 | SIRT1 activator | ↓ Atherosclerosis and endothelial dysfunction | Limited specificity, off-target effects | 125,126 |
| E1231 | SIRT1 activator | ↓ Atherosclerosis and plasma cholesterol level | NA | 127 |
| SRT3025 | SIRT1 activator | ↓ PCSK9 expression and LDL receptor expression | NA | 128 |
| ***Synthetic small molecules: clinical trials*** | | | | |
| SRT2104 | Highly specific SIRT1 activator | Well tolerated in healthy volunteers | NCT00933062 | 152 |
| | | ↓ Plasma cholesterol, LDL and triglyceride levels in elderly volunteers | NCT00964340 | 153 |
| | | No change in plasma glucose or insulin levels in patients with T2DM | NCT01018017 | 154 |
| | | ↓ Arterial stiffness; no change in blood pressure in smokers | NCT01031108 | 155,174 |
| SRT2379 | Highly specific SIRT1 activator | Well tolerated in healthy volunteers | NCT01416376 | 156 |
| SRT3025 | Highly specific SIRT1 activator | Tested in healthy volunteers; trial stopped owing to QT interval prolongation | NCT01340911 | – |
| ***NAD$^+$ boosters: preclinical studies*** | | | | |
| Nicotinamide | Reported as endogenous sirtuin inhibitor as well as sirtuin activator | ↓ Atherosclerosis, vascular inflammation and lipoprotein oxidation | NA | 139 |

Table 2 (cont.) | **Sirtuin-activating compounds and NAD⁺ boosters tested in atherosclerosis**

| Compound | Sirtuin target | Effect on atherosclerosis | Additional information | Refs |
|---|---|---|---|---|
| ***NAD⁺ boosters: preclinical studies (cont.)*** | | | | |
| Nicotinamide mononucleotide | Activates SIRT1 | ↓ Endothelial dysfunction, liver triglyceride levels, hypertension and vascular remodelling<br>↑ Insulin sensitivity | Tested only in aged or hypertensive mice | 140,141, 146,175 |
| Niacin | Not reported | ↓ Plasma LDL, VLDL and lipoprotein(a) levels and vascular inflammation<br>↑ Plasma HDL levels | NA | 142,176 |
| Nicotinamide riboside | Increases NAD⁺ level and sirtuin activity | ↓ LDL | Tested only in high-fat-diet-fed mice | 143 |
| NAMPT activators | Increases NAD⁺ level | Not reported | NA | 144 |
| CD38 inhibitors | Effect is dependent on NAD⁺ and sirtuins | ↓ Hypertension, vascular remodelling and VSMC senescence | NA | 146 |
| PARP inhibitors | Many effects are independent of NAD⁺ and sirtuins | ↓ Atherosclerosis | Likely to act on DNA damage repair and cell death | 147 |
| ***NAD+ boosters: clinical trials*** | | | | |
| Niacin | Not reported | No change in risk of cardiovascular morbidity or death | Controversial trial outcomes | 158,159 |
| Nicotinamide riboside | Not reported | ↓ Blood pressure and vascular stiffness; well tolerated in healthy volunteers | Ongoing for hypertension: NCT03821623, NCT04040959 and NCT04112043 | 160 |
| Nicotinamide mononucleotide | Not reported | Not reported | Ongoing for hypertension: NCT04903210 | – |

NA, not applicable; NAMPT, nicotinamide phosphoribosyltransferase; PARP, poly(ADP-ribose) polymerase; PCSK9, proprotein convertase subtilisin/kexin type 9; T2DM, type 2 diabetes mellitus; VSMC, vascular smooth muscle cell.

features of age-dependent tissue deterioration[117]. These findings suggest that the beneficial effects of resveratrol are more pronounced in animals with metabolic imbalances. Similarly, the SIRT1 activator SRT1720 delays age-related metabolic diseases and improves survival and healthspan in HFD-fed mice by increasing oxidative metabolism in multiple target organs[118,119]. By contrast, in vivo studies of STACs in atherosclerosis are surprisingly limited (TABLE 2). Resveratrol reduces atherosclerosis formation in *Apoe*$^{-/-}$ mice through its lipid-lowering and anti-inflammatory properties[120–123], but does not augment the anti-atherogenic effect of atorvastatin[124]. Therefore, whether the anti-atherosclerotic effects of resveratrol are due to sirtuin activation is unclear. Similarly, SRT1720 attenuates angiotensin II-induced atherosclerosis in *Apoe*$^{-/-}$ mice, with an associated reduction in vascular inflammation[125], and reverses endothelial dysfunction and inflammation in aged mice[126]. The small-molecule SIRT1 activator E1231 reduces atherosclerotic plaque development in *Apoe*$^{-/-}$ mice[127], whereas SRT3025 provides atheroprotection by reducing hepatic proprotein convertase subtilisin/kexin type 9 (PCSK9) secretion and increasing LDL receptor expression in the liver[128].

Although many STACs mimic the transcriptional or molecular changes induced by calorie restriction[117], it is difficult to prove in vivo whether their activity is dependent solely on SIRT1 or involves other sirtuins. For example, resveratrol also activates SIRT5, but inhibits SIRT3, and might have many other non-specific, cellular targets[129], and the effects of resveratrol on antioxidative defence, vasodilatation and activation of autophagy might depend only partially on SIRT1 (REF.[130]). In addition, resveratrol directly inactivates phosphodiesterase, which initiates a signalling pathway that leads to SIRT1 activation[131]. Resveratrol also upregulates NAMPT in human aortic VSMCs, which could promote sirtuin activity[132]. Owing to these limitations and uncertainties, only the newer SIRT1 activators (such as SRT2104, SRT2379 and SRT3025) with superior pharmacokinetic and pharmacodynamic properties have entered clinical trials.

A series of synthetic SIRT6 activators have been developed, including: UBCS039, which binds to a SIRT6-specific acyl channel pocket[8]; isoquercetin, a quercetin-based compound, which binds to and activates SIRT6 with increased specificity[9]; and 4H-chromen, which binds similarly to quercetin[10]. Two other small molecules (MDL-800 and MDL-801) have been identified as SIRT6 activators that increase the deacetylase activity of SIRT6 by up to 22-fold[11]. However, the allosteric binding mode of MDL-801 to SIRT6 has been questioned[133], and none of these SIRT6 activators has yet entered clinical trials.

***Sirtuin-inhibiting compounds.*** In addition to STACs, sirtuin-inhibiting compounds (STICs) have been developed for various conditions, including neurological disorders[134] and cancer. For example, small-molecule inhibitors of SIRT1 and SIRT2 reduce tumour growth by sensitizing tumour cells to chemotherapy[135]. SIRT6 inhibitors also potentiate the antitumour effects of other anticancer drugs[136,137] and exert immunosuppressive effects[137,138], and most inhibit SIRT6 deacetylase activity by competitive inhibition with the acetylated substrate[115]. Although not documented, the protective role of SIRT1 and SIRT6 in atherosclerosis would suggest that exacerbation of risk factors and progression of cardiovascular disease could be substantial and unpredictable adverse effects of STICs. Therefore, sirtuin inhibition would be unfavourable in atherosclerosis.

***$NAD^+$ boosters.*** As well as small molecules, three other approaches have been used to activate sirtuins through enlarging the $NAD^+$ pool: by boosting $NAD^+$ production through supplementation with NAD precursors, by increasing levels of enzymes required for $NAD^+$ synthesis (such as NAMPT) and by inhibiting other $NAD^+$-consuming enzymes (such as PARP family proteins and CD38) (TABLE 2).

Support for the first approach comes from studies showing that dietary nicotinamide supplementation reduces atherosclerosis, oxidation of apolipoprotein B-containing lipoproteins and vascular inflammation in *Apoe*$^{-/-}$ mice[139]. Similarly, supplementation with nicotinamide mononucleotide (NMN) reverses endothelial cell dysfunction and increases arterial SIRT1 activity in aged mice[140], and improves lipid plasma profile, insulin sensitivity and mitochondrial oxidative metabolism[141]. These studies strongly suggest that NMN has anti-ageing and anti-atherosclerotic effects, although its safety profile and effect on atherosclerosis are unclear. The $NAD^+$ precursor nicotinamide riboside and niacin (or vitamin $B_3$; a combination of nicotinamide and nicotinic acid) also reduce plasma cholesterol levels in HFD-fed mice[142,143], but whether this effect is dependent on $NAD^+$ and sirtuins is unclear. The second approach involves NAMPT activation to increase the production of NMN, and the NAMPT activator SBI-797812 has been shown to increase $NAD^+$ levels in the liver of mice[144]. The third approach targets the age-dependent decline in $NAD^+$ levels through downregulation of other $NAD^+$-consuming enzymes, such as CD38 (also known as ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 1), which is the main enzyme involved in NMN degradation in vivo. Improvement in age-related mitochondrial dysfunction following CD38 downregulation is at least partially due to increased SIRT3 activity owing to the greater availability of $NAD^+$ (REF.[145]). Supplementation with a CD38 inhibitor reduces hypertension, vascular remodelling and VSMC senescence in angiotensin II-treated mice[146]. PARP family proteins are also $NAD^+$-consuming enzymes, and PARP inhibitors have been successfully tested in atherosclerosis in mice. However, the anti-atherosclerotic effect of these agents might result from several different actions, not mediated via $NAD^+$ or sirtuins, including DNA damage repair, cell death, immune cell functions and lipid metabolism[147].

### *Clinical trials*

Currently, >20 clinical trials are being conducted on the safety and efficacy of resveratrol in cardiovascular diseases, including peripheral arterial disease, coronary artery disease, metabolic syndrome and diastolic heart failure. Resveratrol affects multiple molecular targets associated with cardioprotection[148], decreases LDL levels and inhibits platelet aggregation in patients with stable coronary artery disease[149]. Moreover, resveratrol decreases the expression of endothelial adhesion molecules and inflammatory biomarkers (such as plasma cytokines) in healthy volunteers[150]. In patients at high risk of cardiovascular disease, daily consumption of a grape resveratrol-containing nutraceutical for 1 year improved levels of inflammatory and fibrinolytic biomarkers[151].

Although resveratrol has demonstrated beneficial cardiovascular effects in some trials, others have found low bioavailability after oral intake, disturbing changes in plasma lipid levels and other, non-specific adverse effects[148]. Nevertheless, these trials suggest that sirtuin activation could be a valuable approach to the prevention of atherosclerosis and clinical events, particularly for next-generation small-molecule modulators of sirtuins that are far more potent and selective than resveratrol. To date, only small-molecule activators of SIRT1 have been studied in clinical trials, although expectations are high for SIRT6 activators given their potential health benefits.

The SIRT1 activator SRT2104 has been studied in 14 clinical trials, ranging from type 2 diabetes, inflammatory diseases (such as sepsis and psoriasis) and muscular atrophy. The first-in-human study of SRT2104 showed good tolerability[152] and decreased serum cholesterol, LDL and triglyceride levels after 28 days in a cohort of elderly (aged 60–80 years) volunteers, but no significant effect was observed on the oral glucose tolerance test response[153]. In patients with type 2 diabetes given 0.25 g, 0.5 g, 1.0 g or 2.0 g of SRT2104, the observed lack of effect on glucose or insulin control might have been caused by large variability in pharmacokinetics, which were not proportional to the dose[154]. Indeed, an additional study in patients with type 2 diabetes showed inconsistent, and predominantly neutral, effects of SRT2104 on endothelial function, with no discernible effects on plasma lipid levels[155]. However, SRT2104 was associated with a moderate improvement in arterial stiffness without changes in blood pressure in otherwise healthy smokers[155]. Similarly, the SIRT1 activator SRT2379 was found to be well tolerated in healthy volunteers but, despite showing promising anti-inflammatory effects in preclinical models, did not demonstrate a similar effect in endotoxin-induced inflammation in humans[156]. Consequently, further clinical development of SRT2379 was stopped. Similarly, SRT3025 was tested in healthy male volunteers for the treatment of metabolic diseases, but the trial was stopped owing to QT interval prolongation[157].

To date, >100 trials have been conducted with niacin to test its therapeutic potential in cardiovascular disease;

however, a meta-analysis published in 2019 revealed no association between niacin and reduced risk of cardiovascular morbidity and mortality[158]. In particular, the AIM-HIGH trial[159] showed that administration of extended-release niacin had no incremental benefit to statin therapy in patients with atherosclerotic cardiovascular disease and atherogenic dyslipidaemia. Clinical trials with the $NAD^+$ precursor nicotinamide riboside showed a good tolerability profile in healthy volunteers, and secondary outcomes suggested that this agent could reduce blood pressure and arterial stiffness[160], and more studies investigating the use of nicotinamide riboside for the treatment of hypertension and arterial stiffness[161–163], and a clinical trial with NMN in patients with hypertension[164], are currently underway. Successful preclinical studies of CD38 and PARP inhibitors in animal models of atherosclerosis are promising, although, to date, these drugs have undergone clinical trials only for some types of cancer.

## Future perspectives and concerns

Considerable efforts have been made over the past 15 years to develop potent, selective and safe sirtuin modulators. Early clinical data with some SIRT1 activators in atherosclerosis were less promising than expected, so improvements in pharmacokinetics and identification of activators of other sirtuins, particularly SIRT6, are needed. The ability of SIRT6 to modulate chromatin and deacylate multiple long-chain fatty acyl groups also opens new possibilities for targeting SIRT6 with small molecules. However, caution is necessary. Although sirtuin activators and $NAD^+$ boosters show promising results on surrogate end points — such as inflammatory markers, LDL levels and platelet function — no strong evidence exists that these approaches reduce the progression of atherosclerosis in humans or prevent clinical events. In addition, whether the effects of these agents are mediated through sirtuin activation or other pathways is unclear, and longer treatment regimens are required to assess safety.

Another point of concern is the pro-angiogenic effects of certain sirtuins (such as SIRT1 and SIRT6) and $NAD^+$ boosters (such as MNM and resveratrol)[7], where sirtuin activators could promote intraplaque angiogenesis and plaque vulnerability, thereby increasing the risk of clinical complications. Sirtuin activators might also stimulate cell proliferation and neovascularization and, therefore, carry a substantial risk of promoting tumour growth. Nevertheless, sirtuins also inhibit cell senescence and apoptosis, both of which are tumour-suppressor functions, and sirtuins can also repress the oncogene *MYC*[165], so the tumorigenic effects of sirtuin activators require future study.

Several questions also remain unanswered about the pharmacokinetic properties, tissue distribution and intracellular concentration of $NAD^+$ boosters. Specifically, studies are needed to investigate whether oral administration of $NAD^+$-boosting molecules can achieve therapeutic $NAD^+$ levels in a particular subcellular compartment or tissue, and which mechanisms (sirtuin-dependent or sirtuin-independent) underlie any beneficial effect in cardiovascular disease. Notwithstanding these concerns, the exciting discoveries on sirtuins and $NAD^+$ metabolism pave the way for future research into the development of new sirtuin-modulating agents and translation into human disease therapy.

## Conclusions

Sirtuins are important guardians of healthspan, protecting cells from stress and preventing genomic instability. Loss of sirtuins results in cellular dysfunction, senescence or cell death, all of which can promote arterial ageing. Endothelial cell dysfunction, vascular remodelling, hypertension and atherosclerosis are characterized by reduced sirtuin levels, whereas increasing sirtuin expression or activity has been shown to reduce disease development in animal models.

Although most sirtuins show beneficial effects in cells contained in atherosclerotic plaques and in experimental models of atherosclerosis, much is still unknown. Atherosclerosis affects the expression of many sirtuins; some might be protective and others might have opposing or synergistic effects. The difficulty lies in predicting the effects of therapeutic activation or inhibition of a single or multiple sirtuins on atherosclerosis and determining whether selectivity for a single sirtuin is desirable or not.

Despite the remarkable cardiovascular protective effects of sirtuins on disease-related cellular processes, clinical trials with sirtuin-modulating agents in cardiovascular disease have not shown reductions in clinical events. In particular, many compounds targeting SIRT1 have not demonstrated reproducible outcomes and reduce only a subset of risk factors without substantial disease-related improvement, and SIRT6 activators have not yet reached clinical trials. The use of $NAD^+$ boosters could provide an alternative solution to increase sirtuin activity in age-related diseases, but the pharmacokinetic profile and therapeutic potential of these agents in cardiovascular disease are unknown. Further understanding of the pleiotropic effects of sirtuins and their various targets is needed for the development of new compounds and to design new mechanisms of sirtuin modulation for the treatment of cardiovascular disease.

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**Author contributions**
M.O.J.G. researched data for the article. Both authors discussed its content and wrote the manuscript. M.R.B. reviewed and edited the manuscript before submission.

**Competing interests**
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