Forum Review Article

Extranuclear sirtuins and metabolic stress

Mahmoud S. Elkhwanky and Jukka Hakkola

Research Unit of Biomedicine, Pharmacology and Toxicology, University of Oulu, Oulu, Finland; and Medical Research Center Oulu, Oulu University Hospital and University of Oulu, Oulu, Finland;

Correspondence: Prof. Jukka Hakkola, Research Unit of Biomedicine, Pharmacology and Toxicology, University of Oulu, POB 5000, FI-90014 University of Oulu, Finland. Tel: +358-924-485235, E-mail: jukka.hakkola@oulu.fi

Running title: Sirtuins and metabolic stress

Words: 7141

References: 144

Illustrations

-Gray scale: 0

-Color: 6 (online 6, hard copy 0)

Keywords: SIRT2, SIRT3, SIRT4, SIRT5, metabolic disease, fasting

Abstract

Significance: Extranuclear sirtuins in cytosol (SIRT2) and mitochondria (SIRT3, SIRT4 and SIRT5) are key regulators of metabolic enzymes and the antioxidative defense mechanisms. They play an important role in the adjustment of metabolic pathways in alterations of the nutritional status. **Recent Advances:** Recent studies have shown that in addition to lysine deacetylation, sirtuins catalyze several different lysine deacylation reactions, removal of lipid modifications and ADP-ribosylation. Large-scale studies have revealed hundreds of target proteins regulated by different sirtuin modifications. **Critical Issues:** Sensing of the metabolic state and regulation of the sirtuin function and expression is a critical component of the machinery optimizing cellular functions in the switch from fed to fasting condition. Overfeeding, obesity and metabolic diseases cause metabolic stress that dysregulates the sirtuins which may play a role in the pathogenesis and complications of metabolic diseases such as type 2 diabetes, fatty liver disease and cardiac diseases. In the current review we will discuss the significance of the extranuclear sirtuins as metabolic regulators and in protection against the reactive oxygen species, and also how these sirtuins are regulated by metabolic status and their putative role in metabolic diseases. **Future Directions:** To efficiently utilize sirtuins as drug targets for treatment of the metabolic diseases, better understanding of the sirtuin functions, targets, regulation and cross-talk is needed. Furthermore, more studies in humans are needed to confirm the many observations mainly made in animal and cell models so far.

Introduction

Sirtuins (SIRTs) are a family of enzymes with crucial roles in the control of cellular metabolism (133). Continuously increasing evidence indicates that sirtuins are needed for metabolic adaptations in energy homeostasis in response to changes in nutritional status. Adaptation to varying nutritional conditions is one of the very fundamental functions developed during evolution. Evolutionarily, the challenge has been the scarcity of food sources, and the human metabolic system is well orchestrated to cope with the metabolic pressure induced by fasting periods. In contrast, today's problems of overfeeding, physical inactivity and obesity induce significant difficulties for the metabolic homeostasis, cause cellular metabolic stress, and disturb the regulatory mechanisms tuned to secure the energy supply during the caloric shortage, thus resulting in development of diabetes, fatty liver disease and other components of the metabolic syndrome (39).

There are seven members in the mammalian sirtuin family. Sirtuins have different predominant subcellular localizations and they can be divided into nuclear (SIRT1, SIRT6 and SIRT7), mitochondrial (SIRT3, SIRT4, SIRT5) and cytosolic (SIRT2) forms, although this division is not unconditional and some sirtuins are detected in more than one cell compartment, and there can be also shuttling between the different cellular localizations (95). Nuclear sirtuins catalyze modifications of both histone and nonhistone proteins, including many transcription factors and coactivators, and play an important role in the control of transcriptional regulation (95). Instead, the extranuclear sirtuins in the mitochondria and cytosol especially target enzymes involved in the metabolic reactions and the antioxidative, protective defense mechanisms (63,95).

While the sirtuins were originally thought mainly to catalyze lysine deacetylation (52), more recent studies have revealed a much broader range of activities including several different lysine deacylation reactions, removal of lipid modifications and ADP-ribosylation (30,42,76,116). These protein modifications have turned out to be a significant additional layer in the regulation that can drastically affect the target protein function. Large-scale studies have identified thousands of such modified sites in hundreds of proteins, indicating broad potential for sirtuins to regulate cell functions (45,86,91,100). As major metabolic regulators, the expression and activity of sirtuins themselves need to be strictly controlled (10). A complex system sensing nutritional level and cellular energy state aims to maintain metabolic homeostasis at optimal level.

Sirtuins are NAD^+ dependent enzymes, and their function is therefore directly controlled by the cellular redox state (105). Additionally, the sirtuins are extensively regulated by numerous other mechanisms including transcriptional and post-transcriptional regulation of expression (10). Dysregulation of sirtuins by metabolic stress induced by overfeeding and unhealthy diet can be anticipated to participate in the pathogenesis of metabolic diseases by disrupting the basic metabolic pathways and by compromising the defense against oxidative stress. In the current review, we will focus on the role of extranuclear sirtuins in the adaptive mechanisms in response to metabolic stress. We will discuss the mechanisms of sensing the metabolic state, the significance of mitochondrial and cytosolic sirtuins in metabolic homeostasis, and how these enzymes may be involved in the progression of metabolic diseases.

Sirtuins sense the cellular metabolic and energetic state through NAD^+

All living organisms have developed pathways by which they can sense and respond to the fluctuations in the available nutrient levels and metabolic stress (61). A nutrient-sensor may sense the nutrient availability directly by binding with the nutrient, or indirectly through a metabolite that reflects the nutrient availability (79,135). Among these metabolic sensors, the sirtuin protein family plays an important role. In this chapter, we will explain briefly how sirtuins sense the metabolic and energetic state inside the cells through NAD^+ availability and how in turn the NAD^+-dependent activation of sirtuins regulates critical functions required for cellular adaptation to the current metabolic state.

NAD^+

NAD^+ is an essential cofactor in metabolism (19,132). During the oxidation of glucose, fatty acids and amino acids, the NAD^+ acts as a hydride acceptor, forming the reduced form NADH. The NADH is then translocated to mitochondria through the malate-aspartate and glycerol phosphate shuttles (7,82). In mitochondria, the NADH is re-oxidized by donating the hydride ion to the complex I in the electron transport chain to finally generate the high-energy molecule ATP (107,142). The major NAD^+/NADH pools exist in the cytosol and mitochondria (141). The NAD^+/NADH ratio affects the overall redox state of the cell. Importantly, the NAD^+/NADH redox state is responsive to the perturbations in the major metabolic pathways such as glycolysis, the malate-aspartate shuttle, the tricarboxylic acid (TCA) cycle, oxidative respiration, and oxidative stress (32,93,142). Furthermore, fasting and exercise have been shown to increase the NAD^+ level (16). Similarly, caloric restriction (CR) in rodent models also increases the NAD^+ level in several tissues such as liver, muscles, and white adipose tissue (16,40). In contrast, high-fat diet (HFD) causes a significant decline in the NAD^+ level (18). Additionally, it has been shown that the NAD^+ level declines with aging in several organs such as heart, liver, kidney, and lungs (9). Moreover, NAD^+ is the precursor for the production of $NADP^+$ through phosphorylation by NAD^+ kinase (74). Importantly, the $NADP^+$/NADPH ratio plays a role in several biosynthetic pathways and in protection of cells against reactive oxygen species (ROS) (74).

Maintenance of the intracellular NAD^+ homeostasis

Maintenance of the NAD^+ homeostasis is crucial for healthy cells. Mounting studies have demonstrated that the changes in the NAD^+/NADH ratio are associated with several pathological conditions such as metabolic syndrome, cardiovascular diseases, aging, and cancer (32,141). A recent study demonstrated that a decline in the nuclear NAD^+ level can disrupt the balance between the nuclear- and the mitochondrial-encoded OXPHOS subunits (37). Importantly, this imbalance could be reversed by caloric restriction and raising the NAD^+ level (37). Therefore, boosting cellular NAD^+ level serves as a potential target for treating mitochondrial disorders and aging (114) . Cells aim to secure the NAD^+ balance by increasing the NAD^+ production by synthesizing NAD^+ either through *de novo* or salvage pathways (8,25,50). However, the NAD^+ salvage pathway is considered the most important for maintaining the cellular NAD^+ level (102).

The *de novo* synthesis of NAD^+ starts with the essential amino acid tryptophan (Trp) which is converted to NAD^+ after several steps (for a review see ref (114)). The salvage pathway is considered the primary source of NAD^+ biosynthesis by catalytic conversion of several NAD^+ metabolites such as nicotinic acid (NA), nicotinamide (NAM), and nicotinamide riboside (NR) (114). An example of the salvage pathway is the conversion of nicotinic acid to nicotinic acid mononucleotide (NAMN) by nicotininc acid phosphoribosyltransferase (NAPRT), which is subsequently converted to NAD^+ by the enzymes, nicotinamide mononucleotide adenylyltransferase (NMNAT) and NAD synthase (NADS) (114). Another example is the conversion of NAM and NR to nicotinamide mononucleotide (NMN) by the action of nicotinamide phosphoribosyltransferase (NAMPT) and nicotinamide riboside kinase (NRK), respectively. The NMN is then finally converted to NAD^+ by the action of NMNAT (114).

It has been reported that *Nampt*$^{+/-}$ mice, having reduced expression of NAMPT, the key enzyme synthesizing NAD^+ from NA, have impaired glucose-stimulated insulin secretion (103). Importantly, the impairment in the insulin secretion could be rescued by NMN supplementation, indicating that maintaining the NAD^+ homeostasis is crucial for pancreatic function (103). Additionally, Yoshino et al. demonstrated that the NAMPT activity is reduced by HFD and aging, leading to lower NAD^+ level, which could trigger the pathogenesis of type 2 diabetes (136). NR is a naturally occurring NAD^+ precursor, which has been reported to increase NAD^+ level and in

turn, increase the activity of both SIRT1 and SIRT3, finally leading to improved mitochondrial function in HFD-induced obesity (18).

Furthermore, pharmacological approaches have been developed to boost the NAD^+ level by either inhibition of the enzymes consuming NAD^+ such as poly(adenosine diphosphate-ribose) polymerase (PARP) or CD38, or by supplementation with an NAD^+ precursor such as NA, NMN or NR (4,6,18,136).

Sirtuins as sensors for the NAD^+

All sirtuins have a conserved NAD^+ binding domain (137) and they can act as energy sensors through being sensitive to the NAD^+/NADH ratio (19). Therefore, a change in the NAD^+ level in response to different energetic challenges such as fasting or overfeeding will affect the sirtuin activity (33,128) (Fig.1). Sirtuins catalyze the removal of acyl group and/or adding ADP-ribose residue to protein by hydrolysis of NAD^+ to generate NAM and O-acetyl-ADP-ribose. Importantly, NAM may serve as an inhibitor of sirtuins; in addition to NAD^+, the sirtuin activity is thus regulated by the NAM level (19).

Cross-talk between sirtuins and other energy sensors: AMPK and PGC-1a

Sirtuins have been reported to interact with other energy sensors such as AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α). AMPK is an essential cellular energy sensor (43). AMPK is activated in response to a rise in AMP/ATP and ADP/ATP ratios resulting from high consumption of the high energy molecules ATP. High level of AMP is a characteristic of energetic stress states such as glucose starvation of cultured cells (43). Several studies have reported activation of AMPK in the liver, skeletal muscle and heart of the mice and rats during caloric restriction; however, there are also studies that have failed to confirm this (for a review see (14)). AMPK activation promotes recovery of ATP level by inhibition of the ATP-consuming anabolic pathways and by enhancement of the catabolic pathways generating ATP (14,43).

Cross-talk of AMPK and SIRT1 is well-established, and these two factors play a synergistic role in the promotion of mitochondrial function through PGC-1α (15). Furthermore, SIRT1 may be involved in AMPK activation (49,96). Another sirtuin involved in AMPK activation is mitochondrial SIRT3, which has been shown to activate AMPK in several cell types

(36,51,65,89). The mechanism may involve deacetylation of the AMPK upstream kinase LKB1 (36). Moreover, the mitochondrial sirtuins may regulate the AMPK activity by modulating the ATP synthesis, as has been shown for SIRT4 (48). AMPK in turn increases cellular NAD^+ levels and also regulates expression of several sirtuins (12,17). Overexpression of AMPK in mouse hepatocytes induced SIRT1-3 and 6, but repressed SIRT5 mRNAs (12). Metformin, the frontline drug therapy against type 2 diabetes and a well-established AMPK activator, partly repeated the effects of AMPK overexpression. Indeed, SIRT1 and SIRT2 mRNA and protein were induced by metformin; however, in contrast to AMPK, metformin reduced SIRT3 expression (11,12,22). Interestingly, the SIRT5 protein, but not the mRNA, was repressed by metformin both in cultured hepatocytes and in mouse liver *in vivo* (12). Another level of regulation involves direct phosphorylation of sirtuins by AMPK, and SIRT2 has been reported to be phosphorylated by AMPK (99). Overall, there appears to be very extensive interaction between AMPK and the sirtuin family that is perfectly logical considering that these factors are among the most important energy sensory systems in cells (Fig.2).

PGC-1α is known to be the master regulator of mitochondrial biogenesis and energy expenditure in the whole body. PGC-1α is induced in conditions of energy demand, for instance during fasting, exercise, and cold (34,120). In turn, it regulates a number of transcription factors involved in many metabolic pathways such as hepatic gluconeogenesis, and fatty acid synthesis and oxidation (34,120).

Sirtuins and AMPK work in close interaction with PGC-1α to regulate energy homeostasis. Both AMPK and SIRT1 promote PGC-1α function through phosphorylation and deacetylation, respectively (53,104). However, the effect may be context-specific, and in the liver, SIRT1 induces gluconeogenic genes and glucose output through PGC-1α, but does not regulate the effects of PGC-1α on mitochondrial genes (104). On the other hand, in brown adipose tissue and muscle, SIRT1-mediated deacetylation of PGC-1α improved mitochondrial function (64). Furthermore, SIRT2 may deacetylate PGC-1α in adipocytes and thus regulate β-oxidation and mitochondrial function (62). SIRT3, but not SIRT2, was reported to induce PGC-1α expression in HIB1B brown adipocytes, and this was associated with increased phosphorylation of CREB, a transcriptional regulator of PGC-1α (111). A recent study also reported direct interaction of SIRT3 and PGC-1α and stabilization of PGC-1α protein by SIRT3 in mouse pre-osteoblastic

MC3T3-E1 cells (28). However, the mechanism would require further study, especially considering that SIRT3 has mainly mitochondrial and PGC-1α mainly nuclear localization. Sirtuins may also regulate PGC-1α indirectly through AMPK activity (48). In hepatocytes, PGC-1α induces expression of both SIRT3 and SIRT5, but not the other sirtuin forms, in an estrogen-related receptor α (ERRα) and peroxisome proliferator-activated receptor (PPAR) α-dependent manner (12,60). This suggests that SIRT3 and SIRT5 may complement each other in activating mitochondrial functions in response to metabolic demands. Specific regulation of the two sirtuins promoting mitochondrial function agrees well with the role that PGC-1α has in stimulating the mitochondrial function and biogenesis.

The role of extranuclear sirtuins in metabolism and metabolic disorders

SIRT2, the main cytosolic sirtuin

SIRT2 is the only sirtuin primarily located in the cytosol, although it has also been found in the nucleus and mitochondria (71,119). Originally, SIRT2 was reported to be a tubulin deacetylase protein (87). In agreement with this function, the suppression of SIRT2 in cardiomyocytes by advanced glycation end products (AGEs) leads to hyperacetylation of α-tubulin and finally triggers diabetic cardiomyopathy (138). Therefore, SIRT2 activation might be a potential mechanism against diabetic cardiomyopathy. Later studies have shown that, in addition to tubulin, SIRT2 deacetylates many other proteins in both the cytosol and the nucleus (119). In addition to deacetylation, SIRT2 has been shown to catalyze demyristoylase activity (118). Both activities can modify numerous proteins involved in many physiological functions. SIRT2 expression is detected in a wide range of mice tissues, but particularly in metabolically relevant organs, such as muscle, liver, testes, pancreas, kidney and adipose tissue; however, the highest expression is found in the brain (56,80).

Although SIRT2 is thought to be predominantly a cytosolic protein, many of the currently established functions are related to the modification of nuclear factors. SIRT2 can be translocated to the nucleus and it has an important epigenetic role (57). Furthermore, a nucleus-specific SIRT2 isoform has been reported, but with no deacetylate activity (98). SIRT2 has been demonstrated to deacetylate several histones and it is involved in the regulation of the cell cycle, DNA repair, and transcription (57). As an example of histone deacetylation, SIRT2 deacetylates the H4K16 and regulates chromatin structure during cell cycle (57). Additionally, during mitosis SIRT2 level was shown to be induced and the protein was translocated to the nucleus (29). Importantly, *Sirt2*$^{-/-}$ mice have greater DNA damage occurrence and genome instability, which make them prone to develop different types of cancers (110).

It has been demonstrated that SIRT2 expression is regulated in response to the changes in cellular energetic state (122). The SIRT2 expression is repressed in the high energy state and induced during caloric restriction. In mice, long-term caloric restriction has been shown to enhance the SIRT2 protein level, specifically in the white adipose tissue and kidney, but not in the liver or brain (122). Furthermore, short-term fasting increases the SIRT2 mRNA and protein level in both the white and the brown adipose tissues in mice (123). Additionally, cold exposure in mice

induces the SIRT2 level in the brown, but not in the white adipose tissue (123). In humans, treatment of obese individuals with hypocaloric diet for 18 months increased both SIRT1 and SIRT2 in the blood mononuclear cells (26). On the other hand, obesity in humans and HFD-treatment in mice downregulated the SIRT2 protein level in the visceral white adipose tissue compared with the lean controls (62). Altogether, these studies support the hypothesis that the SIRT2 expression is regulated in response to the energetic state and may act as an energy sensor. Consequently, SIRT2 enables cells to adapt to the changes in energetic state and plays a key role in the regulation of various metabolic processes such as adipogenesis, hepatic gluconeogenesis and insulin action, as well as inflammation and ROS protection (38).

SIRT2 is the most abundant sirtuin in adipose tissue; however, its role in adipogenesis has only recently emerged (56,123). Interestingly, SIRT2 expression was found to be decreased during differentiation in the mouse 3T3-L1 preadipocytes and in the adipose tissue *in vivo* (56,123). Moreover, overexpression of SIRT2 in the mouse 3T3-L1 preadipocytes led to inhibition of adipogenesis at early stage (56,123). SIRT2-mediated inhibition of the adipogenesis was not due to defects in insulin and/or IGF-1 signaling pathways, but was mainly conducted via deacetylation of the forkhead box (FOXO) 1, leading to its retention in the nucleus and repression of the *Pparγ* gene transcription (56,123). Moreover, SIRT2 overexpression in cultured adipocytes has been shown to increase lipolysis both in the presence and absence of insulin (56,123). Further studies are required to reveal whether SIRT2 can interact with FOXO1 in other tissues; an important target tissue would be the liver, where FOXO1 is a key player in the regulation of gluconeogenesis (56). Interestingly, it has been shown that in addition to SIRT2, SIRT1 inhibits the adipogenesis in the 3T3-L1 preadipocytes by directly acting as a PPARγ corepressor (94). Surprisingly, adipocyte-specific deletion of SIRT1 protects mice against metabolic dysfunction during chronic treatment with HFD (81). The adipocyte-specific SIRT2 deletion is still needed to further unravel the physiological role of SIRT2 in the adipose tissue. Additionally, SIRT2 has been shown to inhibit the *de novo* fatty acid synthesis in the liver by deacetylation and inhibition of the ATP-citrate lyase (ACLY), the key enzyme responsible for the conversion of cytosolic citrate to acetyl-CoA, the building block for fatty acid synthesis (69,124). Therefore, activation of SIRT2 in the liver might be one way to reduce liver steatosis (124). Altogether, SIRT2 could be a potential therapeutic target for regulation of adipogenesis and obesity.

Recently, SIRT2 has been demonstrated to play a crucial role in the processes maintaining glucose homeostasis and insulin sensitivity (54,99). SIRT2 has been reported to enhance the hepatic gluconeogenesis during glucose deprivation by acting on different targets in the liver (54). One of the important SIRT2 targets is phosphoenolpyruvate carboxykinase (PEPCK), catalyzing a rate limiting step in the gluconeogenesis pathway. Deacetylation of PEPCK by SIRT2 leads to its increased stability (54). Another, potentially relevant target could be PGC-1α, a key activator of gluconeogenic genes such as *Pepck* and *G6pase*. At least in adipose tissue SIRT2 can deacetyate PGC-1α (62). Interestingly, in a very recent study, Zhang et al. reported that treatment with SIRT2 inhibitor sirtinol (which also inhibits SIRT1) could reduce the hepatic glucose output *in vitro* and *in vivo* (139). Sirtinol had little effect in SIRT2-knockdown cells, suggesting that SIRT2 does indeed mediate the effect. The repression of glucose output was due to hyperacetylation and finally degradation of PEPCK1 (139). Inhibition of gluconeogenesis by SIRT2 inhibitors could putatively be a useful mechanism for treatment of type 2 diabetes (139). However, more studies are needed in relevant disease models.

Recent studies have shown cross-talk between sirtuins and the insulin signaling pathway in several organs. Both SIRT1 and SIRT2 were reported to deacetylate and thus regulate FOXO1, which is a target of the PI3K-AKT pathway (44,56). SIRT2 has also been reported to enhance insulin signaling in insulin responsive cells by direct interaction with AKT, a key factor in the insulin signaling pathway (99). Moreover, SIRT2 could deacetylate and destabilize the glucokinase regulatory protein (GKRP), the major interacting partner for glucokinase (92). This might promote glucokinase export to the cytosol where it catalyzes phosphorylation of glucose to glucose-6-phosphate, and thus enhances the glucose uptake (92). Therefore, SIRT2 was thought to increase insulin sensitivity and to reduce insulin resistance; on the other hand, as discussed above, SIRT2 has been reported to deacetylate PEPCK, leading to increase in its stability and activation of gluconeogenesis (54,139). Clearly, more studies evaluating the role of SIRT2 in hepatic glucose metabolism are needed. In contrast, SIRT2 expression has been reported to be increased in insulin-resistant C2C12 skeletal muscles cells (3,27). Interestingly, inhibition of SIRT2 in these cells improved the insulin-stimulated glucose uptake and increased phosphorylation of AKT and GSK3B, suggesting that SIRT2 may negatively affect the skeletal muscle glucose uptake (3). A different point of view was introduced by a study evaluating the role of SIRT2 in glucose uptake during mitoenergetic dysfunction (68). During mitochondrial

dysfunction, the cells cope with the energy crisis by increasing the glycolytic flux to sustain cellular ATP levels, which requires increased glucose uptake (68). Interestingly, it was demonstrated that mitochondrial dysfunction in C2C12 myoblasts triggers LKB1-mediated activation of AMPK, which in turn activates the mTOR-RAPTOR and GLUT1-mediated glucose uptake (68). This process required SIRT2, and glucose uptake was greatly reduced by chemical inhibition of SIRT2 (68). The authors proposed that AMPK activation stimulates SIRT2 phosphorylation enhancing SIRT2 activity; however, the SIRT2 phosphorylation status was not directly studied (68). Nevertheless, SIRT2 phosphorylation by AMPK has been reported (99). In summary, the role and significance of SIRT2 in insulin signaling in major metabolic tissues is still to be characterized in detail.

The production of ROS, which is primarily derived from the cellular respiration in mitochondria, increases during caloric restriction, in metabolic dysregulation and with aging. (13,83). The ROS constitutes of very reactive compounds such as superoxide ion (O_2^{*}), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^{*}), derived from incomplete reduction of molecular oxygen (13,83). Normally, well-controlled, antioxidative defense mechanisms protect important macromolecules such as proteins and DNA from oxidative damage (13,83). Imbalance between the production of ROS and the antioxidative defense causes oxidative stress (13,83). Importantly, the oxidative stress has been implicated in several chronic diseases such as cardiovascular diseases, obesity, and diabetes (13,83).

Sirtuins have emerged as key players regulating the antioxidative capacity of cells; however, their exact roles are still to be unraveled (1). SIRT2 is a central regulator of the defense mechanism against the ROS. SIRT2 has been shown to deacetylate and activate FOXO3a, a transcriptional activator of superoxide dismutase 2 (SOD2), which in turn reduces the ROS level (122). Interestingly, SIRT2 promotes apoptosis under very high ROS levels (122), and this might be very important to avoid transformation of cells as a result of accumulation of ROS-mediated DNA mutations. Furthermore, under oxidative stress, SIRT2 may activate glucose-6-phosphate dehydrogenase (G6PD), a key enzyme in the pentose phosphate pathway, producing more NADPH (125). NADPH plays a role in the maintenance of cellular redox potential and is used to keep ROS scavenger glutathione (GSH) in its reduced form. Additionally, SIRT2 may deacetylate and activate phosphoglycerate mutase (PGAM2), a glycolytic enzyme indirectly regulating

G6PD and thus NADPH level (127). Interestingly, despite the antioxidative function of SIRT2, inhibition of SIRT2 in the heart has a protective role against ischemic-reperfusion injury (77). A recent study reported that SIRT2 deacetylates nuclear factor erythroid 2-related factor 2 (NRF2), resulting in destabilization and reduced level of NRF2 (131). NRF2 downregulation in turn was found to reduce iron export. Although not studied in the report, regulation of NRF2 may also have significant effects on the defense mechanisms against oxidative stress as NRF2 is activated by oxidative stress and plays a crucial role in the transcriptional activation of the defense machinery (75). On the other hand, it has been reported that SIRT2 may positively regulate NRF2 expression (20); clearly, further research is needed also in this field.

Additional mechanisms connecting SIRT2 with the regulation of longevity have recently been suggested (88). North et al. demonstrated that the stability of the mitotic checkpoint kinase (BUBR1), which has emerged as a key factor in the regulation of normal aging, is under the control of SIRT2 (88). Deacetylation of the lysine K668 inhibits BUBR1 proteasomal degradation and increases its stability. Moreover, overexpression of SIRT2 in mice hypomorphic for BUBR1 (*BubR1*$^{H/H}$) increases the median lifespan, with a greater effect in male mice (88). Additionally, a pharmacological inducer of the NAD^+ synthesis ameliorates several-age related diseases in mice and restores the BUPR1 protein abundance (88). These results suggest, at least provisionally, that SIRT2 could be targeted for treatment of age-related diseases and to increase the healthspan in mammals (88).

In conclusion, SIRT2 appears to respond sensitively to changes in nutritional status, and in turn, it tunes metabolic functions in the major metabolic tissues (Fig.3) and enhances the antioxidative defense. However, there are still many discrepancies in the current data. Therefore, at the moment it is not even clear whether activation or inhibition of SIRT2 would be a beneficial strategy for treatment of type 2 diabetes and other metabolic diseases.

Mitochondrial sirtuins

SIRT3, the main promoter of the mitochondrial energy metabolism

SIRT3 is a mitochondrial matrix protein (73,108,109). It has wide tissue distribution, but the expression is predominant in mitochondria-rich tissues with important roles in metabolism such as liver, brown adipose tissue, muscle, heart and brain (73,111). Large-scale analysis of

acetylome in liver mitochondria in wild type as well as in SIRT3 knockout mice has shown that SIRT3 is a dominant, but not an exclusive, deacetylase enzyme in mitochondria and targets thousands of acetylation sites in hundreds of proteins (45,101). Evaluation of the target proteins reveals major role of SIRT3 in the regulation of mitochondrial energy metabolism, and the primary pathways affected by SIRT3 included oxidative phosphorylation, fatty acid oxidation, ketogenesis, branched chain amino acid catabolism, the citric acid cycle, and the urea cycle (101), all functions contributing to the maintenance of energy homeostasis. Furthermore, SIRT3 regulates the acetylation status of multiple proteins within a given pathway that may allow more efficient control over the pathway (45). Interestingly, however, SIRT3 knockout animals do not display an obvious metabolic phenotype without metabolic challenge despite the mitochondrial hyperacetylation (73). Instead, in the livers of fasting mice the whole-body SIRT3 knockout caused significant abnormalities in several key elements of energy metabolism including fatty acid oxidation, ATP levels and ketone body production (46,112), suggesting that SIRT3 participates in the regulation of the adaptive rather than the basal energy metabolism. Curiously, tissue-specific knockouts in the liver or muscle did not affect the whole-body metabolic homeostasis although hyperacetylation was observed in the corresponding tissues (35). This suggests complex effect of SIRT3 on metabolic homeostasis, perhaps involving inter-tissue communication. Alternatively, there may be experimental explanations, due to strain differences or other factors, for the phenotypic discrepancy between the tissue-specific and the whole-body SIRT3 knockouts.

SIRT3 responds to the nutritional status both through gene expression and NAD^+ dependency. Fasting and caloric restriction can upregulate SIRT3 in the liver, brown adipose tissue and muscle, and this coincides with deacetylation of the SIRT3 target sites (45,46,89,111,129). This suggests a role for SIRT3 as a metabolic switch enabling metabolically active tissues to react to the nutritional stress. In contrast to the nutrient shortage, high-fat feeding repressed SIRT3 expression in the liver and muscles (5,58,89). SIRT3 level in skeletal muscle was also downregulated by both insulin resistance and insulin deficiency (134). In summary, SIRT3 expression appears to be strongly affected by metabolic stress. Induction during fasting is obviously a physiologically important response enabling the body to adapt and maintain optimal energy homeostasis during periods of starvation or food sparsity. In the liver, this implicates facilitating the use of lipids and amino acids as energy source. In contrast, SIRT3 depression by

HFD and diabetes may represent a pathological mechanism potentially contributing to the metabolic disturbances. Indeed, SIRT3 knockout mice have impaired insulin signaling and glucose tolerance, reduced oxygen consumption, and they display accelerated obesity, hyperlipidemia, and steatohepatitis during HFD (47,55). Thus in metabolic diseases, SIRT3 may be part of a vicious circle in which SIRT3 is repressed by HFD and diabetes that in turn aggravates the metabolic disturbances.

Interestingly, SIRT3 was reported to physically interact with, deacetylate and activate the nicotinamide mononucleotide adenylyltransferase 3 (NMNAT3), the rate-limiting enzyme for mitochondrial NAD^+ biosynthesis (134). This suggests the interesting possibility that SIRT3, NMNAT3 and NAD^+ form a positive feedback cycle. SIRT3-NMNAT3 interaction is crucial for SIRT3-mediated anti-hypertrophic effect (134).

Mitochondrial energy metabolism produces significant amounts of ROS. Due to the potential toxic effect of ROS it is important to control and limit the oxidative stress. Logically, in conjunction with the effect of promotion of oxidative phosphorylation, SIRT3 plays another important role in the detoxification of ROS. Similar to SIRT2, SIRT3 has been shown to activate the antioxidant machinery in the mouse heart by inducing the expression of superoxide dismutase 2 (SOD2) and catalase through deacetylation of the transcription factor FOXO3a (115). On the other hand, according to several studies the main mechanism of SOD2 activation by SIRT3 is direct deacetylation and activation of the enzymatic function (24,97,117). SIRT3 also deacetylated and activated mitochondrial isocitrate dehydrogenase 2 (IDH2). This in turn increased NADPH levels and the ratio of reduced-to-oxidized glutathione (GSH/GSSG) in mitochondria enabling neutralization of ROS (113). Mitochondria are both the initiator and one of the first targets of oxidative stress, and excess ROS may lead to membrane depolarization. A recent study by Yang et al. reported that SIRT3 activity can be activated by loss of mitochondrial membrane potential (130). A pool of SIRT3 was found to be associated with ATP synthase subunit ATP5O. This interaction was dissociated upon mitochondrial matrix pH reduction in association with membrane depolarization and resulted in deacetylation of a cohort of SIRT3 target proteins. Sequestering of SIRT3 by ATP5O may provide a rapid way of activating SIRT3 activity upon mitochondrial toxicity. SIRT3 was also needed for recovery of the membrane

potential. In another study, SIRT3 has been reported to deacetylate the regulatory component of the mitochondrial permeability transition pore, cyclophilin D (41).

Reduction of SIRT3 expression by HFD and metabolic diseases could compromise the mitochondrial antioxidant defense system that may contribute to the disease complications. One example of this involves pancreatic β-cells. Oxidative stress and inflammation induced by HFD and type 2 diabetes cause β-cell dysfunction accompanied by reduced SIRT3 expression both in mice and in humans (23,144). SIRT3 deficiency further impairs the ROS detoxification and reduces the glucose-stimulated insulin secretion (23,144). In contrast, SIRT3 overexpression appears to have a beneficial effect (59,144).

Collectively, the current evidence indicates that SIRT3 is a major regulator of mitochondrial function with a crucial role in adjustment to metabolic stress in conditions of caloric restriction (Fig. 4). Furthermore, SIRT3 coordinates the stimulation of oxidative phosphorylation and the antioxidative defense system. Disruption of SIRT3 regulation by overnutrition can be estimated to be an integral part of the pathogenesis in metabolic diseases and in complications such as cardiac disease.

SIRT4, the mitochondrial metabolic break and the gatekeeper of the lipid metabolism

SIRT4 is a mitochondrial matrix protein expressed abundantly in metabolically relevant tissues including the liver, brain, heart, kidney and pancreatic β-cells (42). Compared with the other mitochondrial sirtuins, the enzymatic activity of SIRT4 is less established. SIRT4 appears to be quite promiscuous in its enzymatic function and it catalyzes ADP-ribosylation and lysine deacetylation and lipoamidation, although there is some controversy about the efficiency of the different activities (42,67,76). Mathias et al. suggested that SIRT4 catalyzes more efficiently lipoyl removal than deacetylation (76). The significance of the ADP-ribosylase activity has also been questioned (31).

Recently, SIRT4 has been shown to catalyze the removal of novel lysine modifications: methylglutaryl (MG)-lysine, hydroxymethylglutaryl (HMG)-lysine, and 3-methylglutaconyl (MGc)-lysine (2,121). These modifications are produced nonenzymatically by leucine catabolism

intermediates and they may provide a mechanism for inhibitory feedback. SIRT4 in turn facilitates leucine oxidation by removal of the modifications (2,121).

SIRT4 has been reported to inhibit glutamate dehydrogenase (GDH) activity through ADP-ribosylation (42). GDH converts glutamate to TCA cycle intermediate α-ketoglutarate. Through the inhibition of GDH, SIRT4 repressed glucose and amino acid stimulated insulin secretion from β-cells (42). Another mechanism that may play a role involves the newly identified, SIRT4-regulated MG-lysine, HMG-lysine, and MGc-lysine modifications affecting the leucine catabolism. Through the regulation of leucine level, SIRT4 may affect the allosteric regulation of GDH by leucine (2). Caloric restriction repressed SIRT4 activity, although it did not affect the protein level, which promoted amino acid-stimulated insulin secretion (42).

In cultured fibroblasts and in the liver *in vivo*, SIRT4 represses pyruvate dehydrogenase activity (PDH) through lipoamidase activity (76). SIRT4 hydrolyzed the lipoamide cofactors from the E2 component dihydrolipoyllysine acetyltransferase, which is required for PDH activity. PDH catalyzes conversion of pyruvate to acetyl CoA, thus interconnecting glycolysis and TCA cycle. In fact, SIRT4 appears to limit TCA cycle substrate availability at multiple points, and SIRT3 and SIRT4 seem to have opposing roles in the regulation of the TCA cycle (42,76,90).

Several studies have addressed the role of SIRT4 in hepatic lipid metabolism. SIRT4 plays an important role in determination between anabolic and catabolic lipid homeostasis (67) (Fig. 5). SIRT4 knockout mice hepatocytes have higher rate of β-oxidation (66,85). SIRT4 expression is depressed by fasting (48,66), and therefore reduced SIRT4 activity promotes the use of fatty acid oxidation as energy source in nutrient deprivation state. The process involves several interconnected mechanisms. SIRT4 promotes ATP synthesis, and SIRT4 knockdown was found to decrease cellular ATP level and increase the ADP/ATP ratio (48). Surprisingly, this associated with increased oxygen consumption, which appears to be explained by SIRT4-mediated regulation of adenine nucleotide translocator 2 uncoupling protein (48). Reduced ATP synthesis activates AMPK kinase through the increased amounts of AMP and ADP. AMPK phosphorylates and inactivates acetyl CoA carboxylase (ACC), an enzyme catalyzing acetyl-CoA conversion to malonyl-CoA, thus preventing fatty acid synthesis (48). On the other hand, SIRT4 deacetylates and inhibits maloynyl CoA decarboxylase, catalyzing the reverse reaction (67). Thus during the fed condition, SIRT4 stimulates lipid synthesis, while the SIRT4 repression during fasting

reverses the process and activates fatty acid oxidation. SIRT4 repression also induces expression of many genes involved in fatty acid oxidation, suggesting signaling from mitochondria to nucleus (66,85). The process requires SIRT1, and SIRT4 knockdown induced SIRT1 expression (66,85). Important transcriptional regulators of mitochondrial biogenesis, PGC-1α and ERRα, and several PGC-1α-regulated, nuclear-encoded, mitochondrial genes were also induced by SIRT4 knockdown (48,85). This involves AMPK-dependent induction of PGC-1α (48). SIRT1 also activates PGC-1α, and SIRT1 activation plays a role in peroxisome proliferator-activated receptor α (PPARα) coactivation by PGC-1α and the subsequent induction of fatty acid oxidation genes (66). Although most studies have been performed in liver, SIRT4 inhibition also promotes β-oxidation in muscle (85).

SIRT4 is obviously an important regulator of mitochondrial metabolism by promoting lipid synthesis, reducing carbon source availability of the TCA cycle, and by regulating uncoupling of oxidative phosphorylation. Reduction of SIRT4 under nutrient deprivation coordinates cells to utilize fatty acids as energy source, and this complements the opposite effects mediated by SIRT3 (and perhaps SIRT5) induction. In concordance, SIRT4 expression was induced in the livers of mouse models of type 2 diabetes and in human liver samples with non-alcoholic fatty liver disease, suggesting involvement in the metabolic disease (85,126).

SIRT5, the regulator of the urea cycle and what else?

SIRT5 has wide tissue distribution, but the highest expression was found in the tissues relevant for metabolic functions, i.e., liver, skeletal muscle, heart, kidney and brain (84). SIRT5 is predominantly a mitochondrial sirtuin localized to the mitochondrial matrix (84). However, an isoform with an alternative C-terminus has been identified, which is targeted, in addition to the mitochondria, also to the cytosol (78). In agreement with this, SIRT5 function is not limited to mitochondria: it has also cytosolic targets (91).

SIRT5 has low deacetylation activity. Instead, it catalyzes several less studied lysine modifications including desuccinylation, demalonylation and deglutarylation. Several large screening studies have addressed the occurrence of succinyl, maloynyl and glutaryl modifications in the liver and the significance of SIRT5 activity on the corresponding deacylation reactions

(86,91,100,116). SIRT5 appears to be a major desuccinylase enzyme and targets more than half of the mitochondrial succinylated proteins (100). Succinylation has been shown to affect protein function of 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2), the pyruvate dehydrogenase complex and the succinate dehydrogenase as a proof of functional significance of this protein modification (91,100). Pathway analysis of SIRT5 targets identified fatty acid β-oxidation, branched chain amino acid catabolism, the citric acid cycle, ATP synthesis and ketone body synthesis as the most enriched target pathways (100). Interestingly, there is rather conflicting data on the overlap of the mitochondrial acetylome and succinylome. Rardin et al. reported 80% overlap between the sites of lysine acetylation and succinylation with 24% overlap of SIRT3 and SIRT5 target sites (100). On the other hand, Park et al. suggested only very limited overlap between lysine acetylation and succinylation (91).

Besides the liver, succinylation appears to play an important role in the heart, and the SIRT5 protein level was also found to be high in the heart (106). In the heart, SIRT5 especially targets fatty acid oxidation, but also several other components of energy metabolism were found to be under the control of SIRT5-mediated succinylation (106). SIRT5 knockout mice also developed hypertrophic cardiomyopathy (106).

In the context of lysine malonylation, SIRT5 was found to target proteins involved in gluconeogenesis and glycolysis, and SIRT5 knockout in primary hepatocytes was found to diminish glycolytic flux (86). SIRT5 appears to be the only sirtuin with deglutarylase activity (116). Also glutarylation may be involved in numerous mitochondrial functions; however, carbamoyl phosphate synthase 1 (CPS1), the rate-limiting enzyme in urea cycle, is the most established target at the moment (116).

Characterization of whole-body metabolism in germline *Sirt5*$^{-/-}$ mice displayed rather modest abnormalities both under regular chow or HFD (34). The main finding was elevated serum ammonia during fasting (34). This agrees well with the well-established importance of CPS1 as a SIRT5 target protein. Indeed, desuccinylation, demalonylation and deglutarylation all seem to target urea cycle (17,22,29). There is coordination in the regulation of urea cycle by SIRT5 and SIRT3 and they regulate the two consecutive steps in the process, i.e., CPS1 and ornithine transcarbamoylase, respectively (11). Enhancement of the urea cycle is needed when amino acids are used for gluconeogenesis during fasting. Coordinated function of SIRT3 and SIRT5 in the

regulation of ureagenesis agrees well with the regulation of both sirtuin genes by PGC-1α, a fasting-activated transcriptional regulator (2).

SIRT5 targets several components of the electron transport chain (140). It was first originally reported that SIRT5 has a suppressive effect on cellular respiration (91). However, several more recent studies have observed positive effects on oxidative phosphorylation (12,140,143). Therefore it was rather surprising, considering that oxidative phosphorylation is a major source of ROS, that there was a higher ROS level in the SIRT5 knockout murine embryonic fibroblasts (143). In fact, SIRT5 is another sirtuin form involved in the antioxidative defense (Fig. 6). Similar to SIRT3, also SIRT5 activates the function of IDH2 and thus increased NADPH production and GSH/GSSG ratio (143). Also another NADPH-producing enzyme, glucose-6-phosphate dehydrogenase (G6PD), was activated by SIRT5. However, the mechanisms are different: IDH2 is activated by desuccinylation and G6PD by deglutarylation (143). Oxidative stimuli seem to activate the function, but not the expression of SIRT5 (143). SIRT5 also desuccinylates and activates the ROS detoxifying enzyme superoxide dismutase 1 (SOD1) and, at least in the brain, regulates the SOD2 expression (70,72).

While many studies have now identified a large number of proteins modified by SIRT5 and it seems to be involved in many aspects of mitochondrial function including the urea cycle, energy metabolism and ROS detoxification, the overall importance of this enzyme still remains rather poorly understood. The current evidence does not suggest any indispensable role in unchallenged metabolic conditions. However, SIRT5 may be important under fasting to maintain optimal metabolic homeostasis.

Concluding remarks

Although the sirtuins' target proteins, the modifications catalyzed and their functional consequences are only partially characterized, it is now clear that the sirtuins in the mitochondria and cytosol play important roles in sensing the metabolic state and adjusting the metabolic pathways by modulating functions of the key metabolic enzymes. Especially, the sirtuins are important in nutritional switch from the fed to the fasting state. Furthermore, they play an important role in defense against the oxidative stress.

Obesity and HFD-induced metabolic stress cause dysregulation of the sirtuin system that probably plays an important role in the pathogenesis of metabolic diseases. This may be mediated by inappropriate function of the metabolic pathways and/or through the increased oxidative stress. Furthermore, abnormal expression of sirtuins has been observed in animals having metabolic diseases such diabetes and fatty liver disease, which may contribute to the development of disease complications such as cardiac diseases. Sirtuins may thus have an important role both in the early and the late stages of the metabolic diseases. Although the data from human studies is quite limited, the existing data suggests that sirtuins are dysregulated similarly as in the experimental animals by obesity and metabolic diseases. Obviously, this means that the sirtuins may be useful drug targets. Since most extranuclear sirtuins, apart from SIRT4, are repressed in the metabolic diseases, sirtuin activators, rather than inhibitors, may offer a viable approach. Indeed, such molecules are under development (21). However, the picture is complicated and in some situations also inhibition may be useful, as exemplified by the reduction of hepatic glucose output by SIRT2 inhibition. To utilize the full therapeutic potential, better understanding of the sirtuin functions is required. Furthermore, to support the promising prospects for sirtuin-mediated pharmacotherapy, more clinical research in humans is required.

In many cases, the different sirtuins appear to have complementary functions and also extensive cross-talk. For example, SIRT3 and SIRT5 seem to have synergistic effect on urea cycle function, while SIRT3 and SIRT4 may have the opposite influence on TCA cycle. This agrees well with the regulatory patterns of these sirtuins during fasting. There also seems to be cross-talk between sirtuins at different cell compartments as demonstrated by the effect of SIRT4 knockdown on SIRT1 expression and function. This level of regulation is currently poorly characterized and the mechanisms are not well understood, and much further research is thus clearly required.

Acknowledgements: The original research of the authors has been financially supported by the grants from the Academy of Finland (grant 286743),) and the Novo Nordisk Foundation (grant NNF15OC0015846) and the Diabetes research Foundation.

Author discloser statement: No competing financial interest exist.

Abbreviations:

ACC: Acetyl CoA carboxylase

ACLY: ATP-citrate lyase

ADP: Adenosine diphosphate

AGEs: Advanced glycation end products

AKT: Thymoma viral proto-oncogene 1

AMP: Adenosine monophosphate

AMPK: AMP-activated protein kinase

ATP5O: ATP synthase subunit O

ATP: Adenosine triphosphate

CD38: Cluster of differentiation 38

CR: Caloric restriction

CaMKK2: Calcium/calmodulin-dependent protein kinase kinase 2

ERRα: Estrogen-related receptor α

FOXO: Forkhead box

G6PD: Glucose-6-phosphate dehydrogenase

G6pase: Glucose-6-phosphtase

GDH: Glutamate dehydrogenase

GKRP: Glucokinase regulatory protein

GLUT: Glucose transporter

GSH: Glutathione, reduced form

GSK3B: Glycogen synthase kinase-3 beta

GSSG: Glutathione, oxidized form

H2O2: Hydrogen peroxide

HBEpiCs: Human bronchial epithelial cells

HFD: High-fat diet

HMGCS2: 3-hydroxy-3-methylglutaryl-CoA synthase 2

IDH2: Isocitrate dehydrogenase 2

IGF-1: Insulin-like growth factor 1

LKB1: liver kinase B1

NA: Nicotinic acid

NAD: Nicotinamide adenine dinucleotide

NADP: Nicotinamide adenine dinucleotide phosphate

NAM: Nicotinamide

NAMN: nicotinic acid mononucleotide

NAMPT: Nicotinamide phosphoribosyltransferase

NMN: Nicotinamide mononucleotide

NMNAT3: Nicotinamide mononucleotide adenylytransferase 3

NR: Nicotinamide riboside

NRF2: Nuclear factor erythroid 2–related factor 2

OXPHOS: Oxidative phosphorylation

PARPs: Poly(adenosine diphosphate-ribose) polymerase

PDH: Pyruvate dehydrogenase

PEPCK: Phosphoenolpyruvate carboxykinase

PGAM2: Phosphoglycerate mutase

PGC-1α: Peroxisome proliferator-activated receptor γ coactivator-1α

PI3K: Phosphatidylinositol-4,5-bisphosphate 3-kinase

PPARα: Peroxisome proliferator-activated receptor α

PPARγ: Peroxisome proliferator-activated receptor γ

ROS: Reactive oxygen species

SOD2: Superoxide dismutase 2

SIRT(s): Sirtuin(s)

TCA: Tricarboxylic acid

mTOR-RAPTOR: Mechanistic target of rapamycin-Regulatory-associated protein of mTOR

References:

1. Aguilaniu H, Gustafsson L, Rigoulet M and Nyström T. Asymmetric inheritance of oxidatively damaged proteins during cytokinesis. *Science* 299: 1751-1753, 2003.

2. Anderson KA, Huynh FK, Fisher-Wellman K, Stuart JD, Peterson BS, Douros JD, Wagner GR, Thompson JW, Madsen AS, Green MF, Sivley RM, Ilkayeva OR, Stevens RD, Backos DS, Capra JA, Olsen CA, Campbell JE, Muoio DM, Grimsrud PA and Hirschey MD. SIRT4 Is a Lysine Deacylase that Controls Leucine Metabolism and Insulin Secretion. *Cell Metab* 25: 838-855.e15, 2017.

3. Arora A and Dey CS. SIRT2 negatively regulates insulin resistance in C2C12 skeletal muscle cells. *Biochim Biophys Acta Mol Basis Dis* 1842: 1372-1378, 2014.

4. Bai P, Cantó C, Oudart H, Brunyánszki A, Cen Y, Thomas C, Yamamoto H, Huber A, Kiss B, Houtkooper RH, Schoonjans K, Schreiber V, Sauve AA, Menissier-De Murcia J and Auwerx J. PARP-1 inhibition increases mitochondrial metabolism through SIRT1 activation. *Cell Metab* 13: 461-468, 2011.

5. Bao J, Scott I, Lu Z, Pang L, Dimond CC, Gius D and Sack MN. SIRT3 is regulated by nutrient excess and modulates hepatic susceptibility to lipotoxicity. *Free Radic Biol Med* 49: 1230-1237, 2010.

6. Barbosa MTP, Soares SM, Novak CM, Sinclair D, Levine JA, Aksoy P and Chini EN. The enzyme CD38 (a NAD glycohydrolase, EC 3.2.2.5) is necessary for the development of diet-induced obesity. *FASEB J* 21: 3629-3639, 2007.

7. Berg J, Tymoczko J and Stryer L. Section 18.5 Many Shuttles Allow Movement Across the Mitochondrial Membranes. In: *Biochemistry. 5th edition.*: New York: W H Freeman, 2002.

8. Bogan KL and Brenner C. Nicotinic acid, nicotinamide, and nicotinamide riboside: A molecular evaluation of NAD+ precursor vitamins in human nutrition. *Annu Rev of Nutr* 28: 115-130, 2008.

9. Braidy N, Guillemin GJ, Mansour H, Chan-Ling T, Poljak A and Grant R. Age related changes in NAD+ metabolism oxidative stress and sirt1 activity in wistar rats. *PLoS ONE* 6, 2011.

10. Buler M, Andersson U and Hakkola J. Who watches the watchmen? Regulation of the expression and activity of sirtuins. *FASEB J* 30: 3942-3960, 2016.

11. Buler M, Aatsinki S, Izzi V and Hakkola J. Metformin reduces hepatic expression of SIRT3, the mitochondrial deacetylase controlling energy metabolism. *PloS one* 7: e49863-e49863, 2012.

12. Buler M, Aatsinki S, Izzi V, Uusimaa J and Hakkola J. SIRT5 is under the control of PGC-1Î± and AMPK and is involved in regulation of mitochondrial energy metabolism. *FASEB J* 28: 3225-37, 2014.

13. Burton GJ and Jauniaux E. Oxidative stress. *Best Pract Res Clin Obstet Gynaecol* 25: 287-299, 2011.

14. Canto C and Auwerx J. Calorie restriction: is AMPK a key sensor and effector? *Physio (Bethesda)* 26: 214-224, 2011.

15. Canto C and Auwerx J. PGC-1alpha, SIRT1 and AMPK, an energy sensing network that controls energy expenditure. *Curr Opin Lipidol* 20: 98-105, 2009.

16. Cantó C and Auwerx J. Caloric restriction, SIRT1 and longevity. *Trends in Endocrinol and Metabo* 20: 325-331, 2009.

17. Canto C, Gerhart-Hines Z, Feige JN, Lagouge M, Noriega L, Milne JC, Elliott PJ, Puigserver P and Auwerx J. AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. *Nature* 458: 1056-1060, 2009.

18. Cantó C, Houtkooper RH, Pirinen E, Youn DY, Oosterveer MH, Cen Y, Fernandez-Marcos PJ, Yamamoto H, Andreux PA, Cettour-Rose P, Gademann K, Rinsch C, Schoonjans K, Sauve AA and Auwerx J. The NAD+ precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet-induced obesity. *Cell Metab* 15: 838-847, 2012.

19. Canto C, Menzies KJ and Auwerx J. NAD(+) Metabolism and the Control of Energy Homeostasis: A Balancing Act between Mitochondria and the Nucleus. *Cell Metab* 22: 31-53, 2015.

20. Cao W, Hong Y, Chen H, Wu F, Wei X and Ying W. SIRT2 mediates NADH-induced increases in Nrf2, GCL, and glutathione by modulating Akt phosphorylation in PC12 cells. *FEBS Lett* 590: 2241-2255, 2016.

21. Carafa V, Rotili D, Forgione M, Cuomo F, Serretiello E, Hailu GS, Jarho E, Lahtela-Kakkonen M, Mai A and Altucci L. Sirtuin functions and modulation: from chemistry to the clinic. *Clin Epigenetics* 8: 61-016-0224-3. eCollection 2016, 2016.

22. Caton PW, Nayuni NK, Kieswich J, Khan NQ, Yaqoob MM and Corder R. Metformin suppresses hepatic gluconeogenesis through induction of SIRT1 and GCN5. *J Endocrinol* 205: 97-106, 2010.

23. Caton PW, Richardson SJ, Kieswich J, Bugliani M, Holland ML, Marchetti P, Morgan NG, Yaqoob MM, Holness MJ and Sugden MC. Sirtuin 3 regulates mouse pancreatic beta cell function and is suppressed in pancreatic islets isolated from human type 2 diabetic patients. *Diabetologia* 56: 1068-1077, 2013.

24. Chen Y, Zhang J, Lin Y, Lei Q, Guan KL, Zhao S and Xiong Y. Tumour suppressor SIRT3 deacetylates and activates manganese superoxide dismutase to scavenge ROS. *EMBO Rep* 12: 534-541, 2011.

25. Chi Y and Sauve AA. Nicotinamide riboside, a trace nutrient in foods, is a Vitamin B3 with effects on energy metabolism and neuroprotection. *Curr Opin Clin Nutr Metab Care* 16: 657-661, 2013.

26. Crujeiras AB, Parra D, Goyenechea E and Martínez JA. Sirtuin gene expression in human mononuclear cells is modulated by caloric restriction. *Eur J Clin Invest* 38: 672-678, 2008.

27. Dimauro I, Scalabrin M, Fantini C, Grazioli E, Beltran Valls MR, Mercatelli N, Parisi A, Sabatini S, Di Luigi L and Caporossi D. Resistance training and redox homeostasis: Correlation with age-associated genomic changes. *Redox Biol* 10: 34-44, 2016.

28. Ding Y, Yang H, Wang Y, Chen J, Ji Z and Sun H. Sirtuin 3 is required for osteogenic differentiation through maintenance of PGC-1α-SOD2-mediated regulation of mitochondrial function. *Int. J. Biol. Sci* 13: 254-264, 2017.

29. Dryden SC, Nahhas FA, Nowak JE, Goustin A- and Tainsky MA. Role for human SIRT2 NAD-dependent deacetylase activity in control of mitotic exit in the cell cycle. *Mol Cell Biol* 23: 3173-3185, 2003.

30. Du J, Zhou Y, Su X, Yu JJ, Khan S, Jiang H, Kim J, Woo J, Kim JH, Choi BH, He B, Chen W, Zhang S, Cerione RA, Auwerx J, Hao Q and Lin H. Sirt5 is a NAD-dependent protein lysine demalonylase and desuccinylase. *Science* 334: 806-809, 2011.

31. Du J, Jiang H and Lin H. Investigating the ADP-ribosyltransferase activity of sirtuins with NAD analogues and 32P-NAD. *Biochemistry (N Y)* 48: 2878-90, 2009.

32. Eto K, Tsubamoto Y, Terauchi Y, Sugiyama T, Kishimoto T, Takahashi N, Yamauchi N, Kubota N, Murayama S, Aizawa T, Akanuma Y, Aizawa S, Kasai H, Yazaki Y and Kadowaki T. Role of NADH shuttle system in glucose-induced activation of mitochondrial metabolism and insulin secretion. *Science* 283: 981-985, 1999.

33. Feldman JL, Dittenhafer-Reed KE, Kudo N, Thelen JN, Ito A, Yoshida M and Denu JM. Kinetic and structural basis for Acyl-group selectivity and NAD+ dependence in sirtuin-catalyzed deacylation. *Biochemistry (N Y)* 54: 3037-3050, 2015.

34. Fernandez-Marcos PJ and Auwerx J. Regulation of PGC-1a, a nodal regulator of mitochondrial biogenesis. *Am J Clin Nutr* 93, 2011.

35. Fernandez-Marcos PJ, Jeninga EH, Canto C, Harach T, de Boer VC, Andreux P, Moullan N, Pirinen E, Yamamoto H, Houten SM, Schoonjans K and Auwerx J. Muscle or liver-specific Sirt3 deficiency induces hyperacetylation of mitochondrial proteins without affecting global metabolic homeostasis. *Sci Rep* 2: 425, 2012.

36. Fu J, Jin J, Cichewicz RH, Hageman SA, Ellis TK, Xiang L, Peng Q, Jiang M, Arbez N, Hotaling K, Ross CA and Duan W. trans-(-)-epsilon-Viniferin increases mitochondrial sirtuin 3 (SIRT3), activates AMP-activated protein kinase (AMPK), and protects cells in models of Huntington Disease. *J Biol Chem* 287: 24460-24472, 2012.

37. Gomes AP, Price NL, Ling AJY, Moslehi JJ, Montgomery MK, Rajman L, White JP, Teodoro JS, Wrann CD, Hubbard BP, Mercken EM, Palmeira CM, De Cabo R, Rolo AP, Turner N, Bell EL and Sinclair DA. Declining NAD+ induces a pseudohypoxic state disrupting nuclear-mitochondrial communication during aging. *Cell* 155: 1624-1638, 2013.

38. Gomes P, Fleming Outeiro T and Cavadas C. Emerging Role of Sirtuin 2 in the Regulation of Mammalian Metabolism. *Trends Pharmacol Sci* 36: 756-768, 2015.

39. Grundy SM. Metabolic syndrome update. *Trends Cardiovasc Med* 26: 364-373, 2016.

40. Guarente L. Sirtuins as potential targets for metabolic syndrome. *Nature* 444: 868-874, 2006.

41. Hafner AV, Dai J, Gomes AP, Xiao CY, Palmeira CM, Rosenzweig A and Sinclair DA. Regulation of the mPTP by SIRT3-mediated deacetylation of CypD at lysine 166 suppresses age-related cardiac hypertrophy. *Aging (Albany NY)* 2: 914-923, 2010.

42. Haigis MC, Mostoslavsky R, Haigis KM, Fahie K, Christodoulou DC, Murphy A, Valenzuela DM, Yancopoulos GD, Karow M, Blander G, Wolberger C, Prolla TA, Weindruch R, Alt FW and Guarente L. SIRT4 Inhibits Glutamate Dehydrogenase and Opposes the Effects of Calorie Restriction in Pancreatic Î² Cells. *Cell* 126: 941-954, 2006.

43. Hardie DG, Ross FA and Hawley SA. AMPK: A nutrient and energy sensor that maintains energy homeostasis. *Nat Rev Mol Cell Biol* 13: 251-262, 2012.

44. Hariharan N, Maejima Y, Nakae J, Paik J, Depinho RA and Sadoshima J. Deacetylation of FoxO by Sirt1 plays an essential role in mediating starvation-induced autophagy in cardiac myocytes. *Circ Res* 107: 1470-1482, 2010.

45. Hebert AS, Dittenhafer-Reed KE, Yu W, Bailey DJ, Selen ES, Boersma MD, Carson JJ, Tonelli M, Balloon AJ, Higbee AJ, Westphall MS, Pagliarini DJ, Prolla TA, Assadi-Porter F, Roy S, Denu JM and Coon JJ. Calorie restriction and SIRT3 trigger global reprogramming of the mitochondrial protein acetylome. *Mol Cell* 49: 186-199, 2013.

46. Hirschey MD, Shimazu T, Goetzman E, Jing E, Schwer B, Lombard DB, Grueter CA, Harris C, Biddinger S, Ilkayeva OR, Stevens RD, Li Y, Saha AK, Ruderman NB, Bain JR, Newgard CB, Farese RV, Alt FW, Kahn CR and Verdin E. SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation. *Nature* 464: 121-5, 2010.

47. Hirschey MD, Shimazu T, Jing E, Grueter CA, Collins AM, Aouizerat B, StanÄ• Ã¡kovÃ¡ A, Goetzman E, Lam MM, Schwer B, Stevens RD, Muehlbauer MJ, Kakar S, Bass NM,

Kuusisto J, Laakso M, Alt FW, Newgard CB, Farese RV, Kahn CR and Verdin E. SIRT3 deficiency and mitochondrial protein hyperacetylation accelerate the development of the metabolic syndrome. *Mol Cell* 44: 177-90, 2011.

48. Ho L, Titus AS, Banerjee KK, George S, Lin W, Deota S, Saha AK, Nakamura K, Gut P, Verdin E and Kolthur-Seetharam U. SIRT4 regulates ATP homeostasis and mediates a retrograde signaling via AMPK. *Aging (Albany NY)* 5: 835-849, 2013.

49. Hou X, Xu S, Maitland-Toolan KA, Sato K, Jiang B, Ido Y, Lan F, Walsh K, Wierzbicki M, Verbeuren TJ, Cohen RA and Zang M. SIRT1 regulates hepatocyte lipid metabolism through activating AMP-activated protein kinase. *J Biol Chem* 283: 20015-20026, 2008.

50. Houtkooper RH, Cantó C, Wanders RJ and Auwerx J. The secret life of NAD+: An old metabolite controlling new metabolic signaling pathways. *Endocr Rev* 31: 194-223, 2010.

51. Huh JE, Shin JH, Jang ES, Park SJ, Park DR, Ko R, Seo DH, Kim HS, Lee SH, Choi Y, Kim HS and Lee SY. Sirtuin 3 (SIRT3) maintains bone homeostasis by regulating AMPK-PGC-1beta axis in mice. *Sci Rep* 6: 22511, 2016.

52. Imai S, Armstrong CM, Kaeberlein M and Guarente L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* 403: 795-800, 2000.

53. Jager S, Handschin C, St-Pierre J and Spiegelman BM. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. *Proc Natl Acad Sci U S A* 104: 12017-12022, 2007.

54. Jiang W, Wang S, Xiao M, Lin Y, Zhou L, Lei Q, Xiong Y, Guan K- and Zhao S. Acetylation Regulates Gluconeogenesis by Promoting PEPCK1 Degradation via Recruiting the UBR5 Ubiquitin Ligase. *Mol Cell* 43: 33-44, 2011.

55. Jing E, Emanuelli B, Hirschey MD, Boucher J, Lee KY, Lombard D, Verdin EM and Kahn CR. Sirtuin-3 (Sirt3) regulates skeletal muscle metabolism and insulin signaling via altered mitochondrial oxidation and reactive oxygen species production. *Proc Natl Acad Sci U S A* 108: 14608-14613, 2011.

56. Jing E, Gesta S and Kahn CR. SIRT2 Regulates Adipocyte Differentiation through FoxO1 Acetylation/Deacetylation. *Cell Metab* 6: 105-114, 2007.

57. Jing H and Lin H. Sirtuins in epigenetic regulation. *Chem Rev* 115: 2350-2375, 2015.

58. Kendrick AA, Choudhury M, Rahman SM, McCurdy CE, Friederich M, Van Hove J,L.K., Watson PA, Birdsey N, Bao J, Gius D, Sack MN, Jing E, Kahn CR, Friedman JE and Jonscher KR. Fatty liver is associated with reduced SIRT3 activity and mitochondrial protein hyperacetylation. *Biochem J* 433: 505-14, 2011.

59. Kim M, Lee JS, Oh JE, Nan J, Lee H, Jung HS, Chung SS and Park KS. SIRT3 Overexpression Attenuates Palmitate-Induced Pancreatic beta-Cell Dysfunction. *PLoS One* 10: e0124744, 2015.

60. Kong X, Wang R, Xue Y, Liu X, Zhang H, Chen Y, Fang F and Chang Y. Sirtuin 3, a new target of PGC-1alpha, plays an important role in the suppression of ROS and mitochondrial biogenesis. *PLoS One* 5: e11707, 2010.

61. Krejcí A. Metabolic sensors and their interplay with cell signalling and transcription. *Biochem Soc Trans* 40: 311-323, 2012.

62. Krishnan J, Danzer C, Simka T, Ukropec J, Walter KM, Kumpf S, Mirtschink P, Ukropcova B, Gasperikova D, Pedrazzini T and Krek W. Dietary obesity-associated hif1a activation in adipocytes restricts fatty acid oxidation and energy expenditure via suppression of the Sirt2-NAD+ system. *Genes Dev* 26: 259-270, 2012.

63. Kumar S and Lombard DB. Mitochondrial sirtuins and their relationships with metabolic disease and cancer. *Antioxid Redox Signal* 22: 1060-77, 2015.

64. Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P, Geny B, Laakso M, Puigserver P and Auwerx J. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. *Cell* 127: 1109-1122, 2006.

65. Lai Y-, Tabima DM, Dube JJ, Hughan KS, Vanderpool RR, Goncharov DA, St. Croix CM, Garcia-Ocanã A, Goncharova EA, Tofovic SP, Mora AL and Gladwin MT. SIRT3-AMP-Activated Protein Kinase Activation by Nitrite and Metformin Improves Hyperglycemia and Normalizes Pulmonary Hypertension Associated with Heart Failure with Preserved Ejection Fraction. *Circulation* 133: 717-731, 2016.

66. Laurent G, de Boer, V. C. J., Finley LWS, Sweeney M, Lu H, Schug TT, Cen Y, Jeong SM, Li X, Sauve AA and Haigis MC. SIRT4 Represses Peroxisome Proliferator-Activated Receptor Â Activity To Suppress Hepatic Fat Oxidation. *Mol Cell Biol* 33: 4552-4561, 2013.

67. Laurent G, German NJ, Saha AK, de Boer V,C.J., Davies M, Koves TR, Dephoure N, Fischer F, Boanca G, Vaitheesvaran B, Lovitch SB, Sharpe AH, Kurland IJ, Steegborn C, Gygi SP, Muoio DM, Ruderman NB and Haigis MC. SIRT4 coordinates the balance between lipid synthesis and catabolism by repressing malonyl CoA decarboxylase. *Mol Cell* 50: 686-98, 2013.

68. Liemburg-Apers DC, Wagenaars JA, Smeitink JAM, Willems PHGM and Koopman WJH. Acute stimulation of glucose influx upon mitoenergetic dysfunction requires LKB1, AMPK, Sirt2 and mTOR-RAPTOR. *J Cell Sci* 129: 4411-4423, 2016.

69. Lin R, Tao R, Gao X, Li T, Zhou X, Guan K-, Xiong Y and Lei Q-. Acetylation stabilizes ATP-citrate lyase to promote lipid biosynthesis and tumor growth. *Mol Cell* 51: 506-518, 2013.

70. Lin Z, Xu H, Wang J, Lin Q, Ruan Z, Liu F, Jin W, Huang H and Chen X. SIRT5 desuccinylates and activates SOD1 to eliminate ROS. *Biochem Biophys Res Commun* 441: 191-5, 2013.

71. Liu G, Park SH, Imbesi M, Nathan WJ, Zou X, Zhu Y, Jiang H, Parisiadou L and Gius D. Loss of NAD-Dependent Protein Deacetylase Sirtuin-2 Alters Mitochondrial Protein Acetylation and Dysregulates Mitophagy. *Antioxid Redox Signal*, 2016.

72. Liu L, Peritore C, Ginsberg J, Shih J, Arun S and Donmez G. Protective role of SIRT5 against motor deficit and dopaminergic degeneration in MPTP-induced mice model of Parkinson's disease. *Behav Brain Res* 281: 215-221, 2015.

73. Lombard DB, Alt FW, Cheng HL, Bunkenborg J, Streeper RS, Mostoslavsky R, Kim J, Yancopoulos G, Valenzuela D, Murphy A, Yang Y, Chen Y, Hirschey MD, Bronson RT, Haigis M, Guarente LP, Farese RV,Jr, Weissman S, Verdin E and Schwer B. Mammalian Sir2 homolog SIRT3 regulates global mitochondrial lysine acetylation. *Mol Cell Biol* 27: 8807-8814, 2007.

74. Love NR, Pollak N, Döllea C, Nierea M, Chen Y, Oliverid P, Amaya E, Patel S and Ziegler M. NAD kinase controls animal NADP biosynthesis and is modulated via evolutionarily divergent calmodulin-dependent mechanisms. *Proc Natl Acad Sci U S A* 112: 1386-1391, 2015.

75. Lu MC, Ji JA, Jiang ZY and You QD. The Keap1-Nrf2-ARE Pathway As a Potential Preventive and Therapeutic Target: An Update. *Med Res Rev* 36: 924-963, 2016.

76. Mathias RA, Greco TM, Oberstein A, Budayeva HG, Chakrabarti R, Rowland EA, Kang Y, Shenk T and Cristea IM. Sirtuin 4 is a lipoamidase regulating pyruvate dehydrogenase complex activity. *Cell* 159: 1615-1625, 2014.

77. Matsushima S and Sadoshima J. The role of sirtuins in cardiac disease. *Am J of Physiol-Heart Circ Physiol* 309: H1375-H1389, 2015.

78. Matsushita N, Yonashiro R, Ogata Y, Sugiura A, Nagashima S, Fukuda T, Inatome R and Yanagi S. Distinct regulation of mitochondrial localization and stability of two human Sirt5 isoforms. *Genes to cells* 16: 190-202, 2011.

79. Mattaini KR and Vander Heiden MG. Glycosylation to adapt to stress. *Science* 337: 925-926, 2012.

80. Maxwell MM, Tomkinson EM, Nobles J, Wizeman JW, Amore AM, Quinti L, Chopra V, Hersch SM and Kazantsev AG. The Sirtuin 2 microtubule deacetylase is an abundant neuronal protein that accumulates in the aging CNS. *Hum Mol Genet* 20: 3986-3996, 2011.

81. Mayoral R, Osborn O, McNelis J, Johnson AM, Oh DY, Izquierdo CL, Chung H, Li P, Traves PG, Bandyopadhyay G, Pessentheiner AR, Ofrecio JM, Cook JR, Qiang L, Accili D and

Olefsky JM. Adipocyte SIRT1 knockout promotes PPARgamma activity, adipogenesis and insulin sensitivity in chronic-HFD and obesity. *Mol Metab* 4: 378-391, 2015.

82. Menzies KJ, Zhang H, Katsyuba E and Auwerx J. Protein acetylation in metabolism-metabolites and cofactors. *Nat Rev Endocrinol* 12: 43-60, 2016.

83. Merksamer PI, Liu Y, He W, Hirschey MD, Chen D and Verdin E. The sirtuins, oxidative stress and aging: An emerging link. *Aging* 5: 144-150, 2013.

84. Nakagawa T, Lomb DJ, Haigis MC and Guarente L. SIRT5 Deacetylates Carbamoyl Phosphate Synthetase 1 and Regulates the Urea Cycle. *Cell* 137: 560-570, 2009.

85. Nasrin N, Wu X, Fortier E, Feng Y, Bare' OC, Chen S, Ren X, Wu Z, Streeper RS and Bordone L. SIRT4 regulates fatty acid oxidation and mitochondrial gene expression in liver and muscle cells. *J Biol Chem* 285: 31995-2002, 2010.

86. Nishida Y, Rardin MJ, Carrico C, He W, Sahu AK, Gut P, Najjar R, Fitch M, Hellerstein M, Gibson BW and Verdin E. SIRT5 Regulates both Cytosolic and Mitochondrial Protein Malonylation with Glycolysis as a Major Target. *Mol Cell* 59: 321-32, 2015.

87. North BJ, Marshall BL, Borra MT, Denu JM and Verdin E. The human Sir2 ortholog, SIRT2, is an NAD+-dependent tubulin deacetylase. *Mol Cell* 11: 437-444, 2003.

88. North BJ, Rosenberg MA, Jeganathan KB, Hafner AV, Michan S, Dai J, Baker DJ, Cen Y, Wu LE, Sauve AA, Van Deursen JM, Rosenzweig A and Sinclair DA. SIRT2 induces the checkpoint kinase BubR1 to increase lifespan. *EMBO J* 33: 1438-1453, 2014.

89. Palacios OM, Carmona JJ, Michan S, Chen KY, Manabe Y, Ward 3rd. JL, Goodyear LJ and Tong Q. Diet and exercise signals regulate SIRT3 and activate AMPK and PGC-1alpha in skeletal muscle. *Aging* 1: 771-783, 2009.

90. Parihar P, Solanki I, Mansuri ML and Parihar MS. Mitochondrial sirtuins: emerging roles in metabolic regulations, energy homeostasis and diseases. *Exp Gerontol* 61: 130-141, 2015.

91. Park J, Chen Y, Tishkoff DX, Peng C, Tan M, Dai L, Xie Z, Zhang Y, Zwaans BM, Skinner ME, Lombard DB and Zhao Y. SIRT5-mediated lysine desuccinylation impacts diverse metabolic pathways. *Mol Cell* 50: 919-930, 2013.

92. Park J-, Kim T-, Jo S-, Kim M- and Ahn Y-. Acetylation of glucokinase regulatory protein decreases glucose metabolism by suppressing glucokinase activity. *Sci Rep* 5, 2015.

93. Patterson GH, Knobel SM, Arkhammar P, Thastrup O and Piston DW. Separation of the glucose-stimulated cytoplasmic and mitochondrial NAD(P)H responses in pancreatic islet ß cells. *Proc Natl Acad Sci U S A* 97: 5203-5207, 2000.

94. Picard F, Kurtev M, Chung N, Topark-Ngarm A, Senawong T, Machado De Oliveira R, Leid M, McBurney MW and Guarente L. Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. *Nature* 429: 771-776, 2004.

95. Poulose N and Raju R. Sirtuin regulation in aging and injury. *Biochimica et Biophysica Acta (BBA) - Mol Bas of Dis* 1852: 2442-55, 2015.

96. Price NL, Gomes AP, Ling AJ, Duarte FV, Martin-Montalvo A, North BJ, Agarwal B, Ye L, Ramadori G, Teodoro JS, Hubbard BP, Varela AT, Davis JG, Varamini B, Hafner A, Moaddel R, Rolo AP, Coppari R, Palmeira CM, de Cabo R, Baur JA and Sinclair DA. SIRT1 is required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function. *Cell Metab* 15: 675-690, 2012.

97. Qiu X, Brown K, Hirschey MD, Verdin E and Chen D. Calorie restriction reduces oxidative stress by SIRT3-mediated SOD2 activation. *Cell Metab* 12: 662-667, 2010.

98. Rack JGM, Vanlinden MR, Lutter T, Aasland R and Ziegler M. Constitutive nuclear localization of an alternatively spliced sirtuin-2 isoform. *J Mol Biol* 426: 1677-1691, 2014.

99. Ramakrishnan G, Davaakhuu G, Kaplun L, Chung W-, Rana A, Atfi A, Miele L and Tzivion G. Sirt2 deacetylase is a novel AKT Binding partner critical for AKT activation by insulin. *J Biol Chem* 289: 6054-6066, 2014.

100. Rardin MJ, He W, Nishida Y, Newman JC, Carrico C, Danielson SR, Guo A, Gut P, Sahu AK, Li B, Uppala R, Fitch M, Riiff T, Zhu L, Zhou J, Mulhern D, Stevens RD, Ilkayeva OR, Newgard CB, Jacobson MP, Hellerstein M, Goetzman ES, Gibson BW and Verdin E. SIRT5 regulates the mitochondrial lysine succinylome and metabolic networks. *Cell Metab* 18: 920-933, 2013.

101. Rardin MJ, Newman JC, Held JM, Cusack MP, Sorensen DJ, Li B, Schilling B, Mooney SD, Kahn CR, Verdin E and Gibson BW. Label-free quantitative proteomics of the lysine acetylome in mitochondria identifies substrates of SIRT3 in metabolic pathways. *Proc Natl Acad Sci U S A* 110: 6601-6606, 2013.

102. Revollo JR, Grimm AA and Imai S-. The NAD biosynthesis pathway mediated by nicotinamide phosphoribosyltransferase regulates Sir2 activity in mammalian cells. *J Biol Chem* 279: 50754-50763, 2004.

103. Revollo JR, Körner A, Mills KF, Satoh A, Wang T, Garten A, Dasgupta B, Sasaki Y, Wolberger C, Townsend RR, Milbrandt J, Kiess W and Imai S-. Nampt/PBEF/Visfatin Regulates Insulin Secretion in ß Cells as a Systemic NAD Biosynthetic Enzyme. *Cell Metab* 6: 363-375, 2007.

104. Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM and Puigserver P. Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. *Nature* 434: 113-118, 2005.

105. Ruggieri S, Orsomando G, Sorci L and Raffaelli N. Regulation of NAD biosynthetic enzymes modulates NAD-sensing processes to shape mammalian cell physiology under varying biological cues. *Biochim Biophys Acta* 1854: 1138-49, 2015.

106. Sadhukhan S, Liu X, Ryu D, Nelson OD, Stupinski JA, Li Z, Chen W, Zhang S, Weiss RS, Locasale JW, Auwerx J and Lin H. Metabolomics-assisted proteomics identifies succinylation and SIRT5 as important regulators of cardiac function. *Proc Natl Acad Sci U S A* 113: 4320-4325, 2016.

107. Sazanov LA. A giant molecular proton pump: Structure and mechanism of respiratory complex I. *Nat Rev Mol Cell Biol* 16: 375-388, 2015.

108. Schwer B, Bunkenborg J, Verdin RO, Andersen JS and Verdin E. Reversible lysine acetylation controls the activity of the mitochondrial enzyme acetyl-CoA synthetase 2. *Proc Natl Acad Sci U S A* 103: 10224-10229, 2006.

109. Schwer B, North BJ, Frye RA, Ott M and Verdin E. The human silent information regulator (Sir)2 homologue hSIRT3 is a mitochondrial nicotinamide adenine dinucleotide-dependent deacetylase. *J Cell Biol* 158: 647-657, 2002.

110. Serrano L, Martínez-Redondo P, Marazuela-Duque A, Vazquez BN, Dooley SJ, Voigt P, Beck DB, Kane-Goldsmith N, Tong Q, Rabanal RM, Fondevila D, Muñoz P, Krüger M, Tischfield JA and Vaquero A. The tumor suppressor SirT2 regulates cell cycle progression and genome stability by modulating the mitotic deposition of H4K20 methylation. *Genes Dev* 27: 639-653, 2013.

111. Shi T, Wang F, Stieren E and Tong Q. SIRT3, a mitochondrial sirtuin deacetylase, regulates mitochondrial function and thermogenesis in brown adipocytes. *J Biol Chem* 280: 13560-7, 2005.

112. Shimazu T, Hirschey MD, Hua L, Dittenhafer-Reed KE, Schwer B, Lombard DB, Li Y, Bunkenborg J, Alt FW, Denu JM, Jacobson MP and Verdin E. SIRT3 deacetylates mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase 2 and regulates ketone body production. *Cell Metab* 12: 654-661, 2010.

113. Someya S, Yu W, Hallows WC, Xu J, Vann JM, Leeuwenburgh C, Tanokura M, Denu JM and Prolla TA. Sirt3 mediates reduction of oxidative damage and prevention of age-related hearing loss under caloric restriction. *Cell* 143: 802-812, 2010.

114. Srivastava S. Emerging therapeutic roles for NAD(+) metabolism in mitochondrial and age-related disorders. *Clin Transl Med* 5: 25-016-0104-7. Epub 2016 Jul 27, 2016.

115. Sundaresan NR, Gupta M, Kim G, Rajamohan SB, Isbatan A and Gupta MP. Sirt3 blocks the cardiac hypertrophic response by augmenting Foxo3a-dependent antioxidant defense mechanisms in mice. *J Clin Invest* 119: 2758-2771, 2009.

116. Tan M, Peng C, Anderson KA, Chhoy P, Xie Z, Dai L, Park J, Chen Y, Huang H, Zhang Y, Ro J, Wagner GR, Green MF, Madsen AS, Schmiesing J, Peterson BS, Xu G, Ilkayeva OR, Muehlbauer MJ, Braulke T, Mühlhausen C, Backos DS, Olsen CA, McGuire PJ, Pletcher SD, Lombard DB, Hirschey MD and Zhao Y. Lysine glutarylation is a protein posttranslational modification regulated by SIRT5. *Cell Metab* 19: 605-617, 2014.

117. Tao R, Coleman MC, Pennington JD, Ozden O, Park SH, Jiang H, Kim HS, Flynn CR, Hill S, Hayes McDonald W, Olivier AK, Spitz DR and Gius D. Sirt3-mediated deacetylation of evolutionarily conserved lysine 122 regulates MnSOD activity in response to stress. *Mol Cell* 40: 893-904, 2010.

118. Teng Y-, Jing H, Aramsangtienchai P, He B, Khan S, Hu J, Lin H and Hao Q. Efficient demyristoylase activity of SIRT2 revealed by kinetic and structural studies. *Sci Rep* 5, 2014.

119. Vaquero A, Scher MB, Dong HL, Sutton A, Cheng H-, Alt FW, Serrano L, Sternglanz R and Reinberg D. SirT2 is a histone deacetylase with preference for histone H4 Lys 16 during mitosis. *Genes Dev* 20: 1256-1261, 2006.

120. Villena JA. New insights into PGC-1 coactivators: Redefining their role in the regulation of mitochondrial function and beyond. *FEBS J* 282: 647-672, 2015.

121. Wagner GR, Bhatt DP, O'Connell TM, Thompson JW, Dubois LG, Backos DS, Yang H, Mitchell GA, Ilkayeva OR, Stevens RD, Grimsrud PA and Hirschey MD. A Class of Reactive Acyl-CoA Species Reveals the Non-enzymatic Origins of Protein Acylation. *Cell Metab* 25: 823-837.e8, 2017.

122. Wang F, Nguyen M, Qin FX- and Tong Q. SIRT2 deacetylates FOXO3a in response to oxidative stress and caloric restriction. *Aging Cell* 6: 505-514, 2007.

123. Wang F and Tong Q. SIRT2 suppresses adipocyte differentiation by deacetylating FOXO1 and enhancing FOXO1's repressive interaction with PPARgamma. *Mol Biol Cell* 20: 801-808, 2009.

124. Wang Q, Jiang L, Wang J, Li S, Yu Y, You J, Zeng R, Gao X, Rui L, Li W and Liu Y. Abrogation of hepatic ATP-citrate lyase protects against fatty liver and ameliorates hyperglycemia in leptin receptor-deficient mice. *Hepatology* 49: 1166-1175, 2009.

125. Wang Y-, Zhou L-, Zhao Y-, Wang S-, Chen L-, Liu L-, Ling Z-, Hu F-, Sun Y-, Zhang J-, Yang C, Yang Y, Xiong Y, Guan K- and Ye D. Regulation of G6PD acetylation by SIRT2 and KAT9 modulates NADPH homeostasis and cell survival during oxidative stress. *EMBO J* 33: 1304-1320, 2014.

126. Wu T, Liu YH, Fu YC, Liu XM and Zhou XH. Direct evidence of sirtuin downregulation in the liver of non-alcoholic fatty liver disease patients. *Ann Clin Lab Sci* 44: 410-418, 2014.

127. Xu Y, Li F, Lv L, Li T, Zhou X, Deng C-, Guan K-, Lei Q-L and Xiong Y. Oxidative stress activates sirt2 to deacetylate and stimulate phosphoglycerate mutase. *Cancer Res* 74: 3630-3642, 2014.

128. Yamaguchi S and Yoshino J. Adipose tissue NAD+ biology in obesity and insulin resistance: From mechanism to therapy. *Bioessays*, 2017.

129. Yang H, Yang T, Baur JA, Perez E, Matsui T, Carmona JJ, Lamming DW, Souza-Pinto NC, Bohr VA, Rosenzweig A, de Cabo R, Sauve AA and Sinclair DA. Nutrient-sensitive mitochondrial NAD+ levels dictate cell survival. *Cell* 130: 1095-1107, 2007.

130. Yang W, Nagasawa K, Munch C, Xu Y, Satterstrom K, Jeong S, Hayes SD, Jedrychowski MP, Vyas FS, Zaganjor E, Guarani V, Ringel AE, Gygi SP, Harper JW and Haigis MC. Mitochondrial Sirtuin Network Reveals Dynamic SIRT3-Dependent Deacetylation in Response to Membrane Depolarization. *Cell* 167: 985-1000.e21, 2016.

131. Yang X, Park SH, Chang HC, Shapiro JS, Vassilopoulos A, Sawicki KT, Chen C, Shang M, Burridge PW, Epting CL, Wilsbacher LD, Jenkitkasemwong S, Knutson M, Gius D and Ardehali H. Sirtuin 2 regulates cellular iron homeostasis via deacetylation of transcription factor NRF2. *J Clin Invest* 127: 1505-1516, 2017.

132. Yang Y and Sauve AA. NAD+ metabolism: Bioenergetics, signaling and manipulation for therapy. *Biochim Biophys Acta Prot Proteomics* 1864: 1787-1800, 2016.

133. Ye X, Li M, Hou T, Gao T, Zhu WG and Yang Y. Sirtuins in glucose and lipid metabolism. *Oncotarget* 8: 1845-1859, 2017.

134. Yechoor VK, Patti M, Ueki K, Laustsen PG, Saccone R, Rauniyar R and Kahn CR. Distinct pathways of insulin-regulated versus diabetes-regulated gene expression: an in vivo analysis in MIRKO mice. *Proc Natl Acad Sci U S A* 101: 16525-30, 2004.

135. Yi W, Clark PM, Mason DE, Keenan MC, Hill C, Goddard III WA, Peters EC, Driggers EM and Hsieh-Wilson LC. Phosphofructokinase 1 glycosylation regulates cell growth and metabolism. *Science* 337: 975-980, 2012.

136. Yoshino J, Mills KF, Yoon MJ and Imai S-. Nicotinamide mononucleotide, a key NAD + intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice. *Cell Metab* 14: 528-536, 2011.

137. Yuan H and Marmorstein R. Structural basis for sirtuin activity and inhibition. *J Biol Chem* 287: 42428-42435, 2012.

138. Yuan Q, Zhan L, Zhou Q-, Zhang L-, Chen X-, Hu X- and Yuan X-. SIRT2 regulates microtubule stabilization in diabetic cardiomyopathy. *Eur J Pharmacol* 764: 554-561, 2015.

139. Zhang M, Pan Y, Dorfman RG, Yin Y, Zhou Q, Huang S, Liu J and Zhao S. Sirtinol promotes PEPCK1 degradation and inhibits gluconeogenesis by inhibiting deacetylase SIRT2. *Sci Rep* 7: 7-017-00035-9. Epub 2017 Jan 31, 2017.

140. Zhang Y, Bharathi SS, Rardin MJ, Lu J, Maringer KV, Sims-Lucas S, Prochownik EV, Gibson BW and Goetzman ES. Lysine desuccinylase SIRT5 binds to cardiolipin and regulates the electron transport chain. *J Biol Chem* 292: 10239-10249, 2017.

141. Zhao Y and Yang Y. Real-time and high-throughput analysis of mitochondrial metabolic states in living cells using genetically encoded NAD+/NADH sensors. *Free Radic Biol Med* 100: 43-52, 2016.

142. Zhao Y and Yang Y. Profiling metabolic states with genetically encoded fluorescent biosensors for NADH. *Curr Opin Biotechnol* 31: 86-92, 2015.

143. Zhou L, Wang F, Sun R, Chen X, Zhang M, Xu Q, Wang Y, Wang S, Xiong Y, Guan KL, Yang P, Yu H and Ye D. SIRT5 promotes IDH2 desuccinylation and G6PD deglutarylation to enhance cellular antioxidant defense. *EMBO Rep* 17: 811-822, 2016.

144. Zhou Y, Chung ACK, Fan R, Lee HM, Xu G, Tomlinson B, Chan JCN and Kong APS. Sirt3 Deficiency Increased the Vulnerability of Pancreatic Beta Cells to Oxidative Stress-Induced Dysfunction. *Antioxid Redox Signal*, 2017.

Figure legends:

Figure 1: Sirtuins sense the nutritional and metabolic state through NAD^+ availability. Sirtuins are sensitive to the cellular NAD^+ level. High NAD^+ level during conditions of high-energy demand such as fasting and exercise can activate the extranuclear sirtuins in cytosol and mitochondria, and consequently regulates several metabolic pathways to maintain the cellular energy homeostasis. On the other hand, high-fat diet, metabolic and oxidative stress, and aging decrease NAD^+ level. Low NAD^+ is associated with dysregulation of sirtuin activities and pathological conditions such as mitochondrial diseases, metabolic syndrome and vascular diseases. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

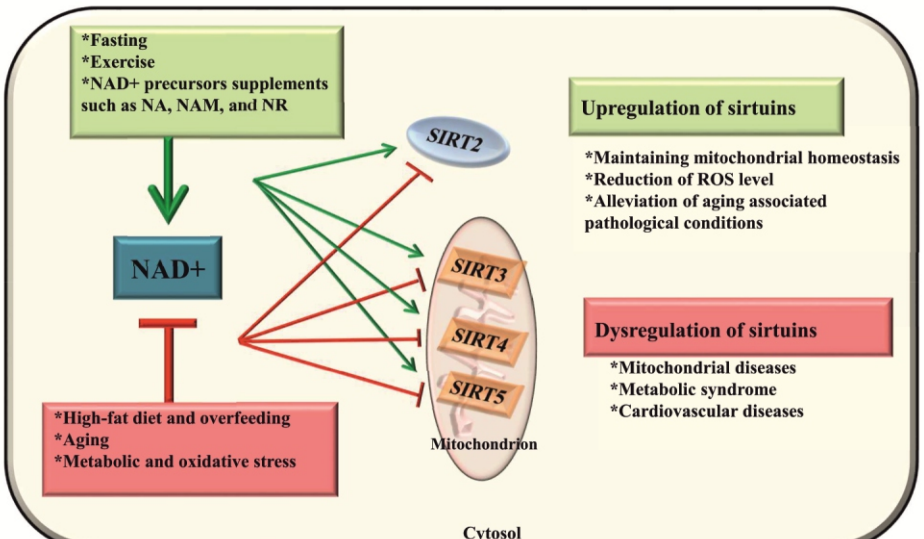
Figure 2: Fasting and exercise regulate extranuclear sirtuins through multiple pathways. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

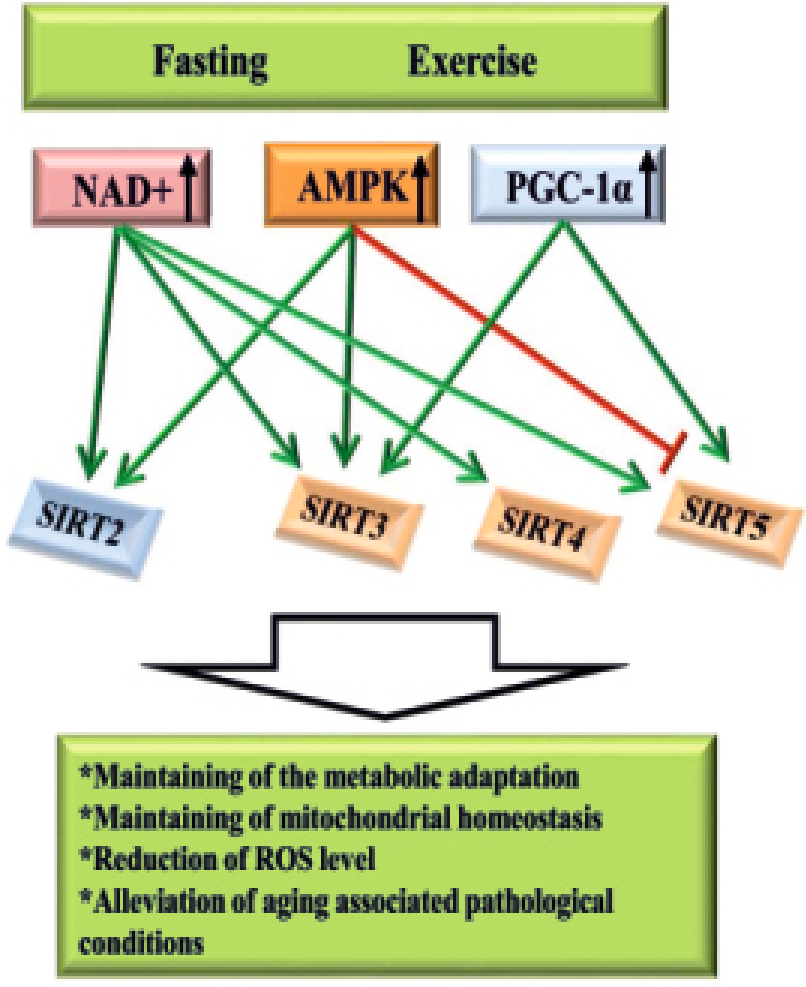
Figure 3: SIRT2 maintains the energy homeostasis through regulation of several metabolic pathways such as adipogenesis, glucose metabolism, fatty acids synthesis and hepatic glucose production. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

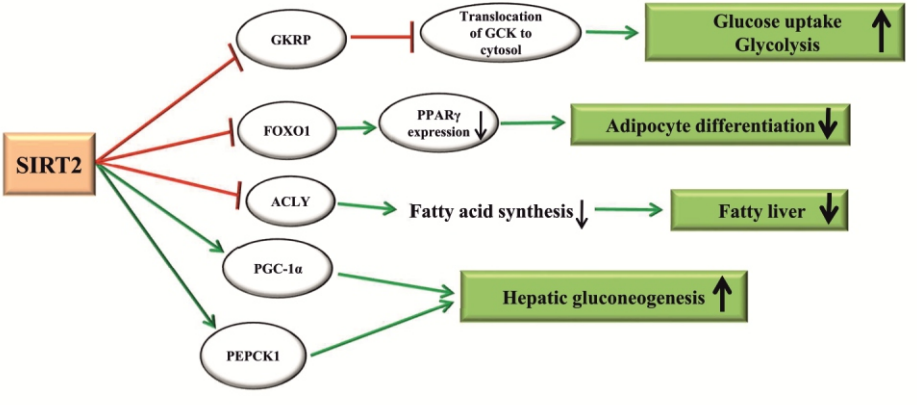
Figure 4: SIRT3 is a major regulator of the mitochondrial functions and plays a key role in adaptation to the physiological metabolic stress such as fasting. Repression of SIRT3 by HFD and diabetes is probably involved in the metabolic disturbances. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

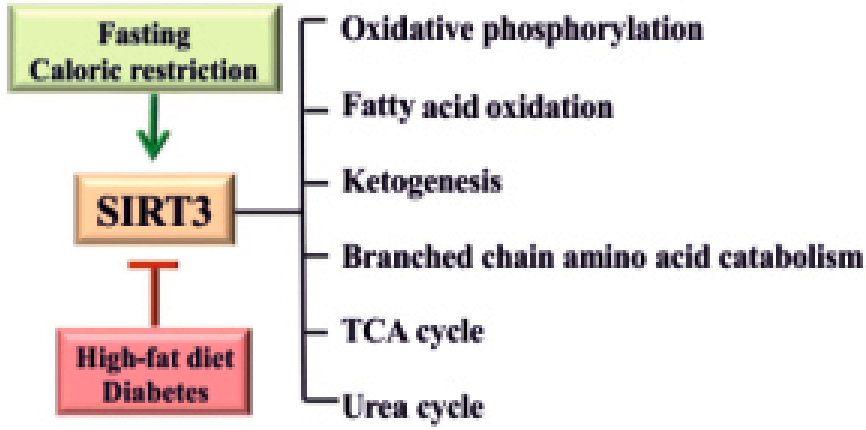
Figure 5: Role of SIRT4 in maintenance of the lipid homeostasis. A) In fed condition, SIRT4 deacetylates and inhibits the maloynyl CoA decarboxylase (MCD), catalyzing the conversion of the malonyl-CoA to acetyl-CoA, thus promoting the opposite reaction and fatty acid synthesis in liver. **B**) During fasting, the repression of the SIRT4 promotes β-oxidation through several mechanisms. Repression of ATP production activates AMPK, which in turn phosphorylates and inhibits the acetyl CoA carboxylase (ACC), the enzyme catalyzing acetyl-CoA conversion to malonyl-CoA, thus preventing fatty acid synthesis. Furthermore, SIRT4 repression indirectly induces synthesis of several β-oxidation genes. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

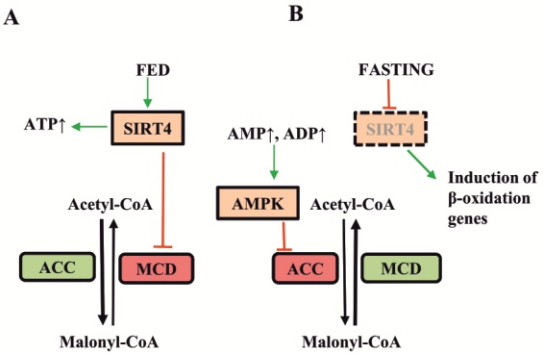
Figure 6: Sirtuins control the antioxidative defense system. Mitochondrial energy metabolism produces high levels of potentially toxic ROS. SIRT2, SIRT3 and SIRT5 all play important roles in activation of the protective mechanisms against ROS. Many of the regulatory mechanisms are shared by the different sirtuins. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

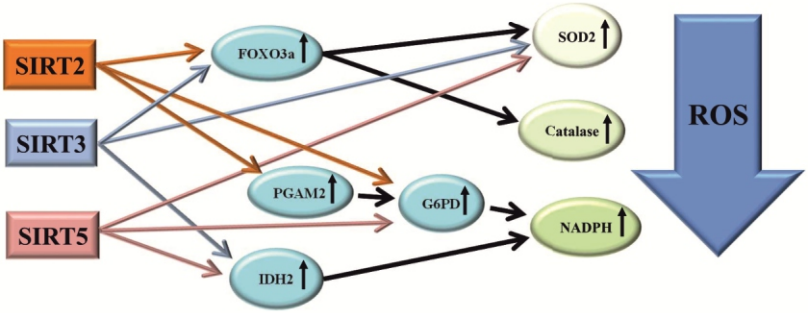

*Fasting
*Exercise
*NAD+ precursors supplements such as NA, NAM, and NR
NAD+
*High-fat diet and overfeeding
*Aging
*Metabolic and oxidative stress
SIRT2
SIRT3
SIRT4
SIRT5
Mitochondrion
Cytosol
Upregulation of sirtuins
*Maintaining mitochondrial homeostasis
*Reduction of ROS level
*Alleviation of aging associated pathological conditions
Dysregulation of sirtuins
*Mitochondrial diseases
*Metabolic syndrome
*Cardiovascular diseases


Fasting
Exercise
NAD+
AMPK
PGC-1α
SIRT2
SIRT3
SIRT4
SIRT5
*Maintaining of the metabolic adaptation
*Maintaining of mitochondrial homeostasis
*Reduction of ROS level
*Alleviation of aging associated pathological conditions


SIRT2
GKRP
Translocation of GCK to cytosol
Glucose uptake
Glycolysis
FOXO1
PPARγ expression
Adipocyte differentiation
ACLY
Fatty acid synthesis
Fatty liver
PGC-1α
PEPCK1
Hepatic gluconeogenesis


Fasting
Caloric restriction
SIRT3
High-fat diet
Diabetes
Oxidative phosphorylation
Fatty acid oxidation
Ketogenesis
Branched chain amino acid catabolism
TCA cycle
Urea cycle


A
FED
ATP↑
SIRT4
Acetyl-CoA
ACC
MCD
Malonyl-CoA
B
FASTING
AMP↑, ADP↑
SIRT4
Induction of
β-oxidation
genes
AMPK
Acetyl-CoA
ACC
MCD
Malonyl-CoA


SIRT2
SIRT3
SIRT5
FOXO3a
PGAM2
IDH2
G6PD
SOD2
Catalase
NADPH
ROS