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Role of Sirtuin 1 in metabolic regulation

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Sirtuin proteins are an evolutionarily conserved family of NAD$^+$-dependent protein deacetylases. Originally discovered in yeast as gene-silencing proteins, they subsequently emerged as key regulators of life span in yeast and other lower species. Recent identification and characterization of mammalian Sirtuin homologs have revealed a widespread spectrum of biological activities in gene regulation linked to cell survival, cell apoptosis and metabolism. These findings have stimulated several drug discovery efforts. Here, we review current knowledge of the biological functions and possible pharmacological implications of Sirtuin 1, the most characterized member of the mammalian Sirtuin family, in glucose and fat metabolism.

Introduction

Silent information regulator 2 (Sir2) proteins are an evolutionarily conserved family of NAD$^+$-dependent protein deacetylases [1–3]. Sir2 proteins were first characterized in yeast, where they were shown to act as histone deacetylases [4] that silenced transcription at silent mating type loci [5], telomeres [6] and the ribosomal DNA (rDNA) [7,8]. Sir2-mediated silencing of the rDNA locus suppressed recombination in the rDNA [4,9] and the production of extrachromosomal rDNA circles [4,9], which reduce replicative yeast life span [9,10]. Additional copies of the *Sir2* gene extended life span in yeast [11], worms [12] and flies [13], whereas deletion of the Sir2 gene reduced life span in yeast [9]. Furthermore, calorie restriction (CR), the most consistent regimen to extend longevity in different species, required the *Sir2* genes for life span extension in yeast and flies [11,13]. Bioinformatics studies identified seven homologs of the yeast Sir2 protein in mammals, called Sirtuins 1–7 (SIRT1–7) [14,15]. The seven isoforms differ in their subcellular localization and in the amino-terminal and carboxy-terminal protein sequences flanking the catalytic core domain. Most sirtuins (SIRT1, SIRT2, SIRT3 and SIRT5) deacetylate lysine residues from protein substrates [16]. The deacetylase reaction requires NAD$^+$ and produces deacetylated protein, nicotinamide (NAM) and *O*-acetyl-ADP-ribose. SIRT4 and SIRT6 mediate the ADP-ribosylation of protein substrates using NAD$^+$ as a donor [17–19]. Several outstanding reviews have been written on the role of sirtuins in aging and metabolism [20–23], on the biochemical properties of sirtuins [16,24] and on the pharmacological modulation of sirtuin activities [25,26]. This review provides a simplified picture of the many partly opposing regulatory roles of SIRT1 in energy metabolism and highlights that SIRT1 is an attractive drug target for metabolic disease.

Mitochondria are a major target of metabolic sirtuin regulation

Several sirtuins, in particular SIRT1, SIRT3, SIRT4 and SIRT5, regulate mitochondrial functioning, and this has major metabolic implications. SIRT1 upregulated mitochondrial biogenesis in several tissues, including skeletal muscle [27–29], brown fat [28,29], liver [29], coronary arterial endothelial cells [30] and renal proximal tubule cells [31]. Mitochondrial biogenesis was, at least in part, mediated by proliferator-activated receptor-coactivator-1 α (PGC-1α) [27–31], peroxisome proliferator-activated receptor α (PPARα) [32], nuclear respiratory factor-1 and mitochondrial transcription factor A [30], and this was reflected by an induction of electron transport and oxidative phosphorylation genes [28,30,31], increased mitochondrial DNA content [30,31] and elevated mitochondrial respiration rates and ATP levels [31].

SIRT3, SIRT4 and SIRT5 were found to localize in mitochondria. SIRT3 deacetylated and thereby stimulated various mitochondrial

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matrix proteins, including acetyl-CoA synthase 2, glutamate dehydrogenase and isocitrate dehydrogenase 2 [33–35]. Activation of these mitochondrial enzymes generates tricarboxylic acid cycle intermediates that promote mitochondrial electron transport and ATP production. In addition, SIRT3 enhanced mitochondrial electron transport via deacetylation of NDUFA9, a complex I subunit. SIRT3 knockout mice displayed an approximately 50% reduction in basal ATP levels in heart, liver and kidney, underscoring the important role of SIRT3 in mitochondrial energy metabolism [36].

SIRT4 inhibited amino-acid-induced and glucose-induced insulin secretion from pancreatic β cells through ADP-ribosylation of mitochondrial glutamate dehydrogenase [17,18]. SIRT4 downregulation seems to play a part in amino-acid-stimulated insulin secretion during CR [17].

SIRT5 stimulates the initial step of the urea cycle by deacetylating carbamoyl phosphate synthetase 1 (CPS1) in mitochondria. Deacetylation of CPS1 was triggered by fasting-induced increments in NAD^+ concentrations. SIRT5 knockout mice failed to upregulate CPS1 activity and showed elevated blood ammonia during fasting [37]. Similar effects were observed during CR and on high-protein diet, showing that SIRT5 plays an important part in ammonia detoxification during fasting, CR and high-protein diet [37].

Regulation of SIRT1 activity

Regulation of SIRT1 activity by nutritional status

CR and fasting were both reported to upregulate SIRT1 protein in mammals in several tissues, including liver, skeletal muscle and fat [38–43]. Discordant results were reported by two studies, in which CR led to no change or a downregulation of SIRT1 protein levels in liver [43,44] and skeletal muscle [44].

Rodgers *et al.* [45] found that SIRT1 protein expression in liver is specifically regulated by glucose and pyruvate and not by feeding-related hormones such as insulin or glucagon. Glucose reduced SIRT1 protein concentrations, whereas pyruvate augmented SIRT1 protein concentrations in cultured hepatocytes. Fasting increased pyruvate concentrations in mouse liver [45] consistent with a pyruvate-induced rise in SIRT1 protein expression. Fasting also raised the levels of NAD^+, a required cofactor for sirtuin activity, in liver [45] and skeletal muscle [27].

SIRT1 protein expression during fasting seems to be upregulated by a protein complex consisting of the forkhead transcription factor FoxO3A and p53. Consistent with a role for p53 in SIRT1 gene transcription, p53-deficient mice failed to increase SIRT1 expression in liver or muscle after overnight fasting [38].

Regulation of SIRT1 activity by NAD^+ metabolism

SIRT1 activity has been shown to be regulated in yeast and mammalian cells by NAD^+, NADH and the $[NAD^+]/[NADH]$ ratio. NAD^+ is a required cofactor for the deacetylation reaction of yeast and mammalian sirtuins [2,4]. The estimated concentration of free nuclear NAD^+ approximates the K_m values of several sirtuins, supporting that free cellular NAD^+ is regulatory for SIRT1 activity [46]. Fasting upregulated the NAD^+ concentrations in liver and skeletal muscle concomitant with an increase in SIRT1 enzyme activity [27,45]. This contrasts with the regulation of NAD^+ in yeast, in which CR did not affect NAD^+ concentrations but lowered NADH concentrations [47]. NADH competitively inhibited NAD^+-stimulated yeast Sir2 and human SIRT1 activities in biochemical assays [47]. In contrast to yeast, CR and fasting have not been reported to modulate NADH concentrations in mammalian tissues [27,38,40,45]. The $[NAD^+]/[NADH]$ ratio might further determine sirtuin activity. An increased $[NAD^+]/[NADH]$ ratio led to SIRT1-mediated inhibition of skeletal muscle gene expression [48] and might underlie yeast life span extension by CR [47].

The NAD^+ salvage pathway plays an important part in regulating SIRT1 deacetylase activity. During the deacetylation reaction, NAD^+ is hydrolyzed to NAM and *O*-acetyl-ADP-ribose [49,50]. NAM was found to be a strong inhibitor of yeast Sir2 and SIRT1 deacetylase activities [48,51–54]. The NAD^+ salvage pathway regenerates NAD^+ from NAM and thus promotes sirtuin activity not only by providing NAD^+ but also by reducing NAM. Overexpression of PNC1, the rate-limiting enzyme of the NAD^+ salvage pathway in yeast, extended yeast replicative life span [54]. Nicotinamide phosphoribosyltransferase (NAMPT) is the rate-limiting enzyme of the mammalian NAD^+ salvage pathway [55]. Forced expression of NAMPT correlated positively with SIRT1 activity [55]. NAMPT activity was found to depend on nutritional status. NAMPT levels increased in several cell lines in response to serum and glucose deprivation [56], in livers of starved rats [56] and in skeletal muscle of fasted mice [57]. AMP-activated protein kinase (AMPK), which senses low energy levels in cells, was shown to activate NAMPT in skeletal muscle in response to glucose deprivation, which in turn upregulated the $[NAD^+]/[NADH]$ ratio [57].

Regulation of circadian rhythms by SIRT1

The physiology and behavior of mammals oscillates in a circadian manner, which is roughly a 24-hour cycle. The primary circadian clock in mammals is located in the hypothalamic suprachiasmatic nucleus (SCN), but subsidiary oscillators exist in most peripheral tissues. Circadian rhythms are endogenous but adjusted to the environment by external cues called zeitgebers (timing cues). Light–dark cycles are the predominant zeitgeber for the SCN pacemaker, whereas food intake is a strong zeitgeber for subsidiary clocks in many peripheral tissues [58,59]. The SCN is believed to synchronize peripheral oscillations by imposing rest–activity rhythms and, thus, feeding–fasting cycles. Circadian control of metabolism is reflected by cyclic expression of metabolic genes [60,61] and diurnal variations in blood glucose and triglycerides [62]. High-fat diets can amplify circadian variations in glucose tolerance and insulin sensitivity [62], locomotor activity [63] and expression of genes controlling fuel use [63]. Importantly, disruption of circadian cycles in mice led to obesity and the metabolic syndrome [64].

The CLOCK and BMAL-1 proteins form heterodimeric transcription factors that control circadian gene expression in the SCN and in peripheral tissues. The CLOCK–BMAL-1 complex promotes expression of several circadian genes, including cryptochrome (CRY) genes and period (PER) genes in mammals. CLOCK was found to work as an acetyltransferase targeting BMAL-1 and histone H3 at circadian promoters [65]. CLOCK-mediated histone acetylation and BMAL-1 acetylation were both required for circadian gene expression [65,66]. SIRT1 was found to bind the CLOCK–BMAL-1 chromatin complex at circadian promoters [67,68] and to antagonize the acetyltransferase activity of CLOCK [67,68]. SIRT1 was required for high-magnitude oscillations of

several core clock genes, including BMAL-1, RORγ, PER2 and CRY1 [68]. Inhibition of SIRT1 activity led to disturbances in the circadian cycle and acetylation of BMAL-1 and histone H3 at circadian promoters [67]. SIRT1-dependent deacetylation of BMAL-1 was enhanced by NAD^+ and attenuated by NAM. SIRT1 thus seems to connect cellular metabolism with the circadian clock through its NAD^+ dependence [67,68].

The NAMPT protein levels and NAD^+ concentrations displayed circadian oscillations in mouse liver and white adipose tissue that were controlled by the SIRT1–CLOCK–BMAL-1 complex [69,70]. CLOCK–BMAL-1 stimulated transcription of NAMPT, the rate-limiting enzyme in NAD^+ biosynthesis [69], whereas SIRT1 abolished CLOCK–BMAL-1 stimulated transcription of NAMPT, the rate-limiting enzyme in NAD^+ biosynthesis [69], whereas SIRT1 abolished CLOCK–BMAL-1-stimulated NAMPT transcription [70].

Effects of SIRT1 on energy balance

Physiological insights into the *in vivo* role of SIRT1 on energy balance came from a recent study of transgenic mice moderately overexpressing SIRT1 from bacterial artificial chromosomes (SirBACO mice). SirBACO mice exhibited a physiological regulation of the SIRT1 transgene as the SIRT1 protein levels were moderately increased twofold to threefold relative to control littermates, and the SIRT1 protein distribution pattern resembled that of the endogenous protein in most tissues. SirBACO mice on a standard diet had reduced energy expenditure, reduced body temperature and reduced food intake. Body weight and body fat content were not altered. When SirBACO mice were made obese by a high-fat diet or crossed onto a genetically obese $Lepr^{db/db}$ background, SIRT1 overexpression did not alter energy expenditure or energy balance [71].

Discordant results were obtained from a different SIRT1-overexpressing transgenic line, in which the SIRT1 coding sequence was knocked into the β-actin gene. Mice that were hemizygous for the SIRT1 transgene expressed normal levels of β-actin and strongly upregulated levels of SIRT1 protein in several tissues. In contrast to SirBACO mice, SIRT1 knock-in mice were hypermetabolic, as reflected by increases in food intake and energy expenditure, reduced body weight and decreased body fat content [72]. The SIRT1 knock-in phenotype, however, is difficult to interpret because the transgene was expressed off the β-actin locus, potentially causing developmental and metabolic defects.

Germline SIRT1 knockout mice have been generated, but straightforward conclusions cannot be drawn because these mice display several developmental defects that might contribute to their metabolic phenotype [73].

Knocking down SIRT1 by intraperitoneal administration of antisense DNA oligomers (ASO) reduced food intake and body weights in lean and high-fat-diet-induced obese rats. Decreased food intake was not linked to hypothalamic neurons, which displayed normal levels of SIRT1 mRNA, but to a concomitant reduction in adipose mass and reduced plasma leptin levels [74].

Recent genome-wide association and linkage analysis studies have shown that genetic variations in the *SIRT1* gene correlate with the risk for obesity [75,76], further underscoring SIRT1's role in energy balance. Table 1 summarizes the reported actions of SIRT1 on energy balance.

Regulation of lipid and cholesterol metabolism by SIRT1

SIRT1 stimulates fat catabolism in liver

SIRT1 promotes fat catabolism in hepatocytes, as reflected by the development of fatty livers in liver-specific *SIRT1* knockdown and knockout mice [32,77]. *SIRT1* knockdown mice failed to upregulate fatty acid oxidation genes or repress lipogenic genes in response to fasting [77]. Likewise, liver-specific SIRT1 knockout mice showed downregulation of several fatty acid β-oxidation genes, many of which were targets of the nuclear receptor PPARα [32]. More detailed mechanistic studies demonstrated that SIRT1-induced expression of these PPARα target genes required SIRT1-mediated deacetylation of PGC-1α, an important transcriptional coactivator of PPARα [27,32,45,78].

ASO-induced SIRT1 knockdown did not cause hepatic steatosis of rats on a high-fat diet despite a 77% reduction in SIRT1 mRNA expression in liver [74]. This, however, does not necessarily oppose the above results because the prevailing SIRT1 protein levels might still have been sufficient to prevent hepatic steatosis.

SIRT1 stimulates fat catabolism in skeletal muscle

SIRT1 was also shown to upregulate fatty acid β-oxidation in skeletal muscle under fasting conditions. Fasting triggered SIRT1 protein expression in skeletal muscle, which in turn led to deacetylation of PGC-1α, a prerequisite for PGC-1α activity and enhanced expression of PGC-1α-regulated fatty acid β-oxidation genes [27]. The putative SIRT1 activators resveratrol and SRT1720 also caused upregulation of fatty acid β-oxidation and mitochondrial biogenesis genes in mouse skeletal muscle through SIRT1-mediated deacetylation of PGC-1α [28,29,79], and SIRT1 was found to mediate adiponectin-induced mitochondrial biogenesis in skeletal muscle [80]. This might play a part in the development of insulin resistance in obese subjects, which frequently display reduced plasma adiponectin levels and adiponectin resistance

TABLE 1

SIRT1 actions on energy balance

Animal model	Body weight	Food intake	Body fat content	Energy expenditure (VO_2)	Body temperature	Locomotor activity	Refs
SirBACO	=	↓	=	↓	↓	↓	Banks *et al.* [71]
SIRT1 knock-in into β-actin gene	↓	↑	↓	↑			Bordone *et al.* [72]
Germline SIRT1 knockout	↓	↑		↑		↓	Boily *et al.* [73]
Liver-specific SIRT1 knockout	↑	=					Purushotham *et al.* [32]
ASO SIRT1 knockdown	↓	↓	↓				Erion *et al.* [74]

[80]. AMPK has been proposed to activate SIRT1-dependent induction of mitochondrial and lipid utilization genes in skeletal muscle in response to fasting or exercise [81]. The underlying mechanism seems to be an AMPK-induced increase in NAMPT activity and cellular NAD^+ levels [57,82].

SIRT1 stimulates fat catabolism in adipose tissue

In white fat cells, SIRT1 was shown to inhibit fat storage in fasted but not in fed mice. SIRT1 interacted with the nuclear corepressor NCoR to repress peroxisome proliferator-activated receptor γ (PPARγ), a nuclear receptor that promotes fat storage [83].

Knockdown of SIRT1 in rats by ASO decreased their white adipose mass. The mRNA levels of the adipogenic genes PPARγ, PPARγ2 and CCAAT/enhancer binding protein alpha were all reduced in adipose tissue. Reduced expression of these factors, which drive adipocyte differentiation, might cause the loss of adipose mass. SIRT1 knockdown in adipocytes also resulted in lowered plasma concentrations of leptin and adiponectin, which are both synthesized in fat cells. This reduction in plasma adiponectin levels is consistent with the stimulation of adiponectin synthesis by SIRT1 [71]. It remains to be clarified, however, why SIRT1 knockdown downregulates PPARγ [74], in opposition to previous studies [83].

In addition to SIRT1's potential role in white adipocyte differentiation, a recent study in our group suggested that SIRT1 might influence brown adipocyte differentiation by silencing a myogenic transcriptional signature in brown preadipocytes [84].

Potential role of adiponectin in SIRT1-induced fat catabolism

One potentially important mechanism accounting for the systemic upregulation of fatty acid oxidation by SIRT1 is adiponectin. Adiponectin signals through at least two receptors, AdipoR1 and AdipoR2, in skeletal muscle and liver to activate AMPK and PPARα [85]. AMPK and PPARα both upregulate fatty acid β-oxidation and mitochondrial biogenesis in these tissues. SIRT1 was shown to deacetylate FoxO1, which in turn stimulated adiponectin gene transcription in white adipocytes. Accordingly, SirBACO mice displayed elevated plasma adiponectin levels, whereas plasma levels of other adipokines such as resistin, PAI-1, TNF-α and leptin were unchanged. Consistent with enhanced adiponectin signaling, SirBACO mice showed elevated phospho-AMPK levels in liver, muscle and white fat and displayed increased mRNA levels of PPARα and its target genes, carnithine palmitoyl transferase 1 and AdipoR2 [71].

SIRT1 stimulates reverse cholesterol transport

Cholesterol is a major risk factor for cardiovascular disease and is the major component of atherosclerotic plaque. Cholesterol accumulation within atherosclerotic plaque is an early pathogenic event that occurs when cholesterol influx into the arterial wall exceeds cholesterol efflux. Increased influx of cholesterol into the arterial wall is followed by an increased influx of macrophages, which take up oxidized and aggregated low-density lipoprotein (LDL) cholesterol. Cholesterol that is synthesized in extrahepatic tissues or acquired from lipoproteins is returned to the liver for excretion in a process called reverse cholesterol transport. The initial step in reverse cholesterol transport is thought to be efflux of cholesterol from cell membranes to acceptor particles in the interstitial fluid. This can happen through the free diffusion of cholesterol molecules across cell membranes and incorporation into lipid-phospholipid-albumin complexes or through the interaction of high-density lipoprotein (HDL) with cell surface binding sites. The latter mechanism operates mainly in macrophages and other cholesterol-loaded cells. Liver secretes the HDL particles as pre-HDL complexes consisting mainly of phospholipid and apolipoprotein A1. Cholesterol-loaded HDL particles are ultimately returned to the liver for excretion of cholesterol into bile. Current strategies to reduce coronary heart disease are aimed primarily at reducing the influx of cholesterol into the arterial wall by lowering plasma LDL cholesterol concentrations. In a considerable proportion of patients, plasma LDL concentrations cannot be lowered to a level that would stop progression of disease. There is, therefore, great interest in strategies that enhance cholesterol efflux from the arterial wall and promote its transport to the liver for excretion.

Liver X receptors (LXRs) are nuclear receptors that function as cholesterol sensors and regulate whole-body cholesterol homeostasis. The LXRs function as heterodimers with retinoid X receptors, which can be activated by ligands for LXRs such as oxysterols. LXRα is highly expressed in liver, adipose tissue, intestine and macrophages, where it regulates reverse cholesterol transport. LXRα stimulates expression of the ATP-binding cassette transporters A1 (ABCA1) and G1 (ABCG1), both of which mediate the efflux of cholesterol from peripheral tissues to apolipoprotein A1 to form HDL.

Li *et al.* [86] found that SIRT1 deacetylated LXRα at a single conserved lysine (K432) to increase promoter activity of *ABCA1* and upregulate its expression. Macrophages and hepatocytes subcultured from germline SIRT1 knockout mice showed a diminished response to LXR ligand-induced expression of ABCA1 and reduced cholesterol efflux compared with the corresponding wild-type cells. Consistent with impaired reverse cholesterol transport, germline SIRT1 knockout mice displayed decreased plasma HDL cholesterol concentrations. SIRT1-induced deacetylation not only increased LXRα transcriptional activity but also paradoxically targeted LXRα for subsequent ubiquitination and degradation. Interestingly, the proteasome inhibitor MG132 repressed LXRα-induced ABCA1 promoter activity to the same extent as the sirtuin inhibitor NAM, and the combination of NAM and MG132 did not result in a further inhibition. This suggested that ubiquitination and degradation of LXRα by the proteasome contribute to the induction of LXRα target genes [86].

ASO-mediated SIRT1 knockdown in rats impaired reverse cholesterol transport [74]. Plasma HDL cholesterol levels, as well as the levels of the cholesterol export and uptake proteins ABCA1 and scavenge receptor B1, were reduced in SIRT1 knockdown rats [74].

In contrast to the above studies, liver-specific SIRT1 knockout mice exhibited no signs of impaired reverse cholesterol transport, at least in liver. Expression of the major cholesterol uptake genes, cholesterol synthesis genes and cholesterol efflux genes was not altered in SIRT1 knockout livers. Cholesterol 7-alpha-hydroxylase, the rate-limiting enzyme in the conversion of cholesterol to bile acids, and other enzymes in the bile acid synthesis pathway were reduced in SIRT1 knockout livers, suggesting that the accumulation of hepatic cholesterol resulted from diminished bile synthesis [32].

Summary

Under fasting conditions, SIRT1 protein levels and NAD^+ concentrations rise along with SIRT1 enzyme activity. SIRT1 promotes fat

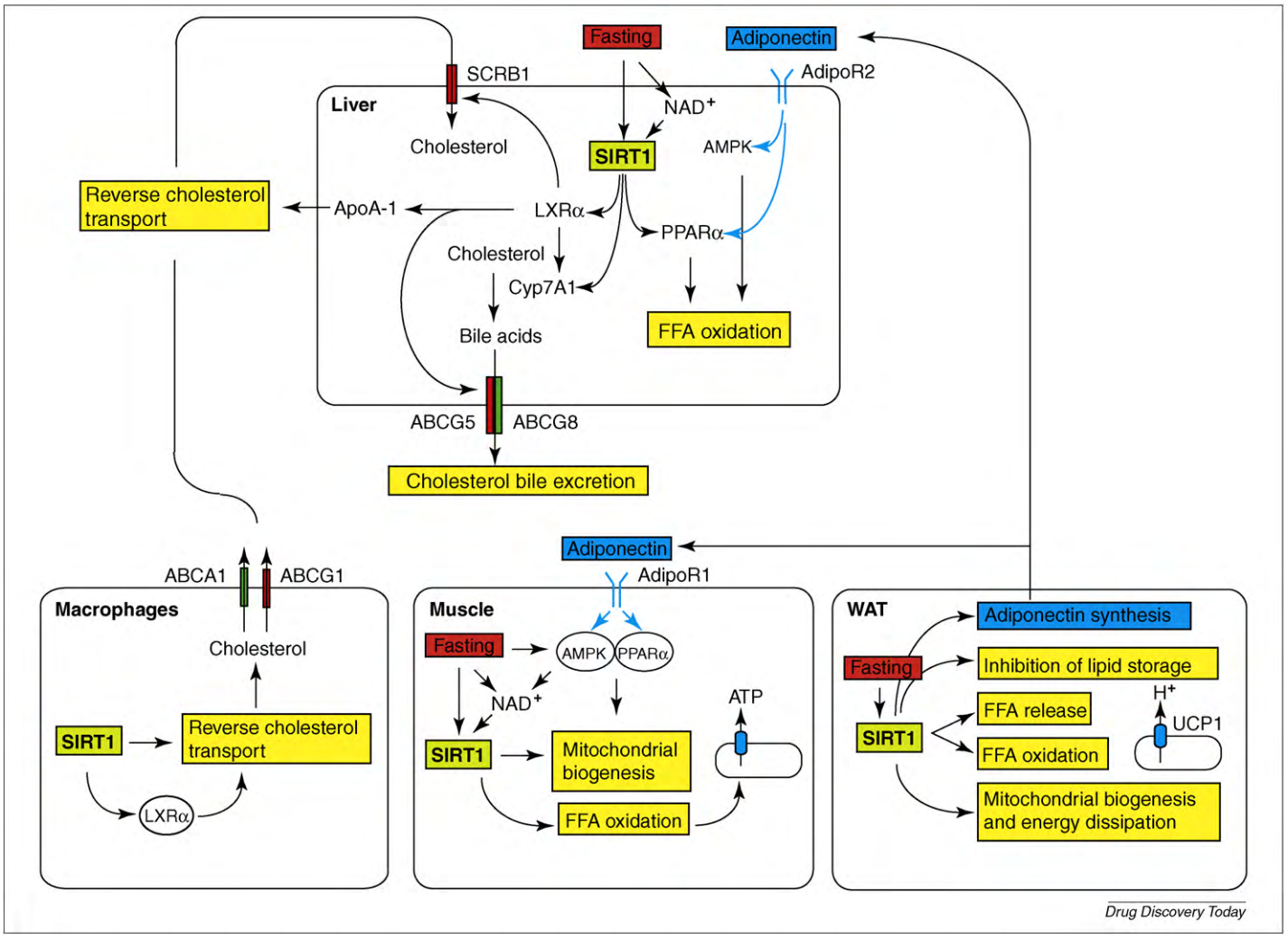

FIGURE 1

SIRT1 promotes fat and cholesterol catabolism. Fasting upregulates SIRT1 protein expression in liver, skeletal muscle and white fat and increases the levels of NAD^+ in skeletal muscle and liver, in turn leading to enhanced SIRT1 enzyme activity. AMPK activity is upregulated during fasting and accounts for the increase in NAD^+ levels in skeletal muscle. SIRT1 activates the nuclear receptor LXRα to drive reverse cholesterol transport (RCT) and cholesterol bile acid excretion. RCT is a process whereby cholesterol is carried on high-density lipoprotein (HDL) particles from macrophages and other peripheral tissues to the liver. SIRT1 inhibits lipogenesis and stimulates free fatty acid release from white adipocytes by blocking PPARγ. SIRT1 also promotes fatty acid oxidation, mitochondrial biogenesis and energy dissipation in muscle, fat and liver cells. This involves direct activation of PPARα by SIRT1 and secondary activation of PPARα and AMPK by SIRT1-mediated adiponectin synthesis and signaling. AdipoR1, adiponectin receptor 1; AdipoR2, adiponectin receptor 2; WAT, white adipose tissue.

and cholesterol catabolism in liver, skeletal muscle and adipose tissue by modulating the expression and activity of several transcription factors and coactivators such as PGC-1α, PPARα, PPARγ and LXRα (Fig. 1). SIRT1 also seems to activate fatty acid oxidation systemically by promoting adiponectin synthesis. Concomitantly with the induction of fat catabolism, SIRT1 stimulates mitochondrial oxidative capacity and energy dissipation in skeletal muscle and fat tissue through direct modulation of factors controlling mitochondrial biogenesis. Table 2 summarizes the reported actions of SIRT1 on fat and cholesterol catabolism.

Regulation of glucose metabolism by SIRT1

SIRT1 improves insulin sensitivity

SirBACO mice on a high-fat diet or crossed onto obese, leptin-receptor-deficient *Lepr*$^{db/db}$ mice displayed reduced plasma glucose and insulin levels and improved glucose tolerance compared to nontransgenic obese controls. Hyperinsulinemic–euglycemic clamps detected increased insulin-stimulated glucose disposal and enhanced insulin-suppressed hepatic glucose production in SirBACO mice relative to wild-type littermates. Consistent with improved insulin sensitivity, plasma insulin levels were lowered in high-fat-fed SirBACO and SirBACO, *Lepr*$^{db/db}$ mice relative to nontransgenic controls. These changes in glucose homeostasis were independent of body weight and body fat content. Transgenic mice expressing a catalytically inactive SIRT1 transgene displayed no differences in glucose tolerance compared with nontransgenic controls, demonstrating that the deacetylase activity of SIRT1 was required for its insulin-sensitizing effects [71]. Islet β-cell mass, β-cell insulin content and glucose-induced insulin secretion from isolated pancreatic islets were similar in SirBACO and control mice. This indicated that improved peripheral insulin signaling accounted for improved glucose homeostasis.

TABLE 2

SIRT1 effects on lipid and cholesterol metabolism

Animal model	Liver TG/Chol	Plasma TG	Plasma FFA	Plasma cholesterol	Plasma leptin	Plasma adiponectin	Refs
SirBACO mice						↑	Banks *et al.* [71]
SIRT1 knock-in into the β-actin gene		=	↓	↓	↓	↓	Bordone *et al.* [72]
Adenoviral SIRT1 overexpression				↑			Rodgers and Puigserver [77]
Adenoviral SIRT1 knockdown	↑			↓			Rodgers and Puigserver [77]
Liver-specific SIRT1 knockout	↑	=	↑	(↓)	=		Purushotham *et al.* [32]
Germline SIRT1 knockout				↓			Boily *et al.* [73]
ASO SIRT1 knockdown	=	=	↓	↓	↓	↓	Erion *et al.* [74]

Adiponectin increases hepatic insulin sensitivity [87]. SIRT1 deacetylated the forkhead transcription factor FoxO1, which in turn activated adiponectin transcription in 3T3-L1 adipocytes [71,88]. In line with this observation, SirBACO mice had elevated plasma adiponectin levels compared with wild-type mice.

SIRT1 also improved insulin sensitivity by downregulating the expression of protein tyrosine phosphatase 1b (PTP1B) [89], a major inhibitor of the insulin receptor [90]. SIRT1 protein expression was downregulated and PTP1B expression was upregulated in skeletal muscle of insulin-resistant, diet-induced obese mice. SIRT1 overexpression restored normal levels of PTP1B and rescued the insulin-evoked phosphorylation of several proteins of the insulin-receptor signaling cascade in skeletal muscle. SIRT1 was shown to deacetylate histone H3 at the PTP1B promoter, which might cause chromatin modifications that block PTP1B transcription [89]. The mechanisms whereby insulin resistance reduces SIRT1 expression remain to be elucidated.

SIRT1 upregulates hepatic gluconeogenesis

Despite its insulin-sensitizing and glucose-lowering effects in insulin-resistant animal models, SIRT1 also stimulates hepatic gluconeogenesis under fasting conditions [45]. As indicated above, fasting elevated SIRT1 protein levels and NAD^+ concentrations in mouse liver. SIRT1 deacetylated PGC-1α in cultured hepatocytes and this further enhanced PGC-1α-driven expression of gluconeogenic genes [45]. To confirm these findings *in vivo*, mice were injected with adenoviruses expressing small hairpin RNA directed against SIRT1. SIRT1 knockdown increased PGC-1α acetylation levels and reduced gluconeogenic gene expression and hepatic glucose output under fasting conditions. This was associated with a moderate improvement in plasma glucose levels, glucose tolerance and insulin sensitivity in lean wild-type mice and obese $Lepr^{db/db}$ mice [77].

FoxO1 promotes gluconeogenesis and its deacetylation by SIRT1 is a prerequisite for its gluconeogenic activity [91]. A highly conserved LXXLL motif (where L is a leucine and X any amino acid) at amino acids 459–463 of murine FoxO1 was shown to mediate its interaction with SIRT1. Mutation of the LXXLL motif disrupted the physical interaction of FoxO1 with SIRT1, increased FoxO1 acetylation levels and lowered the capability of FoxO1 to induce gluconeogenic gene expression. In obese and diabetic $Lepr^{db/db}$ mice, viral overexpression of FoxO1 with a mutation in the LXXLL motif improved glucose homeostasis and reduced gluconeogenic gene expression [91].

A recent study indicated that SIRT1 promotes FoxO1-driven gluconeogenesis during late (>18 hours) fasting [92]. In the first six hours of fasting, glucagon stimulated gluconeogenic gene expression by triggering dephosphorylation and nuclear translocation of CREB-regulated transcription coactivator 2 (CRTC2, also known as TORC2) [93]. Glucagon also led to acetylation of CRTC2 at lysine 628 by the histone acetyltransferase p300, which protected CRTC2 from ubiquitination and degradation. After 18 hours of fasting, hepatic SIRT1 protein accumulated and downregulated CRTC2 protein by deacetylation. CRTC2 accounted for gluconeogenic gene expression during the first six hours of fasting, whereas FoxO1 sustained gluconeogenesis after 18 hours of fasting, when CRTC2 was degraded by SIRT1-mediated deacetylation and ubiquitination [92].

Consistent with the above studies, ASO-induced SIRT1 knockdown led to a moderate reduction in fasting blood glucose levels in obese rats. Hyperinsulinemic–euglycemic clamp studies detected a reduction in hepatic glucose production, whereas peripheral glucose disposal in muscle and white adipose tissue was not altered. SIRT1 knockdown increased the acetylation levels of proteins regulating gluconeogenesis, such as FoxO1, PGC-1α and STAT3 in liver. Acetylation of all these factors diminishes hepatic gluconeogenesis [74,94,95]. Gluconeogenic gene expression was decreased in SIRT1 knockdown rats.

In contrast to the above demonstrations that SIRT1 can upregulate hepatic gluconeogenesis, conditional liver-specific SIRT1 knockout mice did not display defects in hepatic gluconeogenesis [32]. This might reflect the existence of redundant SIRT1-independent mechanisms to sustain hepatic gluconeogenesis.

SIRT1 improves glucose-induced pancreatic insulin secretion

In addition to its actions on hepatic gluconeogenesis and insulin signaling, SIRT1 also seems to upregulate pancreatic insulin secretion. Transgenic mice overexpressing SIRT1 selectively in pancreatic β-cells (BESTO mice) showed augmented pancreatic glucose-stimulated insulin secretion [96]. The plasma glucose and insulin levels were not changed in fed or fasted BESTO mice, but when challenged with a high glucose load in an intraperitoneal glucose tolerance test, BESTO mice reduced glucose levels more efficiently and had higher plasma insulin levels than their nontransgenic siblings. We showed previously that mitochondrial respiratory chain function is crucial for pancreatic glucose-stimulated insulin secretion [97]. SIRT1 seems to modulate insulin secretion via uncoupling protein 2 (UCP2). UCP2 is located in the inner mitochondrial membrane,

where it disrupts the electrochemical proton gradient, lowers ATP production and thereby impairs insulin secretion [98]. Microarray analyses in the mouse pancreatic MIN6 β-cell line revealed SIRT1 reduces expression of UCP2 [96]. Knocking down SIRT1 or inhibiting its activity with NAM indeed increased UCP2 expression and blunted glucose-stimulated insulin secretion in the rat INS1 and mouse MIN6 β-cell lines [99]. An extracellular form of NAMPT (eNAMPT), which is secreted from adipocytes, works as a systemic NAD^+ biosynthetic enzyme and improved glucose-induced pancreatic β-cell insulin secretion [100]. It remains to be shown, however, that alterations in systemic NAD^+ biosynthesis activate sirtuins in pancreatic β-cells.

Summary

SIRT1 has paradoxical roles in glucose homeostasis (Fig. 2). In the fasted state, SIRT1 promotes hepatic gluconeogenesis through deacetylation of PGC-1α and FoxO1. In the fed state, however, SIRT1 re-enforces pancreatic insulin secretion and insulin signaling. The insulin-sensitizing effects of SIRT1 involve regulation of adiponectin synthesis and PTP1B expression. SIRT1's opposing effects were manifest in SirBACO mice. Hepatocytes isolated from these mice showed increased expression of gluconeogenic genes upon stimulation with cAMP, but SirBACO mice were more sensitive to insulin-mediated repression of hepatic glucose production [71]. SIRT1, therefore, seems to preserve glucose homeostasis under fasted and fed conditions. Table 3 provides a summary of the reported effects of SIRT1 on glucose metabolism.

Pharmacological modulation of SIRT1 activity

Several strategies have been developed in the past to modulate SIRT1 enzyme activity. These include direct modulation of SIRT1 enzyme activity, enhancement of NAD^+ biosynthetic pathways and release of SIRT1 inhibition by NAM. Small-molecule activators of SIRT1 enzyme activity have been identified in compound library screens, which were based on a fluorescent deacetylation assay, performed on human SIRT1 using a fluorophore-tagged synthetic

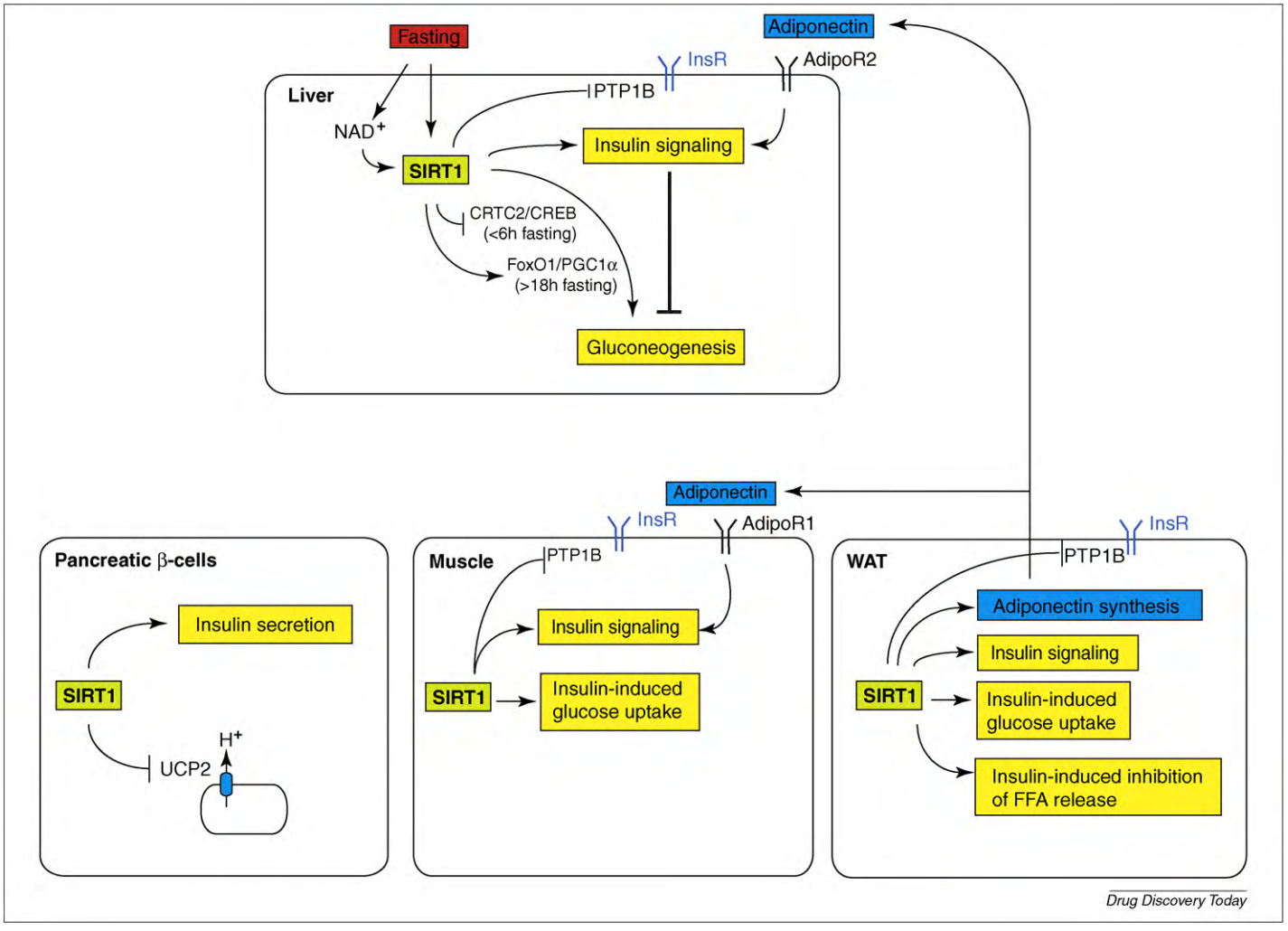

FIGURE 2

SIRT1 preserves glucose homeostasis. Fasting induces SIRT1 protein expression and increases NAD^+ concentrations in liver, leading to enhanced SIRT1 enzyme activity. SIRT1 sustains hepatic gluconeogenesis during late fasting by inducing a switch from CRTC2-CREB- to FoxO1-PGC-1α-driven gluconeogenesis. SIRT1 also promotes insulin signaling in liver, muscle and white adipose tissue (WAT) by decreasing the levels of protein tyrosine phosphatase 1B (PTP1B) and by raising the plasma levels of adiponectin. SIRT1-improved insulin signaling in turn represses hepatic gluconeogenesis, promotes glucose disposal in muscle and fat cells and inhibits free fatty acid release from fat cells under fed conditions. SIRT1 further stimulates glucose-stimulated insulin secretion from pancreatic β-cells by blocking expression of the mitochondrial uncoupling protein 2 (UCP2).

TABLE 3

SIRT1 effects on glucose homeostasis

Animal model	Plasma glucose	Plasma insulin	Glucose tolerance	Insulin sensitivity	Hepatic glucose production	Glucose disposal in WAT/muscle	Refs
SirBACO mice	↓	↓	↑		↓		Banks *et al.* [71]
SIRT1 knock-in into the β-actin gene	↓	↓	↑				Bordone *et al.* [72]
Adenoviral SIRT1 overexpression	↓						Liu *et al.* [92]
Pancreatic β-cell specific SIRT1 overexpression	=	=	↑				Moynihan *et al.* [96]
Adenoviral SIRT1 knockdown	↓		↑	↑	↓		Rodgers and Puigserver [77]
Liver-specific SIRT1 knockout	↑	(↑)					Purushotham *et al.* [32]
ASO SIRT1 knockdown	↓	=			↓	=	Erion *et al.* [74]
Germline SIRT1 knockout	↑	=		=		=	Boily *et al.* [73]

p53 peptide substrate [79,101]. These screens identified several active compounds. Of these, resveratrol and SRT1720 are the most intensively studied. Their potency to activate SIRT1 enzyme activity was measured in a high-throughput mass spectrometry assay, which employed the same fluorophore-tagged p53 peptide used for the high-throughput screens [79]. The potency was measured as (i) the concentration of compound increasing SIRT1 enzyme activity by 50% and (ii) the percentage maximum activation achieved at the highest doses of compound tested [79]. On the basis of both parameters, SRT1720 was found to be more potent than resveratrol. The metabolic effects of resveratrol and SRT1720 have been explored in several metabolic animal models and cell-culture-based systems. Resveratrol and SRT1720 enhanced mitochondrial fatty acid β-oxidation and mitochondrial biogenesis in skeletal muscle [28,29] and liver [102], protected mice on a high-fat diet from developing obesity [28,29] and improved glucose balance and insulin sensitivity in several diabetic animal models and cell-culture-based systems [28,29,79,89,102–104]. Resveratrol and SRT1720 are the only reported sirtuin-enhancing drugs that have been tested in animal models and have provided clear-cut metabolic benefits.

Recent biochemical *in vitro* studies, however, have generated some confusion regarding the mechanism of action of resveratrol and SRT1720 because neither of them activated SIRT1-mediated deacetylation of peptides and native full-length protein targets of SIRT1 in the absence of the fluorophore tag [105–107]. SIRT1 activation by resveratrol and SRT1720 was observed only when a fluorophore tag was covalently attached to the peptide substrate, as in the initial compound screens [105–107]. The fluorophore tag reduced the binding affinity of the p53 peptide substrate to SIRT1 [105], and in the presence of resveratrol, fluorophore-containing substrates bound more tightly to SIRT1 [105]. SRT1720 was found to interact directly with fluorophore-containing p53 peptides but not with native p53 peptides, suggesting that this interaction promotes higher affinity of fluorophore-containing substrates to SIRT1 [107]. The mechanism of SIRT1 activation by resveratrol and SRT1720 only in the presence of a fluorophore-tagged peptide substrate remains unclear, seems to strictly depend on substrate structure and can still be based on allosteric regulation, as initially proposed [79,101].

The reported metabolic effects of resveratrol and SRT1720 have been correlated with enhanced SIRT1-dependent deacetylation of protein substrates in animal models and in several cell-based assays [28–31,83,92,101–104,108]. It is conceivable that the biochemical *in vitro* assays used might not reproduce the cellular environment in which SIRT1 functions. These compounds could also cause secondary activation of SIRT1 *in vivo*. Further studies are needed to resolve the mechanism whereby resveratrol and SRT1720 increase SIRT1 activity *in vivo*.

Pacholec *et al.* [107] also reported multiple off-target activities of resveratrol and SRT1720. Resveratrol's reported effects on energy expenditure, insulin sensitivity, glucose tolerance and physical endurance [28,29,102] might indeed also be explained by AMPK activation [109], which can subsequently stimulate SIRT1 [57,82]. As indicated above, however, many of the reported metabolic effects of resveratrol and SRT1720 have been shown to correlate with and depend on SIRT1 activity and might not be accounted for by off-target activities. Pacholec *et al.* [107] also questioned the reported metabolic effects of SRT1720. They observed that SRT1720 at an oral dose of 30 mg/kg did not alter plasma glucose levels or mitochondrial mass in $Lep^{ob/ob}$ mice fed a high-fat diet, but fasting insulin levels were lowered, indicating an upregulation in insulin sensitivity [107]. An oral dose of 100 mg/kg reduced weight gain and food intake and resulted in death of the mice [107]. These observations, however, contrast with two studies in which SRT1720 at an oral dose of 100 mg/kg improved plasma glucose levels and augmented mitochondrial mass in three different diabetic animal models [29,79]; they also oppose another study, in which SRT1720 at a higher dose of 200 mg/kg did not affect food intake, body weight or survival of the mice [110]. Thus, the tolerability and pharmacological efficacy of SRT1720 in improving hyperglycemia and energy homeostasis in diabetic and obese animal models have been reported by different laboratories and contrasts with the lack of efficacy and putative toxicity of SRT1720 described by Pacholec *et al.* [107]. The reasons for the disparate findings in the study by Pacholec *et al.* are unclear. The quality of the compounds used in that study cannot be ascertained because of the lack of chemical characterization data and so it is possible that the quality of the compounds used might have affected selectivity, toxicity and pharmacological activity of SRT1720.

A second strategy to enhance SIRT1 activity is to de-repress the inhibition of sirtuins by NAM [53,111]. NAM reverses the

deacetylation reaction by binding to an enzyme-bound substrate intermediate formed from NAD^+ and the peptide substrate [52]. Small molecules that bind to the NAM binding pocket within the active site can antagonize NAM inhibition of deacetylase activity [53]. One such antagonist, isonicotinamide, increases the deacetylation reaction rate of yeast Sir2 [53]. Evidence for the effectiveness of the NAM de-repression strategy in mammalian cells or tissues, however, is very limited. Isonicotinamide has been shown to inhibit fat cell differentiation in mesenchymal stem cells [112,113] consistent with SIRT1-mediated inhibition of PPARγ [83]. Potent functional analogs of isonicotinamide that de-repress NAM inhibition have yet to be identified, and their ability to activate sirtuins in animals needs to be shown.

A third SIRT1 activation strategy is to support NAD^+ metabolism. Several studies in yeast and mammalian cells have shown that increasing the cellular NAD^+ content promotes sirtuin activity [55,114,115]. Cellular NAD^+ content can be increased by external application of NAD^+ [114] or by administration of the NAD^+ precursor molecules NAM [116], nicotinamide mononucleotide [100], nicotinic acid [116,117] or nicotinamide ribose [118,119]. All of these precursors effectively increased the NAD^+ content in several mammalian cell types and tissues by utilizing different NAD^+ biosynthetic pathways [100,116–119]; however, it remains to be studied whether these small molecules can also enhance sirtuin activities in animal models.

Sirtuin-inhibiting compounds have also been identified and shown to be effective against neurodegeneration [120], cancer [121,122] and HIV transcription [123]. These small-molecule inhibitors display varying degrees of selectivity for mammalian sirtuins. The first reported Sirtuin inhibitors, Sir two inhibitor napthol (sirtinol) [124] and splitomicin, [125] were identified in yeast screens. More potent sirtinol and splitomicin derivatives have been synthesized [122,123,126]. Other structural classes of small-molecule sirtuin inhibitors include indoles [127], adenosine mimetics [128], suramin derivatives [129] and phloroglucinols [130].

Future outlook

The NAD^+-dependent deacetylase SIRT1 is an ancient, evolutionarily conserved metabolic sensor that preserves energy homeostasis in response to nutrient deprivation. The majority of studies on SIRT1 regulation reported that fasting and CR triggered SIRT1 activity in several tissues by increasing SIRT1 protein levels and NAD^+ concentrations [27,38,40–43,45]. In support of the fasting-induced upregulation of SIRT1 activity are studies showing fasting-induced upregulation of NAMPT [56,57] and AMPK [57,82], both of which increased cellular NAD^+ concentrations and SIRT1 activities [57,82]. Under fasting conditions, SIRT1 was consistently found to promote fat and cholesterol catabolism [27–29,79,83,86] and to drive hepatic gluconeogenesis [45,77,91,92]. Under fed conditions, SIRT1 has consistently been reported to exert a glucose-lowering effect and to benefit glucose balance in insulin-resistant and diabetic states [28,29,71,79,102], despite its gluconeogenic effect. The glucose-lowering effect of SIRT1 has been attributed to direct and indirect enhancement of insulin-receptor signaling [71,89]. Three main pharmacological strategies have been developed to upregulate SIRT1 activity in yeast and mammalian cells: (i) allosteric regulation of SIRT1 enzyme activity [28,29,79,102], (ii) upregulation of the cellular NAD^+ content [55,100,114,115] and (iii) de-repression of NAM-mediated sirtuin inhibition [53,111]. The putative allosteric SIRT1 activators resveratrol and SRT1720 are, at present, the only approach that has been tested in metabolic animal models and has yielded clear-cut metabolic benefits [28,29,79,102]. Three independent studies have questioned whether resveratrol and SRT1720 are direct activators of SIRT1 [105–107], but there is evidence from numerous studies that their metabolic effects correlate with and depend on SIRT1 activities [28–31,83,92,101–104,108,131]. Further studies are necessary to understand how these compounds work *in vivo*. The activation of SIRT1 by resveratrol and SRT1720 seems to strictly depend on substrate structure [105,107] but might still be based on allosteric regulation. The search for allosteric modulators of SIRT1 activity requires the development of novel high-throughput SIRT1 deacetylation assays that are based on native substrates. Strategies to increase NAD^+ content and to de-repress sirtuin activity from NAM inhibition have been effective in increasing sirtuin activity in yeast and in mammalian-cell-based systems [55,114,115]. These strategies, however, have not been evaluated yet in animal models. Future work is required to obtain proof of concept in animal models, to ascertain their therapeutic value, and to identify specific and more potent chemical analogs. That SIRT1 can be pharmacologically targeted makes SIRT1 a very interesting area for further research.

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