Slowing ageing by design: the rise of NAD$^+$ and sirtuin-activating compounds

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Abstract | The sirtuins (SIRT1–7) are a family of nicotinamide adenine dinucleotide (NAD$^+$)-dependent deacylases with remarkable abilities to prevent diseases and even reverse aspects of ageing. Mice engineered to express additional copies of SIRT1 or SIRT6, or treated with sirtuin-activating compounds (STACs) such as resveratrol and SRT2104 or with NAD$^+$ precursors, have improved organ function, physical endurance, disease resistance and longevity. Trials in non-human primates and in humans have indicated that STACs may be safe and effective in treating inflammatory and metabolic disorders, among others. These advances have demonstrated that it is possible to rationally design molecules that can alleviate multiple diseases and possibly extend lifespan in humans.

The past 100 years have witnessed unprecedented advances in our ability to prevent and treat disease. Unfortunately, most medicines are designed to only treat one specific condition while ignoring other comorbidities. Thus, although the inhabitants of most nations are living longer, healthspan is not increasing. In the United Kingdom, for example, the percentage of men's lifespan spent in poor health has risen from 20% to 40% between 1995 and 2006 (REF. 1). Recent progress in longevity research, however, may soon enable doctors to treat diseases that affect multiple organs with one medicine (or just a few in combination), to significantly increase healthspan and compress the morbid period of our lives.

"To eat when you are sick is to feed your sickness" wrote Hippocrates, the father of Western medicine, almost 2,400 years ago. Today, his views seem remarkably prescient. Calorie restriction without malnutrition is considered the gold standard in biogerontology as the most robust way to delay ageing and age-related diseases. Since the discovery almost 80 years ago that calorie restriction can extend the lifespan of rats[2], great strides have been made in understanding why reducing calorie intake results in profound health benefits. Early theories, which included a delay in development and reduced metabolic rate, were subsequently ruled out owing to inconsistent experimental observations[3].

Numerous studies of simple and complex model organisms over the past 20 years have lent credence to the idea that a set of evolutionarily conserved longevity pathways are responsible for the effects of calorie restriction on lifespan[4] (FIG. 1). Dozens of genes and pathways have now been uncovered that compress the period of morbidity and extend the lifespan of model organisms, from yeast to rodents. Major signalling targets include insulin/insulin-like growth factor 1 (IGF1) signalling, target of rapamycin (TOR), adenosine monophosphate-activated protein kinase (AMPK) and the nicotinamide adenine dinucleotide (NAD$^+$)-dependent sirtuin deacylases[5,6]. It is believed that these pathways evolved to sense and respond to the nutritional environment and to promote cellular defence mechanisms in the face of extrinsic adversity (FIG. 1).

Interestingly, several naturally occurring molecules can activate these survival pathways and extend lifespan in rodents[7,8]. Rapamycin, a drug first discovered in a bacterium from Easter Island, reduces organ transplant rejection by inhibiting mechanistic TOR (mTOR). Rapamycin is thought to extend lifespan by mimicking diets that have low levels of essential amino acids such as methionine or tryptophan[9,10]. Another potential longevity drug is metformin, an AMPK-activating molecule from the Hellebore buttercup plant that is a frontline therapy for type 2 diabetes[11]. Clinical trials testing the ability of these molecules to slow aspects of ageing are underway or in the planning stages[12]. As discussed below, a promising approach to discovering longevity drugs is to design compounds *de novo* based on a molecular understanding of longevity. By designing increasingly potent compounds that activate sirtuins, it has been possible to pharmacologically mimic some of the physiological effects of calorie restriction both in animals and humans, with the ultimate goal of creating medicines that treat multiple age-related diseases and prevent many others.

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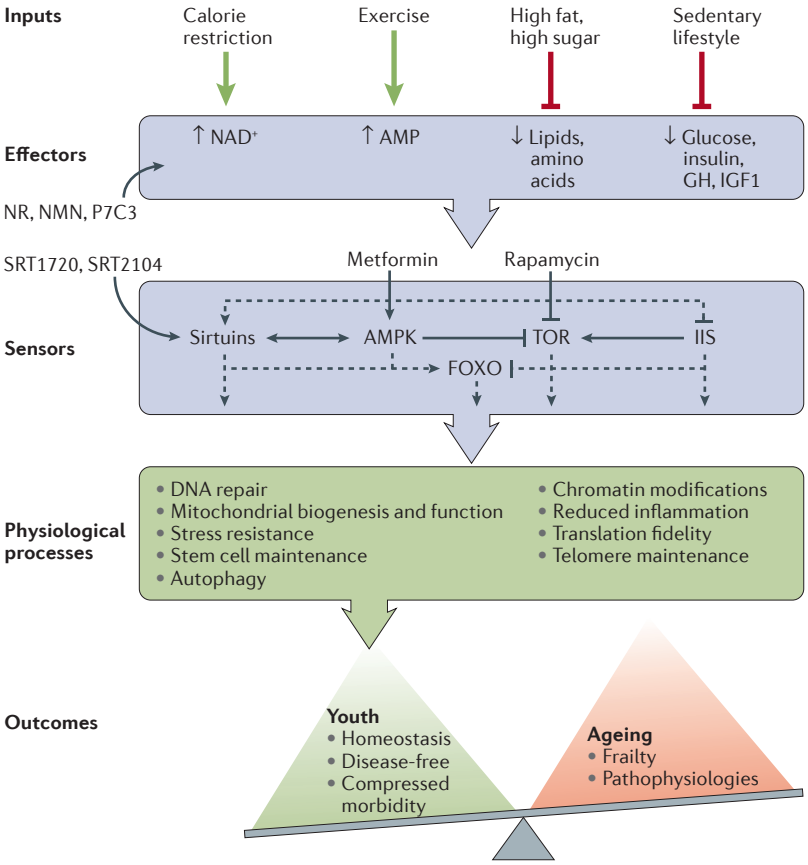


Figure 1 | **Nutrient-responsive signalling pathways that maintain health and extend lifespan.** Calorie or dietary restriction increases the concentrations of metabolic effectors such as nicotinamide adenine dinucleotide (NAD^+) and AMP while reducing the concentrations of glucose, amino acids and lipids. Exogenous administration of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN) or the nicotinamide phosphoribosyltransferase (NAMPT) activator P7C3 can increase NAD^+ levels. Calorie restriction also reduces the concentrations of the hormonal effectors insulin, insulin-like growth factor 1 (IGF1) and growth hormone (GH). These effectors stimulate or inhibit the activity of metabolic sensors such as the sirtuins (SIRTs), AMP kinase (AMPK), target of rapamycin (TOR), insulin–IGF1 signalling (IIS) and forkhead box O (FOXO) transcription factors. Sirtuin-activating compounds (STACs) such as SRT1720 and SRT2104 can directly activate SIRT1, whereas rapamycin is a direct inhibitor of TOR. Metformin indirectly activates AMPK. These metabolic sensors regulate downstream activities such as DNA repair, mitochondrial biogenesis and function, stress resistance, stem cell and telomere maintenance, autophagy, chromatin modifications, reduced inflammation, and translation fidelity. The net effect is to tip the scale in favour of homeostasis and compressed morbidity, resulting in a disease-free, more youthful-like state.

The yeast silent information regulator 2 (*SIR2*) gene, which gave sirtuins their name, was first shown to extend lifespan in yeast almost 20 years ago, making sirtuins one of the first families of longevity genes to be discovered[13–16]. Since then, multiple lines of evidence, from yeast to humans, have implicated sirtuins in mediating the health benefits of dietary restriction and exercise. Although sirtuins have attracted considerable attention, they have also attracted controversy, including finally settled debates about how sirtuins activators work and even whether sirtuins have a role in ageing[17,18]. In this Review, we discuss the trials and tribulations of sirtuin research, which have constructively led to a clearer understanding of sirtuin structure, enzymatic activity and biological roles. We describe the ongoing quest to discover and develop safe and potent sirtuin-activating compounds (STACs) as means to combat multiple age-associated diseases. The use of such compounds in clinical trials is discussed in the context of the challenges that lie ahead if they are to reach their full potential in medicine.

Replicative ageing
In yeast, the number of daughter cells produced by a mother cell before senescence.

Redox reactions
Oxidation–reduction (redox) reactions involving the transfer of electrons between two chemical species.

Sirtuins in metabolism and longevity

Sirtuins were initially discovered in the budding yeast *Saccharomyces cerevisiae* by virtue of their essential function in gene silencing[19] and subsequently found to dictate 'replicative lifespan', which is defined by the number of daughter cells a mother cell can produce[15]. Sir2 silences transcription at three loci: the *HM* mating-type loci, telomeres and the ribosomal DNA (rDNA). As cells age, the Sir2–Sir3–Sir4 complex relocalizes away from telomeres and the mating-type locus to accumulate at the rDNA locus, ostensibly to slow the formation and toxic accumulation of extrachromosomal rDNA circles (ERCs) that cause replicative ageing in yeast[20,21]. Inserting an extra copy of *SIR2* (but not *SIR3* or *SIR4*) into the yeast genome suppresses ERC formation and extends its lifespan[22]. This finding was corroborated by an unbiased genome-wide association study that identified the *SIR2* locus as the most significant regulator of yeast replicative lifespan[23]. In 2000, Sir2 was reported to have histone deacetylase activity, which results in chromatin compaction and requires NAD^+, a cofactor also required for redox reactions and whose levels change in response to nutrients and stress[24,25]. This striking discovery raised the intriguing possibility that yeast Sir2 and its homologues might act as nutrient and metabolic sensors that relay nuclear changes in the NAD^+/NADH ratio to alter both transcription and genome stability[24].

Sirtuins in other species were rapidly identified by virtue of their homology to yeast Sir2, specifically to the conserved central catalytic core[26]. In the nematode *Caenorhabditis elegans*[27] and the fruitfly *Drosophila melanogaster*[28], sirtuins were shown to control stress resistance and longevity, although the experimental approaches and magnitude of the effects have been debated[18] (Supplementary information S1 (box)). By now, numerous research groups have shown that sirtuin overexpression in the nematode[29–31] and the fruitfly[32,33] results in a reproducible increase in longevity.

On the heels of the work performed in these organisms, there has been considerable effort to determine the role of the seven mammalian sirtuins (SIRT1–7) and to find small molecules to modulate their activity for pharmaceutical purposes. There are many points at which to intervene. Sirtuins are regulated at the level of transcription, translation, protein stability and oxidation. Sirtuins are also regulated by protein–protein interactions, natural inhibitors such as nicotinamide, microRNAs, localization within the cell and within organelles, as well as by substrate and co-substrate availability[34–36]. Similar to the yeast sirtuins, the mammalian sirtuins SIRT1, SIRT6 and SIRT7 act as transcription regulators[37,38]. These sirtuins also serve many additional roles, including the control of energy metabolism, cell survival, DNA repair, tissue regeneration, inflammation, neuronal signalling and even

circadian rhythms (reviewed in REFS 39,40). SIRT1, which is predominately a nuclear protein, deacetylates histones H3, H4 and H1 (REFS 37,38) but also modifies more than 50 non-histone proteins[41], including transcription factors and DNA repair proteins. Examples of transcription factors regulated by SIRT1 include p53 (REFS 42,43), nuclear factor-κB (NF-κB)[44], peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α)[45] and sterol regulatory element-binding protein (SREBP)[46]. Examples of SIRT1-regulated DNA repair proteins include Ku70 (REFS 47,48), poly(ADP-ribose) polymerase 1 (PARP1)[49] and Werner syndrome ATP-dependent helicase (WRN)[50,51]. Other mammalian sirtuins localize to various subcellular compartments where they target non-histone proteins[41]: SIRT2 is cytosolic; SIRT3, SIRT4 and SIRT5 are mitochondrial; and SIRT6 and SIRT7 are nuclear (FIG. 2). Adding to the complexity, there is a growing list of chemical reactions that the mammalian sirtuins catalyse, including demalonylation, desuccinylation, decrotonylation, depropionylation, delipoamidation, other long-chain fatty acid deacylations and mono-ADP-ribosylation[52–56], which are generally referred to as deacylation reactions, not to be confused with deacetylation.

During sirtuin-mediated deacetylation of target Lys residues, NAD^+ is converted to nicotinamide (NAM) and *O*-acetyl-ADP-ribose[57]. NAM then acts as a sirtuin inhibitor by binding to the C-pocket of sirtuins, which lies adjacent to the NAD^+-binding pocket[58,59]. NAM remains one of the most effective and widely used sirtuin inhibitors. Recent studies have also revealed a link between NAM and its methylated form 1-methylnicotinamide (MNA) and health. In the nematode, both NAM and MNA increase longevity, and so does overexpression of the nematode gene required for formation of MNA, *anmt-1*, which is a homologue of the mammalian NAM-*N*-methyltransferase (NNMT), via a pathway seemingly independent of sirtuin activity[30]. In mammals, knockdown of NNMT protects against diet-induced obesity[60], although a more recent study found that liver NNMT is increased by calorie restriction and seemed to improve glucose and cholesterol metabolism[61]. Additional studies are needed to understand the potential benefits of sirtuin-mediated by-products of NAD metabolism in mammals.

Although the results from experiments in which sirtuins were upregulated genetically or pharmacologically are generally promising (TABLE 1), the large number of pathways in which sirtuins are involved is a potential double-edged sword. Sirtuins interact with all the major conserved longevity pathways, for example AMPK[62] and insulin–IGF1 signalling[63–68], including targets such as protein kinase A (PKA)[63], mTOR[64–66], forkhead box O (FOXO)[67] and IGF1 (REF. 68) (FIG. 1). Whether the multiple pathways in which sirtuins are involved will prove to be advantageous or deleterious for therapy in humans is unclear, but in the past 10 years, numerous sirtuin-overexpressing mouse strains have been generated, and at least two of them live longer. Notably, however, a transgenic mouse with high levels of *Sirt1* overexpression recapitulates many of the benefits of calorie restriction, including increased metabolic activity, reduced blood lipid levels and improved glucose metabolism, but not a longer lifespan[69]. Another transgenic mouse with moderate overexpression of *Sirt1* is protected from inflammation, liver cancer, diabetes and hepatic steatosis, but it too does not live longer[70,71]. A brain-specific *Sirt1*-overexpressing mouse strain (BRASTO) has a 9–16% longer mean lifespan, depending on the sex of the mice, and a significant increase in maximal longevity[72]. Male mice ubiquitously overexpressing *Sirt6* live ~15% longer than wild-type mice, although this effect is not observed in females[73]. *Sirt6*-overexpressing mice are also resistant to hypoxia and to the detriments of a high-fat diet[74]. In humans, loss of SIRT6 activity has been directly implicated in the progression of ductal pancreatic adenocarcinoma, a highly fatal form of pancreatic cancer[75]. These findings indicate that molecules that activate SIRT1 and/or SIRT6 in humans may provide both broad health benefits and potent antitumour activities[76]. Achieving this therapeutic potential, however, would require a detailed molecular understanding of how sirtuins can be activated.

Sirtuin-activating compounds

The search for molecules that activate sirtuins began more than a decade ago. In the past 3 years, many of the challenges facing the development of STACs as medicines have been overcome, including resolving technical issues (see below), increasing the bioavailability of STACs and determining which diseases to prioritize in clinical trials.

How do sirtuin activators work? The first STACs were discovered for SIRT1 in 2003, the most potent of which was resveratrol. This initial discovery was important because it proved that allosteric activation of sirtuins was possible[77]. High-throughput screening and medicinal chemical efforts have since identified more than 14,000 STACs from a dozen chemical classes, including stilbenes (for example, resveratrol), chalcones (for example, butein) and flavones (for example, quercetin) from plants[77]. Synthetic STACs include imidazothiazoles (for example, SRT1720)[78], thiazolopyridines (for example, STAC-2), benzimidazoles (for example, STAC-5) and bridged ureas (for example, STAC-9)[79,80] (BOX 1). All of these chemical classes activate SIRT1 by lowering the K_m value of the substrate through a K-type allosteric activation mechanism. Interestingly, recent work demonstrated that SIRT6 deacetylase activity can be activated by endogenous fatty acids at the amino terminus of the enzyme[56], hinting that SIRT6 may also be amenable to activation *in vivo* by synthetic molecules. This finding presents an exciting possibility given the ability of SIRT6 to enhance DNA repair, to modulate the metabolism of cancer cells to prevent malignancy, and to extend mouse lifespan[73,75,81–84].

The original fluorescence-based screens used to discover STACs were criticized because STACs only seemed to activate SIRT1 when bulky and hydrophobic fluorescent moieties were attached to the peptide substrates[85,86]. The most critical paper concluded that resveratrol and the chemically distinct STACs SRT1720 and SRT2104 were simply binding to the hydrophobic fluorophores[17]. This challenge, which spanned a few years, led to the discovery that SIRT1 has several structural and

Hepatic steatosis
Also known as fatty liver, is a term used to describe the accumulation of fat in the liver cells.

Allosteric activation
Activation of an enzyme by binding of a ligand, which enhances the binding of substrates at other binding sites.

K_m
Michaelis constant, which reflects the affinity of an enzyme for its substrate. The K_m is measured as the substrate concentration at which the reaction rate is half of its maximum rate.

K-type allosteric activation
Refers to the major type of allosteric activation, in which the main feature that is altered is the Michaelis constant (K_m).

positional requirements of amino acids that are adjacent to the acetylated Lys residue for enabling substrate recognition[87] and SIRT1 activation[79,85,86]. A major clue came from the discovery that the activation of SIRT1 is favoured when a large hydrophobic residue is present on the carboxy-terminal side of the acetylated Lys[88]. A study performed on natural peptide substrates revealed that bulky hydrophobic amino acids (Trp, Tyr or Phe) at the +1 and +6 positions relative to the acetyl-Lys could substitute for the fluorescent groups in the original assays[89]. These findings indicated that SIRT1 prefers specific hydrophobic amino acids in locations adjacent to the target Lys for substrate recognition. The same study also identified a mutation in *SIRT1* (substitution of Glu230 with Lys) that

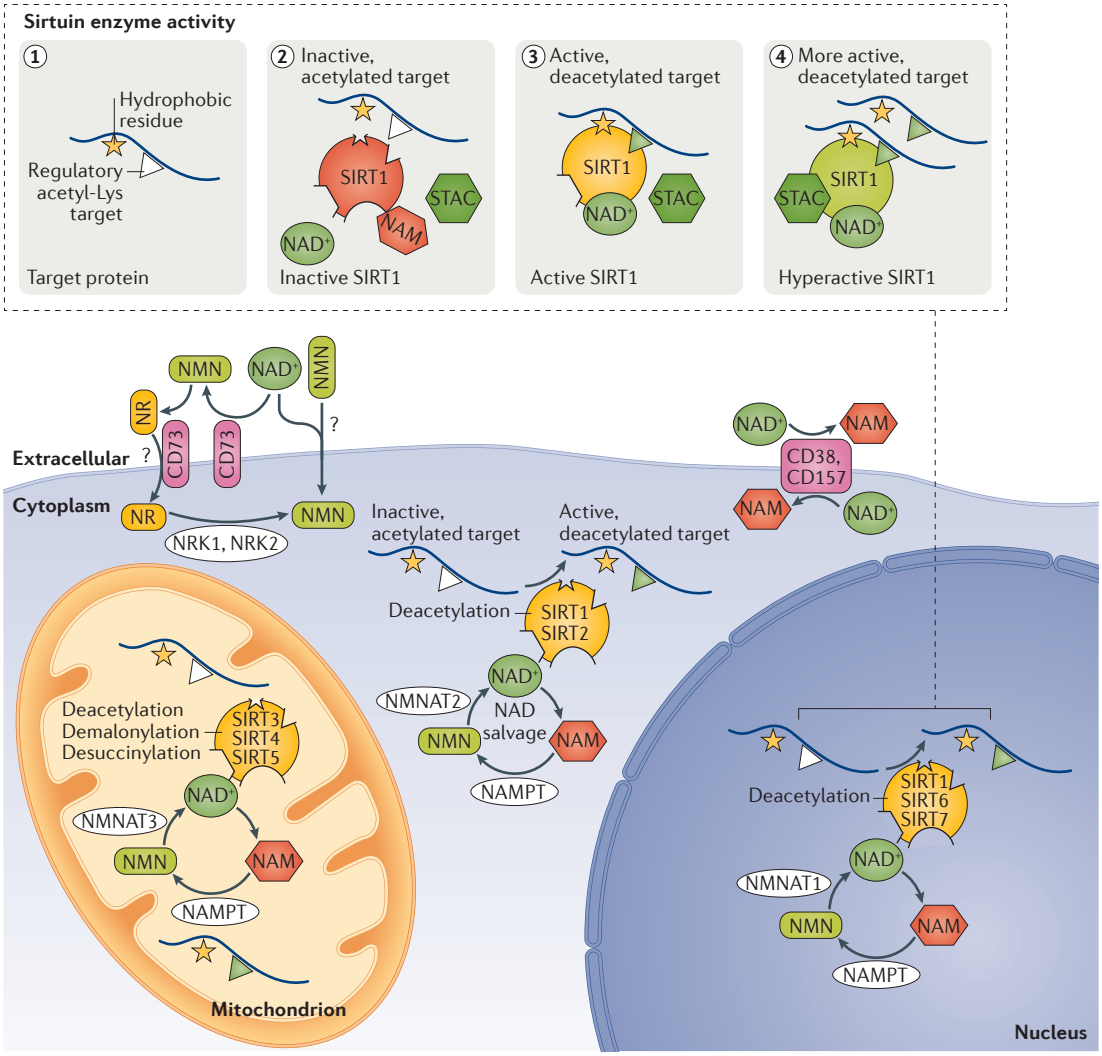


Figure 2 | **Localization, enzymatic activity and modulation of sirtuins by small molecules.** The mammalian sirtuins (SIRT1–7) are a family deacylases that are localized to various cellular compartments. SIRT1, SIRT6 and SIRT7 are localized primarily to the nucleus, whereas SIRT3, SIRT4 and SIRT5 are present in the mitochondria. SIRT1 (in some cell types) and SIRT2 are localized to the cytosol. Their activities are regulated by nicotinamide dinucleotide (NAD^+), which is synthesized *de novo* from Trp, Asp or niacin (not shown). NAD^+ is also recycled by the NAD salvage pathway from nicotinamide (NAM) by nicotinamide phosphoribosyltransferase (NAMPT), which converts NAM to nicotinamide mononucleotide (NMN). NMN is converted to NAD^+ by nicotinamide mononucleotide adenylyltransferase1 (NMNAT1), NMNAT2 or NMNAT3, which are also capable of converting nicotinic acid mononucleotide (NaMN) to NAD^+. Additional NAD^+ precursors used in the NAD salvage pathway include nicotinamide riboside (NR) and nicotinic acid riboside, which are converted to NMN and NaMN, respectively (not shown) by nicotinamide riboside kinase 1 (NRK1) and NRK2. The cell membrane-bound glycohydrolases CD38 and CD157 degrade NAD^+ to NAM[135,142]. CD73 converts NAD^+ to NMN and NMN to NR; it is a transmembrane protein that may also act as a NR transporter[177,178]. There is evidence to indicate that NAD^+ and NMN can be directly transported across the cell membrane in some cell types, although the mechanism is not yet known. The inset shows that SIRT1 activation is stimulated by the presence of a hydrophobic amino acid adjacently to the target Lys (panel 1). When NAM levels are sufficiently high, it binds into the carboxy-terminal pocket of SIRT1 and inhibits it[58] (panel 2). SIRT1 utilizes NAD^+ as a co-substrate to promote the binding and deacetylation of specific protein substrates (panel 3). Sirtuin-activating compounds (STACs) activate SIRT1 by binding to the STAC-binding domain and primarily lowering the K_m for the peptide substrate, thereby increasing its catalytic activity (panel 4).

Table 1 | **Sirtuins and sirtuin-activating compounds in stress resistance, calorie restriction, exercise and ageing**

Species	Sirtuin knockout shortens longevity	Sirtuin overexpression extends longevity	STACs extend longevity	Sirtuins mediate the effects of CR on longevity	Sirtuin expression or activity altered by CR or fasting	Sirtuin implicated in ageing-stress pathways	Sirtuin expression or activity altered by exercise
Yeast (*Saccharomyces cerevisiae*)	Yes (20–50%)[15,22]	Yes (30–50%)[15,22]	• Resveratrol (30–58%)[77] • Resveratrol failed[86]	• Sir2 is essential[132,180] • Hst2-dependent[181] • Sir2-independent[182,183]	• Yes[132,180] • NAD^+ levels unchanged[184,185]	>100 citations	Not tested
Worm (*Caenorhabditis elegans*)	Yes (~5–10%)[157,186]	• *High expression (2.1 line 50%)[27] • Low gene copy number (10–25%)[29,30,157,187,188] • Eleven independent low copy overexpressing lines (~5–15%)[188]	• Resveratrol (~10–65%)[157,159,189,190] • SRT1720 failed[158]	• Essential[31,186] • Non-essential[191,192]	Not tested	>40 citations	Not tested
Fruit fly (*Drosophila melanogaster*)	• Yes (~50%)[193] • No change[194,195]	• *Yes (~18–29%)[28,161] • Fat-body specific dSir2 (REFS 32,196) • Dose-dependent[197]	• Resveratrol (8–29%)[157,197] • Resveratrol failed[190]	• *Essential[28] • Fat-body specific dSir2 (REFS 32,196)	Yes[195,197]	>24 citations	Not tested
Honey bee (*Apis mellifera*)	Not tested	Not tested	Resveratrol (33–38%)[166]	Not tested	Not tested	Not tested	Not tested
Fish (*Nothobranchius furzeri* and *Nothobranchius guentheri*)	Not tested	Not tested	Resveratrol (27–59%)[163–165]	Not tested	Not tested	Not tested	Not tested
Mouse (*Mus musculus*)	• Elevated embryonic and postnatal lethality[198] • Yes (~20–50%)[68,199,200]	• Brain *Sirt1* (9–16%)[72] • *Sirt6* in males (9–17%)[73]	• Resveratrol in animals fed with high-fat diets[167] • SRT1720 (~8–22%)[124] • SRT2104 in males (~10%)[125]	Essential[199,200]	>100 citations	>800 citations	>100 citations

Multiple studies from the same laboratory were removed to limit citations. CR, calorie restriction; Hst2, homologous to Sir2; NAD^+, nicotinamide adenine dinucleotide; Sir2, silent information regulator 2; SIRTs, sirtuins; STACs, sirtuin-activating compounds. *Disputed by REF. 18.

prevented its activation by resveratrol[89] and, strikingly, also by more than 100 synthetic STACs from multiple chemical scaffolds[89], indicating that both natural and synthetic STACs activate SIRT1 by a common mechanism.

According to Zorn and Wells[90], "one of the simplest ways to activate an enzyme with a small molecule is to bind to an allosteric site within the catalytic domain of that enzyme and induce a conformational change which changes the affinity of that enzyme for its native substrate." Evidence from crystal structures and enzymological and biophysical studies has indicated that STACs function not by binding within the catalytic domain but by binding to a relatively rigid helix-turn-helix N terminus called the STAC-binding domain (SBD)[91]. Deletion studies of human SIRT1 have indicated that the N terminus is a key mediator of allosteric activation. The N terminus is necessary for activation by resveratrol and by synthetic STACs, which physically bind to this region[89], and itself can activate SIRT1 in *trans*[92]. These data indicate that the N-terminal domain may function to activate SIRT1 in *trans* when SIRT1 is a dimer, or in *cis* to enhance substrate binding to the SIRT1 catalytic core[92].

Recent crystallographic and amino acid substitution studies of human SIRT1 have identified the residues with which STACs come in contact and suggest an activation mechanism that is consistent with a 'bend-at-the-elbow' model[91]. STACs bind to the α-helical SBD in the N terminus, allowing the domain to flip over the site of interaction between the substrate and catalytic core[91] (FIG. 3a). Next to the SBD are the hinge residues Arg234 and Glu230, located within a polybasic linker (KRKKRK), which allow the STAC-bound SBD to interact with the catalytic domain through a salt bridge formed between the guanidinium group of the SBD and the carboxylate group of Asp475 and hydrogen bonds to His473 and Val459 (FIG. 3b). In this model, the negatively charged Glu230 makes contacts with the positively charged Arg446, which explains why the SIRT1 substitution of Glu230 to Lys (a negative to positive charge) blocks SIRT1 activation and why subsequently replacing Arg446 with Glu (a positive to negative charge) restores the ability of STACs to activate SIRT1.

Additional clues to the mechanism of the allosteric activation of sirtuins have emerged from studies of the yeast Sir2 protein, which is the catalytic component of a Sir2–Sir3–Sir4 complex. Molecular dynamic simulations have indicated that the substrate-binding channel toggles between an open and closed conformation, with Sir4

HOMA index
The homeostatic model assessment (HOMA) index is a clinical measure used to predict the function of pancreatic β-cells and insulin resistance.

maintaining the N-terminal helix in an active conformation[89]. Interestingly, Glu230 of mammalian SIRT1 structurally aligns with a similar negatively charged residue in yeast Sir2 (Asp223), which is required for gene silencing[93].

***Specificity of SIRT1 activators* in vivo.** Resveratrol is a nonspecific compound, meaning it interacts with numerous proteins within the cell[94]. In addition to SIRT1, reported targets of resveratrol include AMPK[95,96], phoshodiesterases[97], F1-ATPase[98], complex III of the mitochondrial electron transport chain[99], PARP1 and Tyr-tRNA synthetase[100]. Elucidating which effects of resveratrol are mediated by SIRT1 and which by other effectors has been a considerable task; for example, whether resveratrol acts on SIRT1 or AMPK. Metformin, a pro-longevity drug that activates AMPK and produces similar physiological effects to resveratrol, increases NAD^+ levels by activating the NAD salvage pathway enzyme nicotinamide phosphoribosyltransferase (NAMPT) (FIG. 2) and increases the NAD^+/NADH ratio, both of which increase SIRT1 activity[96,101]. Other studies have shown that resveratrol acts on SIRT1 to activate AMPK by deacetylating and activating the AMPK kinase LKB1 (also known as STK11)[102–105]. Thus, SIRT1 can activate AMPK and AMPK can activate SIRT1. Recent evidence suggests that resveratrol can activate both AMPK and SIRT1 *in vivo*; however, the predominant target is highly dose-dependent[105].

Box 1 | **The hunt for sirtuin activators**

As soon as yeast cells were shown to live longer with an extra copy of the sirtuin-encoding gene silent information regulator 2 (*SIR2*), a molecule that can activate sirtuins was quickly sought after. Generally, although activators are rare and much harder to study than inhibitors, they have distinct advantages: they are less prone to be rendered ineffective by residual enzymatic activity, they often have greater target specificity within an enzyme family and they elicit fewer side effects[90]. To date, the sirtuin that has received the most attention as a drug target is SIRT1. The first generation of sirtuin-activating compounds (STACs) were a group of related plant polyphenols that included, among others, quercetin, butein and resveratrol, which is a molecule found in red wine[77]. These molecules lower the binding affinity of SIRT1 for the substrate to increase enzymatic activity by tenfold[77]. Resveratrol rapidly became the molecule of choice to test SIRT1 activation because it was potent, nontoxic, naturally available and inexpensive. Many studies have since shown that resveratrol can extend maximal lifespan in yeast[154–156], worms[157,158], flies[159–161], fish[162–165] and even honeybees[166] (TABLE 1). In mice, resveratrol protects against many of the deleterious effects of a high-calorie diet, delays age-related diseases, increases exercise endurance, and can mimic many of the transcriptional changes caused by calorie restriction[167–171]. These findings have led to the prevailing model in which SIRT1 imparts many of the benefits of a calorie restriction diet[69–71,136]. Additional screens for more potent, soluble and efficacious STACs have been successful[172,173].

In mice, STACs mimic calorie restriction, increase mitochondrial function, protect from fatty liver and muscle wasting, and have strong antidiabetic, cardioprotective and anti-inflammatory effects in disease models[78]. STACs can also extend a mouse's lifespan by up to 15%, even when initially given at 1 year of age[123–125]. In dozens of studies, the effects of STACs were shown to be either partially or completely SIRT1-dependent (reviewed in REF. 174). For example, in mice, the ability of resveratrol to prevent skin tumours is blunted in the *Sirt1* germline knockout[175], and mitochondrial function in skeletal muscles is not induced in a *Sirt1*-knockout strain[105]. Furthermore, synthetic STACs that are much more specific such as SRT1720 and SRT2104 in mice increase mitochondrial mass in muscle and liver[124,125]. Mechanistically, STACs trigger the deacetylation of peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α) to increase mitochondrial biogenesis; these effects were also shown to be SIRT1-dependent[176].

The identification of a SIRT1 mutation that blocked STAC activation *in vitro*[89] has provided the perfect means to test whether STACs alter physiology by activating SIRT1 directly. In human HeLa cells and in mouse primary myocytes engineered to express the E230K SIRT1 mutation, resveratrol and synthetic STACs (SRT1720 and SRT2014) failed to induce the expected changes in mitochondrial mass, gene expression and cellular ATP concentrations. These results provided strong evidence that these STACs work by directly activating SIRT1 (REF. 89). To address the question of how STACs function *in vivo*, it would be interesting to repeat these experiments in a mouse carrying a SIRT1 E230K knock-in mutation.

Human and non-human primate studies. Studies using rodents have demonstrated that STACs promote health during ageing or metabolic stress, including protection from cancer, neurodegeneration, cardiovascular disease and diabetes, and even lifespan extension. Because regulatory agencies currently do not consider ageing a disease, the first sirtuin activator is most likely to be used clinically to treat an age-related disease such as diabetes, non-alcoholic fatty liver disease or an inflammatory disease (FIG. 4).

The big question is whether sirtuin activators will work in humans[106]. Resveratrol as a proof-of-concept molecule is providing valuable clues. Even before it was shown to activate SIRT1, epidemiological studies in the 1980s suggested that resveratrol may explain aspects of the 'French paradox', the phenomenon whereby the French have a low incidence of cardiovascular disease even though they consume high amounts of saturated fats[107]. However, the validity of the French paradox has since been challenged[108]. A conglomerate of laboratories at the National Institute on Aging in the United States evaluated the benefits of resveratrol in controlled studies in non-human primates[109–111]. Following a 2-year high-fat and high-sugar feeding regimen, they determined that a 2-year resveratrol treatment prevented high-fat and high-sugar-induced arterial wall inflammation and increased arterial pulse wave velocity[110], which are a major cause of cardiovascular morbidity and mortality in Western societies[112]. Additional studies from the National Institute on Aging using the same experimental approach found that resveratrol led to improvements in insulin sensitivity of visceral adipose tissue compared with control monkeys[109]. Moreover, preservation of pancreatic β-cell morphology and maintenance, and expression of essential β-cell transcription factors were observed[111].

At least six clinical studies have now evaluated the safety and effects of resveratrol in humans (TABLE 2). The majority of studies observed improvements in glucose metabolism and in multiple indicators of protection from cardiovascular disease[113–118]. In one randomized double-blind crossover study, a group of healthy obese men were administered resveratrol (150 mg per day) or placebo for 30 days. The men receiving resveratrol exhibited a significantly reduced resting metabolic rate, reduced systolic blood pressure and improved HOMA index[118]. Muscle samples from those patients showed increased SIRT1 and PGC1α protein expression, and increased AMPK activity, mitochondrial respiration and fatty acid oxidation[118].

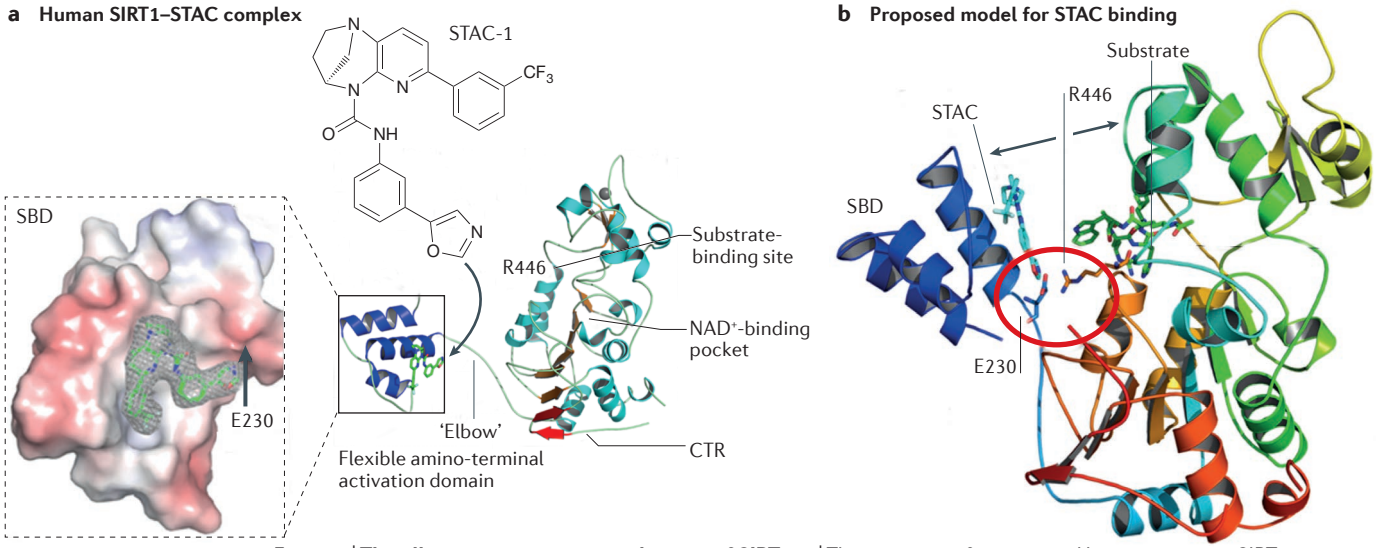


Figure 3 | **The allosteric activation mechanism of SIRT1. a** | The structure of a truncated human sirtuin 1 (SIRT1) in complex with a synthetic sirtuin-activating compound (STAC) showing the binding site of STAC-1 at the amino-terminal STAC-binding domain (SBD). STAC binding promotes the interaction of the SBD with the central catalytic domain to lower the K_m for the substrate, thereby increasing SIRT1 activity. The carboxy-terminal regulatory segment (CTR) stabilizes the deacetylase activity. Substitution of Glu230 (E230) with a Lys residue impairs enzymatic activation by reducing the electrostatic interaction between Glu230 on the SBD and Arg446 on the substrate-binding site. **b** | Model of how STACs activate SIRT1. Structural analysis and mutagenesis indicated that the amino acids Glu230 and Arg446 form a salt bridge (red circle) to facilitate binding of the amino terminus of SIRT1 with its catalytic core (movement indicated by arrows). The protein ribbon is rainbow-coloured from blue at the amino terminus to red at the carboxyl terminus. A STAC1 and an acetylated p53 peptide substrate are shown in cyan and green, respectively. Adapted from REF. 91, Nature Publishing Group.

Another study in non-obese men, however, found that resveratrol failed to provide any measurable physiological improvements[117]. More recently, a phase II study evaluating resveratrol in patients with Alzheimer disease showed that resveratrol can delay cognitive decline in the ability to perform daily tasks[119], a finding consistent with studies of resveratrol and SIRT1 in mouse models of Alzheimer disease[120]. A comprehensive summary and meta-analysis of clinical data has been recently published, the conclusion of which is that the human data are consistent with rodent studies, although more studies are warranted[121].

The reason for variability in the efficacy of resveratrol in clinical trials is not yet known[106]. One explanation is the choice of human subjects. Studies that have carefully prescreened patients for advanced age and/or for insulin resistance have seen the greatest benefit of resveratrol, suggesting that SIRT1 activation restores homeostasis and is most effective in the context of dysfunctional physiology[114]. In mice, for example, the strongest effects of resveratrol are on obese mice. Another confounding issue is that resveratrol and many of the early STACs are hydrophobic, with low solubility and bioavailability. Thus, the efficacy of STACs probably depends on the formulation, the size of the chemical particles and whether the compounds are taken with food[106]. Nevertheless, for resveratrol to have metabolic benefits it might not need to be available to all tissues: a recent study using rats found that resveratrol activates SIRT1 in the gut endothelium to stimulate the secretion of the metabolic hormone glucagon-like peptide 1, thereby reducing glucose levels without even having to enter the circulation[122].

More than 14,000 synthetic STACs have been synthesized to date, and dozens of these have been tested in animal models of type 2 diabetes, colitis, neurodegeneration, fatty liver and atherosclerosis, among others[78,89,123–127]. Currently, STACs are in their fifth generation[78–80] and have more than 1,000-fold greater potency *in vitro* than resveratrol, with an EC_{50} in the low nanomolar range. The STAC named SRT2104, which mimics aspects of calorie restriction and extends male mouse lifespan[125], has advanced through multiple phase I trials with few, if any, side effects[128–130] to phase II trials (TABLE 2). Two SRT2104 clinical trials in elderly volunteers and otherwise healthy smokers showed a slight reduction in body weight, a 15–30% improvement in the cholesterol ratio and a 19% decrease in triglyceride levels[129,130]. A separate study of patients with the inflammatory condition plaque-type psoriasis showed a significant reduction in disease manifestation following 84 days of oral administration of 500 or 1,000 mg per kg SRT2104 (REF. 131). Pharmaceutical companies are continuing to develop STACs that have improved pharmaceutical properties and to conduct additional clinical trials (J. Ellis, personal communication).

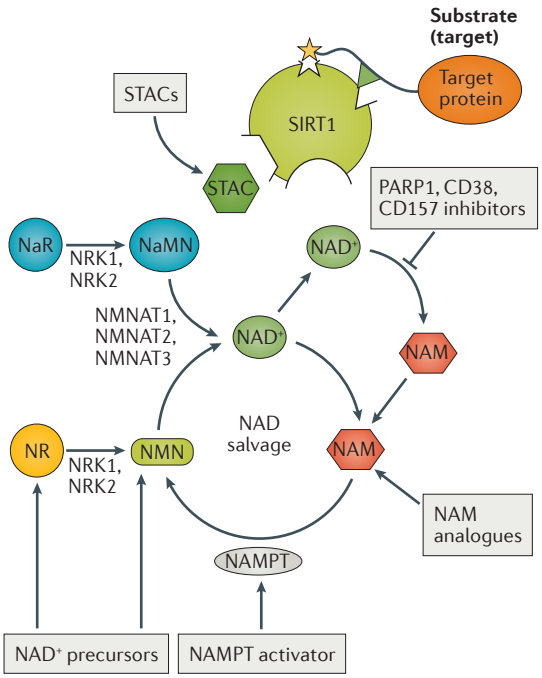
NAD^+ boosters as sirtuin activators

It has been known since 2003 that upregulation of the NAD salvage pathway, which recycles NAD^+ from NAM, can extend lifespan and mimic calorie restriction in yeast[132,133]. The gene responsible for the rate-limiting step in the NAD salvage pathway in yeast, *PNC1* (which encodes nicotinamidase), is activated by diverse stresses and calorie restriction, resulting in greater flux through

Bioavailability
The degree and rate at which a substance is absorbed and is made available at the site of physiological activity.

EC_{50}
The concentration of substrate that elicits a half-maximal enzymatic response.

Plaque-type psoriasis
The most common form of the disease, which is manifested as raised, red patches covered with a silvery white build-up of dead skin cells or scale.



Target proteins	Tissues and pathologies
FOXO NF-κB	**Brain** • Alzheimer disease • Neurodegenerative disease
STAT5 EF2	**Eye** • Macular degeneration • Keratoconjunctivitis sicca
AKT TGFβ	**Skin** • Psoriasis • Severe plaque-type psoriasis
FOXO UCP2	**Pancreas** • Glucose sensing • Hyperinsulinaemia • Metabolic syndrome
PGC1α FOXO	**Muscle** • Type 2 diabetes • Sarcopaenia • Cardiovascular disease
LXR PPARα	**Liver** • Metabolic syndrome • Hepatic steatosis
PPARγ MYC	**Fat** • Inflammatory cytokines • Metabolic syndrome
IL-8 MMP9	**Lung** • Chronic obstructive pulmonary disease
HNGB1 NLRP3	**Blood** • Sepsis • Endotoxin-induced inflammation

Figure 4 | **Sirtuin activation and its disease relevance.** Sirtuin activity can be stimulated through various mechanisms: allosterically by sirtuin-activating compounds (STACs), such as resveratrol, SRT1720 and SRT2104, which lower the K_m for the target proteins; increasing nicotinamide adenine dinucleotide (NAD^+) levels by providing its precursors nicotinamide riboside (NR) or nicotinamide mononucleotide (NMN); inhibiting the glycohydrolases CD38 or CD157 (with apigenin, quercetin, GSK 897-78c)[142,143]; inhibiting poly(ADP-ribose) polymerases (PARPs)[34]; activating nicotinamide phosphoribosyltransferase (NAMPT) with P7C3 (REF. 152); or by providing nicotinamide (NAM) analogues, such as methyl-NAM. Nicotinamide riboside kinase 1 (NRK1) and NRK2 convert NR and nicotinic acid riboside (NaR) is to NMN and nicotinic acid mononucleotide (NaMN), respectively. NaMN and NMN are converted to NAD^+ by nicotinamide mononucleotide adenylyltransferase 1 (NMNAT1), NMNAT2 and NMNAT3. Sirtuins directly or indirectly target more than 100 signalling proteins with relevance to human disease in a variety of tissues ranging from brain to blood (reviewed in REF. 41). Although STACs have therapeutic potential, certain diseases, such as cancer in certain contexts and Parkinson disease, may benefit from sirtuin inhibition (reviewed in REF. 179). EF2, elongation factor 2; FOXO, forkhead box O; HNGB1, high-mobility group box 1; IL, interleukin; LXR, liver X receptor; MMP9, matrix metalloproteinase 9; NF-κB, nuclear factor-κB; NLRP3, NOD-, LRR- and pyrin domain-containing 3; PGC1α, peroxisome proliferator-activated receptor-γ co-activator 1α; PPAR, peroxisome proliferator-activated receptor; STAT5, signal transducer and activator of transcription 5; TGFβ, transforming growth factor-β; UCP2, mitochondrial uncoupling protein 2.

the pathway and increased Sir2 activity. This early observation helped to explain how diverse stresses such as calorie restriction, amino acid restriction, heat stress and osmotic stress extend yeast lifespan.

In mammals, the homologue of PNC1 is NAMPT, which also catalyses the rate-limiting step in NAD salvage. NAMPT catalyses the formation of nicotinamide mononucleotide (NMN) from NAM, which is then converted to NAD by nicotinamide mononucleotide adenylyltransferase 1 (NMNAT1), NMNAT2 and NMNAT3 (FIG. 4). Nicotinamide riboside, a naturally occurring precursor of NAD^+, enters the salvage pathway after being converted to NMN by the nicotinamide riboside kinase (NRK) enzymes. CD38 and its homologue CD157 are glycohydrolases that degrade NAD^+ (REF. 134). As humans and mice age, levels of NAD^+ decline, possibly because the consumption of NAD^+ by CD38 outweighs its synthesis by the kynurenine pathway[135].

NAD-boosting molecules constitute a newer class of STACs gaining attention as a way to restore NAD^+ levels in elderly individuals and potentially activate all seven sirtuins with a single compound. Examples of NAD-boosting molecules include NMN, nicotinamide riboside[67,136–141] and inhibitors of CD38 such as apigenin[142], quercetin[142] and GSK 897-78c[143]. In rodents, these molecules have been administered via various routes, including intraperitoneal, gavage and in drinking water, at doses ranging from 100 to 1,000 mg per kg per day[67,136–141] for more than 6 months without any apparent adverse side effects[140]. The effects of NAD-boosting STACs on physiology are surprisingly broad, with improvements in glucose metabolism and mitochondrial function, and the

recovery from injury of the heart, ears and eyes[67,144–147] (FIG. 4). Nicotinamide riboside supplementation in mice starting at 24 months of age (a very advanced aged for mice), resulted in a modest (~5%) yet significant increase in longevity[141]. Nicotinamide riboside also prevents high-fat diet-induced glucose dysregulation[145] and protects mice from DNA damage[148,149], noise-induced hearing loss[150], cardiac injury[151] and stem cell-niche depletion[141]. Treatment with the related molecule NMN also protected 2-year old mice against a high-fat diet[147] and restored youthful levels of mitochondrial function, ATP production and insulin sensitivity in muscle, ostensibly through SIRT1 activation[137,147]. Other indicators of inflammation and muscle wasting were also reduced[137,147]. Other NAD^+ precursors such as nicotinic acid adenine dinucleotide or nicotinic acid riboside have yet to be tested but may be equally or even more efficacious. Non-naturally occurring derivatives of these compounds are also worth exploring given that the natural compounds have relatively low stability and short half-lives[145].

Targeting the enzymes that regulate NAD^+ levels, such as CD38, CD157 and NAMPT, may also be worth exploring for their therapeutic potential (FIG. 4). For example, a recent study indicated that a class of neuroprotective compounds (for example, P7C3) work by allosterically activating NAMPT[152]. This finding is consistent with a study showing that using NMN to increase NAD^+ can protect neurons from the degeneration caused by SARM1, a protein that triggers a precipitous decline in NAD^+ levels[101]. It will be interesting to test the effects of P7C3 and related molecules and whether they might have benefits similar to or better than the currently available STACs.

Future perspectives

The sirtuins have generated a considerable amount of excitement and scrutiny over the past 20 years. There is now consensus that sirtuins underlie aspects of calorie restriction and that they are key regulators of ageing and age-related diseases. Moreover, STACs can prevent and treat a variety of diseases in model organisms and in humans by directly binding to and activating SIRT1.

The path forwards now seems clear. For many years, synthetic STACs have been sufficiently potent to justify their use in the clinic but proved to be limiting in their bioavailability. Now with more soluble and specific compounds available[89,91,123,125,127], STACs are re-entering clinical trials. Currently, given the promising results in patients with psoriasis treated with SRT2014 (REF. 131), further clinical trials for this disease seems to be a good path to follow. Compounds that raise NAD^+ levels, such as nicotinamide riboside and NMN, also show great promise as calorie restriction mimetics to treat numerous age-related conditions, and possibly extend lifespan. Other possible trials include those to treat rare diseases such as a the DNA damage syndromes trichothiodystrophy or Cockayne syndrome (which are both alleviated by increased NAD^+ levels in mice), an inflammatory disorder such as psoriasis, or metabolic diseases such as type 2 diabetes or non-alcoholic fatty liver disease.

Although many questions have been resolved in recent years, many still remain. For example, the physiological roles of the newly described activities of the sirtuins — namely, demalonylation, desuccinylation, decrotonylation, depropionylation and delipoamidation — remain unclear[55]. The pharmacokinetics and pharmacodynamics

Table 2 | **Clinical trials of sirtuin-activating compounds (2000–2016)**

Sirtuin-activating compound	Phase I (safety)	Phase II (safety and efficacy)	Phase III–IV (pivotal and post-marketing)
Resveratrol or trans-resveratrol	• Cardiovascular: NCT01564381, NCT02415114, NCT02616822, NCT01842399, NCT01185067 • Cancer: NCT00256334 • Diabetes: NCT01677611, NCT02502253 • Neuropathy: NCT01339884, NCT01126229, NCT01321151, NCT01640197	• Cardiovascular: NCT01449110, NCT01564381, NCT01842399, NCT02409537, NCT01615445, NCT02062190 • Diabetes: NCT02114892, NCT01158417, NCT02549924, NCT02247596, NCT01451918, NCT01714102, NCT02216552 • Neuropathy: NCT01339884, NCT02621554, NCT01504854, NCT02062190	• Cardiovascular: NCT01914081 • Pulmonary: NCT02245932 • Diabetes: NCT01997762, NCT02244879, NCT02219906, NCT02216552, NCT02766803 • Inflammation: NCT01492114, NCT02244879, NCT02475564, NCT02433925, NCT02130440 • Neuropathy: NCT02621554, NCT02336633, NCT00678431, NCT01219244
SRT2104	• Cardiovascular: NCT01031108, NCT01702493 • Diabetes: NCT01031108 • Inflammation: NCT01453491, NCT01014117	• Inflammation: NCT01154101 • Diabetes: NCT01018017, NCT00937326	None
Nicotinamide riboside	• Phamacokinetics: NCT02689882, NCT02300740, NCT02721537, NCT02712593, NCT02191462 • Cardiovascular: NCT02678611	• Diabetes: NCT02303483	None

Clinical trials of sirtuin-activating compounds are listed in the table with their unique ClinicalTrials.gov identifier number. At the time of this publication, we excluded studies with unknown status.

of NAD precursors and how they are affected by the route of delivery need clarification. In addition, identifying the NAD^+ pools responsible for eliciting their benefits, both within the body and within subcellular compartments, is important. Moreover, are other sirtuins in addition to SIRT1 and SIRT6 amenable to allosteric activation? And what are the transport mechanisms for NAD^+ precursors across cell membranes into the bloodstream and into cells[153]? This last question is important given new data indicating that there is an intracellular form of NAMPT (iNAMPT) and an extracellular form (eNAMPT), which is secreted from fat tissue and controls both systemic and hypothalamic NAD^+ bioavailability[153]. Perhaps the most practical question is whether STACs will ever be approved as a drug to treat ageing or age-related diseases in humans. With the elderly population increasing worldwide and health-care costs threatening the global economy, the answer to that question cannot come soon enough.

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Competing interests statement
The authors declare competing interests: see Web version for details.

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