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Dietary restriction and Sirtuin 1 in metabolic health: connections and divergences

Carles Cantó[1,2]

[1]*Nestlé Institute of Health Sciences, Lausanne, CH-1015, Switzerland*

[2]*École Polytechnique Fédérale de Lausanne, Lausanne, CH-1015, Switzerland*

Aging is the major risk factor for a constellation of multifactorial diseases, including insulin resistance, diabetes and cardiovascular complications. Dietary restriction has been shown to delay or prevent the manifestation of age-related health decline, extending lifespan in most species tested to date. Given the scarce willingness of human subjects to adhere to chronic dietary restriction exercises, there has been an interest in deciphering the molecular mechanisms triggering the adaptations to dietary restriction. In this context, Sirtuin 1 (SIRT1), a NAD^+-dependent deacetylase enzyme, has been proposed to act as a key mediator of the adaptations to nutrient deprivation in eukaryotes, and SIRT1 activating compounds have been often referred to as 'dietary restriction mimetic' molecules. Here, we will discuss the convergences and divergences between the effects of dietary restriction and SIRT1 activation, based on the recent advances in the field. As of now, most evidences indicate that SIRT1 is required, but not sufficient to trigger dietary-restriction induced adaptations.

Dietary restriction: Sirtuin 1: Mouse models: Insulin resistance: Aging

Due to medical and pharmaceutical advances, developed societies are confronted with the fact that human subjects are living longer, leading to an increased prevalence of diverse age-related diseases, such as type 2 diabetes or cardiovascular complications. Therefore, there is an intense interest in developing strategies to allow people not only to live longer, but also to age healthily, a concept often referred to as healthspan.

To date, dietary restriction (DR) is the most well-known non-pharmacological intervention improving age-related health complications. In 1935, McCay *et al.* pioneered DR-related research when they tested how retarded growth, via dietary limitation, affected the ultimate size of the animal's body and their life span[(1)]. At the end of their studies, the authors concluded: 'in retarded group individuals of both sexes attained extreme ages beyond those of either sex that grew normally'. The effect was especially dramatic in male rats. Hence, it was demonstrated for the first time that DR without malnutrition prolongs mean and maximal lifespan in rats compared with *ad libitum* feeding.

DR is now usually defined as a moderate (normally, 20–40 %) reduction in dietary intake as compared with an *ad libitum* diet, without compromising the maintenance of all essential nutrients[(2,3)]. Since the discovery of McCay *et al.* the effects of DR on health and lifespan extension have been shown to stretch all along the evolutionary scale, ranging from yeast to human subjects[(3)]. Up to a 50 % increase in maximum lifespan has been reported in dietary restricted yeast, rotifers, spiders, worms, flies, fish, mice and rats[(2)]. Two major studies have been performed in Rhesus monkeys[(4,5)]. While an impact of DR on maximal lifespan could only be observed in the first of them[(4)], both studies have shown a delayed onset of age-associated pathologies in dietary-restricted monkeys[(4,5)]. In both rodent and primate animal models, DR promotes increased insulin sensitivity and prevents against age-related cardiovascular

Abbreviations: BAT, brown adipose tissue, DR, dietary restriction; KO, knockout, SIRT1, Sirtuin 1; STAC, SIRT1 activating compounds.

Corresponding author: C. Cantó, email carlos.cantoalvarez@rd.nestle.com

complications and cancer[6]. Importantly, DR is also protected against age-related mitochondrial dysfunction, a hallmark for multiple metabolic complications[6,7].

Despite these potential benefits, there are a few aspects that need to be taken into account when considering the implementation of DR regimens. When performed from early stages in life, and in almost any specie tested to date, DR dramatically reduces fertility and promotes growth retardation[3]. In primates, DR has also been reported to lead to aggressiveness and stereotyped behaviours[8]. Further complicating the picture, the effects of DR might have a strong genetic component. Using one of the largest groups of recombinant inbred strains of mice presently available, the ILSXISS[9], Liao *et al.* tested the hypothesis that the lifespan response to DR would fluctuate depending on the genetic variations across these genotypes[10]. Contrary to the extensive contemporary literature, at least half of the strains showed lifespan shortening by DR, rather than lifespan lengthening[10,11]. Other points that are presently under debate include the degree of severity of the DR regime, the optimal age of onset of such regime and how the basal metabolic state of individuals might affect the effectiveness of the intervention[3].

Most studies to date suggest that DR can positively impact on human longevity, especially by reducing the risk of developing age-associated complications[12]. In several studies conducted in overweight human subjects, DR has been shown to improve a number of health outcomes, including a reduction in several cardiac risk factors[13–15], an improvement in insulin-sensitivity[16], and enhanced mitochondrial function[17]. Additionally, prolonged DR has also been found to reduce oxidative damage[18–20]. This way, findings of initial human clinical trials appear to support the promise of DR raised by animal studies, at least in overweight adults. This said, one must take into account that human patients generally display a poor adherence to DR regimens. With this in mind, the scientific community has long pursued efforts to identify or generate DR-mimetic compounds/interventions. To do so, however, a precise understanding of the underlying mechanisms of the action of DR is needed. In this review, we will discuss how the activation of Sirtuin 1 (SIRT1), a NAD^+-dependent deacetylase enzyme, has been hypothesised to be key for DR-induced health benefits and the present standpoint on whether diverse SIRT1 activating strategies can truly be considered DR-mimetic.

Dietary restriction and silent information regulator 2/Sirtuin 1: connecting the dots

To understand the molecular mechanisms by which DR prevents age-related diseases, it could be important to first understand what triggers age-related physiological decline. Aging leads to a constellation of molecular alterations, including accumulative oxidative damage, inflammation, mitochondrial dysfunction, lack of protein turnover or increased covalent modification of proteins[2]. Given the multifactorial nature of aging, it was surprising to find that single-gene mutations markedly contribute to extend lifespan in diverse models including yeast, worms and flies[3]. Many of these longevity-extending mutations decrease the activity of molecular pathways activated by nutrients, such as the insulin signalling pathway, suggesting that they promote a physiological state similar to that experienced during DR[3].

It was particularly exciting to find that a protein called silent information regulator 2 (*SIR2*) could modulate yeast replicative lifespan[21]. *SIR2* was initially identified as a gene that silenced the extra copies of the mating-type information in yeast[22]. Simultaneous work by independent groups established that the *SIR2* gene product was a protein, Sir2, containing a NAD^+-dependent enzymatic histone deacetylase activity[23–26]. The deacetylation reaction catalysed by Sir2 is coordinated with the cleavage of NAD^+ into nicotinamide and 1-O-acetyl-ADP-ribose[26]. Given that multiple metabolic paths use NAD^+, or its reduced form NADH, as a cofactor, it was proposed that Sir2 could act as a metabolic sensor, capable of modulating gene expression according to the metabolic state of the cell[27].

Increasing the dosage of *SIR2* increased replicative lifespan in yeast by 30 %, while its deletion shortened lifespan by 50 %. Several studies indicated that Sir2 could be a critical mediator of the effects of DR on yeast lifespan[28,29]. For example, *SIR2* overexpression was enough to increase replicative lifespan in a similar, non-additive, manner to DR[29]. In an opposite fashion, DR was unable to increase lifespan in yeast where the gene coding for *SIR2* was deleted[29].

Following these initial discoveries, similar observations were made in nematodes and flies. The *Caenorhabditis elegans* genome contains four sirtuin genes, among which *Sir-2·1* is the most homologous to yeast *SIR2*. *Sir-2·1* overexpression was enough extended nematode lifespan[30–32]. The fly *Drosophila melanogaster* has five sirtuin homologues, and *dSir2* also extends lifespan when overexpressed[33,34]. However, these findings were recently challenged and attributed to a poor control of the genetic background[35]. Indeed, when the transgenic worms and flies were backcrossed to identical backgrounds, the lifespan extension was lost[35], although a small but significant effect has still been reported under similar conditions for *Sir-2·1* overexpression in *C. elegans*[36,37].

Irrespective of the ultimate effects on maximal lifespan, *Sir-2·1* and *dSir2* have been identified as critical regulators of the response to nutritional stress induced by DR. Indeed, when *dSir2* was deleted, DR failed to extend lifespan in flies[33,38]. Similarly, worms lacking *Sir-2·1* did not live longer upon DR, and displayed shorter lifespan when exposed to hydrogen peroxide, heat stress, or UV radiation[39]. These results suggest that Sir2 homologues are key regulators of the metabolic and transcriptional adaptations to DR.

SIRT1 is the closest mammalian homologue of the yeast Sir2. While initially described as a transcriptional silencing enzyme via histone deacetylation, the actions of SIRT1 have unfolded far beyond histone modifications[40]. In the past decade, a large number of non-histone deacetylation targets have been identified,

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including key orchestrators of mitochondrial and lipid oxidation gene expression as the peroxisome-proliferator activated receptor γ coactivator 1α[41,42] and FOXO3a[43,44]. For an extended list of identified SIRT1 targets, the reader is referred to other recent reviews[40,45]. The deacetylation of these targets by SIRT1 prompts their transcriptional activity on promoters encoding for genes aimed to extract energy through mitochondrial respiration[40]. Given the dual localisation of SIRT1, either in the cytoplasm and/or the nucleus, it is not surprising that SIRT1 targets have also been identified in both compartments[40]. This way, by sensing changes in NAD^+ levels, SIRT1 is activated upon nutrient stress and triggers metabolic adaptations favouring energy production through non-carbohydrate energy sources and via oxidative processes. Recently, SIRT1 has been shown to trigger the mitochondrial unfolded protein response, which optimises mito-nuclear communication in order to ensure mitochondrial fitness through the efficient repair of damaged mitochondria[37]. Hence, SIRT1 activation could also contribute to alleviate mitochondrial function decay upon aging.

Dietary restriction and Sirtuin 1 in mammals (I): gain-of-function models

In addition to the prevention of age-related diseases, DR is known to promote a vast range of physiological and behavioural changes in mice. These effects include a reduction in body weight by a decrease in fat mass, enhanced insulin sensitivity and increased efficiency for energy production[3]. SIRT1 expression increases in many mammalian cells and tissues upon glucose deprivation or DR[40,46,47]. This, together with the data on lower eukaryotes, raised the hypothesis that forced SIRT1 activation could be used as a DR-mimetic strategy. In this section, we will discuss several approaches used to increase SIRT1 activity and whether they truly resemble the effects of DR.

The first SIRT1 gain-of-function model reported displayed several DR-like features: they were leaner and had improved glucose tolerance[48]. One particularity of this model is that the overexpression of the SIRT1 protein occurred predominantly in the brain and in white- and brown-adipose tissues (BAT), but not in liver or muscle[48]. Not much later, two mouse models for whole-body moderate SIRT1 overexpression were generated[49,50]. In one of them, generated by the Serrano laboratory[49], the moderate overexpression of SIRT1 led to increased BAT function and thermogenic function, even on a low fat diet, which caused a modest increase in insulin sensitivity[51]. The involvement of SIRT1 on thermogenic functions was further strengthened by the second mouse model of moderate SIRT1 overexpression, generated by the Accili laboratory, where SIRT1 was shown to improve white adipose tissues 'browning' upon pharmacological or physiological adrenergic stimulation[52]. In both models, however, no major effects on hepatic or muscle metabolism were observed under low-fat diets, despite a 2–4-fold increase in SIRT1 levels in both tissues[49–51]. Both mouse models, however, showed that a mild overexpression of SIRT1 prevented against high-fat diet induced hyperglycemia, insulin resistance and fatty liver, despite no significant differences in body weight[49,50]. This was largely attributed to a protective effect of SIRT1 against high-fat diet-induced liver insulin resistance and inflammation[49,50]. In line with this, liver-specific overexpression of SIRT1 is enough to effectively protect against insulin resistance in both dietary and genetic mouse models of obesity[53].

While initially surprising, the absence of effects of SIRT1 overexpression in muscle mitochondrial content and insulin sensitivity have been elegantly confirmed in mice with a selective overexpression of SIRT1 in muscle tissue[54]. However, it has recently been suggested that a 100-fold overexpression of SIRT1 in skeletal muscle can lead to decreased muscle mass, cross-sectional area and a shift towards a more oxidative, slow-twitch, muscle fibre types[55]. Another recent report shows how a high, non-physiological, whole body SIRT1 overexpression prompts enhanced mitochondrial content in muscle, together with an improvement of whole-body glucose tolerance[56]. These observations suggest that endogenous SIRT1 is enough to account for the basal maintenance of mitochondrial content under physiological conditions in skeletal muscle, and that certain transcriptional programs might be forcedly enhanced under situations of massive SIRT1 overexpression.

Mice overexpressing SIRT1 in the β-cell display enhanced glucose-stimulated insulin secretion, even though whole-body moderate SIRT1 overexpression did not have any major effect on mice insulinemia on chow diet[45,49–51]. These discrepancies could be attributed to differential SIRT1 overepression levels. However, whether SIRT1 overexpression at massive levels really mimics physiological SIRT1 activation upon DR is, unclear. Indeed, a caveat when comparing all these models is that higher SIRT1 levels do not necessarily have to correlate with SIRT1 activity, for example in situations where NAD^+ might become rate-limiting, as can be the case during aging[37,57]. In this sense, it is interesting to note that the Serrano laboratory evaluated the impact of moderate SIRT1 overexpression on the aging process. While SIRT1 overexpression protected against diverse age-related pathologies including insulin resistance, osteoporosis, impaired wound healing and cancer, no effects on mouse lifespan were observed[58].

So, is SIRT1 overexpression comparable with DR? At a glance, one can clearly identify some overlaps (e.g. amelioration of glycemic profiles), but also some major discrepancies between both models. In most cases[49–51], but not all[48], SIRT1 overexpression does not lead to reduced body weight. This is expected, as mice are not in a limited energy intake scenario. In some models, SIRT1 ovexpression enhanced insulin sensitivity under normal diet conditions[48,51], which would go in line with the expected DR-like effect. This increase in insulin sensitivity has been attributed to higher insulin-stimulated glucose uptake in BAT[51]. The positive influence of SIRT1 on BAT function and thermogenesis, however, is unlikely to be a feature of DR. Upon prolonged low food accessibility, organisms will tend to optimise ATP

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synthesis by decreasing mitochondrial proton leak (i.e. uncoupled respiration), which is the key feature of BAT through the expression of the Uncoupling Protein 1. This is a phenomenon observed even at the cellular level upon glucose deprivation[59]. Some studies indeed indicate that DR in mice leads to reductions in BAT function[60]. Reduced BAT function generally manifested as lower energy expenditure, which is characteristic in rodents and human subjects upon DR regimes[19,61]. This is opposite to the higher energy expenditure observed in some SIRT1 transgenic mouse lines[48,51]. The impact of DR on behavioural traits is also not mimicked by SIRT1 overexpression, as DR promotes an increase in activity and foraging behaviour[62], while daily activity was lower in some SIRT1 transgenic lines[50,51].

SIRT1 biology has also been studied through the use of SIRT1 activating compounds (STAC), among which resveratrol and SRT1720 might be the most well-known. The ability of these compounds to specifically and directly activate SIRT1, however, is still a matter of debate[63–65]. Irrespectively of whether the action is direct or not, both compounds lead to SIRT1 activation and promote very overlapping effects in mice, including a large increase in mitochondrial biogenesis in skeletal muscle and BAT, enhanced energy expenditure and protection against high-fat diet-induced obesity[66,67]. In addition, STAC granted improved insulin sensitivity and a longer lifespan when mice were submitted to a high-fat diet[68,69]. In the case of SRT1720, treated animals displayed enhanced lifespan even on chow diet conditions[70].

Other compounds used to activate SIRT1 are those aimed to elevate NAD^+ bioavailability. This includes compounds that inhibit alternative NAD^+ consuming activities, such as PARP-1[71,72] or CD38[73], or that enhance NAD^+ synthesis, such as nicotinamide mononucleotide[74,75] or nicotinamide riboside[72,76,77]. In agreement with the ability of these strategies to increase NAD^+ availability, all these compounds led to higher SIRT1 activity and ameliorations in glucose homeostasis. In the case of nicotinamide riboside or PARP inhibition, higher energy expenditure, decreased body weight gain upon high-fat feeding and enhanced mitochondrial biogenesis have also been reported[71,72,76,77].

It is interesting that NAD^+ boosting strategies and STAC both converge at preventing high-fat diet-induced body weight gain, a phenomenon never observed in models of moderate SIRT1 overexpression[49–51]. Also, STAC promote dramatic increases in mitochondrial biogenesis, especially in skeletal muscle[66,67], which, again, were not observed upon moderate SIRT1 overexpression[51]. Similarly, increases in skeletal muscle mitochondrial content are not generally seen upon DR, even though this is still a matter of debate[78]. Therefore, all the earlier results indicate that, despite a significant overlap in the effects, there are remarkable differences between the different SIRT1 activating strategies. In some cases, such as the regulation of energy expenditure, the effect of SIRT1 activation might be even opposite to those of DR.

The earlier observations argue that forced SIRT1 activation and DR might lead to similar health benefits, yet not necessarily through similar means. This, however, does not rule out that SIRT1 participates in the adaptations to DR. This aspect can be tested through, at least, two strategies. For example, one could test the interaction between SIRT1 overexpression and the response to DR. In this sense, the overexpression of SIRT1 in skeletal muscle does not seem to alter the effects of DR on body weight, body composition or insulin sensitivity[79]. A possible explanation might be that endogenous SIRT1 levels could be enough to warrant full adaptability to DR in this tissue. A second, more conclusive, strategy is to evaluate the effects of DR in SIRT1 loss-of-function models, as discussed in the next section.

Dietary restriction and Sirtuin 1 in mammals (II): loss-of-function models

The whole-body deletion of SIRT1 leads to high prenatal lethality in inbred mice[80,81]. The very few pups that were born displayed marked cardiac and neurological problems, leading to death very early in the postnatal period[80,81]. In order to bypass this situation, SIRT1 deletion was performed in outbred mice[80]. These mice were viable and displayed a marked hypermetabolism due to decreased energy production efficiency, which, in turn, impeded the proper adaptation of metabolic rates to DR[82]. DR is generally associated with behavioural changes at the level of food foraging activity. These changes, however, did not happen when DR was performed on SIRT1 deficient mice[62]. The role of SIRT1 in the influence of DR on mouse lifespan has been examined recently. For this purpose, SIRT1 knockout (KO), SIRT1 heterozygous and control wild-type mice, aged between 2 and 5 months old, were subjected to either 40 % DR or *ad libitum* diets. The results indicated that DR failed to increase lifespan in SIRT1 KO mice[83]. Despite a trend for decreased maximum lifespan under DR conditions, DR extended the lifespan of both SIRT1 heterozygous or wild-type mice in a comparable manner[83]. These results evoke two concepts: (1) SIRT1 is required in outbred mouse stocks for the lifespan extension caused by DR; and, (2) as suggested by the overexpression models, SIRT1 endogenous levels are largely enough to mediate DR-induced adaptations, since even a 50 % reduction in SIRT1 levels did not have a major impact on the lifespan extension promoted by DR.

One caveat of the earlier studies is that SIRT1 deficient mice display multiple defects on the basal state, including dwarfism, sterility, craniofacial abnormalities and several inflammatory conditions[80,83]. Therefore, all these abnormalities could lead them to premature death irrespectively of feeding conditions. Given such detrimental effects on early development and global health, ensuring non-limiting SIRT1 levels in key central and peripheral tissues might have been naturally selected during evolutionary processes. Interestingly, an inducible model has been developed in order to genetically ablate SIRT1 exclusively in adulthood[56]. SIRT1 deletion in adult mice did not result in any overt phenotype or metabolic

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alteration[56], making it a perfect model to precisely evaluate the impact of SIRT1 on DR-induced adaptations, even though efforts in this direction have not yet been reported.

While the requirement for SIRT1 in DR-induced adaptations in mice seems clear, there are still a number of open questions. For example: do all tissues require SIRT1 expression to allow DR-induced metabolic adaptations? Few efforts have been done in this direction, yet enough to conclude that this is the case in skeletal muscle. When submitted to a 60 % reduction in energy intake, mice display a marked increase in insulin sensitivity and insulin-induced glucose uptake in peripheral tissues[84]. However, SIRT1 deletion specifically in skeletal muscle is enough to largely block the effects of DR on insulin-induced glucose disposal in skeletal muscle[84]. In liver or adipocytes, where SIRT1 levels were similar between muscle-specific SIRT1 KO and control mice, DR-induced benefits on insulin signalling were comparable[84]. This testifies that DR only failed to promote metabolic adaptations in skeletal muscle, where SIRT1 had been specifically deleted. Mechanistically, the authors proposed that DR improves the efficiency of insulin to trigger phosphoinositide 3-kinase signalling and that this is due to SIRT1-mediated deacetylation and inactivation of Stat3, a negative transcriptional regulator of the phosphoinositide 3-kinase regulatory subunits p55α and p50α[84]. Hence, DR would enhance insulin signalling potency in skeletal muscle by increasing the expression of phosphoinositide 3-kinase regulatory subunits in a SIRT1-dependent manner.

The effects of DR have also been explored in liver-specific SIRT1 KO mice. In this case, liver-specific SIRT1 KO mice had similar body weight loss and fat reduction as control mice upon DR, and no major alterations were observed after the examination of different metabolic parameters, including fasting glycemia, insulinemia and glucose tolerance tests[46]. Hence, SIRT1 ablation in the liver does not seem to alter the physiological adaptation to DR. In part, this might be explained by the fact that, contrary to most tissues reported, SIRT1 expression is paradoxically decreased in livers upon DR[46]. As described earlier, SIRT1 has a key role in adipose tissue biology, where it enhances the action of 'browning' stimuli[51,52] and prevents high-fat diet induced metabolic complications, at least at early stages[85,86]. Hence, it will be interesting to evaluate how SIRT1 deficiency in adipose tissues impacts on DR-induced metabolic adaptations.

The adaptation of mice to DR also relies on changes at the level of the central nervous system. In order to evaluate the role of SIRT1 expression in the brain in the adaptations to DR, mice lacking SIRT1 specifically in neurons, astrocytes and glial cells were generated[87]. Wild-type and brain-specific SIRT1 KO mice lost weight similarly after a 7-month 40 % reduction in energetic intake. DR dramatically increased the insulin sensitivity of wild-type animals[87]. However, DR induced only a modest improvement in insulin sensitivity in brain-specific SIRT1 KO mice[87], suggesting that SIRT1 expression in the brain is required to achieve the full benefits on insulin sensitivity conferred by DR. When placed on monitored running wheels, wild-type animals dramatically up-regulated their activity in response to DR. In contrast, while brain-specific SIRT1 KO mice were slightly more active than control mice when fed *ad libitum*, their activity markedly decreased upon DR[87]. Therefore, SIRT1 activity in the brain is required to modulate several metabolic and behavioural adaptations to DR. A caveat of this model is that brain-specific SIRT1 KO mice, as whole-body SIRT1 deficient mice, display numerous abnormalities at the basal state, including dwarfism and a reduced somatotropic signalling (i.e. lower circulating growth hormone and insulin-like growth factor-1 levels)[87], which call for caution when interpreting the effects of DR in this model.

Conclusions and perspectives

Overall, most studies in genetically engineered mouse models indicate that SIRT1 is necessary to trigger many of the metabolic and behavioural adaptations to DR, even though the role of SIRT1 mediating these effects might differ from tissue to tissue. This fits nicely with the hypothesis that SIRT1 acts as an evolutionary conserved sensor of nutrient stress, promoting adaptations aimed to improve energy production efficiency. In line with this, SIRT1 activity directly impinges on many molecular pathways linked to longevity and that critically regulate metabolic adaptation to energy stress, including the FOXO family of transcription factors[3,88] and AMP-activated protein kinase signalling[89].

Most results to date, however, also indicate that SIRT1 activation does not act *per se* a DR-mimetic. Several features of DR are not mimicked, or even opposed, by diverse SIRT1 gain-of-function models. Nonetheless, one should take into account the limitations of these models. For example, moderate SIRT1 overexpression might be aligned with the increases in SIRT1 observed in white adipose tissues or BAT upon DR, but, in fact, tissues such as brain or liver do not show increases in SIRT1 content on dietary restricted mice[46,87]. STAC might push SIRT1 activity beyond physiological limits and might activate additional paths, such as the AMP-activated protein kinase, which could explain the strong effects on skeletal muscle mitochondrial biogenesis[89]. Therefore, two aspects will be worthy of our attention in the years to come: (1) the key elements influencing endogenous SIRT1 activity upon DR in diverse tissues and (2) a global landscape of the cooperation between SIRT1 and other pathways upon DR. While referring to SIRT1 activating strategies as 'DR-mimetic' might be misleading, the data in mouse models highlight that they bear an undeniable therapeutic potential for the amelioration of metabolic and age-related diseases. Novel approaches, such as enhancing NAD^+ synthesis via nicotinamide mononucleotide or nicotinamide riboside supplementation are providing spectacular effects in the management of glucose homeostasis[74,76], and their true potential in human subjects still needs to be unveiled.

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Authorship

C. C. is the sole author of the paper.

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