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Are sirtuins viable targets for improving healthspan and lifespan?

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Abstract

Although the increased lifespan of our populations illustrates the success of modern medicine, the risk of developing many diseases increases exponentially with old age. Caloric restriction is known to retard ageing and delay functional decline as well as the onset of disease in most organisms. Studies have implicated the sirtuins (SIRT1–SIRT7) as mediators of key effects of caloric restriction during ageing. Two unrelated molecules that have been shown to increase SIRT1 activity in some settings, resveratrol and SRT1720, are excellent protectors against metabolic stress in mammals, making SIRT1 a potentially appealing target for therapeutic interventions. This Review covers the current status and controversies surrounding the potential of sirtuins as novel pharmacological targets, with a focus on SIRT1.

Few — if any — pharmacological therapies consistently delay ageing and extend lifespan across taxa. Such a therapy is highly desirable as it is likely to delay or prevent the onset of diseases that are inexorably linked to the ageing process, such as dementia, cancer, diabetes

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Competing interests statement

The authors declare no competing financial interests.

FURTHER INFORMATION

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mellitus, osteoporosis and vascular disease. To date, most interventions have been based on targeting processes that are hypothesized to contribute directly to ageing, such as oxidative stress and hormone depletion, but these approaches have generally failed¹.

More recently, attempts have been made to harness the recognized benefits of caloric restriction on longevity and preservation of health. Caloric restriction is the only intervention that is known to retard ageing in most organisms and delay the onset of disease and functional decline in mammals. Research from the 1930s by Clive McCay and colleagues^{2–4} established the effectiveness of caloric restriction in extending the lifespan of rats. Subsequent studies have demonstrated that sustained reductions in caloric intake can increase maximum lifespan in a range of species⁵. Caloric restriction therefore represents the most robust intervention in ageing research, and because caloric restriction is so successful at promoting health and longevity in laboratory animals there has been interest in the potential for caloric restriction to extend lifespan in humans⁶. Although there is debate as to whether caloric restriction will be as effective in humans as it is in shorter-lived research models^{7,8}, data from non-human primates suggest that caloric restriction can improve the quality of life, reduce the risk of disease and delay mortality⁹.

Of course, the major hurdle for caloric restriction in humans is the inability of most people to voluntarily reduce their caloric intake by an amount that is likely to influence their ageing while maintaining adequate nutrition. Thus, there has been an increasing focus on developing pharmacological agents that can replicate the beneficial effects of caloric restriction on longevity without the need for changing dietary intake. Such agents have been termed caloric restriction mimetics (CRMs)⁶. Cellular processes that are implicated in ageing demonstrate considerable overlap across species, which implies that interventions including CRMs might be usefully evaluated in short-lived animals as a screen for potential therapies in humans¹⁰. Various candidate CRMs are already under investigation in animal models^{6,11}. Fortunately, caloric restriction has been shown to increase lifespan even when applied late in life, albeit with diminished effect¹², so a true CRM may offer tangible benefits to middle-aged and older individuals. If the beneficial effects of caloric restriction could be extrapolated to humans, it would generate a greater improvement in lifespan than most — if not all — other interventions currently in practice or under investigation¹³ (BOX 1).

Several signalling pathways have been reported to mediate and/or modulate the effects of caloric restriction on ageing¹⁴. Various individual components of these pathways have been validated as targets for drug development through genetic manipulation studies in model organisms. One intriguing target to emerge from such studies is sirtuin 1 (SIRT1), a protein that functions at a regulatory crossroad between nutrient sensing, energy metabolism and genome stability^{15–18}. SIRT1 is one of seven mammalian sirtuins, which comprise a conserved family of NAD-dependent deacetylases and ADP-ribosyltransferases that was named after the founding member, the *Saccharomyces cerevisiae* Sir2 (silent information regulator 2) protein.

Deletion of Sir2 in lower organisms appears to interfere with the beneficial effects of caloric restriction in some experimental settings^{19–21}, but Sir2-independent lifespan extension in

response to caloric restriction has also been demonstrated in yeast and worms^{22,23}. Increased expression of *Sir2* homologues has been found to be sufficient to extend lifespan in yeast²⁴, worms²⁵ and flies^{20,26}. However, a recent study did not replicate these findings in worms or flies, leading the authors to suggest that the earlier studies had not controlled adequately for the genetic background, and to question the potential role of *Sir2* in modulating lifespan²⁷. The two laboratories that initially reported these findings have each performed studies using improved controls for the genetic background, with the results supporting their original conclusions that *Sir2* overexpression extended lifespan, albeit with a diminished effect in worms^{26,28}. Thus, sirtuins have emerged as an intriguing but controversial class of enzymes among the potential mediators of caloric restriction.

SIRT1 is an unusual target for drug development because it exerts many different and unrelated effects that are relevant to health and could have a role in lifespan modulation via caloric restriction. These include: promoting insulin sensitivity²⁹, modulating circadian rhythms^{30,31}, improving genome stability¹⁷, suppressing tumours³², reducing inflammation³³, protecting against neurodegenerative diseases^{34,35} and even controlling anxiety in mice³⁶. However, this may ultimately be beneficial, or even necessary, given the multifactorial pathogenesis of ageing and ageing-associated diseases^{13,37}.

The first SIRT1 activator to be widely studied was resveratrol, which is a small polyphenol that was identified using an *in vitro* screen³⁸. Resveratrol has been found to have many effects that are consistent with SIRT1 activation and promising from a drug development standpoint; for example, it improves insulin sensitivity, inhibits tumour growth, suppresses inflammation, promotes cardiovascular health and protects against neurodegenerative diseases^{39–42}. Moreover, resveratrol mimics transcriptional profiles associated with caloric restriction^{43,44} and prevents early mortality in obese mice³⁹. However, resveratrol has many targets in mammalian cells, and its ability to activate SIRT1 *in vitro* is dependent on the use of fluorescent substrates, calling its mechanism of action into question^{45,46}.

The development of sirtuin-activating compounds (STACs) with improved bioavailability and specificity for sirtuin activation is a growing field in medicinal chemistry, and SIRT1 activators have recently been described that are 1,000 times more effective *in vitro* than resveratrol⁴⁷. Like resveratrol or overexpression of SIRT1, the novel STAC SRT1720 increases healthspan, improves insulin sensitivity and alleviates other harmful effects of obesity in mice^{48,49}, and several STACs have already entered clinical trials (see the ClinicalTrials.gov website). Nevertheless, SRT1720 and several other STACs also exhibit substrate-specific effects on SIRT1 activity *in vitro*, fuelling continued debate about their mechanism of action^{50–52}. Despite the uncertainty surrounding the pharmacological manipulation of SIRT1 activity, studies in knockout animals have shown that the absence of SIRT1 causes metabolic derangements⁵³ and infertility⁵⁴, and impairs normal cognitive function⁵⁵, whereas its overexpression induces various protective effects against metabolic and other stresses (TABLE 1), making it clear that this enzyme has a major role in mammalian physiology.

Thus, improving our understanding of the myriad functions of SIRT1 and the development of pharmacological interventions that specifically target SIRT1 may have applications in the prevention and treatment of human age-related diseases, and perhaps even ageing itself.

Mammalian sirtuins

Without a doubt, sirtuins and drugs that act on sirtuins have become hot topics in biomedical research during the past decade^{13,56,57}, as demonstrated by the almost exponential growth in the number of manuscripts appearing in scientific publications. Over the past 30 years, sirtuins have emerged from a seminal observation on gene silencing in yeast⁵⁸ to become the centre of a heated debate about their functions, roles in drug responses and potential as targets for therapeutic applications in humans.

Mammalian sirtuins are differentially located within the cellular compartments and have different biochemical activities and molecular masses (TABLE 1). SIRT1 and SIRT6 are predominately found in the nucleus (SIRT1 is also found in the cytosol), whereas SIRT7 is located within the nucleolus⁵⁹. SIRT2 is predominantly located in the cytoplasm^{60,61}, whereas SIRT3, SIRT4 and SIRT5 are localized to the mitochondria⁶². Regarding their biochemical properties, SIRT1, SIRT2, SIRT3 and SIRT6 exhibit NAD-dependent deacetylase activity, although their catalytic efficiency and substrate specificities vary^{61,63}. SIRT4 and SIRT6 are ADP-ribosyltransferases^{57,64,65}. Intriguingly, SIRT5 was recently shown to have desuccinylase and demalonylase activity that appeared to be more physiologically relevant than its modest deacetylase activity, leading to a potential redefinition of sirtuins as 'deacylases' rather than deacetylases⁶⁶. SIRT7 was described as a tumour suppressor p53 deacetylase⁶⁷, but this finding contradicted an earlier study⁵⁹ and has not been followed up in detail, leaving some doubt as to the catalytic activity of this sirtuin (TABLE 1).

The biochemical and biological functions of sirtuins are coupled to the metabolic state of a cell or tissue via their dependence on NAD⁺^{68,69}. The mitochondrial sirtuins, SIRT3–SIRT5 (REFS 59,70), contribute to the regulation of ATP production, metabolism, apoptosis and cell signalling⁷¹. SIRT3 is responsible for deacetylating the majority of acetylated mitochondrial proteins; SIRT4 ADP-ribosylates substrates including glutamate dehydrogenase; and SIRT5 may demalonylate, desuccinylate and possibly deacetylate a variety of substrates including carbamoyl phosphate synthase 1 (REFS 64,66,72,73).

The literature on mitochondrial sirtuins is not as extensive as for SIRT1. However, recent reports illustrate the importance of SIRT3, SIRT4 and SIRT5 in the regulation of antioxidant defences, energy metabolism and other aspects of mitochondrial biology^{74,75}. Interestingly, SIRT3 and SIRT6 have also been implicated as potential regulators of longevity (BOX 2). Furthermore, a growing number of mitochondrial proteins are being found to be regulated via acetylation and/or deacetylation^{72,75,76}, and no other class of deacetylase has been described in the mitochondria, indicating that mitochondrial sirtuins are likely to regulate a multitude of mitochondrial processes, from energy production to apoptosis. Although a detailed discussion of the biology and biochemistry of each of the seven mammalian sirtuins

is beyond the scope of this Review, this subject has been covered extensively in the literature^{57,70,77,78}.

SIRT1 deacetylates lysine residues in histone 1, histone 3 and histone 4, indicating a potential role for SIRT1 in the age-dependent regulation of transcription and genomic stability via chromatin modification^{68,79,80}. However, many of the roles of SIRT1 that are attractive for therapeutic purposes involve deacetylation of nonhistone targets to regulate metabolism and metabolic diseases. SIRT1 sits at the crossroads of nutrient (energy) sensing and various adaptive pathways that regulate stress resistance and metabolism, suggesting that it might be well positioned to modulate healthspan during caloric restriction (FIG. 1). In line with this role, sudden or prolonged changes in nutrient availability (for example, fasting or caloric restriction) or food quality (for example, a high-fat diet) evoke pronounced changes in the activity and expression of SIRT1 and its targets. Furthermore, induced overexpression of SIRT1 (tissue-specific or general; TABLE 2) or treatment with structurally unrelated chemical activators of SIRT1 (TABLE 3) results in benefits including protection against insulin resistance induced by a high-fat diet, improved cardiac function and protection from ischaemic injuries, suppression of multiple tumour types and improved vascular function^{29,32,81–86}.

Given the controversy surrounding the pharmacological activation of SIRT1, and the promiscuous effects of resveratrol in particular, it is important to consider that SIRT1-independent mechanisms may be responsible for the outcomes listed in TABLE 3. In contrast to the effects of increased SIRT1 activity, loss of SIRT1 function impairs energy metabolism and cognition while blocking some of the benefits of caloric restriction on insulin sensitivity and possibly also on lifespan^{16,53,55}. Examples of key pathways controlled by SIRT1 that are likely to be implicated in its beneficial effects on metabolism and ageing include: downregulation of p53 activity^{63,87}; suppression of nuclear factor- κ B (NF- κ B)-mediated inflammatory pathways⁸⁸; modulation of forkhead box protein O (FOXO) transcription factors^{21,89,90}; suppression of adipogenesis pathways mediated by peroxisome proliferator-activated receptor- γ (PPAR γ)⁹¹; activation of PPAR γ co-activator 1 α (PGC1 α), thus promoting fat mobilization and increasing mitochondrial size and number^{92,93}; and promotion of insulin secretion through the suppression of mitochondrial uncoupling protein 2 in pancreatic β -cells⁹⁴.

There are many other targets of SIRT1 that are involved in stress responses, inflammation, DNA repair and circadian rhythms, as well as a myriad of regulatory proteins of energy utilization that place SIRT1 as a key node for metabolic and stress response regulation, and support a role for SIRT1 in ageing in humans^{37,57,95} (FIG. 1). Consistent with studies in animals, genetic variation in the human *SIRT1* locus has been correlated with an increased incidence of obesity and type 2 diabetes⁹⁶, and possibly also longevity⁹⁷.

SIRT1, resveratrol and disease

Given the many different targets and pathways that are known to be modulated by SIRT1, activation of this enzyme has been suggested to have beneficial effects in several disease processes, many of which have been studied using the small polyphenolic STAC resveratrol.

Preparations containing resveratrol have been a part of traditional Chinese and Japanese medicine for millennia. However, the molecule itself was first isolated from white hellebore in 1940 (REF. 98), and received little attention until 1997 when it was identified in a screen for cyclooxygenase inhibitors and shown to have cancer-chemopreventive activity in mice⁴¹. In 2003, resveratrol was the top hit in another screen designed to identify activators of sirtuin enzymes, and was subsequently shown to extend lifespan in yeast³⁸.

Resveratrol has similarly been shown to extend lifespan in worms⁹⁹ and flies, in a Sir2-dependent manner. These findings have been disputed^{46,100} but they have also been reproduced at least once for all three organisms^{101–103}. An additional study showed that resveratrol extends lifespan in a short-lived species of fish¹⁰⁴. In rodents, two independent studies have concluded that the transcriptional response to resveratrol strongly resembles the response induced by caloric restriction^{43,44}. Despite these similarities, neither resveratrol nor overexpression of SIRT1 has been found to extend lifespan in mice^{44,83}, whereas caloric restriction does¹⁰⁵. However, resveratrol does restore normal lifespan in mice that are fed a high-fat diet to induce obesity and features of diabetes^{39,44}.

Heightened interest in resveratrol has led to the discovery of additional targets beyond sirtuins and cyclooxygenases. These include several kinases, the oestrogen and aryl hydrocarbon receptors, cytochrome P450 enzymes, quinone reductase 2 (REF. 13), the F1-ATPase¹⁰⁶ and, most recently, phosphodiesterases (PDEs)¹⁰⁷. Resveratrol also activates AMP-activated protein kinase (AMPK). AMPK activation can be a downstream consequence of SIRT1 activation¹⁰⁸. However, activation of AMPK by resveratrol does not require SIRT1 (REF. 109) nor is it a direct effect of the molecule³⁹. Together, these observations have caused considerable confusion as to the precise mechanism (or mechanisms) of action of resveratrol. In particular, there has been an intense debate surrounding the importance of sirtuin enzymes in mediating the effects of resveratrol.

Although many of the effects of resveratrol that are observed in rodents are consistent with sirtuin activation, and many of its effects in cell culture can be abrogated by SIRT1 inhibition, the *in vitro* effect of resveratrol on SIRT1 activity has been questioned and been found to be highly substrate-dependent^{45,46}. Specifically, the initial report that identified resveratrol as an activator of SIRT1 used the Fluor-de-Lys assay, which relies on the use of a fluorescently tagged substrate. Surprisingly, when a similar assay was performed using a non-fluorescent substrate, activation of SIRT1 by resveratrol was not observed^{46,108}. This has alternatively been interpreted to suggest that the activation of SIRT1 in the Fluor-de-Lys assay is artefactual, or that the fluorescent moiety better mimics bulky and/or hydrophobic substrates or the involvement of SIRT1 binding partners. In support of the contention that endogenous substrates could behave as predicted by the Fluor-de-Lys assay, a class of molecules that bind at the same site as resveratrol activate SIRT1 against substrates that contain only natural amino acids⁵⁰.

However, the same has not been shown for resveratrol itself; notably, scientists from Amgen and Pfizer have shown that resveratrol did not activate SIRT1 *in vitro* in the presence of native substrates (such as p53 and PGC1 α), calling further into question its ability to directly activate SIRT1 (REFS 52,110). Based in part on these findings, the possibility has been

raised that SIRT1 activation by resveratrol could occur indirectly via the activation of AMPK or another unknown mechanism^{13,111–113}. Recently, Park *et al.*¹⁰⁷ have provided support for the possibility of an AMPK-dependent mechanism by showing that resveratrol can inhibit cyclic AMP-specific PDEs and by delineating a multistep pathway by which increased cAMP triggers activation of AMPK (see below).

Directly determining SIRT1 activity in the tissues of animals treated with resveratrol (and other potential SIRT1 modulators) has proven to be challenging. In most cases, deacetylation of downstream targets, such as PGC1 α , FOXO or the NF- κ B subunit p65 (also known as RELA), has been used as a proxy. Although these assays do provide some evidence for increased SIRT1 activity, they do not confirm that direct binding to SIRT1 is the mechanism of action of these compounds, nor do they exclude the alternative possibility that acetyltransferases are inhibited. Resolving this debate and developing a rapid and reliable SIRT1 activity assay remain important challenges for the field.

Regardless of the controversy about its mode of action, resveratrol has been confirmed to have numerous health benefits in various species, as discussed below.

Metabolic disease

Resveratrol substantially improves metabolism in mice and protects them from the negative consequences of an obesogenic diet, including insulin resistance and decreased lifespan³⁹. Although resveratrol does cause weight loss at higher doses¹¹⁴, insulin sensitivity and lifespan are restored even by lower doses that do not cause weight loss³⁹. In addition, resveratrol prevents the development of fatty liver, provides numerous cardiovascular benefits (discussed in more detail below), increases motor coordination and improves bone health^{44,115}. Whether or not mice are fed a high-fat diet, resveratrol causes a striking increase in endurance and improves tolerance to cold temperatures, suggesting improved mitochondrial function¹¹⁴. Indeed, increased mitochondrial function has been reported in skeletal muscle, brown fat and in the liver following resveratrol treatment, and these changes may contribute to many of the beneficial effects that have been reported for resveratrol^{39,114}.

The primary driver of increased mitochondrial biogenesis in resveratrol-treated mice appears to be the transcriptional co-activator PGC1 α , which is a direct deacetylation target of SIRT1 and a ‘master regulator’ of mitochondrial biogenesis^{39,114}. Deacetylation of PGC1 α has been reported in multiple tissues of resveratrol-treated mice, and transcription of many of its target genes is increased. There is also a shift towards more oxidative fibre types in skeletal muscle, which is a well-known effect of PGC1 α activation¹¹⁴. Intriguingly, overexpression of PGC1 α in skeletal muscle is sufficient to confer increased lifespan and slow many age-related changes¹¹⁶, supporting the idea that this may be a key mechanism by which resveratrol confers beneficial effects on metabolism. However, given the large number of targets that have been identified for both resveratrol and SIRT1, an enormous amount of work remains before definitive mechanisms can be assigned to the observed benefits^{95,115}.

Importantly, many of the effects of resveratrol are blocked in mice that are deficient for AMPK, including the increases in metabolic rate, insulin sensitivity, mitochondrial biogenesis and endurance¹¹⁷. AMPK stimulation by resveratrol may be secondary to SIRT1

activation, as SIRT1 deacetylates and activates the upstream kinase liver kinase B1 (REF. 108). However, AMPK activation by resveratrol can also occur independently from SIRT1 (REF. 109), and could even lie upstream of SIRT1, as AMPK stimulates the expression of nicotinamide phosphoribosyltransferase (NAMPT), which leads to increased production of NAD — the co-substrate for SIRT1 (REF. 113). SIRT1-independent activation of AMPK by resveratrol has been suggested to occur as a result of direct inhibition of oxidative phosphorylation in mitochondria, leading to a rise in levels of intracellular AMP¹¹¹. Such an effect would require a very high concentration of resveratrol in tissues and would have to be transient, given the increased mitochondrial activity observed in resveratrol-treated mice^{114,117}.

A stronger possibility is that resveratrol might trigger the activation of AMPK through its recently described ability to directly inhibit cAMP-specific PDEs¹⁰⁷. This effect occurs at lower doses than those required to inhibit electron transport, although the concentrations at which *in vitro* effects were reported still exceed the plasma concentrations of resveratrol that are observed *in vivo*¹¹⁸. The report¹⁰⁷ describes a multistep mechanism leading from cAMP activation to AMPK activation and, impressively, shows that many of the benefits of resveratrol are recapitulated using rolipram, which is a specific inhibitor of PDE4 — the major PDE isoform in muscle. Interfering with this mechanism prevents resveratrol-induced deacetylation of PGC1 α in cells, and rolipram reduces PGC1 α acetylation *in vivo*. Based on this evidence, Park *et al.*¹⁰⁷ place SIRT1 downstream of AMPK, although the requirement for SIRT1 in the metabolic benefits of resveratrol is not directly tested. In addition, AMPK can phosphorylate PGC1 α directly¹¹⁹, thus potentially triggering downstream effects without any requirement for SIRT1.

Unfortunately, owing to the poor viability of SIRT1, along with developmental and metabolic abnormalities in SIRT1-null mice^{54,120}, it is difficult to perform experiments to definitively test the dependence of resveratrol on SIRT1 for its effects on metabolism. Determining the role of SIRT1 has become increasingly important, as it has been appreciated that SIRT1 and AMPK are mutually reinforcing and interdependent for their full effects¹¹².

To address this problem, Price *et al.*¹²¹ recently used a tamoxifen-inducible strategy to delete SIRT1 in adult animals, thus bypassing many of the caveats associated with constitutive knockout models. Interestingly, SIRT1 deletion was sufficient to block many of the effects of resveratrol on mitochondrial biogenesis and mitochondrial function in skeletal muscle. Conversely, the experiments revealed improvements in glucose homeostasis following resveratrol treatment even in animals that lacked SIRT1. In agreement with previous studies¹¹⁷, the effects of resveratrol were found to be correlated with AMPK activation *in vivo*, and required AMPK *in vitro*. However, two observations suggested that SIRT1 might be upstream rather than downstream of AMPK in the mediation of these effects. First, SIRT1 was required for the activation of AMPK by lower doses of resveratrol (although increasing the dose led to SIRT1-independent AMPK activation). Second, overexpression of SIRT1 was sufficient to activate AMPK and trigger mitochondrial biogenesis *in vivo*.

These findings suggest that SIRT1 may activate AMPK directly or through another mechanism, such as protein kinase A-dependent phosphorylation¹²². Alternatively, the absence of SIRT1 might dampen the ability of AMPK to respond to other independent stimuli. Further *in vivo* studies will be required to fully elucidate the roles of SIRT1 and AMPK in each of the beneficial effects of resveratrol, along with the upstream mechanisms that contribute to their activation.

At present, only a limited number of studies have been carried out on the effects of resveratrol in humans but several encouraging reports have been published, suggesting that at least some of the metabolic benefits seen in mice may be similarly observed in humans¹²³. Brasnyo *et al.*¹²⁴ reported that administration of a low dose of resveratrol (5 mg) twice daily for 4 weeks improved insulin sensitivity and decreased oxidative stress. Timmers *et al.*¹¹⁸ reported the results of a recent study of 11 obese male patients, showing that 30 days of resveratrol treatment (150 mg per day) led to improved insulin sensitivity and reduced blood glucose levels, blood pressure and circulating concentrations of triglycerides and alanine transaminase. This was associated with decreased intrahepatic fat content, mitochondrial respiration (in muscle) and AMPK phosphorylation, and increased SIRT1 and PGC1 α protein content. Crandall *et al.*¹²⁵ showed in a small pilot study (seven female patients and three male patients; body mass index (BMI) 29 ± 5 ; 73 ± 3 years of age) that high doses of resveratrol improved insulin sensitivity in older individuals with impaired glucose tolerance. Thus, the results to date from clinical studies confirm that the beneficial metabolic effects of resveratrol in humans are similar to those seen in animal experiments.

Cardiovascular disease

In the early 1990s, the findings that Southern Europeans consuming a Mediterranean diet suffer from a relatively low incidence of coronary heart disease, despite having a diet relatively rich in saturated fats (dubbed the 'French paradox'), unleashed a quest for the dietary factor responsible for the cardioprotection⁶. The subsequent discovery of resveratrol in red wine, which is an important constituent of Mediterranean diets, led to extensive research into the protective effects of resveratrol on the cardiovascular system. Given the importance of macro- and microvascular processes in ageing and age-related diseases as well as in disability¹²⁶, resveratrol may also affect ageing via such processes. Accruing evidence shows that resveratrol can activate several interrelated cellular pathways in the cardiovascular system, all of which may contribute to its cardioprotective effects.

For example, resveratrol was effective in suppressing plaque development in various animal models of athero-genesis^{127,128}. Resveratrol was demonstrated to confer diverse cellular and molecular effects *in vitro*, including inhibition of low-density lipoprotein (LDL) oxidation¹²⁹, inhibition of calcification of vascular smooth muscle cells¹³⁰, regulation of vascular smooth muscle proliferation^{129,131} and migration¹³², attenuation of cellular reactive oxygen species production¹³², reversal of age-associated changes in the secretory profile of vascular smooth muscle cells¹³³ and upregulation of endothelial nitric oxide production¹³⁴, all of these effects are compatible with its anti-atherogenic activity *in vivo*.

Previous studies suggest that resveratrol can also suppress platelet aggregation^{135,136}, which may attenuate atherogenesis and protect against recurrent myocardial infarction. Resveratrol,

both *in vivo* and at nutritionally relevant concentrations *in vitro*, was demonstrated to exert anti-inflammatory effects including inhibition of NF- κ B activation, upregulation of PPAR γ , downregulation of inflammatory gene expression and inhibition of chemokine secretion, inhibition of monocyte chemotaxis and attenuation of leukocyte adhesiveness to endothelial cells, all of which may contribute to its cardioprotective effects^{44,137–139}.

At high concentrations, resveratrol can act as an anti-oxidant. More importantly, resveratrol can upregulate the expression of several major cellular antioxidant enzymes (including superoxide dismutase, glutathione peroxidase and haem oxygenase) in the cardiovascular system^{140–142}, which results in significant attenuation of oxidative stress in pathophysiological conditions. Resveratrol also down-regulates the expression of NADPH oxidases, which are major sources of free radical production in the cardiovascular system^{143,144}. Mitochondrial oxidative stress is an important mechanism for the development of vascular pathologies in both diabetes and ageing^{138,139,145}. Thus it is significant that in the vasculature resveratrol was demonstrated to be a potent inhibitor of mitochondrial generation of reactive oxygen species¹⁴⁶. The aforementioned effects are likely to contribute to the resveratrol-mediated attenuation of vascular oxidative stress in animal models of metabolic diseases and ageing^{44,143,146}.

Resveratrol was also shown to upregulate endothelial nitric oxide synthase and increase nitric oxide bioavailability^{143,147}, which improves vasodilator function in various models of human diseases that are known to be associated with increased cardiovascular risk (for example, diabetes mellitus, metabolic syndrome and hypertension). Importantly, resveratrol was shown to confer protective effects in a porcine model of metabolic syndrome and chronic myocardial ischaemia; it improved endothelial function and myocardial perfusion, lowered levels of C-reactive protein and improved glucose tolerance¹⁴⁸.

The cardiovascular benefits of resveratrol have been recently documented in humans. In one clinical study, oral administration of resveratrol resulted in an acute dose-related improvement in endothelium-dependent vaso-dilation, which was correlated with an increase in plasma concentrations of resveratrol, directly demonstrating for the first time in humans that resveratrol can improve flow-mediated dilation acutely in at-risk population groups¹⁴⁹. In addition, a low dose of resveratrol (over a period of 3 months) was reported to have beneficial effects on left-ventricle diastolic function, endothelial function and LDL-cholesterol levels while protecting against unfavourable haemorheological changes in patients with coronary artery disease¹⁵⁰.

Recent studies suggest that impaired nitric oxide bioavailability leads to dysregulation of mitochondrial biogenesis in the vasculature^{151,152}, which is likely to contribute to cellular energetic imbalance, oxidative stress and endothelial dysfunction in ageing and metabolic diseases. Thus the findings that resveratrol can promote mitochondrial biogenesis in endothelial cells, similarly to its effects in parenchymal tissues^{39,114}, may open new avenues for the development of novel pharmacological approaches to promote vascular health both in patients with diabetes and in the aged population. Other cardioprotective effects of resveratrol include inhibition of vascular smooth muscle cell proliferation^{152,153}, which has therapeutic relevance for the treatment and/or prevention of pulmonary hypertension¹³¹ and

neointima formation¹²⁹. On the basis of the synergistic inhibitory effects of resveratrol on vascular smooth muscle cell proliferation, macrophage activation and platelet stimulation, the use of resveratrol-eluting coatings for the prevention of in-stent stenosis is being considered¹⁵⁴.

The molecular targets of resveratrol that mediate its proven multifaceted cardioprotective effects are the subject of ongoing investigations. On the basis of evidence accumulated during the past decade, the general picture that emerges is that many of the pathways involved in resveratrol-induced cardiovascular protection are under the control of evolutionarily conserved master regulators of cellular stress resistance, redox homeostasis and cellular energetics. On the basis of the structural similarity of resveratrol to the synthetic oestrogen diethylstilbestrol, the cellular effects of resveratrol were explained by stating that it is a phytoestrogen¹⁵⁵. Given the suspected cardioprotective benefits of oestrogens, this idea appeared to be appealing and stimulated several studies suggesting that certain cardiovascular effects induced by high doses of resveratrol may indeed involve activation of the oestrogen receptor^{156,157}. Yet there are many studies extant that dispose of the notion that activation of the oestrogen receptor is the major function of resveratrol, including reports showing that resveratrol binds to oestrogen receptor- α and oestrogen receptor- β with an affinity approximately 100,000-fold lower than estradiol¹⁵⁸, and that resveratrol actually acts as an oestrogen receptor antagonist¹⁵⁹.

Conversely, there is strong evidence that overexpression of SIRT1 exerts protective effects both in cardiac myocytes^{81,160} and in vascular cells^{152,161}, and the available data suggest that many of the beneficial cardiovascular effects of resveratrol are mediated by pathways that require the presence of functional SIRT1. For example, resveratrol was reported to regulate the expression of manganese superoxide dismutase in cardiac myocytes¹⁶² and endothelial cells¹⁴⁶ via a SIRT1-dependent pathway, which acts to reduce oxidative stress.

Genetic depletion of SIRT1 also abrogates the protective effect of resveratrol in a mouse model of diabetic cardiomyopathy¹⁶³. There is solid evidence that SIRT1 mediates the inhibitory effect of resveratrol on NF- κ B activity⁸⁸. Recent studies demonstrate that both resveratrol treatment and SIRT1 overexpression in cultured endothelial cells decrease the expression and activity of tissue factor (which is a key initiator of coagulation) via inhibition of NF- κ B activation¹⁶⁴. It is therefore reasonable to hypothesize that other structurally different pharmacological agents that activate SIRT1 *in vitro* might confer similar cardioprotective effects to those induced by resveratrol or SIRT1 overexpression. The available data suggest that this is the case. For example, treatment with SRT1720, a synthetic molecule that triggers potent SIRT1 activation *in vitro*⁴⁷, as well as deacetylation of SIRT1 substrates *in vivo* (see below), significantly reduced the number of ischaemic foci and attenuated inflammatory gene expression in the hearts of mice that were fed a high-fat diet⁴⁹.

Furthermore, SIRT1 is required for resveratrol-mediated induction of mitochondrial biogenesis and attenuation of mitochondrial oxidative stress in cardiovascular cells^{146,152}. In cultured vascular smooth muscle cells, resveratrol treatment *in vitro* was shown to modulate angiotensin signalling via a SIRT1-dependent pathway¹⁶⁵. Resveratrol treatment *in vivo* was

also shown to ameliorate the cardiovascular effects of angiotensin II¹⁶⁶. Recent studies demonstrate that in human vascular endothelial cells, SIRT1 activation by resveratrol upregulates the transcription factor Krüppel-like factor 2, which confers vasoprotective effects¹⁶⁷. Furthermore, the protective effects of resveratrol against myocardial damage induced by the antitumour agent doxorubicin are prevented by pharmacological inhibition of SIRT1 (REFS 168,169). Although such evidence strongly supports the view that resveratrol — either directly or indirectly — activates and/or upregulates SIRT1 in the cardiovascular system, there are likely to be other molecular targets of resveratrol that contribute to its cardioprotective effects.

It is significant that in endothelial cells¹²⁸ and other cell types^{128,129,170} resveratrol activates nuclear factor erythroid 2-related factor 2 (NRF2) and upregulates NRF2-driven antioxidant systems at lower concentrations than those needed for the activation or induction of SIRT1 *in vitro*^{129,170}. Many of the NRF2 targets (for example, catalase, NAD(P)H:quinone oxidoreductase 1, glutathione peroxidase and haem oxygenase 1) have been demonstrated to confer protective effects on the endothelium under conditions of increased oxidative stress. Importantly, knockdown of NRF2 abrogates resveratrol-mediated reduction of hyperglycaemia-induced mitochondrial and cellular oxidative stress in endothelial cells¹⁷⁰.

The *in vivo* role of resveratrol-induced NRF2 activation in vasoprotection has been recently confirmed using *Nrf2*^{-/-} mice that were fed a high-fat diet¹⁷¹. Because NRF2-driven pathways can be activated *in vitro* by concentrations of resveratrol that are readily achievable *in vivo*, these studies afford a proof of concept that NRF2 activation importantly contributes to the vasoprotective effects of resveratrol. At present, the crosstalk between pathways governed by NRF2 and SIRT1 is not well understood. Thus, further studies are evidently needed to elucidate the interaction — if any — between NRF2 and SIRT1 signalling in the cardiovascular system.

Brain function and neurodegeneration

Resveratrol and overexpression of SIRT1 counteract amyloid- β toxicity in cellular models¹⁷². *SIRT1* gene expression has been reported to be lower in patients with Alzheimer's disease and correlates with the accumulation of micro-tubule-associated protein tau¹⁷³. Resveratrol was shown to confer neuroprotection in animal models of vascular cognitive impairment, including the prevention of neuronal injury as well as behavioural and/or cognitive impairments induced by cerebral ischaemia^{174–177}, and it was also shown to protect neuronal cells against polyglutamine toxicity in cell culture models^{176,178}. Interestingly, the protective effect of resveratrol against ischaemia requires both PPAR α and sirtuins^{176,179}.

Although much of the research on Alzheimer's disease has centred on neuronal pathology, there is accumulating evidence that the pathogenesis of Alzheimer's disease might begin in middle age and be influenced by cardiovascular risk factors and metabolic syndrome¹⁸⁰. Therefore, resveratrol and other SIRT1 activators may have a dual impact in the prevention of dementia: first, by directly acting on brain cells, and second, by preventing metabolic syndrome.

Resveratrol also mitigates lipopolysaccharide- and amyloid- β -induced microglial inflammation by inhibiting the signalling pathways involving Toll-like receptor 4, NF- κ B and signal transducer and activator of transcription 3 (STAT3)¹⁸¹. The precise role of SIRT1 in dementia has been a focus for recent research. SIRT1 increases α -secretase activity, and thus downregulation of SIRT1 might contribute to amyloid- β production¹⁸². However, genetic studies have not reported any linkage between SIRT1 variability and Alzheimer's disease¹⁷². Sirtuins have been implicated in slowing down axonal (Wallerian) and neuronal degeneration in mice^{35,183}, improving synaptic plasticity and cognition^{55,184}, mediating adaptive responses to dietary restriction in the hypothalamus¹⁸⁵, activating monoamine oxidase to lower serotonin levels and increase anxiety³⁶, as well as suppressing amyloid- β production¹⁸⁶ and tau protein aggregation¹⁸⁷.

Emerging evidence suggests that resveratrol treatment or SIRT1 overexpression also have a protective effect in models of Parkinson's disease^{188,189}. One study showed that SIRT2 inhibitors protected against α -synuclein-mediated toxicity *in vitro* and *in vivo* in a *Drosophila melanogaster* model of Parkinson's disease¹⁹⁰, and another report has shown that resveratrol protects cultured neurons from oxidative stress and α -synuclein-mediated toxicity¹⁹¹. More recently, two groups reported neuroprotective effects of SIRT1 *in vitro* and in mouse models of Huntington's disease. One group reported that SIRT1 partially prevented neuronal death and reduced the metabolic consequences of Huntington's disease¹⁹². The second group, using a different mouse model, reported that SIRT1 mediated neuroprotection, and identified CREB (cAMP-responsive element-binding protein)-regulated transcription co-activator 1 (TORC1) as a previously unknown target of SIRT1 deacetylase activity that regulates the transcription of bone-derived neurotrophic factor³⁴.

From a mechanistic standpoint, these new data suggest that SIRT1 may modulate the activity of multiple targets to confer neuroprotection under conditions of stress or disease. The relative importance of TORC1 compared with other targets described for SIRT1 (for example, p53, FOXO or PGC1 α), and whether SIRT1-dependent regulation of TORC1 has a major role in normal brain function, remain open questions. All of these data suggest that SIRT1 has a key role in mediating or promoting metabolic homeostasis in the brain as it does in other tissues.

SIRT1 is found at moderate levels throughout the brain, and it will be fascinating to determine its functions in different structures of the brain as well as how it affects behaviour, learning and memory. Notably, mice lacking SIRT1 were shown to have lower levels of oxidative stress in the brain, raising the possibility that this enzyme could have detrimental effects under some conditions¹⁶. Dissecting the circuitry and signalling involved in the regulation of sirtuin activity will be challenging but crucial in understanding the therapeutic value of sirtuins for treating neurodegenerative disorders.

Inflammation and stress response

Resveratrol has well-established anti-inflammatory and antioxidant activities, which appear to include both SIRT1-independent and SIRT1-dependent effects^{13,193}. Resveratrol inhibits many pathways that mediate inflammation, including signalling through NF- κ B,

extracellular signal-regulated kinase 1 (ERK1) and ERK2, interferon- γ (IFN γ), interleukin-10 (IL-10), cyclooxygenase 1 (COX1) and COX2 (REFS 194–198).

The role of SIRT1 in different aspects of stress responses, from inflammation to genotoxic stress, has been widely studied and may be important in the aetiology of ageing and age-related diseases^{56–78}. The activation of SIRT1 negatively regulates inflammation through its effect on NF- κ B⁸⁸; it physically interacts with the p65 sub unit of NF- κ B and causes its deacetylation, thus inactivating NF- κ B and preventing the induction of its target genes. Under hypoxic stress, SIRT1 induces hypoxia-inducible factor 2 α (HIF2 α) activity by direct deacetylation, marking the initiation of this stress response pathway¹⁹⁹. However, SIRT1 can also deacetylate the related factor HIF1 α , causing its inhibition, to limit glycolysis under hypoxic conditions²⁰⁰. This physiological response to hypoxia is a clear example of how the changing levels of NAD and related metabolites during hypoxia can rewire cellular responses through SIRT1 to preserve energy homeostasis under stress conditions²⁰⁰.

In response to oxidative stress, SIRT1 deacetylates the DNA repair factor KU70. This prevents BCL-2-associated X protein (BAX) from entering the mitochondria, thus inhibiting stress-induced apoptosis and possibly promoting the survival of irreplaceable cells after an insult²⁰¹. SIRT1 also controls the cellular response to stress by regulating FOXOs — an important family of transcription factors. FOXOs act as sensors of the insulin signalling pathway, and in lower organisms they regulate longevity. SIRT1, in response to oxidative stress, deacetylates FOXO1, FOXO3 and FOXO4, which induces cell cycle arrest and resistance to oxidative stress^{89,90,202}. SIRT1 also has an important role in the activation of the heat shock response through the heat shock factor protein 1 (HSF1)²⁰³. Following a heat or protein aggregation challenge, SIRT1 activates HSF1 by direct deacetylation, which promotes the transcription of heat shock response genes²⁰³. Thus, SIRT1 acts as a sensor of various stresses, organizes the survival signals in response to these stresses and helps to preserve metabolic homeostasis.

Synthetic sirtuin activators

Given the controversy surrounding the mechanism of action of resveratrol^{45,46,50,52,110}, there has been considerable interest in designing novel SIRT1 activators that are potentially more potent and specific. Synthetic compounds that are structurally distinct from resveratrol and have potent SIRT1-activating power *in vitro* have recently been described by Sirtris Pharmaceuticals (which has been acquired by GlaxoSmithKline)⁴⁷. These compounds were reported to bind to SIRT1, lowering the Michaelis constant (K_m) for acetylated substrates and resulting in improvements in physiological responses *in vivo* and *in vitro*^{47,189}.

Unfortunately, the novel molecules described by Sirtris have also become embroiled in controversy. Pacholec *et al.*⁵² reported that a number of Sirtris's compounds, including SRT1720, SRT2183 and SRT1460 (re-synthesized by scientists at Pfizer), failed to activate SIRT1 in their hands using various substrates, including those used by Milne and colleagues⁴⁷. Later, it was reported by a second group that SRT1720 and SRT2183 were able to effectively decrease the acetylation of p53 in cells even in the absence of SIRT1, and this was attributed to inhibition of histone acetyltransferase p300 (REF. 51). These

observations led the authors of both studies to conclude that the tested compounds are not actual SIRT1 activators; furthermore, Pacholec *et al.*⁵² showed that *in vitro* SIRT1 activation proceeds through the formation of a complex between the activator and the fluorescent moiety (6-carboxy-tetramethyl-rhodamine (TAMRA)) of the peptide substrate.

Notably, aspects of this study have been questioned by Cen *et al.*⁷⁰, and scientists at Sirtris have since published a paper in response that includes extensive kinetic and biophysical evidence for the direct interaction of the enzyme and its activators²⁰⁴. Importantly, this report shows that there are compounds that activate SIRT1 but show no detectable binding to the TAMRA peptide, which is inconsistent with substrate enhancement but consistent with allosteric activation of the enzyme. The Sirtris report also demonstrates that peptide sequences composed of only natural amino acids can be competent substrates for SIRT1 activation⁷⁰.

Based on their demonstration that resveratrol can inhibit PDEs, Park *et al.*¹⁰⁷ suggested that other SIRT1 activators may also act as PDE inhibitors. Such an effect was also described by Pacholec *et al.*⁵², albeit at concentrations well above those required to activate SIRT1. However, Sirtris has since disclosed that internal testing has excluded PDE inhibition as the mechanism of action for the 'SRT' series of compounds²⁰⁵. As illustrated by this exciting mechanistic debate, there is a clear need to understand and develop reliable assays to test the activation of sirtuins *in vitro* and *in vivo*. Despite the controversy surrounding their mechanism of action, many effects of both resveratrol and the best-studied Sirtris compound, SRT1720, are clearly dependent on SIRT1 in cultured cells^{49,152,162,163,204,206–219}.

Besides Sirtris, various other groups are working on the development of sirtuin activators, and several oxazolo[4,5-b]pyridine and imidazo[1,2-b]thiazole derivatives have been identified as novel activators of SIRT1 (REFS 220,221). In addition, 1,4-dihydropyridine derivatives have been found to activate several of the sirtuins (SIRT1–SIRT3) in a dose-dependent manner²²², raising hopes that less controversial tools may soon be available to study the effects of sirtuin activation.

In vivo studies

Like resveratrol, SRT1720 has been shown to mitigate various negative effects of obesity and high-fat diets in both rats and mice. Indeed, the first publication on SRT1720 showed that the compound had beneficial effects on glucose homeostasis and insulin sensitivity in both diet-induced obese mice and in Zucker fa/fa rats (a model of obesity)⁴⁷. A subsequent further characterization of the effects of SRT1720 *in vivo* confirmed the ability of SRT1720 to protect against the negative effects of diet-induced obesity in mice, and identified a connection to metabolic adaptation in fatty acid and oxidative metabolism through downstream targets of SIRT1 such as PGC1 α and FOXO1 (REF. 48).

Additional evidence for the strong metabolic effects of SRT1720 was shown in a study that examined gene expression profiles in mice and found changes in the expression of genes involved in mitochondrial biogenesis, metabolic signalling and inflammation²²³. A fourth study failed to replicate the earlier results; instead, the authors found that the dose used in

prior studies was toxic, and that a lower dose caused weight gain with no enhancement of mitochondrial function (as reflected by cytochrome *c* oxidase or citrate synthase activity) or improvement in serum glucose levels (although fasting insulin levels were reduced)⁵². Finally, two studies have reported that genes involved in lipogenesis, in particular sterol regulatory element-binding protein (*SREBP*) and its target genes, are affected by SRT1720 in mouse models of genetic or monosodium glutamate-induced obesity^{224,225}. Repression of lipogenic gene expression was associated with reduced hepatic steatosis in both studies.

The reason for the discordant effects observed by Pacholec *et al.*⁵² remains unclear. However, we have recently reported that life-long treatment with SRT1720 improves survival in obese mice, to a similar extent as resveratrol, even at a dose that was previously described as toxic⁴⁹. Moreover, increased survival time was accompanied by a reduction in liver steatosis, improved insulin sensitivity, suppression of inflammation and apoptosis, and normalization of hepatic gene expression profiles. In addition, SRT1720 had beneficial effects on mitochondrial function that are dependent on SIRT1. These findings suggest that two structurally unrelated molecules (resveratrol and SRT1720) that are reported to activate SIRT1 induce similar protective effects *in vivo*.

Clinical trials

Sirtis has initiated multiple clinical studies with three selective non-resveratrol-related SIRT1 activators — SRT2104, SRT2379 and SRT3025 — in the context of inflammatory, metabolic and cardiovascular diseases²²⁶. To date, the treatment of over 400 healthy volunteers and patients with SRT2104 has been reported to be safe and well tolerated. In a double-blind placebo-controlled study, SRT2104 was reported to significantly attenuate the release of the pro-inflammatory cytokines IL-6 and IL-8 as well as the activation of coagulation in response to low-dose endotoxin in healthy male volunteers²²⁷. SRT2104 is currently being studied in the setting of moderate to severe plaque-type psoriasis (see the [ClinicalTrials.gov](https://clinicaltrials.gov) website). SRT2379 has also been assessed for safety and pharmacokinetics in Phase I trials, and is currently being evaluated in the same clinical setting of acute inflammation in response to low-dose endotoxin (see the [ClinicalTrials.gov](https://clinicaltrials.gov) website). SRT3025 is nearing the end of Phase I studies to evaluate its safety and tolerability in healthy volunteers, with its future development under discussion.

Notably, the development of SRT501 — a resveratrol-based formulation — for the treatment of multiple myeloma was suspended when 5 out of 24 individuals developed nephropathy²²⁸. This study involved a very high dose of the formulation (5 g per day), and it was suggested that dehydration secondary to diarrhoea precipitated the nephropathy, which is a normal complication of multiple myeloma²²⁹. Nevertheless, these observations highlight the need for exercising caution in clinical trials.

Challenges for translation to geriatric medicine

The benefits of treating or preventing individual diseases in older people are limited because there are so many competing risks of death and disability once old age is reached²³⁰. Conversely, it has been proposed that by influencing the ageing process it may be possible to delay many age-related diseases and disabilities — the so-called ‘longevity

dividend^{138,139,231,232}. It has been estimated that the gain in life expectancy achieved by delaying ageing with caloric restriction might substantially exceed that achieved by finding a combined cure for cardiovascular disease, cancer and diabetes mellitus²³³ (FIG. 1).

SIRT1 activation appears to provide a possible opportunity for realizing such a longevity dividend in humans. Alterations in SIRT1 activity and expression have been reported to have a role in many age-related conditions including neurodegeneration, cancer, osteoporosis, type 2 diabetes, sarcopaenia, inflammation, frailty and cardiovascular disease^{56,126,145,234}. Such a broad range of diseases is unusual in drug development, where there has mostly been a reductionist focus on a single disease, pathway, target and drug. However, it is consistent with the widespread expression and pleiotropic effects of SIRT1, and parallels other processes implicated in ageing and age-related disease such as oxidative stress and mitochondrial dysfunction. Furthermore, this raises the prospect that STACs may have utility in managing the geriatric syndromes of frailty and functional decline.

It is disappointing that resveratrol does not increase longevity in normal mice, as has been observed in simpler experimental animal models¹⁰³. However, it should be noted that very few primary preventive interventions reduce mortality in humans, despite having a beneficial impact on individual diseases. Moreover, a delay in the onset of age-related diseases is a very valuable clinical outcome that reflects a step towards ‘compression of morbidity’²³⁵. The beneficial effects of resveratrol on age-related changes in insulin sensitivity, cardiovascular risk factors and liver steatosis are very relevant for geriatric medicine. Much of the morbidity and phenotype of old age has a vascular and/or microvascular component¹²⁶, and there is an increasing recognition of the interactions among ageing, metabolic syndrome, insulin resistance, fatty liver and vascular disease. The liver has a central role in mediating the effects of caloric restriction, and age-related changes in liver structure and function have systemic implications^{236,237}. Therefore, it is of note that resveratrol and SRT1720 led to a reversal of liver steatosis in aged mice that were on a high-calorie diet^{129,131}, as well as in mice with Werner Syndrome¹⁵⁵, and that hepatic SIRT1 activity declines with age, possibly secondary to decreased NAD⁺ substrate availability²³⁸.

Although the findings are very preliminary at present, emerging evidence from recent studies suggests that the beneficial effects of resveratrol and the more potent STACs may also be realized in humans^{118,125,149,150,221}. However, translating the effects of resveratrol and other STACs from animal studies into clinical trials and into the marketplace will be challenging. Clinical trials in humans are unlikely to use longevity as an initial primary outcome, so some sort of surrogate outcome or biomarker of ageing is necessary. As yet, there is no established set of biological or clinical biomarkers of ageing so it is more likely that a traditional disease-based outcome will be utilized to establish the efficacy of SIRT1-activating drugs¹. Based on the experiments in mice it would seem that metabolic syndrome, liver steatosis and cardiovascular disease are reasonable options, and several small preliminary studies of resveratrol in humans have reported promising results in these disease areas (discussed above)^{118,131,132}.

STACs may also have a role in managing geriatric syndromes — such as frailty and loss of function — that characterize geriatric patients and generate an enormous burden of

morbidity and disability. Frailty is a complex syndrome that is often defined clinically by features that are indicative of bioenergetic failure: weakness, slowness, inactivity, exhaustion and shrinkage²³⁹. Recent studies suggest that frailty is linked to a progressive dysfunction of cellular bioenergetics, including mitochondrial function²⁴⁰, and that a point mutation in mitochondrial DNA (the mt204 C allele) increases the risk for frailty by twofold²⁴¹. Thus, frailty would appear to be an ideal but as yet untested target for STACs.

Conclusions

There are no known interventions that are proven to substantially slow the ageing process in humans. Indeed, it has been argued that such drugs can never be developed because ageing is caused by a random accumulation of damage, some of which is inevitable and irreversible²³². Nevertheless, ongoing research continues to draw a more complete picture of caloric restriction at the molecular level, which may ultimately allow for the development of pharmacotherapies that confer some of the health benefits of this dietary regimen. Such developments would be particularly appealing to ageing populations in developed countries, as continuous warnings from health-care institutions and governments are ignored in favour of increased calorie consumption and decreased physical activity. Furthermore, compounds that slow the ageing process by forestalling age-related diseases would not only lengthen life but also improve the quality of life and productivity of elderly individuals.

The available evidence strongly suggests that activation of SIRT1 would lead to beneficial outcomes on human health, if not on longevity. As such, we believe that this enzyme remains a viable and potentially very important drug target. In particular, it will be crucial to determine the mechanisms by which compounds such as resveratrol and SRT1720 lead to increased SIRT1 activity, and to determine what portion of the beneficial effects that have been reported for these compounds are mediated by SIRT1. It will also be necessary to further understand the tissue-specific effects of these activators, and determine the true preventive or therapeutic benefits of these molecules — alone or in combination with other agents. We have only begun to understand the biology of the remaining six sirtuins, and there is strong evidence that at least SIRT3 and SIRT6 will also be relevant to the ageing process. Thus, continued study and validation of this family of enzymes is likely to be an important area of research for some time to come.

As researchers continue to elucidate the mechanisms of ageing and their underlying molecular pathways, the range of targets for ageing interventions should continue to increase. Indeed, ongoing research in the field continues to highlight promising compounds that warrant further study^{11,242,243}. A significant portion of this wealth of data is emerging from a programme initiated in 2003 by the National Institute on Aging (NIA) to rigorously test pharmacological and dietary agents that may extend the longevity of mice at three independent sites^{11,244} (see the NIA website). Such efforts may soon result in a wealth of molecules that can influence health and longevity, at least in rodent models.

However, it is important to emphasize that none of the compounds currently under investigation has been definitively shown to effectively delay ageing or age-related diseases in humans. Yet over the past three decades, intensive research into the basic biology of

ageing and the effects of caloric restriction has identified genes and pathways that participate in the regulation of lifespan and healthspan, and are shared by multiple species. These findings have raised the hope for the discovery of targets to develop new therapies to prevent age-related diseases, geriatric syndromes such as frailty and perhaps even influence ageing itself in humans.

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Box 1 The effects of various interventions on human life expectancy

Several lifestyle interventions are currently in practice or being investigated for their ability to enhance lifespan. The effects of such interventions are illustrated in the figure, which was compiled by combining data from unrelated studies on different populations.

Stopping smoking is believed to improve health and decrease mortality. Indeed, a study has shown that stopping smoking at the age of 30 has a significant effect on life expectancy, increasing it by 10 years, although there is a significant benefit in quitting even in later years^{245,246}. Lowering the body mass index (BMI) to what is considered to be within the healthy range is also predicted to improve lifespan, the effect being greater for morbidly obese individuals. However, the estimated effects of BMI are based on observational studies and may not accurately reflect the consequences of deliberate weight loss²⁴⁷.

Exercise also has beneficial effects, with studies suggesting that regular exercise can result in a 4-year increase in life expectancy. This is illustrated in the figure, and the estimate is based on a comparison of the most active individuals to the least active individuals over 50 years of age^{248–250} (the average difference for both genders across three related studies is shown in the figure). Indeed, lifespan extensions of up to 6.1 years have been reported in populations of elite athletes²⁵¹.

Vitamin intake may also affect mortality. Although vitamin A, vitamin E and β -carotene may actually be associated with increased mortality, vitamin C appears to have no effect; selenium tended to decrease mortality but the effect was not conclusive based on the available data²⁵². The effect of wine consumption on lifespan has also been investigated. In a study (in males) of the effects of moderate wine consumption (defined as less than half a glass per day)²⁵³, where life expectancy was calculated at 50 years of age, a 5-year increase in lifespan was recorded. Of this increase, 2 years were attributed to alcohol per se, whereas 3 years were attributed to other components of wine, such as polyphenols (including resveratrol).

Curing age-related diseases will obviously affect life expectancy, but the magnitude of the effect for any one disease is not large. In the figure, the effects of disease cures are based on the estimates of Olshansky and colleagues²³³. In rodents, dietary restriction positively affects lifespan, and the increase in life expectancy shown in the figure is based on a 30% increase in mean lifespan, which is typical of rodent studies²⁵⁴; the current CDC (Centers for Disease Control) estimate of human life expectancy in the United States is 78.5 years. The influence of exercise appears to be greater in rodents than in humans, and this could reflect an inherent difference in the plasticity of lifespan between species. Therefore, an alternative approach for estimating the effect of dietary restriction in humans is to assume it will be ~2.1 times as effective as exercise, as was the case for rats, albeit at a suboptimal level of dietary restriction²⁵⁵.

The available data are insufficient to allow inclusion of human growth hormone supplementation in the figure. However, it is worth noting that even severe growth hormone deficiency does not result in a shorter lifespan when childhood mortality is excluded²⁵⁶. Although the available data suggest that low-dose aspirin lowers total

mortality, the benefits are greater in high-risk individuals, and it is unclear whether this intervention has any utility in healthy or optimally medicated patients^{257–259}. Aggregated trials show no benefit of statins on all-cause mortality over 3 to 4 years of follow-up; however, a 15-year follow-up did reveal a significant beneficial effect in male patients with hypercholesterol-aemia^{226,260}.

Generalized measures of diet quality are also associated with all-cause mortality, although the outcome has not been extrapolated to years gained or lost²²⁷. Note that lifestyle changes, particularly the effects of smoking and obesity, are relevant to only a subset of the population. Thus, the changes in individual life expectancy presented here overestimate the potential impact of these interventions on average human lifespan (data adapted from REF. 13).

Box 2 SIRT3 and SIRT6 as regulators of longevity

Although sirtuin 1 (SIRT1) is the closest homologue of the sirtuin genes that were reported to extend lifespan in lower organisms, the first sirtuin to be associated with longevity in a human population was SIRT3. Rose and colleagues^{261,262} identified a variable enhancer region for *SIRT3*, and showed that individuals carrying the alleles with the lowest enhancer activity were the least likely to survive to advanced ages.

Unfortunately, a larger population study has since failed to prove an association between genetic variability in the vicinity of *SIRT3* and longevity, suggesting that the effect is weak at best²⁶³. However, interest in SIRT3 has been renewed by reports suggesting that it mediates the induction of antioxidant defences and metabolic adaptations during caloric restriction. Caloric restriction reduces oxidative damage in the brain and liver, prevents the loss of neurons and hair cells from the inner ear, and dramatically attenuates age-related hearing loss in mice, and each of these effects requires SIRT3 (REFS 74,264).

SIRT3 is also induced by caloric restriction in white adipose tissue, brown adipose tissue and skeletal muscle, and mediates adaptive changes in hepatic metabolism, including the upregulation of fatty acid oxidation, ketone body production and the urea cycle^{62,75,265–267}. Therefore, SIRT3 is emerging as a key player in the metabolic adaptations to diet and lifestyle that may well influence mammalian lifespan.

SIRT6 has an essential role in postnatal life, and among the sirtuins its deficiency leads to the most dramatic phenotypes. SIRT6-null mice are born with no visible abnormalities but soon after birth they develop a severe metabolic imbalance, hypoglycaemia and growth retardation, and the majority die at approximately 1 month of age²⁶⁸.

Enzymatically, SIRT6 acts as both a deacetylase and an ADP-ribosyltransferase, and a growing number of reports have highlighted roles for this sirtuin in DNA repair, telomere maintenance, genomic stability and cell senescence^{65,268–273}. SIRT6 attenuates nuclear factor- κ B (NF- κ B) signalling by interacting with its p65 (also known as RELA) subunit, and reducing RELA expression partially rescues the shortened lifespan of SIRT6-deficient mice²⁷⁴. In addition, SIRT6 co-represses hypoxia-inducible factor 1 α (HIF1 α) to suppress glucose uptake and glycolysis; the hypoglycaemia that limits lifespan in SIRT6-null mice may be a result of unrestrained HIF1 α expression²⁷⁵.

However, the most impressive demonstration of a link between SIRT6 expression and longevity is a recent report showing that overexpression of SIRT6 extends the lifespan of male mice²⁷⁶. In this study, SIRT6 overexpression lowered serum levels of insulin-like growth factor 1 (IGF1) and increased the expression of insulin-like growth factor-binding protein 1 in male mice, bringing the values closer to those observed in control female mice. By contrast, SIRT6 overexpression in female mice had no further effect on these parameters nor did it affect longevity. Attenuation of IGF1 signalling is associated with increased longevity in many animal models, suggesting that these effects could have a direct role in SIRT6-induced lifespan extension²⁷⁷.

Given its effects on IGF1 signalling and its potential role in regulating genomic stability, SIRT6 might extend lifespan at least in part by acting as a tumour suppressor. This explanation was not favoured by the authors of the study²⁷⁶ because SIRT6-overexpressing mice and control mice displayed a similar incidence and spectrum of tumours; however, further studies will be required to provide a definitive answer. Thus, SIRT6 has a major influence on mammalian physiology, is essential for normal lifespan and may directly influence longevity.

Figure 1. Pleiotropic effects of SIRT1 on age-related diseases

Sirtuin 1 (SIRT1) is a crucial mediator of the physiological adaptive responses to energy availability. Activation of SIRT1 has beneficial effects in several age-related diseases, particularly those associated with metabolic dysregulation. Here, we illustrate the pleiotropy of SIRT1, including just four of the SIRT1 targets and/or regulatory proteins that have been reported to be associated with each particular disease or condition. BMAL1, brain and muscle ARNT-like 1; E2F1, E2F transcription factor 1; eNOS, endothelial nitric oxide synthase; FOXO, forkhead box protein O; HIF1 α , hypoxia-inducible factor 1 α ; KU70, DNA repair factor KU70; LKB1, liver kinase B1; MYOD, myoblast determination protein; NF- κ B, nuclear factor- κ B; p53, tumour suppressor p53; PARP1, poly(ADP-ribose) polymerase 1; PER2, period circadian protein homolog 2; PGC1 α , PPAR γ co-activator 1 α ; RAR β , retinoic acid receptor- β ; RUNX2, runt-related transcription factor 2; SOST, sclerostin; SREBP, sterol regulatory element-binding protein; STAT3, signal transducer and activator of transcription 3; UCP2, uncoupling protein 2.

Table 1

Properties and functions of mammalian sirtuins

Sirtuin	Molecular mass	Cellular localization	Activity	Key regulatory functions
SIRT1	81.7 kDa	Nucleus and cytosol	• Deacetylase	Metabolism, inflammation
SIRT2	43.2 kDa	Cytosol	• Deacetylase	Cell cycle and motility, myelination
SIRT3	43.6 kDa	Mitochondria	• Deacetylase	Fatty acid oxidation, antioxidant defences
SIRT4	35.2 kDa	Mitochondria	• ADP-ribosyl-transferase	Amino acid-stimulated insulin secretion, suppression of fatty acid oxidation
SIRT5	33.9 kDa	Mitochondria	• Deacetylase? • Demalonylase • Desuccinylase	Urea cycle
SIRT6	39.1 kDa	Nucleus	• Deacetylase • ADP-ribosyl-transferase	Genome stability, metabolism
SIRT7	44.8 kDa	Nucleolus	• Deacetylase?	Ribosomal DNA transcription

Table 2Studies of the effects of *SIRT1* gene expression manipulation in mice

Models of increased SIRT1 expression	Key phenotypes	Refs
β -cell-specific SIRT1 overexpression (BESTO, C57Bl/6 strain background)	<ul style="list-style-type: none"> Improved glucose tolerance, enhanced insulin secretion, decreased UCP2 expression and increased ATP production by isolated islet cells Loss of the above phenotypes in both genders by 18-24 months of age, correlating with a decline in serum levels of nicotinamide mononucleotide (NMN; a precursor of NAD); restoration of improved glucose tolerance and insulin secretion by NMN supplementation in female mice only 	94 278
Liver-specific overexpression (adenovirus, BALB/c strain background)	<ul style="list-style-type: none"> Moderate hyperglycaemia and glucose intolerance after 5 hours of fasting; normal glucose and pyruvate tolerance despite increased expression of PEPCK and glucose-6-phosphatase after 19 hours of fasting Decreased PGC1α acetylation Increased serum levels of cholesterol and decreased cholesterol levels in liver after 20 hours of fasting, with some associated changes in gene expression (glucose intolerance and most of the changes in gene expression, but not the effects on cholesterol levels, are blocked by concurrent PGC1α knockdown) 	279
Heart-specific overexpression (2.5-, 7.5- and 12.5-fold; FVB strain background)	<ul style="list-style-type: none"> 2.5- or 7.5-fold overexpression: reduced age-dependent cardiac hypertrophy and dysfunction, protection from paraquat (not tested in mice with 2.5-fold overexpression) 12.5-fold overexpression: increased cardiomyopathy, dysfunction and oxidative stress 	81
7.5-fold overexpression in FVB strain background, backcrossed to C57Bl/6J strain background	<ul style="list-style-type: none"> Decreased infarct size and apoptosis following ischaemia-reperfusion Better functional recovery in Langendorff-perfused hearts 	84
Knock-in at β -actin locus (increased expression in MEFs, brain, white and brown adipose tissue, but not in muscle or liver)	<ul style="list-style-type: none"> Improved glucose tolerance, increased energy expenditure and food intake, decreased body weight and adiposity, delayed reproduction and improved rotarod performance 	29
BAC transgenic mice (two- to fourfold increased expression in MEFs, liver, kidney, thymus, spleen, intestines, muscle and brown adipose tissue, backcrossed to C57Bl/6 strain background)	<ul style="list-style-type: none"> Improved glucose tolerance, increased energy expenditure and food intake, reduced hepatic steatosis, SREBP1c expression and inflammation (reduced expression of IL-6 and TNF), as well as increased expression of MnSOD (on high-fat diet but not on standard diet) Increased expression of NRF1 on either diet, and suppressed NF-κB activity in TNF-stimulated MEFs 	85
BAC transgenic mice (two- to fourfold increased expression in MEFs, liver, kidney, thymus, spleen, intestines, muscle and brown adipose tissue, backcrossed to C57Bl/6 strain background)	<ul style="list-style-type: none"> Increased telomere length and decreased attrition in liver and kidney Increased homologous recombination 	280
BAC transgenic mice (two- to fourfold increased expression in MEFs, liver, kidney, thymus, spleen, intestines, muscle and brown adipose tissue, backcrossed to C57Bl/6 strain background)	<ul style="list-style-type: none"> Reduced DNA damage, CDKN2A expression, osteoporosis as well as spontaneous carcinomas and sarcomas Improved glucose tolerance, wound healing and tightrope performance No effect on lifespan Protection from liver tumours induced by a combination of diethylnitrosamine and a high-fat diet, but not from fibrosarcomas induced by 3-methyl-cholanthrene 	83
BAC transgenic mice (two- to threefold increased expression in brain, pancreas, liver, kidney, thymus, skeletal muscle and heart, as well as white and brown adipose tissue; ~sevenfold increased expression in spleen)	<ul style="list-style-type: none"> Improved glucose tolerance on high-fat diet or db/db background, but not on standard diet Decreased food intake, energy expenditure and activity on standard diet only Increased expression of adiponectin in white adipose tissue, and increased circulating levels on either diet 	82
Intestine-specific overexpression (~sevenfold) in mice with the multiple intestinal neoplasia mutation in the gene encoding APC (APC ^{min/+} mice)	<ul style="list-style-type: none"> Reduced tumour burden and mortality 	32
Three- to fourfold overexpression in bone marrow lymphocyte progenitors and ~twofold overexpression in T and B cells	<ul style="list-style-type: none"> Improved survival and decreased thymic lymphomas following irradiation (MISTO in <i>Tp53</i>^{+/-} mice) 	17

Models of increased SIRT1 expression	Key phenotypes	Refs
(MISTO), or ~tenfold overexpression in brain (NeSTO) (C57Bl/6 and 129/Sv mixed strain background)	• Attenuated changes in gene expression with age (NeSTO)	
NeSTO	• No changes in long-term potentiation or in immediate, spatial or associative memory • Increased synaptic excitability	55
Endothelial cell-specific overexpression	• Protection from impairment of vasorelaxation on high-fat diet, fewer atherosclerotic lesions in <i>Apoe</i> ^{-/-} mice	86
Xenograft-specific overexpression (~fourfold overexpression in HCT116 colon carcinoma cells)	• Reduced tumorigenicity when implanted subcutaneously in female athymic nude mice	281
Neuron-specific overexpression of human SIRT1	• Memory deficit; no effect on damage induced by ischaemia or MPTP	282
Kidney-specific overexpression (proximal tubules)	• Protection from cisplatin-induced acute kidney injury, reactive oxygen species production and apoptosis • Prevention of loss of peroxisomes but not mitochondria, partially preserved MCAD protein expression • No protection from ischaemia-reperfusion injury	283
Islet-specific overexpression (targeted adenovirus, BALB/c strain background)	• Protection from streptozotocin-induced cell loss, hyperglycaemia and glucose intolerance	284
Liver-specific overexpression (adenovirus, <i>Ldlr</i> ^{-/-} mice on high-fat or high-sucrose diet, or ob/ob mice)	• Decreased hepatic steatosis, improved glucose tolerance and insulin sensitivity • Decreased mammalian TORC1 activation and ER stress	285
Smooth muscle-specific overexpression of human SIRT1	• Inhibition of neointima formation and decreased proliferation of vascular smooth muscle cells following carotid artery ligation or wire injury	286

APC, adenomatosis polyposis coli; APOE, apolipoprotein E; BAC, bacterial artificial chromosome; CDKN2A, cyclin-dependent kinase inhibitor 2A; db/db, 'diabetes' mutation in the leptin receptor; ER, endoplasmic reticulum; FVB, Friend virus B-type; IL-6, interleukin-6; LDLR, low-density lipoprotein receptor; MCAD, medium-chain specific acyl-CoA dehydrogenase, mitochondrial; MEF, mouse embryonic fibroblast; MISTO, Mx-cre-dependent IFN-inducible SIRT1 overexpression; MnSOD, manganese superoxide dismutase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NeSTO, Nestin-cre, SIRT1^{STOP} transgenic mice (mice that, when crossed to a brain-specific Cre-driver (Nestin-cre), overexpress SIRT1 in the brain); NF-κB, nuclear factor-κB; NRF1, nuclear respiratory factor 1; ob/ob, 'obese' mutation in the leptin gene; PEPCK, phosphoenolpyruvate carboxylase; PGC1α, PPARγ co-activator 1α; SIRT1, sirtuin 1; SREBP1c, sterol regulatory element-binding protein 1c; TNF, tumour necrosis factor; TORC1, CREB-regulated transcription co-activator 1; TP53, tumour suppressor p53 gene; UCP2, uncoupling protein 2.

Table 3

Effects of long-term resveratrol and SRT1720 supplementation in mice

Models	Dose	Effect on mortality	Positive effects on age-related conditions	Refs
<i>Resveratrol</i>				
Mice fed a high-calorie diet	0.04% (~22 mg per kg per day) from 12 months of age	↓ (31%)	↑ in insulin sensitivity ↓ in liver steatosis ↑ in mitochondrial function (liver) ↑ in motor function	39
Mice fed a high-fat diet	0.2-0.4% for 15 weeks	Not assessed	↑ in mitochondrial function (muscle) ↑ in aerobic capacity ↑ in motor function ↑ in insulin sensitivity	114
Mice	0.01-0.04% from 12 months of age	↔	↓ in osteopaenia ↓ in cataracts ↓ in kidney disease ↑ in vascular function ↑ in motor function	44
Mice	4.9 mg per kg per day from 14 months of age	Not assessed	↑ in insulin sensitivity ↑ in cardiac function	43
Mice	0.015% from 12 months of age	↔	↑ in cognitive function ↓ in cerebrovascular abnormalities	287
Premature ageing mice with Werner syndrome	0.04% from weaning	↔	↑ in insulin sensitivity ↓ in liver steatosis ↓ in oxidative damage	288
Mice	1.5-2.3 mg per kg per day from 6, 12 or 24 months of age	Not assessed	↓ in immunosenescence (CD4 and CD8 T lymphocytes, IL-6 and TNF)	289
Mice (genetically heterogeneous)	0.03-0.12% from 9 months of age	↔	Not assessed	290
Mice	0.05% at 18 months (60 mg per kg per day) and 28 months (46 mg per kg per day)	Not assessed	↓ in oxidative damage (muscle)	291
<i>SRT1720</i>				
Mice fed a high-calorie diet	100 mg per kg from 12 months of age	↓ in mortality (total lifespan increased by 18%)	↑ in insulin sensitivity ↓ in liver steatosis ↑ in pancreatic islet morphology ↑ in motor function ↑ in mitochondrial function	49
Mice fed a high-fat diet	100 mg per kg per day by gavage for 10 weeks	Not assessed	↑ in insulin sensitivity ↑ in citrate synthase activity in muscle	47
Leptin-deficient mice	100 mg per kg per day by gavage for 1 week			
Zucker fa/fa rats	100 mg per kg per day by gavage for 4 weeks			
Mice fed a high-fat diet	100 or 500 mg per kg per day for 20 weeks	Not assessed	↑ in insulin sensitivity ↓ in body weight ↑ in endurance ↑ in fatty acid oxidation	48
ICR (CD1) mice treated with monosodium glutamate	200 mg per kg per day for 10 weeks	Not assessed	↓ in liver steatosis ↓ in serum aminotransferase levels ↓ in hepatic inflammation	224

↔, unchanged; IL-6, interleukin-6; ICR (CD1) mice, an outbred line of mice; TNF, tumour necrosis factor; Zucker fa/fa, a rat model of obesity.