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Sirtuins as potential targets for metabolic syndrome

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Metabolic syndrome threatens health gains made during the past century. Physiological processes degraded by this syndrome are often oppositely affected by calorie restriction, which extends lifespan and prevents disease in rodents. Recent research in the field of ageing has begun to identify important mediators of calorie restriction, offering the hope of new drugs to improve healthspan. Moreover, if metabolic syndrome and calorie restriction are opposite extremes of the same metabolic spectrum, calorie restriction mimetics might provide another therapeutic approach to metabolic syndrome. Sirtuins and other important metabolic pathways that affect calorie restriction may serve as entry points for drugs to treat metabolic syndrome.

In the western world the prevalence of metabolic syndrome in the adult population is approaching one-quarter, probably triggered by high-calorie diets and physical inactivity. It is characterized by a combination of physiological parameters, including obesity, inflammation, high blood pressure and dyslipidaemia (high levels of circulating triacylglycerols and low-density lipoprotein (LDL) cholesterol, and low levels of high-density lipoprotein (HDL) cholesterol)[1–3]. Metabolic syndrome is also associated with dysregulation of glucose homeostasis — that is, glucose intolerance (the inability to clear an orally administered dose of glucose from the blood normally), which is indicative of insulin insensitivity (inability of insulin to promote normal glucose uptake by cells). This dysregulation can be associated with higher levels of blood insulin — a compensation mechanism — and, as the syndrome progresses, increased blood glucose levels and diabetes. Metabolic syndrome was first recognized as a risk factor for cardiovascular disease, and is associated with atherosclerosis. This syndrome also heightens risk for stroke, cancer, arthritis and, of course, diabetes. Lifestyle changes are the first defence in treating metabolic syndrome, followed by pharmacological intervention.

Whereas prediabetic conditions were once thought to be related to ageing, as are type II diabetes and cardiovascular disease, the recent epidemic of metabolic syndrome has afflicted younger adults and even children. Nevertheless, there does seem to be an ageing- or time-dependent component to the progression from metabolic syndrome to diabetes, and the resulting high risk for cardiovascular disease. Moreover, a link can be imagined between metabolic syndrome and our evolutionary strategy for survival.

It is likely that the selected evolutionary strategy in times of food availability was the preferential use of carbohydrates for energy, and the storage of fat, because fat is more reduced and has a higher energy content per unit mass. Thus, animals may have taken advantage of the fact that fat storage was a sign that food was available and leanness was a sign of food scarcity. More specifically, fat cells are known to secrete hormones known as adipokines, so this dietary information could readily be disseminated throughout the body. In times of food availability, the best life strategy would be to reproduce and not worry about future, post-reproductive health deterioration. In times of food scarcity, the opposite strategy would apply. In the western world, where food is abundant, we may therefore be harvesting the consequences of an evolutionary strategy that neglected the long-term health effects of caloric excess.

In this review I explore how recent findings in the study of ageing might have implications for understanding and treating metabolic syndrome. In particular, I focus on the link between SIR2-related proteins (sirtuins) and calorie restriction (CR), and present a hypothesis that metabolic syndrome and CR might lie at opposite ends of the same spectrum. Therefore, findings on how CR works may provide new possibilities for treating metabolic syndrome.

Calorie restriction and metabolic syndrome

Calorie restriction was first described as a reduction in food intake in laboratory rodents of between 20% and 40% of *ad libitum* levels that would extend their lifespan by up to 50%[4]. It now seems that CR works universally to promote survival in organisms ranging from yeast to rodents and, perhaps, primates. As described above, CR may have evolved as an adaptive trait to postpone reproduction during food scarcity to a later time of food availability[5]. If CR thus evolved as a programme, it may be regulated by a relatively small number of genes. Recent findings have linked CR to the *SIR2* gene family, which were first shown to have anti-ageing functions in yeast[6], *Caenorhabditis elegans*[7] and *Drosophila*[8]. The discovery that yeast Sir2 and the mammalian orthologue SIRT1 are NAD^+-dependent deacetylases[9,10] spurred the hypothesis that sirtuins might regulate the pace of ageing in accord with metabolism, and might therefore provide the longevity that results from CR.

Do CR and metabolic syndrome lie at opposite ends of the same spectrum and so involve an overlapping set of regulators? Several considerations suggest that this may be the case. First is the obvious fact that metabolic syndrome is triggered by dietary excess and CR by dietary restriction. Second, many of the physiological parameters that are characteristic of metabolic syndrome (described above) are oppositely affected by CR, which yields improved glucose tolerance (and lower blood glucose and insulin levels), decreased LDL cholesterol and triacylglycerols, and increased HDL cholesterol. Third, whereas metabolic syndrome predisposes to diseases, CR protects against many diseases in rodent models, including cardiovascular disease, cancer, diabetes and neurodegenerative disease[11–13].

Thus, it may be useful to think of metabolic syndrome and CR as lying at opposite ends of a balance, which can be tipped in either direction by diet and physical activity (Fig. 1). Most importantly, this hypothesis

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posits that the regulatory factors that mediate the positive effects of a low-calorie diet may also have direct relevance to at least the glucose intolerance and obesity of metabolic syndrome. Below, I focus on a group of such factors — the sirtuins — and also discuss the transcriptional coactivators PPAR-γ (peroxisome-proliferator-activated receptor-γ) coactivator-1α (PGC-1α) and PGC-1β that are involved in regulating metabolic genes in the liver, muscles and brown fat, and AMP-activated protein kinase (AMPK), which is normally activated in many cell types by a deficit in energy.

Calorie restriction in various organisms

Studies on ageing in yeast mother cells show that Sir2 has at least two activities that might promote longevity for mothers and also confer fitness on daughter cells. First, it represses genome instability in the rDNA repeats and thus slows the formation of toxic rDNA circles[14]. Second, it promotes the asymmetric segregation of oxidatively damaged proteins to mother cells, and thereby resets the full lifespan to the damage-free daughters[15].

A regimen for CR in yeast was described in which mother cells were grown on 0.5% glucose as their carbon and energy source, instead of the usual 2% glucose[16]. Under these conditions of moderate CR, knocking out *SIR2* alone prevented lifespan extension in some yeast strains[17], whereas knocking out *SIR2* and two *SIR2* paralogues was required to block the extension in another strain[18]. Importantly, these effects were observed in strains that also bore deletions in *FOB1*, which prevented the accumulation of rDNA circles and their accompanying short lifespan in Sir2 mutants[17].

Two mechanisms have been shown to upregulate Sir2 activity during the moderate 0.5% glucose CR regimen (Fig. 2). In the first, CR was shown to trigger a metabolic shift from fermentation to respiration, and this increase in respiration was required for life extension[17]. Higher respiration rates resulted in an increase in the NAD^+/NADH ratio and the corresponding activation of Sir2 (ref. 19). In the second, CR was shown to upregulate *PCN1* — which re-synthesizes NAD^+ from nicotinamide and ADP-ribose — and thereby lower the levels of nicotinamide, a potent Sir2 inhibitor[20]. A more severe 0.05% glucose CR regimen also extended the lifespan of mother cells, but in a manner not requiring Sir2 and perhaps invoking the TOR nutrient-sensing pathway[21,22].

In *Drosophila*, CR — achieved by means of a modest reduction in the yeast extract in food — was shown to reduce the expression of the general histone deacetylase, RPD3, which, in turn, resulted in an increase in SIR2 mRNA expression[23]. Moreover, knocking out *Sir2* prevented the longevity induced by CR, and both *Sir2* overexpression and CR gave lifespan extensions that were not additive[8,24]. Finally, in *C. elegans*, the extension in lifespan in *eat* mutants, which are defective in pharangyl pumping of food, seemed to be at least partly dependent on *sir-2.1* (ref. 25). These findings all suggest a key role for sirtuins in mediating effects of moderate CR in lower organisms.

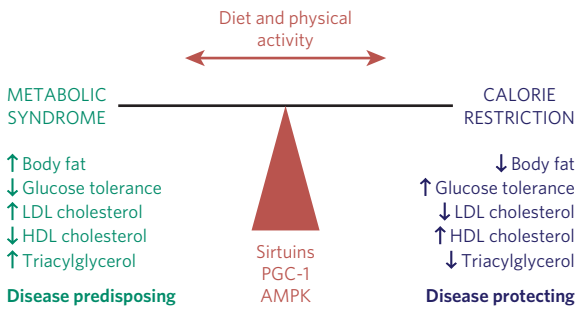

Figure 1 | Metabolic syndrome and calorie restriction are balanced at opposite ends of the same spectrum by diet and physical activity. The regulators shown might be involved in the underlying mechanisms that influence the balance. The reciprocity of phenotypes of metabolic syndrome and calorie restriction and their effects on disease are also indicated.

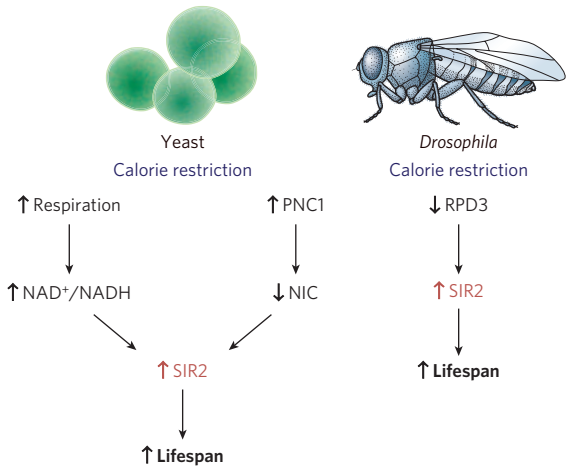

Figure 2 | Pathways of SIR2 activation by moderate calorie restriction in yeast and *Drosophila*. In yeast, two pathways activate SIR2 during CR, one involving an increase in respiration and the NAD^+/NADH ratio, the other an increase in the NAD^+-scavenging pathway enzyme, PNC1, which reduces nicotinamide (NIC) levels. In *Drosophila*, CR represses expression of the class I deacetylase RPD3, thereby activating *Drosophila* SIR2.

Are mammalian sirtuins required for CR-induced effects? In at least one example, the answer seems to be yes. CR mice showed a large increase in physical activity that seems to require SIRT1, because the increase did not occur in *Sirt1*-knockout mice[26]. Also, many of the functions described below for SIRT1, 3, 4 and 7 are consistent with a role for mammalian sirtuins in CR-induced changes in metabolism and increases in stress tolerance.

Functions of SIRT1 in mammalian physiology

The initial characterization of SIRT1 showed that it deacetylates important transcription factors, including p53, forkhead subgroup O (FOXO) proteins and the DNA repair factor KU, thereby increasing the stress resistance of cells by inhibiting apoptosis and increasing repair[27–32]. Moreover, SIRT1 has been linked to both lipid and glucose homeostasis. In white adipose tissue, SIRT1 was shown to inhibit adipogenesis in precursor cells and to reduce fat storage in differentiated cells[33]. One mechanism involved seemed to be inhibition of the nuclear receptor, PPAR-γ, by SIRT1 docking with its negative cofactors NCOR and SMRT at target gene promoters. However, because this mechanism does not explain the lipolysis triggered by CR in adipocytes, other activities may also be important.

SIRT1 can also regulate glucose homeostasis in three different tissues by affecting different targets (Fig. 3). In pancreatic β-cells, SIRT1 is a positive regulator of insulin secretion[34,35]. Insulinoma cells with SIRT1 reduced by RNA inhibition showed impaired insulin secretion, and transgenic mice overexpressing SIRT1 specifically in β-cells had improved glucose tolerance. Lowering SIRT1 in the insulinoma cells activated transcription of the uncoupling protein 2 gene (*Ucp-2*), whereas the SIRT1 transgenic mice showed super-repressed levels of UCP-2. Because UCP-2 encodes a mitochondrial membrane protein that might uncouple ATP synthesis from respiration, its repression by SIRT1 may increase the efficiency of ATP synthesis in β-cells in response to glucose, and thus positively regulate insulin secretion.

SIRT1 was also shown to protect β-cells against oxidative stress in a mechanism proposed to involve deacetylation of FOXO proteins[36]. So this sirtuin might also restrain β-cell loss during ageing and thereby mitigate a catastrophic reduction in insulin production in patients with early-stage diabetes to slow the progression to full-blown disease.

In the liver, SIRT1 seems to regulate gluconeogenesis. In liver cells, this sirtuin bound to and deacetylated the PPAR-γ coactivator PGC-1α[37] (discussed in detail below), thereby activating it. Indeed, SIRT1 levels in the liver were shown to increase markedly after overnight fasting,

resulting in an increase in glucose production. Because PGC-1α and FOXO proteins both regulate genes involved in gluconeogenesis, there are clearly several mechanisms by which SIRT1 could affect glucose production in the liver in times of severe energy limitation. In neurons, SIRT1 seemed not to activate but to repress the activity of PGC-1α[38], revealing the complexity of PGC-1α regulation by this sirtuin.

Finally, SIRT1 might also affect glucose homeostasis by regulating the response of target cells (such as muscle cells) to insulin. This hormone activates a pathway of intracellular kinases that regulate forkhead transcription factors[39,40], which, as mentioned above, are directly regulated by SIRT1. Moreover, PGC-1α activates genes involved not only in gluconeogenesis, but also in mitochondrial biogenesis, fatty acid oxidation and respiration (see below). By regulating the activity of PGC-1α in the muscles and liver, SIRT1 may also influence the abilities of these tissues to respire and metabolize carbohydrates and fats. The regulation of PGC-1α by SIRT1 could thus influence both glucose and lipid homeostasis.

SIRT1 activity during calorie restriction

Does SIRT1 activity increase in all tissues during food limitation? The first indication that the answer to this question may well be no was the finding that fasting in wild-type but not *Sirt1*-knockout mice increased pancreatic UCP-2, implying that a reduction in SIRT1 activity occurred during fasting[34]. Consistent with this was the finding that the NAD^+/NADH ratio decreased in starved panceas, whereas the NAD^+/NADH ratio in the liver increased after fasting[37]. Thus, during periods of acute food shortage, it seems possible that the activity of SIRT1 changes in different directions in different tissues. However, one caveat is that the NAD^+/NADH ratio has not clearly been shown to be the primary determinant of SIRT1 activity in mammals, as opposed to, for example, changes in protein levels.

SIRT1 can increase the stress resistance of cells, so during long-term CR its activity might be expected to rise in all tissues. Indeed, SIRT1 protein levels have been shown to increase during CR in the brain, white adipose tissue, muscles, liver and kidneys[33,41]. However, a decrease in the NAD^+/NADH ratio in the livers of mice during CR has also been reported[42]. A functional assay, described below, was consistent with this latter finding, showing that the activity of another sirtuin, SIRT4, also decreases in the liver during CR. Given these disparate observations, it will be important to study the liver in more detail — for example, comparing transcription profiles of wild-type and *Sirt1*-knockout mice — to determine whether SIRT1 activity rises or falls during CR. If it turns out that SIRT1 activity does change in different directions in different tissues during CR, as seems to be the case in fasting, then pharmacological interventions that activate or repress sirtuins in the whole animal may mimic CR in only a segmental fashion.

Most importantly, in line with the model in Fig. 1, it will be important to determine whether the changes in SIRT1 activity during CR are inverse to changes observed in genetically or dietary-induced obese animals. If so, SIRT1 and perhaps other sirtuins could be potential pharmacological targets not only for diseases of ageing but also for metabolic syndrome.

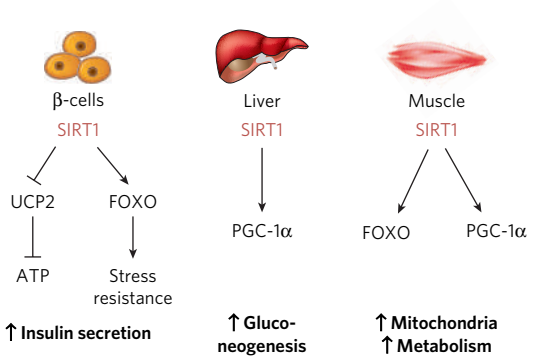

Figure 3 | Influence of SIRT1 on glucose homeostasis in three mammalian tissue types. In β-cells, SIRT1 represses the uncoupling protein gene, *Ucp2*, and thereby increases ATP synthesis and insulin secretion in response to glucose. SIRT1 also protects β-cells against stress-induced apoptosis by increasing activity of the forkhead protein FOXO1. In the liver, SIRT1 deacetylases the coactivator PGC-1α, thereby increasing expression of genes for gluconeogenesis. In the muscles, the effect of SIRT1 on FOXO1 and PGC-1α proteins should result in an increase in mitochondrial biogenesis and metabolism.

Mitochondrial SIRT3 and SIRT4 in metabolism

Mitochondria have figured prominently in at least some models of ageing[43], such as the oxidative damage theory, which proposes that reactive oxygen species generated as a by-product of respiration cause cumulative damage in mitochondria. In addition to providing the 'factory' for respiration and ATP production, mitochondria also house many metabolic pathways. The fact that both SIRT3 and 4 are imported into the mitochondrial matrix[44–46] suggests that these sirtuins might have a role in stress management and metabolism. Although the role of mitochondria in ageing remains putative, the fact that SIRT3, 4 and 5 have all been reported to be mitochondrial proteins provides further support for the potential importance of this organelle in ageing.

SIRT3 was recently shown to deacetylate the mitochondrial enzyme acetyl-coenzyme-A synthetase 2 (AceCS2)[47,48], which converts acetate to acetyl-CoA, thereby allowing the entry of carbon from dietary acetate into central metabolism (Fig. 4). This is a strikingly conserved function, because the sole bacterial sirtuin, CobB, was shown to deacetylate bacterial AceCS[49]. Because the acetylated lysine in AceCS is in the active site, deacetylation activates the enzyme. Notably, CobB is required for bacteria to use acetate as a carbon source. In mammals, whereas SIRT3 deacetylated and activated AceCS2, SIRT1 was reported to deacetylate and activate the cytoplasmic isoform, AceCS1 (ref. 47). Although many studies have shown that SIRT1 is nuclear, its presence in the cytoplasm has been reported in some cell types under certain conditions[35]. These findings all suggest that SIRT3 (and perhaps SIRT1) might regulate the entry of acetate into the tricarboxylic acid cycle and central metabolism. This step might be especially important during times of food limitation in order to both harvest dietary acetate and make use of the acetate that is known to be generated by the liver during ketogenesis[50]. It will be important to demonstrate directly the physiological relevance of these biochemical findings — for example, by studying the effects of different diets in *Sirt3*$^{-/-}$ mice.

SIRT4 also regulates the flow of carbon into central metabolism, in this case from the amino acids glutamate and glutamine (Fig. 4). Biochemical studies of SIRT4 showed that it does not have NAD^+-dependent deacetylase activity, but instead uses NAD^+ to transfer ADP-ribose to protein substrates[46]. The physiologically relevant substrate for this ADP-ribosyltransferase activity turned out to be the mitochondrial enzyme glutamate dehydrogenase (GDH). By ADP-ribosylating GDH, SIRT4 inhibits its activity and blocks the conversion of glutamate (and glutamine, which is converted to glutamate in cells) to the tricarboxylic acid cycle intermediate, α-ketoglutarate.

Importantly, pancreatic β-cells were found to be highly enriched in SIRT4, and knocking out *Sirt4* in both insulinoma cells and mice triggered insulin hypersecretion[46]. This increase seems to be due to the potential use of these amino acids as fuel sources in β-cells lacking SIRT4. Indeed, unlike the wild type, the *Sirt4*-knockout mice secreted insulin in response to glutamine as well as glucose. Thus, SIRT4 functions to repress amino-acid-stimulated insulin secretion (AASIS) in β-cells.

The physiological role of SIRT4 becomes clear when it is considered that amino acids can serve as carbon and energy sources in times of energy limitation. The β-cells of wild-type mice on a CR diet have been shown to secrete insulin in response to glutamine[46]. This qualitative change in insulin responsiveness seemed to be due to downregulation of SIRT4, because GDH was less ADP-ribosylated in mitochondria from CR mice than in those of controls. Similarly, in the liver, GDH was less ADP-ribosylated in CR mice, which would allow for the use of amino

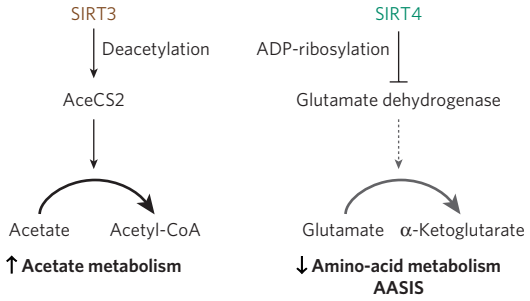

Figure 4 | Functions of SIRT3 and SIRT4 in regulating the entry of acetate or amino acids into central metabolism. SIRT3 deacetylates and activates the mitochondrial enzyme AceCS2, which converts acetate to acetyl-CoA, thereby facilitating use of acetate in metabolism. SIRT4 ADP-ribosylates the mitochondrial enzyme glutamate GDH, which converts glutamate to α-ketoglutarate, thereby repressing the entry of glutamate and glutamine into metabolism and blocking their ability to trigger AASIS.

acids for gluconeogenesis. Thus, whether glutamine and glutamate can be used as fuel sourcces in central metabolism and in AASIS is regulated by SIRT4 according to diet.

It is fascinating to note that SIRT3 and 4 seem to function oppositely with respect to carbon use — SIRT3 promotes the use of acetate, whereas SIRT4 represses the use of glutamate and glutamine. Because both SIRT3 and 4 are likely to be regulated in the same direction in the same cellular compartment by changes in the NAD^+/NADH ratio, their roles seem to be conflicting.

How can we make sense of this? I speculate that the ability to use one or other fuel source during CR is parsed between different tissues (Fig. 5). For example, the metabolism of amino acids to make glucose clearly occurs in the liver. Because some of the amino acids used for gluconeogenesis come from protein breakdown in the muscles, it would make sense to downregulate SIRT4 specifically in the liver to increase GDH activity and amino-acid metabolism (Fig. 5). As amino-acid metabolism generates glucose under these conditions, we can begin to understand teleologically the qualitative shift to AASIS in β-cells, which is also mediated by downregulation of SIRT4.

In a reciprocal fashion, it may be desirable to potentiate the use of dietary acetate as a carbon and energy source in the muscles but not the liver, where it is produced during ketogenesis. Consistent with this idea, AceCS2 is abundant in skeletal muscle and the heart, but almost absent from the liver, and is highly upregulated in muscles during food limitation[51]. Whether SIRT3, which is expressed at very low levels in the muscles, is highly induced in muscle tissue during ketogenic conditions remains to be tested. If so, SIRT3 and 4 might have reciprocal systemic roles in the liver and muscles during food limitation to facilitate the use by each tissue of a fuel source sent by the other for metabolism and generation of energy.

In summary, SIRT3 and 4 clearly have important roles in diet-induced metabolic changes. Because mitochondria are so important in stress and, perhaps, ageing as generators of energy, recipients of damage and regulators of apoptosis[43], it will be interesting to see whether the mitochondrial sirtuins also function in stress management.

Nuclear SIRT6 and SIRT7 and metabolism

Along with SIRT1, SIRT6 and 7 are the other nuclear sirtuins. Interesting functions for SIRT6 emerged from the analysis of the *Sirt6*-knockout mouse, which exhibits genomic instability and a progeroid phenotype[52]. A defect in base excision repair was found that might explain the cell loss leading to the rapid ageing phenotype. However, the mice also showed severe defects in glucose homeostasis and low levels of insulin-like growth factor (IGF-1), which were evident even before the onset of the degenerative phenotypes. In fact, the attrition in lymphocytes that was observed in these mice resulted from a systemic effect, perhaps the defect in glucose homeostasis or IGF-1. Thus, like SIRT1, SIRT6 might have an important role in glucose homeostasis, and further studies should provide important information about whether this sirtuin helps coordinate metabolic changes with diet.

SIRT7 is the only sirtuin shown to be localized in nucleoli[53,54], where it is associated with RNA polymerase I (ref. 54). Indeed, SIRT7 seems to be a positive regulator of rRNA transcription, because its inhibition reduced transcription and its overexpression enhanced it. However, regulation of ribosome biogenesis by sirtuins may be more complex, as SIRT1 has been reported to deacetylate the RNA polymerase factor TAF168 and thereby regulate rRNA transcription in the opposite direction[55]. SIRT7 is highly expressed in many tissues with dividing cells[54]. It will be of interest to determine whether SIRT7 activity decreases in these tissues during CR to restrain ribosome biogenesis and cell growth when energy is limiting. By contrast, SIRT7 may not have an important role in organs consisting of postmitotic cells such as the muscles, heart and brain, because expression of this sirtuin was not observed in these tissues.

Possible links between sirtuins and metabolic syndrome

Because of the properties of SIRT1, 3 and 4 outlined above, it might be useful to consider possible effects on metabolic syndrome of activating or inhibiting these sirtuins in different tissues (Table 1). The cases of SIRT3, 4 and 1 seem to provide examples of increasing complexity. Both SIRT3 and 4 regulate the flow of carbon from acetate and amino acids into metabolism, through which they could contribute to the synthesis of carbohydrate or fat. It may be useful, therefore, to inhibit SIRT3 to block any incorporation of acetate into metabolism for synthesis. The same logic applies to SIRT4, except that in this case it is activation that would reduce entry of glutamate and glutamine into central metabolism — for example, as fuel for gluconeogenesis in the liver. However, this sirtuin may be more complex than SIRT3 — it is the inhibition of SIRT4 in β-cells that might provide at least temporary benefit for glucose intolerance, because it would increase AASIS.

In the case of SIRT1, it seems likely that activation in white adipose tissue would provide benefit by stimulating fat loss. Likewise, activation in β-cells might help early-stage diabetes by increasing insulin production (Table 1). We can also speculate on a beneficial role for SIRT1 activation in muscle to provide stress resistance and prevent muscle loss. However, we will not know whether activation or inhibition of SIRT1 in the liver is useful until we know whether the effects on this tissue observed during fasting apply to long-term CR. It should be possible to test whether regulating SIRT1, 3 and 4 in the indicated directions and tissues brings about the desired effects by generating tissue-specific knockout and transgenic mice for these genes. If such genetically altered mice demonstrate an improved physiological response when challenged with diets high in

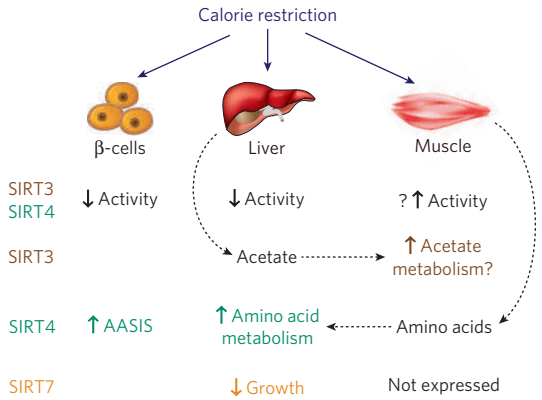

Figure 5 | Model of the effects of SIRT3, SIRT4 and SIRT7 in different tissues during calorie restriction. The indicated direction of change in sirtuin activity is the best surmised on the basis of published data. Question marks indicate that SIRT3 has not yet been tested for induction by CR in muscle. Note the reciprocal effects of SIRT3 and SIRT4 on metabolism of acetate and amino acids in muscle and liver.

fat and carbohydrate, it can be hypothesized that manipulating these sirtuins might benefit humans with metabolic syndrome. However, the pathway to developing drugs to selectively activate or repress a specific sirtuin in a particular tissue will be considerably more challenging than the genetic proof of principle studies.

PGC-1α and PGC-1β

PGC-1α was identified as a coactivator that bound to the nuclear receptor, PPAR-γ, and stimulated fat metabolism and thermogenesis in brown fat cells[56]. This protein has other important roles in the muscles and liver during energy limitation that might be relevant to metabolic syndrome and make PGC-1 proteins attractive targets[57]. In muscles, exercise can induce the β-adrenergic system to activate cyclic-AMP (cAMP)-dependent protein kinase and its transcription factor target, cAMP-responsive element-binding protein (CREB) to upregulate PGC-1α expression. This increase can then drive differentiation of slow twitch fibres, which, unlike fast twitch fibres, make exclusive use of oxidative metabolism for energy production. In these fibres, PGC-1α stimulates transcription of nuclear genes encoding mitochondrial proteins by binding to transcription factors such as nuclear respiratory factors 1 and 2 (NRF-1/2) and oestrogen-related receptor (ERR) proteins. PGC-1α also activates fatty acid oxidation by binding to PPAR-α and δ. The net effect of PGC-1α activity in muscle is therefore an increase in fatty acid oxidation and metabolic activity. Furthermore, PGC-1α mRNA has been shown to be decreased in the muscles of patients with type 2 diabetes[58,59], although it is not yet clear whether this change contributes to disease pathology. Thus, it is a reasonable deduction that activation of PCG-1α in muscle could provide benefit for metabolic syndrome (Table 1).

In the liver, PGC-1α was shown to activate both fatty acid oxidation and gluconeogenesis by binding to transcription factors FOXO1 and hepatocyte nuclear factor-4α (HNF4α)[60]. Thus, logic might suggest that inhibiting its activity in this tissue might help slow the progression from glucose intolerance to diabetes in people with metabolic syndrome (Table 1). One possible complication, however, is that PGC-1α inhibition could lead to steatosis, or fatty liver, due to compromised fat oxidation. This would reduce hepatic insulin sensitivity, thereby countering some of the beneficial effects on glucose output and perhaps leading to other hepatic problems.

In this same tissue, PGC-1β was shown to activate cholesterol and fat synthesis and export to the bloodstream by binding to the lipogenic transcription factors sterol regulatory element binding protein (SREBP) and liver X receptor (LXR)[61]. Therefore, inhibiting PGC-1β in the liver might be of benefit in ameliorating the hyperlipidaemia in patients with metabolic syndrome. However, another report shows that PGC-1β is a coactivator for the forkhead protein FOXA2 (ref. 62). Forkhead proteins are normally repressed by insulin signalling, because it leads to their phosphorylation by AKT (also known as protein kinase B) and retention in the cytoplasm. Indeed, fasting was shown to promote the nuclear localization of hepatic FOXA2, where it increased fatty acid oxidation, glycolysis and ketogenesis, and reduced gluconeogenesis and hepatic fat[63]. These properties suggest that it is the activation of PGC-1β that would cause a hepatic response favourable for metabolic syndrome. Further study will be required to resolve which set of these apparently opposing activities of PGC-1β is most relevant to metabolic syndrome.

So, both SIRT1 and PGC-1 proteins probably have important roles in muscles and the liver (Table 1). The function of sirtuins may be broader and encompass white adipose tissue, β-cells and probably other tissues as well. Both SIRT1 and PGC-1α are upregulated by energy limitation[41], and thereby exert coordinated effects in the liver and muscles during a state of food limitation — for example, upregulation of fatty acid oxidation to provide carbon for gluconeogenesis. However, in the face of energy excess, it might be most efficacious to activate oxidative metabolism in order to reduce fat, but to avoid activating gluconeogenesis, which would exacerbate a pre-diabetic condition. Achieving this aim by pharmacologically modulating PGC-1 proteins or the transcription factors through which they function stands as an important challenge in devising new treatments for insulin insensitivity and obesity.

Table 1 | Functions of various regulators in β-cells, the liver and muscles

Regulator		β-cell	Liver	Muscle
SIRT1	Activity	Increases insulin secretion	Metabolism	Increases stress resistance
	Therapy	Activate	None known*	Activate
SIRT3	Activity			Increases acetate metabolism
	Therapy			Inhibit
SIRT4	Activity	Decreases AASIS	Decreases amino-acid metabolism	
	Therapy	Inhibit	Activate	
PGC-1α	Activity		Increases gluconeogenesis	Increases metabolism
	Therapy		Inhibit†	Activate
PGC-1β	Activity		Increases fat/cholesterol	
	Therapy		Inhibit†	
AMPK	Activity		Decreases fat/cholesterol	Increases fat oxidation
	Therapy		Activate	Activate

*The role of SIRT1 in the liver in CR is not fully understood.
†May involve complications.
Therapy for metabolic syndrome is predicted as activation or inhibition of the indicated sirtuin in the indicated tissue.

AMP-activated protein kinase

Another intriguing regulator of energy homeostasis is AMP-activated protein kinase (AMPK), which senses the AMP/ATP ratio in cells[64,65]. During energy or food limitation, AMP binds to AMPK and renders it a substrate for the activating kinase LBK1 (refs 66–68). In neurons, another Ca^{2+}/calmodulin-sensitive kinase also phosphorylates AMPK on the same residue without the requirement for bound AMP[69].

AMPK is already a prime target for treatment of metabolic syndrome, because one leading drug currently in use, metformin, is thought to work by activating this kinase, although the mechanism is not certain[70]. Although it is beyond the scope of this review to cover all of the known effects of AMPK, several of its targets seem especially pertinent. First, AMPK phosphorylates and inhibits acetyl-CoA carboxylase, which converts acetyl-CoA to malonyl-CoA[71]. The product of this reaction is the building block for fatty acid synthesis in the liver. Malonyl-CoA also blocks fatty acid oxidation in muscles by inhibiting its transport into mitochondria[64,65]. So, activating AMPK leads to inhibition of fatty acid synthesis in the liver and promotion of fatty acid oxidation in muscles (Table 1). Second, AMPK phosphorylates and inhibits 3-hydroxy-3-methylglutaryl-CoA reductase[64,65], which catalyses the committed step in cholesterol synthesis in the liver, so activation of AMPK also leads to a decrease in cholesterol production. Third, AMPK activates the PGC-1α promoter, and its activation will thereby increase metabolism in muscles, as discussed above.

Finally, recent studies have identified another pathway that is relevant to both AMPK and PGC-1α activity in the liver. TORC2 (CREB-regulated transcription coactivator 2) is induced by fasting to enter the nucleus and coactivate CREB, along with the canonical CREB coactivator CBP[72]. TORC2 seems to be especially important in triggering the activation of gluconeogenesis, probably by helping CREB to upregulate expression of PGC-1α, as described above. In addition, nuclear TORC2 triggers a feedback mechanism in which it upregulates expression of the insulin pathway protein, insulin receptor substrate 2 (IRS2), to improve insulin signalling and temper gluconeogenesis[73]. Most importantly, TORC2 can be phosphorylated by AMPK or the related kinase SIK to return it to its inactive, cytoplasmic state[72]. Indeed, knocking out the serine/threonine kinase LKB1 (and thus AMPK activity) in the liver activated TORC2, thereby driving PGC-1α expression and gluconeogen-

esis[74]. Thus, the activation of AMPK may also reduce gluconeogenesis in the liver by inhibiting TORC2.

Although at present there are no known direct connections between AMPK and sirtuins, it would not be surprising to see their emergence, given their common use in adapting an animal's metabolism to the energy needs imposed by its diet. One possible and intriguing intersection would be the regulation of one or more of the AMPK kinases by a sirtuin.

Summary and conclusion

Metabolic syndrome is a major health challenge of the twenty-first century, threatening to reverse historic trends towards ever increasing life- and healthspans in the developed world. We are on the cusp of a molecular understanding of ageing itself, and how it is regulated by diet. This research has dovetailed with studies of obesity, diabetes and metabolic disease to introduce us to some of the critical regulators of metabolic functions in mammals. In this review I have focused on the sirtuins, because they are candidates for regulators that bridge the control of metabolism and ageing. Because of this they, along with other metabolic regulators such as PGC-1 proteins, AMPK, FOXA2 and TORC2, are likely to be important to our understanding of how a low-calorie diet — that is, calorie restriction — promotes longevity and disease resistance. But equally importantly, they might provide insight into metabolic syndrome, because these same regulators may go awry in this pathological state. For this reason, it is possible that the development of drugs that target these metabolic regulators will not only be useful in combating ageing and its associated diseases but will also be effective in treating the insulin insensitivity, obesity and perhaps other symptoms associated with metabolic syndrome. Thus, we can imagine new treatments for diabetes, cardiovascular disease and other ageing-associated diseases that begin well before the onset of any noticeable symptoms. Like low-dose aspirin and the statins, such a class of drugs may vastly improve quality of life and productivity in an ageing cohort of people. Although some have questioned the ethics of anti-ageing research, its potential to mitigate metabolic syndrome and diseases of ageing demands that it proceed as rapidly as possible. ■

Note added in proof: Two recent studies have shown that the plant polyphenol resveratrol activates SIRT1 and mitigates effects of high-calorie and high-fat diets in mice[75,76].

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