OPINION

Caloric restriction mimetics: towards a molecular definition

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Abstract | Caloric restriction, be it constant or intermittent, is reputed to have health-promoting and lifespan-extending effects. Caloric restriction mimetics (CRMs) are compounds that mimic the biochemical and functional effects of caloric restriction. In this Opinion article, we propose a unifying definition of CRMs as compounds that stimulate autophagy by favouring the deacetylation of cellular proteins. This deacetylation process can be achieved by three classes of compounds that deplete acetyl coenzyme A (AcCoA; the sole donor of acetyl groups), that inhibit acetyl transferases (a group of enzymes that acetylate lysine residues in an array of proteins) or that stimulate the activity of deacetylases and hence reverse the action of acetyl transferases. A unifying definition of CRMs will be important for the continued development of this class of therapeutic agents.

Long-term caloric restriction (that is, reduced calorie intake without malnutrition) or intermittent short-term starvation (fasting) has been shown to prolong the mean and the maximum lifespan of yeast, plants, worms, flies and rodents[1–3]. Caloric restriction may also have beneficial effects on longevity in primates and has been shown to promote an increased healthspan — the period of life during which an individual is generally healthy — by reducing the incidence of metabolic diseases, cancer, arteriosclerosis and neurodegeneration[4,5]. It is important to note that, at least in rodents, the healthspan-increasing effects of caloric restriction caused by a continuously low calorie intake also reduce body weight. In principle, this effect can be avoided (or at least limited) by using intermittent fasting regimens, as they have similar healthspan-extending effects but do not affect body weight[6,7]. Intermittent fasting causes only short-term weight loss in non-obese individuals[8] and may help obese patients to reduce body weight[9]. Alternate-day fasting may enable non-obese individuals to obtain the health benefits of caloric restriction without unwanted weight reduction. Taken together, intermittent fasting regimens may offer several advantages, particularly because the molecular consequences of fasting, rather than weight reduction per se, may account for at least some of the observed positive effects of caloric restriction.

Although most obese individuals know that stable weight loss would reduce their risk of developing metabolic syndrome — the deterioration of multiple metabolic parameters that increases the chance of developing cardiovascular disease and other comorbidities — only 20% of overweight individuals are capable of losing 10% of their weight for a period of at least 1 year[10]. Similarly, most normal-weighted, apparently healthy individuals with pre-diabetes, dyslipidaemia or other signs of imminent severe disease are refractory to harsh diets, vigorous exercise, alternate-day fasting or similar strategies to improve their health[10,11]. Natural or synthetic pharmacological agents could theoretically be used to induce the beneficial effect of caloric restriction without provoking its discomfort. Although several prominent caloric restriction mimetics (CRMs) have been suggested for clinical use[3,12], there is no consensus on their biochemical and functional definition.

Acute nutrient depletion in cultured human cells and starvation of rodents are both accompanied by a rapid decrease in the levels of cytosolic acetyl coenzyme A (AcCoA), which leads to the deacetylation of cellular proteins[13]. The specific depletion of AcCoA by genetic or pharmacological means is sufficient to induce a similar level of protein deacetylation and to stimulate autophagy (BOX 1), which is potentially one of the most important mechanisms through which starved cells can adapt to low levels of nutrients[14,15]. Replenishment of AcCoA can avoid this starvation-induced deacetylation and can suppress autophagy, both *in vitro* and *in vivo*, in mammalian cells[13] and in yeast[16].

On the basis of these and other premises that are discussed below, we suggest that there are three ways to mimic the biochemical changes that are physiologically induced by the limitation of nutrients (FIG. 1). First, the nucleocytosolic pool of AcCoA can be reduced by preventing the synthesis of AcCoA in mitochondria and in the cytosol or, alternatively, by preventing its export from mitochondria into the cytosol. Second, the activity of acetyl transferases (also known as transacetylases), which use AcCoA as the donor of acetyl groups, can be suppressed. Third, deacetylases, particularly sirtuins, which catalyse the removal of acetyl groups from a range of cellular proteins, can be activated. The net result of these three manipulations is a reduction in the acetylation level of cellular proteins, both in the cytosol (with the consequent activation of nutrient sensors, resulting in the activation of AMP-activated protein kinase (AMPK) and in the inhibition of mammalian target of rapamycin complex 1 (mTORC1)) and in the nucleus (with the consequent transcriptional and epigenetic reprogramming), which induces numerous changes in cellular biology that ultimately stimulate autophagy[13,16]. Indeed, autophagy is one of the most important alterations elicited by caloric restriction and CRMs[13,17–20] (BOX 1), as it accounts for the well-known longevity-extending effects of caloric restriction[21,22], as well as CRMs, at least in model organisms[19–21]. Overexpression of autophagy-related protein 5 (ATG5; an essential component of the autophagic machinery) was recently reported to increase

Box 1 | **Autophagy in health and disease**

Autophagy begins with the almost simultaneous activation of two kinase complexes: the protein kinase complex organized around UNC51-like kinase 1 (ULK1; which is functionally coupled to the positive autophagy regulator AMP-activated kinase (AMPK) as well as to the negative autophagy regulator mammalian target of rapamycin complex 1 (mTORC1)) and the lipid kinase complex organized around beclin 1 (which is functionally coupled to stress kinases). The activation of these two major kinase complexes facilitates the nucleation of the isolation membrane behind which autophagic cargo is sequestered. Two ubiquitylation-like systems involving multiple essential autophagy gene products (such as autophagy-related protein 3 (ATG3), ATG4, ATG5, ATG7, ATG10 and ATG12) enable the lipidation of proteins from the ATG8 family (which includes microtubule-associated proteins 1A/1B light chain 3 (LC3)); this facilitates the elongation of the autophagic membrane and the stable incorporation of ATG8 proteins (such as LC3) into them. The redistribution of LC3 and its homologues (such as GABARAB (γ-aminobutyric acid receptor subunit-αB)) into autophagosomes is monitored in most cellular assays of autophagy. The closure of the isolation membrane leads to the generation of autophagosomes, which are vesicles that are characterized by two juxtaposed membranes. Autophagosomes then undergo SNARE (soluble NSF (*N*-ethylmaleimide-sensitive factor) attachment protein (SNAP) receptor)-dependent fusion with lysosomes to form autolysosomes, which causes the degradation of the inner membrane and the luminal content by lysosomal hydrolases. Autophagy may be induced by multiple stress-related signals that, in turn, are connected to conventional stress pathways, including those controlled by nutrient sensors (for example, AMPK, mTORC1 and sirtuins), transcription factors (for example, nuclear factor-κB (NF-κB), p53 and signal transducer and activator of transcription 3 (STAT3)) and organelle stress (for example, DNA damage, the unfolded protein response and mitochondrial fission).

In some rare cases, protracted autophagy may cause the death (also known as autosis) of affected cells[150]. However, in most cases autophagy constitutes a cellular strategy to cope with sublethal stress and damage, which means that it enables the cells to mobilize energy reserves (through the catabolism of macromolecules including proteins, lipids and glycogen) and to remove damaged structures (such as aggregates of misfolded proteins and uncoupled mitochondria), hence reducing the probability of apoptotic or necrotic death[127]. For these reasons, autophagy often participates in so-called hormetic responses, in which a first exposure to sublethal stress increases the threshold required for subsequent damage to be lethal. Such hormetic responses (which are involved in the process of hormesis) are exemplified by ischaemic conditioning of the myocardium and — perhaps — by the effects of caloric restriction on ageing. Autophagy is also important for the neutralization of invading intracellular particles (a process known as xenophagy) and the destruction of viral particles (a process known as virophagy). It has a cardinal role in immune reactions, as well as in reducing the unwarranted inflammatory reactions that may cause chronic tissue damage.

lifespan in both male and female C57BL/6 mice, which indicates that the induction of autophagy is sufficient to increase longevity[23].

The purpose of this Opinion article is to provide a unifying hypothesis on the mode of action of CRMs, in both biochemical and functional terms. We propose that CRMs are agents that *in fine* reduce protein acetylation, thereby initiating the autophagic cascade that is beneficial for cellular function (FIG. 2).

Depletion of cytosolic AcCoA

Overnight starvation of rodents or nutrient-free culture of human cells for 2–4 hours causes a reduction in the cellular pool of AcCoA (which is apparent in most tissues)[13,24], which is consistent with the fact that this metabolite has a central role in energy metabolism. Accordingly, AcCoA depletion precedes the depletion of ATP or NADH[13]. AcCoA is produced in mitochondria as a result of glycolysis (from pyruvate, which is transported into the mitochondrial matrix by the mitochondrial pyruvate carrier (MPC) and then converted into AcCoA by pyruvate dehydrogenase (PDH)) or as a result of lipolysis (from fatty acids, which are transported into the matrix by the carnitine *O*-palmitoyl transferase 1 (CPT1), and then subjected to β-oxidation). In addition, AcCoA can be generated by the mitochondrial AcCoA synthetase short-chain family member 1 (ACSS1), which joins acetate and CoA in an ATP-driven reaction. AcCoA formed in the mitochondrial matrix cannot be exported as such; rather, it must be condensed with oxaloacetate into citrate, exported by citrate transport protein (CTP, also known as tricarboxylate transport protein, mitochondrial) into the cytosol and then converted back to oxaloacetate and AcCoA by ATP citrate lyase (ACLY). A fraction of cytosolic AcCoA can also be generated by the cytosolic AcCoA synthetase ACSS2 (FIG. 3).

One of the major changes in cellular physiology that is induced by AcCoA depletion is a reduction in the acetylation levels of cellular proteins[13,25]. Global protein deacetylation that results from AcCoA depletion may be explained by the fact that AcCoA is the sole acetyl group donor for acetyl transferases. Acetyl transferases have a fairly low affinity for AcCoA, in the range of the physiological concentration of AcCoA[26]; by contrast, protein kinases mostly have a high affinity for the donor of phosphate groups, ATP, with a dissociation constant (K_d) that is much lower than the physiological ATP level. This could be used to explain why the global protein phosphorylation level is independent of the intracellular ATP concentration (which is usually in the range of 1–10 mM), whereas the global protein acetylation level is strongly influenced by levels of cytosolic AcCoA (which is usually in the range of 3–10 μM)[27,28]. In addition, AcCoA depletion may reduce the non-enzymatic acetylation of proteins[29]. As the cytosolic and nuclear pools of AcCoA freely communicate, AcCoA depletion may also affect the level of histone acetylation; this has been shown to be the case for mammalian cells depleted of ACLY[30] and yeast cells depleted of the ACSS2 orthologue Acs2p[31].

Both in mammals and in yeast, specific depletion of the nucleocytosolic pool of AcCoA (as opposed to the mitochondrial pool) causes massive induction of autophagy. In addition, the brain-specific knockdown of *Drosophila melanogaster* nucleocytosolic AcCoA synthetase promotes longevity and autophagic protein degradation[13,16]. This induction of autophagy is probably secondary to the reduction of protein acetylation that is caused by limiting the amount of AcCoA (see below).

There are several places to intervene in the AcCoA biosynthetic pathway in order to reduce AcCoA synthesis: inhibition of MPC, PDH, CPT1, CTP, ACSS2 or ACLY (TABLE 1). Knockdown of any of these proteins causes AcCoA depletion and a general reduction in the acetylation of cellular proteins, as well as the induction of autophagy in cultured human cells. Moreover, investigational drugs that inhibit MPC (such as UK5099), CTP (such as 1,2,3-benzenetricarboxylate) and ACLY (such as 3,5-dichloro-2-hydroxy-*N*-(4-methoxy[1,1′-biphenyl]-3-yl)-benzenesulphonamide) also induce the triad of AcCoA depletion, protein deacetylation and autophagy[13]. It should be noted that, although depletion of glucose can induce autophagy, possibly also through the depletion of AcCoA, the inhibition of glycolysis with 2-deoxyglucose fails to do so. Indeed,

inhibition of hexokinase 2 by 2-deoxyglucose results in the autophagy-suppressive activation of mTORC1 (REF. 32), which could explain the toxicity of this compound *in vivo*[33]. As a result, 2-deoxyglucose cannot be considered as a bona fide CRM. By contrast, metformin, which is thought to function as an inhibitor of complex I of the respiratory chain, does induce autophagy[34], although its effects on AcCoA levels and protein acetylation remain to be elucidated.

Inhibitors of the mitochondrial and cytosolic AcCoA synthesis cascade that are used clinically include the CPT1 inhibitor perhexiline, which is approved in Australia and New Zealand for the prophylactic treatment of angina pectoris, and the ACLY inhibitor hydroxycitrate, which is available as an over-the-counter weight loss agent in the United States. Perhexiline was less efficient in inducing autophagy in mice than was hydroxycitrate, which induced autophagy in all investigated organs including the liver, the myocardium and skeletal muscle[13]. This induction of autophagy was obtained just 6 hours after intraperitoneal injection or 48 hours after oral administration of hydroxycitrate. Hydroxycitrate also induced a reduction in body weight without any reduction in food intake in wild-type mice; however, *Atg4b*-knockout animals, which have a major defect in autophagy[35], did not show any weight reduction in response to administration of hydroxycitrate[13]. These results suggest a hitherto unsuspected link between autophagy and weight control. If given at a high dose (≥3 g per day), hydroxycitrate induces weight loss in obese patients, as observed in controlled clinical trials[36]. Importantly, meta-analyses of several clinical trials indicate that hydroxycitrate is inefficient if administered at lower doses[36], which is consistent with the observations in rodents that indicate a minimum effective dose of 0.5–1 mg per day of hydroxycitrate per kg of body weight[37].

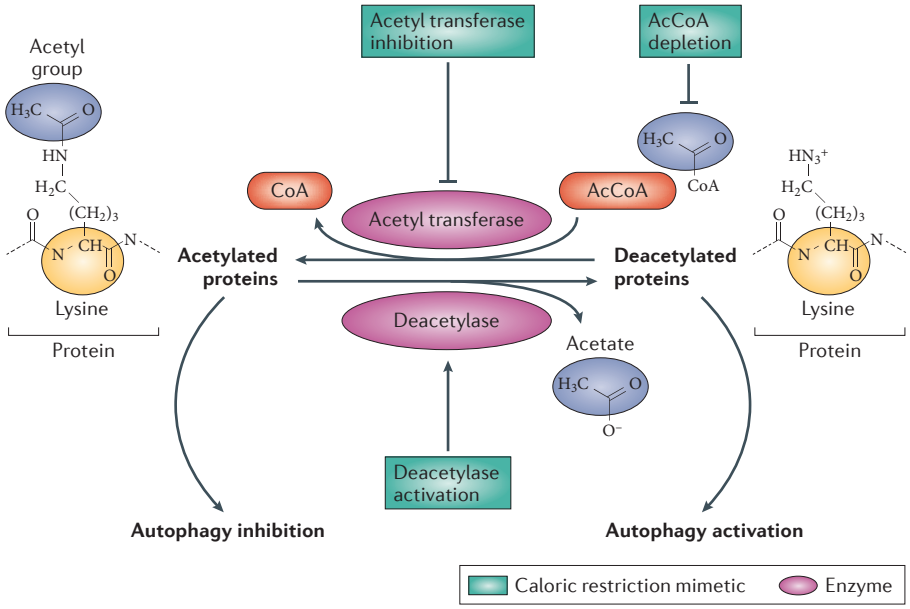

Figure 1 | **General properties of CRMs.** Caloric restriction mimetics can either provoke the depletion of acetyl coenzyme A (AcCoA), inhibit the activity of acetyl transferases or activate deacetylases. The net result of these interventions is the deacetylation of a set of cellular proteins, thereby facilitating the induction of autophagy.

Inhibitors of acetyl transferases

In the human genome, at least 70 gene products are designated as protein acetyl transferases (see the Gene Ontology Consortium website) as they catalyse the post-translational modification of protein acetylation at lysine residues. For historical reasons, the best-characterized proteins in this class are called 'histone acetyl transferases', although many of these enzymes can also acetylate non-histone substrates. Moreover, acetyl transferases can function in multiple distinct (and presumably all) subcellular compartments, not only in the nucleus where they have an important role in epigenetic control[38] but also in the cytosol, mitochondria[39] and endoplasmic reticulum[40]. Thus, the acetylation of metabolic enzymes is involved in bioenergetic control[41,42]. Several natural compounds that have been suggested to have health-promoting effects are able to inhibit acetyl transferases (TABLE 1), although there are no systematic studies to determine the exact inhibitory range.

Natural compounds with potent pro-autophagic effects can inhibit a broad range of acetyl transferases. This applies to curcumin (from the South Asian spice turmeric (*Curcuma longa*), which is one of the principal ingredients of curry powder) and epigallocatechin-3-gallate (EGCG; which is one of the major active compounds in green tea). Curcumin can prolong the lifespan of *Caenorhabditis elegans* and this effect is epistatic to that of thioflavin T, which requires the expression of essential autophagy-relevant genes (*atg-9* and *vps-34*) to be active[43]. In addition, feeding *D. melanogaster* during their healthspan with curcumin extends median and maximum lifespan: this effect is epistatic to dietary restriction and is accompanied by inhibition of the TOR pathway[44], suppression of which is known to cause an autophagy-dependent extension in lifespan[45]. Interestingly, if curcumin is continued to be administered to old animals (flies), it loses its beneficial effects and even shortens lifespan. This suggests that the duration and the time-specific application of CRMs (and potentially also of caloric restriction in general) may be as important as their optimal dosing[44].

According to one report, EGCG augments the lifespan of *C. elegans*[46]. Another report claims that EGCG does not extend the lifespan of *C. elegans* in normal conditions but does so in conditions of thermic or oxidative stress[47]. EGCG prolongs the lifespan of healthy Wistar rats[48]. Moreover, EGCG has neuroprotective effects in rodent models of Alzheimer's disease[49], ischaemic stroke[50] and amyotrophic lateral sclerosis (ALS)[51]. In mice fed a high-fat diet, EGCG inhibits hepatosteatosis and concomitantly increases autophagy-mediated lipolysis[52]. However, as a caveat, it should be noted that curcumin and EGCG have both been classified as antioxidants and may affect multiple pharmacological targets in addition to histone acetyl transferases[53,54], which means that their exact mode of action remains a matter of speculation.

Spermidine — a polyamine contained in all organisms — is another potent natural inhibitor of histone acetyl transferases and induces protein deacetylation and autophagy in many organisms, including mice[19,55]. The concentration of spermidine is highest in sperm and it promotes mating and fertilization efficiency in *C. elegans* and *D. melanogaster*[56]. Spermidine is contained in variable amounts in all food items (with

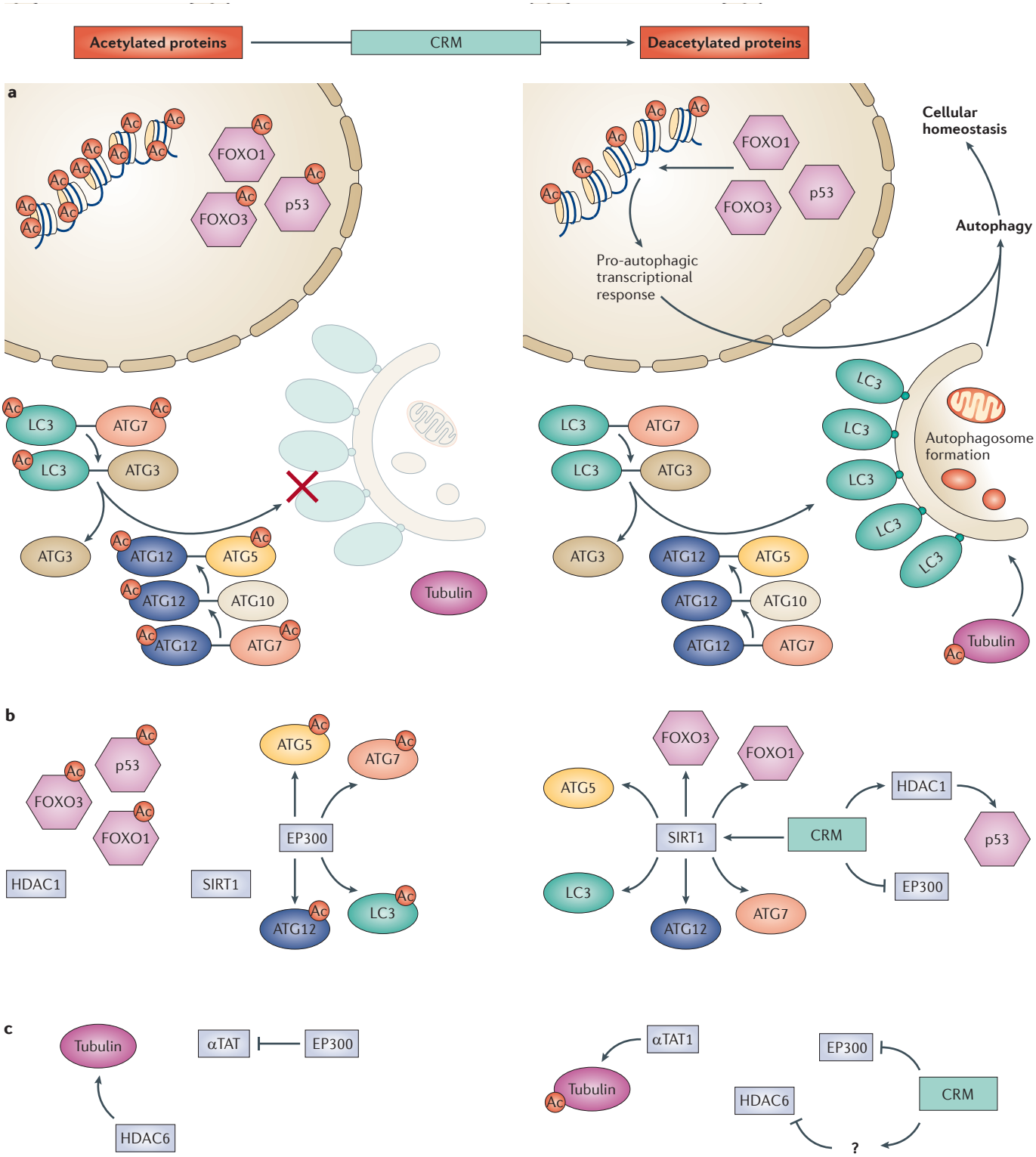

Figure 2 | **Links between protein acetylation and autophagy.** The deacetylation of autophagy-related gene products as well as of key transcription factors mediates the response to caloric restriction mimetics (CRMs), which function via the inhibition of acetyl transferases and/or the activation of deacetylase reactions. **a** | Deacetylation of members of the autophagy-related protein 8 (ATG8) family of proteins (which includes LC3) and of ATG12 ubiquitin-like conjugation systems culminates in the lipidation of LC3 and the initiation of autophagosome formation, which is required for efficient induction of autophagy; this leads to CRM-induced cellular homeostasis. At the same time, the deacetylation of the transcription factors forkhead box protein O1 (FOXO1), FOXO3 and p53 mediates a pro-autophagic transcriptional response. **b** | CRMs mainly function through the inhibition of the acetyl transferase E1A-binding protein p300 (EP300) or through the activation of the deacetylase sirtuin 1 (SIRT1) to induce a pro-autophagic response by the commensurate deacetylation of autophagy-relevant proteins and histones. **c** | The increase in acetylation of tubulin during autophagy induction is explained by the loss of inhibitory acetylation by EP300 on α-tubulin *N*-acetyl transferase 1 (αTAT1), which thus hyperacetylates tubulin in response to nutrient limitation or CRMs. Ac, acetyl group; HDAC, histone deacetylase.

particularly high concentrations in some health-related products such as durian fruit, fermented soybeans and wheatgerm) and is produced by the gut microflora. Increases in polyamine uptake cause an increase in circulating polyamine levels, both in mice and in humans[57]. Continuous supplementation of spermidine to yeast, *C. elegans* or *D. melanogaster* induces autophagy and extends the median and maximal lifespan of these organisms. This effect was shown to be mediated through autophagy, as the deletion or the depletion of essential autophagy-relevant genes abolished the longevity-extending effects of spermidine in yeast, nematodes and flies[19]. Supplementation of spermidine to adult flies avoids the age-dependent decrease in levels of spermidine in the brain and reverses the age-associated memory impairments[58]. This latter effect depends on the induction of autophagy, as the beneficial effect of spermidine on brain function is not seen in flies heterozygous for *Atg7* (REF. 58). Moreover, this effect can be mimicked by neuron-specific expression of a transgene encoding ornithine decarboxylase 1 (Odc1), which is the rate-limiting enzyme for the *de novo* synthesis of polyamines[58]. In addition, outbred female ICR mice fed with probiotic bacteria that have been selected to produce high amounts of polyamines from the precursor arginine (which can be optionally provided as a food supplement) reportedly show an age-dependent survival benefit that is associated with the suppression of chronic low-grade inflammation in the colon, reduced inflammatory cytokines in the circulation and protection against age-induced memory impairments[59,60].

Supplementation of chow with polyamines also reduces the incidence of chemically induced colon cancers *in vivo*, but it accelerates the growth of such cancers after they have developed[61]. Similarly, administration of *N*(1),*N*(4)-bis(2,3-butandienyl)-1,4-butanediamine (MDL 72527), which is an inhibitor of polyamine catabolism, can prevent the colon carcinogenesis that is induced by enterotoxigenic *Bacteroides fragilis* in mice[62]. Systemic administration of spermidine enhances the expression of autophagy markers in all mouse tissue investigated (including liver, heart, muscle and blood vessels)[55] and suppresses arterial ageing at the mechanical and biochemical levels by reversing stiffness, the accumulation of advanced glycation end products and the signs of stress that are mediated by reactive oxygen species[63]. The topical application of spermidine also reduces signs of skin inflammation, for example, in mouse ears painted with 12-*O*-tetradecanoylphorbol-13-acetate (TPA)[64]. Numerous patents and patent applications suggest that the internal or external use of spermidine, alone or in combination with other agents, may be beneficial for cosmetic purposes such as the avoidance or the reversal of skin ageing, hair loss and depigmentation.

However, the overall effects of such regimens — including polyamine supplementation to food[61,65] or drinking water[19] and microbiome-derived polyamine production[60] — on mean and maximum lifespan still require detailed investigation, as the reported studies lack complete lifespan analyses with sufficient numbers of animals. Similarly, pharmacological and genetic studies designed to evaluate the long-term effects of CRMs in mice are often affected by methodological problems such as the absence of detailed ageing protocols, the use of small cohorts that preclude robust statistics and the unusually short lifespans for the untreated control cohort[23,59,60]. Therefore, this type of study should be further standardized using the intervention testing programme of the US National Institute on Aging as an example[66–68].

Depletion of one particular histone acetyl transferase, E1A-binding protein p300 (EP300), by RNA interference potently induces autophagy in cultured human cells[69], and transgenic expression of a dominant-negative EP300 mutant induces autophagy *in vivo* in the mouse heart[70]. Suppression of autophagy by AcCoA-elevating agents (such as the membrane-permeant α-ketoglutarate precursor dimethyl-α-ketoglutarate) critically relies on EP300, therefore the knockdown of EP300 induces autophagy even in conditions in which AcCoA is elevated[13]. EP300 has been shown to directly acetylate and to inhibit several core autophagy proteins including ATG5, ATG7, ATG12 and microtubule-associated proteins 1A/1B light chain 3 (LC3)[69]. Moreover, EP300 can acetylate forkhead box protein O (FOXO) transcription factors to inhibit the transactivation of pro-autophagic genes. These results suggest that EP300 is one of the major regulators of autophagy. Of note, inhibition of EP300 can paradoxically lead to activation of the acetyl transferase α-tubulin *N*-acetyl transferase 1 (αTAT1; also known as MEC17), which hyperacetylates tubulin — an event that is important for autophagy during starvation[71]. It was recently reported that the intracellular localization of EP300 itself is controlled by BAT3 (also known as BAG6), which is a regulator of autophagy. For the induction of autophagy, BAT3 triggers the nuclear translocation of EP300, which causes p53 acetylation in the nucleus; depletion of EP300 from the cytosol deacetylates ATG7, which is required for autophagy. Thus, BAT3 guides EP300 and regulates acetylation on both cytosolic and nuclear targets to modulate autophagy[72].

Several natural compounds have been shown to inhibit the enzymatic activity of EP300; one example is anacardic acid (also known as 6-pentadecyl-salicylic acid; from the nutshell of the cashew (*Anacardium occidentale*)), which competes with AcCoA for binding to EP300 (REF. 73) and induces autophagy[74]. Nonetheless, anacardic acid has multiple other pharmacological targets including inhibitor of nuclear factor-κB kinase (IKK), lipoxygenase 1, xanthine oxidase, tyrosinase and ureases[75], as well as the TIP60 (also known as KAT) family of histone acetyl transferases (HATs), which are known to induce autophagy[76]. This indicates that it may be difficult to explain the mode of action of this putative health-improving agent by its effects on a single target. Garcinol, from the fruit of the Kokum tree (*Garcina indica*), is another nonspecific EP300 inhibitor[77] that also inactivates other enzymes, including 5-lipoxygenase[78] and the transcription factor signal transducer and activator of transcription 1 (STAT1)[79]. Gallic acid (also known as 3,4,5-trihydroxybenzoic acid), which is contained in multiple plants, has a similarly broad action[80], precluding it to be considered as a specific pharmacological agent. C464 is a fairly specific competitive EP300 inhibitor (inhibition constant (K_i) = 400 nM)[81]. According to one study, C464 can inhibit the function of regulatory T cells in mice, thereby stimulating anticancer immune responses[82]. In addition, parenteral administration of C464 induces massive autophagy in the heart, muscle and liver in mice[13]. Future work must determine whether C464 or other EP300-specific inhibitors may constitute leads for the development of a novel class of CRMs.

In addition, acetyl transferases located in mitochondria theoretically may constitute interesting pharmacological targets for selectively stimulating mitochondrial autophagy (also known as mitophagy). Indeed, knockdown of GCN5-like protein 1 (GCN5L1; also known as BLOC1S1), which is a component of the mitochondrial acetyl transferase machinery, causes local protein deacetylation (which apparently is mediated by one particular mitochondrial deacetylase, sirtuin 3 (SIRT3)), resulting in an increase in mitophagy[83]. It should be noted that chemical acetylation of proteins (particularly mitochondrial proteins) has also been suggested to occur[29]; however, this non-enzymatic reaction

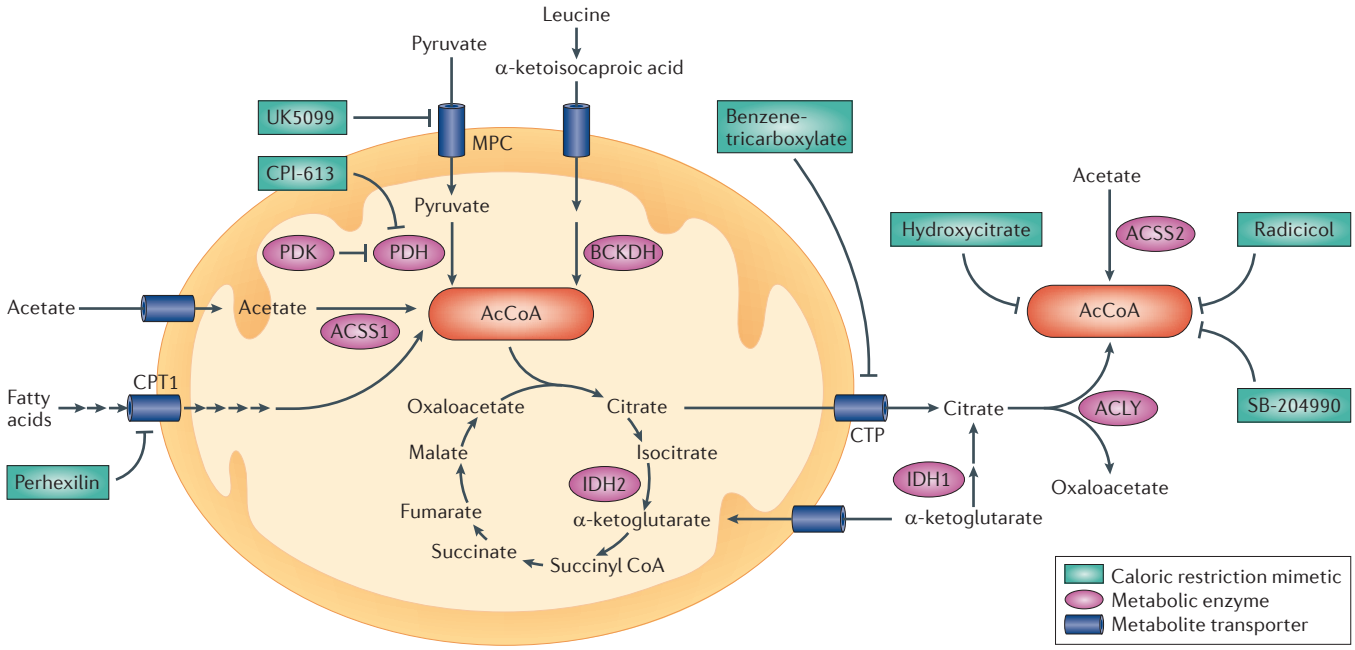

Figure 3 | **AcCoA metabolism and its modulation by pharmacological agents.** The depletion of cytosolic acetyl coenzyme A (AcCoA) is one possible mechanism of caloric restriction mimetics (shown in green). Cytosolic depletion of AcCoA can be achieved by blocking several pathways: the generation of AcCoA in mitochondria (by inhibiting mitochondrial pyruvate carrier (MPC), pyruvate dehydrogenase (PDH) or carnitine *O*-palmitoyl transferase 1 (CPT1)); the export of AcCoA from mitochondria via conversion to and translocation of citrate with its subsequent cytosolic reconversion (by inhibiting citrate transport protein (CTP) or ATP citrate lyase (ACLY)); or the *de novo* generation of AcCoA from acetate within the cytosol (by inhibiting acyl-CoA synthetase short-chain family member 2 (ACSS2)). BCKDH, branched-chain ketoacid dehydrogenase; IDH, isocitrate dehydrogenase; PDK, pyruvate dehydrogenase kinase.

still depends on the availability of AcCoA and may thus be targetable (FIG. 1). Stimulation of mitophagy, which is a mechanism of quality control, might be useful for the treatment of mitochondriopathies, in which the removal of dysfunctional mitochondria is a therapeutic goal[84]. In our opinion, this possibility warrants further in-depth investigation.

Taken together, the information that is currently available suggests that acetyl transferases may constitute targets for the therapeutic induction of general or organelle-specific autophagy. It will be important to generate specific acetyl transferase inhibitors and to avoid the inhibition of some exceptional pro-autophagic enzymes such as the tubulin Lys40 acetyl transferase αTAT1 (REFS 71,85) or the NuA4 acetyl transferase complex that acetylates ATG3 during the induction of autophagy that follows starvation[86].

Activators of protein deacetylases

Protein deacetylases are enzymes that remove acetyl groups from lysine residues in proteins and hence antagonize the effects of acetyl transferases. For historical reasons, most of them are called histone deacetylases (HDACs), although such enzymes tend to deacetylate non-histone proteins as well. Although HDACs are zinc-dependent enzymes, there is another class of deacetylases (which includes the sirtuins) that is zinc independent and uses NAD as an obligatory cofactor; several therapeutic uses of altered NAD levels have been discussed[87]. The transfection-enforced expression of a SIRT1 mutant lacking the nuclear import signal can trigger autophagy as efficiently as the non-mutated protein, which indicates that this deacetylase can stimulate autophagy via non-nuclear effects[55]. Indeed, SIRT1 may directly deacetylate several autophagy-relevant proteins (such as ATG5, ATG7, ATG12, FOXO3 and LC3)[88]. For the optimal induction of autophagy by nutrient depletion, such deacetylases must become activated. Thus, mouse embryonic fibroblasts lacking SIRT1 fail to mount a full autophagic response to starvation[88]. HCT116 colorectal carcinoma cells cultured in nutrient-free conditions activate autophagy to a lesser extent when SIRT1 is depleted by small interfering RNAs (siRNAs) than when SIRT1 is expressed at normal levels[20]. Embryonic stem cells isolated from *Sirt1*$^{-/-}$ mice have an impaired autophagic response to peroxide-induced stress that results in increased cell death and mitochondrial alterations[89]. Therefore, SIRT1-mediated deacetylation may also be essential for protective autophagy induction in mice.

A link between autophagy induction and SIRT1 deacetylatase activity has been found using genetic experiments. Inbred *Sirt1*$^{-/-}$ mice have a similar phenotype to autophagy-incompetent *Atg5*$^{-/-}$ mice[90] in that they have increased perinatal mortality shortly after birth, which reflects the inability of these mice to trigger an adaptive pro-autophagic response during postnatal starvation[88]. Tissues from these *Sirt1*$^{-/-}$ mice are characterized by damaged organelles and by the accumulation of the autophagic substrate sequestosome 1 (SQSTM1; also known as p62), which are both typical markers of a defective autophagic process[88]. However, *Sirt1*$^{-/-}$ mice with an outbred genetic background can reach 24 months of age but have metabolic alterations relative to normal animals[91]. *Sirt1*$^{-/-}$ animals are characterized by functional defects in liver mitochondria (possibly secondary to defective mitophagy), which culminates in insufficient ATP production and maladaptation to nutritional stress. Consequently, these mice are unable to adapt their metabolic rate in response to caloric restriction[91].

Notably, caloric restriction potently induces the expression of SIRT1 and SIRT3 in multiple tissues *in vivo*, which suggests that it has a direct function in the

health-promoting effects induced by caloric restriction[92]. Indeed, *Sirt1*$^{-/-}$ mice do not live longer on a calorically restricted diet[93] and SIRT3 is essential for the protective effect of caloric restriction against age-associated hearing loss[94]. Overexpression of SIRT6 extends the lifespan of male, but not female, mice and reduces insulin-like growth factor 1 (IGF1) serum levels and signalling activity[95], mimicking the effects of caloric restriction on humans, in whom serum IGF1 levels also decrease[96].

Resveratrol is considered to be the first example of a SIRT1 activator, although it has been disputed whether this effect is achieved by direct molecular interactions with the SIRT1 protein causing its allosteric activation[97] or whether it involves indirect mechanisms — for example, an increase in cellular cyclic AMP levels (as a result of the resveratrol-mediated inhibition of phosphodiesterase 4) leading to activation of AMPK[98]. Irrespective of these unknown factors, resveratrol potently stimulates autophagy via SIRT1 in nematodes and mammalian cells[20,55]. Moreover, the resveratrol-induced increase in lifespan is abolished following knockdown of essential autophagy genes[20]. Resveratrol also improves the healthspan of mice that become obese, irrespective of whether the obesity stems from genetic defects in appetite control or a lipid-rich Western-style diet[99]. Moreover, resveratrol exerts beneficial metabolic and inflammatory adaptations in the visceral adipose tissues of rhesus monkeys receiving an obesogenic high-fat, high-sugar diet[100]. Importantly, in aged mice, resveratrol induces in several organs a transcriptional signature similar to that induced by dietary restriction, which ultimately leads to an overall amelioration of age-impaired parameters (including changes in bone mineral density, as well as cardiac and vascular functions) with a net delay in functional decline and ageing[101].

Indeed, a recent meta-analysis showed that resveratrol significantly improves glucose control and insulin sensitivity in patients with diabetes[102], and evidence suggests that resveratrol has exercise-mimetic effects in patients with type 2 diabetes[103]. Pancreatic β-cell dedifferentiation could be prevented by resveratrol supplementation in non-human primates fed a high-fat, high-sugar diet, although resveratrol failed to reverse insulin resistance in these animals[104]. The inhibitory effect of resveratrol on β-cell dedifferentiation has been recapitulated in isolated human islet cells in an *in vitro* model mimicking high-fat, high-sugar conditions. This resveratrol effect was reversed by the addition of the SIRT1 inhibitor EX-527, which suggests that it was indeed mediated by SIRT1 activation[104]. However, the effects of resveratrol on human physiology are controversial and two clinical studies failed to show any major effects[105,106].

Another putative SIRT1 activator, SRT1720, improves whole-body glucose homeostasis and insulin sensitivity in Zucker *fa/fa* rats, as shown by hyperinsulinaemic–euglycaemic clamp studies[107]. Although SRT1720 failed to reduce caloric consumption or weight gain of normal C57BL6 mice fed a high-fat diet, it was able to improve the median and the maximum lifespan of such mice. This was accompanied by a marked reversal of the high-fat-diet-induced structural and functional deficits in the liver (including hepatosteatosis, hepatocyte apoptosis and reduced oxygen consumption) and in the pancreas (including islet hypertrophy), which were associated with a reduction in circulating transaminases (which are indicative of liver damage) and circulating interleukin-6 (which is indicative of inflammation), as well as improved insulin sensitivity[108]. Importantly, C57BL6/J mice fed a standard diet supplemented with SRT1720 had a slightly reduced body weight and fat mass, which was associated with increased lifespan and improved insulin sensitivity[109].

At the biochemical level, SRT1720 was shown to prevent the hyperacetylation of PPARγ coactivator 1α (PGC1α), which is induced by the consumption of a high-fat diet[108]; PGC1α is a direct target of SIRT1 (REF. 110). Mice lacking SIRT1 expression in the liver showed a similar decrease in hepatocyte state 3 respiration in response to high-fat diet as did control mice; however, the capacity of SRT1720 to rescue mitochondrial oxygen consumption was lost following *Sirt1* knockout[108], which indicates that this effect is achieved through an effect on SIRT1.

Taken together, the data suggest that several plant-derived or synthetic sirtuin activators may function as potent CRMs (reviewed in REF. 111) (TABLE 1). Moreover, it seems increasingly plausible that supplementation with natural NAD precursors such as nicotinamide mononucleotide or nicotinamide riboside may also mediate their anti-ageing effects by activating sirtuins[87,112].

Selectivity and drug safety

Despite the encouraging results that suggest that the aforementioned CRMs may be therapeutically useful, several caveats must be noted. Toxicity is one of them. Perhexiline, for example, causes hepatotoxicity and neurotoxicity in a small subset of patients who have impaired perhexiline catabolism[113]. These toxic effects can be avoided by lowering the dose of perhexiline, which highlights the importance of individually adapting treatment regimens to each patient[113]. Moreover, although placebo-controlled, double-blinded trials suggest that hydroxycitric acid is safe to use in humans and without side effects[113], it caused substantial toxicity in the testes of obese Zucker rats[37]. Non-competitive and selective ACLY inhibitors (such as SB204990 and BMS-303141) are being developed as hydroxycitric acid substitutes (and are as effective as hydroxycitric acid at inducing autophagy and AcCoA depletion (F.P. and G.K., unpublished observations)) and should continue to be evaluated in preclinical and clinical studies.

Another major caveat concerns the specificity of several natural CRMs. For example, EGCG[54] and curcumin[53] have multiple molecular targets and may have pleiotropic effects as a result of their suboptimal specificity. Therefore, genetic studies are of particular importance to identify the health-promoting targets of CRMs.

Glossary

Autophagy
A neologism (from the Greek 'auto' (self) and 'phagein' (to eat)) that describes the capacity of cells to sequester portions of their cytoplasm and to subject them to lysosomal degradation.

Caloric restriction
A dietary regimen that is based on low calorie intake without malnutrition.

Caloric restriction mimetics
Pharmaceutical agents that induce the same biochemical alterations as does caloric restriction.

Healthspan
The length of time that an individual is in optimal health.

Hyperinsulinaemic–euglycaemic clamp studies
A physiological test used on whole animals to measure insulin-stimulated glucose uptake by all tissues of the body, hence measuring insulin sensitivity or resistance.

Mitophagy
Specific autophagic removal of mitochondria, which are usually dysfunctional.

Rapamycin
A macrolide antibiotic produced by the bacteria *Streptomyces hygroscopicus* that inhibits a negative regulator of autophagy, namely the mechanistic target of mammalian target of rapamycin complex 1 (mTORC1).

Table 1 | **Biochemical and functional characteristics of CRMs**

Agent (source)	Target	*In vitro* effects	Preclinical and clinical effects that could be related to autophagy	Refs
AcCoA-depleting agents				
UK5099 (synthetic)	MPC	• Inhibition of pyruvate import into mitochondria	• Unknown	151
Perhexiline maleate (synthetic)	CPT1	• Inhibition of mitochondrial uptake of long-chain fatty acids	• Anti-angina pectoris agent • Improvement of hypertrophic cardiomyopathy	152,153
1,2,3-benzene-tricarboxylate (synthetic)	CTP	• Protection against pancreatic β-cell death induced by high levels of glucose and fatty acids • Improvement of sperm motility	• Reduction of insulin secretion in rat islet cells	154,155
Hydroxycitrate (from *Garcinia cambogia* and *Hibiscus subdariffa*)	ACLY	• Reduction of cancer cell stemness and epithelial–mesenchymal transition	• Autophagy-dependent weight loss in mice • Reduction of body weight, insulin resistance and oxidative stress in obese Zucker rats • Antitumour effects on transplantable mouse cancers if combined with lipoic acid • Weight loss in controlled clinical trials	13,36,152, 156,157
Radicicol (from *Diheterospora chlamydosporia)*	ACLY	• Inhibition of glycolytic functions in glioblastoma • Inhibition of adipocyte differentiation and *in vitro* adipogenesis	• Anti-inflammatory action in sepsis in mice • Improved muscle regeneration in mice • Protection against renal and myocardial ischaemia–reperfusion damage in rodents	158–162
SB-204990 (synthetic)	ACLY	• Tumour growth suppression in a variety of cancer cell lines	• Decrease in cholesterol and triglyceride levels in rats • Tumour growth suppression in mice	163,164
CPI-613 (synthetic; a lipoate analogue)	PDH	• Inhibition of PDH E1 subunit and 2-oxoglutarate dehydrogenases	• Antitumour effects in mice	165–167
Acetyl transferase inhibitors				
Curcumin (from *Curcuma longa*)	HATs	• Reduced acetylation of histones and non-histone proteins via nonspecific inhibition of EP300 acetyl transferases • Anti-proliferative effects on several cancer cell lines • Inhibition of WNT–β-catenin and NF-κB pathways	• Improvement in heart function in a rat model of myocardial infarction • Prevention of heart failure and reversal of cardiac hypertrophy • Tumour growth inhibition in an orthotopic model of pancreatic cancer in mice • Inhibition of forestomach, duodenal and colon carcinogenesis in mice • Lifespan extension in *Caenorhabditis elegans* and *Drosophila melanogaster* • Improved glucose tolerance and reduced insulin resistance in *db/db* mice • Prevention of azidothymidine-induced colon cancer in *db/db* mice	168–182
Epigallocatechin-3-gallate (from green tea)	HATs	• Inhibition of acetylation and translocation of NF-κB into the nucleus	• Lifespan extension in *C. elegans* and rats • Neuroprotection in mouse models of Alzheimer's disease, ischaemic stroke and ALS • Inhibition of hepatosteatosis and increase of lipolysis in mice fed a high-fat diet • Suppression of dextran sulphate-induced colitis • Reduced weight gain and insulin resistance in mice receiving an obesogenic diet	48, 183,184
Spermidine (from natural food items such as fermented soybean and wheatgerm)	HATs	• Inhibition of HATs	• Autophagy-dependent lifespan extension in yeast, nematodes and flies • Lifespan extension in mice • Prevention of colon cancer development in mice • Reduction in arterial ageing and ROS-mediated oxidative stress • Reduction in skin inflammation in mice • Reversal of age-mediated memory impairment in flies	19,55,56, 59,61,63,64
Anacardic acid (from *Anacardium occidentale*)	HATs	• Inhibition of EP300/CBP and TIP60 families of acetyl transferases by competing with AcCoA for binding to the active sites	• Tumour growth and angiogenesis inhibition in human cancers xenografted to mice	185,186

Table 1 (cont.) | **Biochemical and functional characteristics of CRMs**

Agent (source)	Target	*In vitro* effects	Preclinical and clinical effects that could be related to autophagy	Refs
Garcinol (from *Garcinia indica*)	HATs	• Inhibition of EP300 and CBP acetyl transferases • Antitumoural effects via inhibition of NF-κB, STAT3, STAT1 • Inhibition of NF-κB-dependent COX2 expression	• Antitumoural effects in a xenograft model of squamous cell carcinoma of the head and neck • Anti-carcinogenesis effect via 5-lipooxygenase inhibition	78,187,188
MB-3 (synthetic)	HATs	• Inhibition of GCN5 family of HATs • Inhibition of acetylation and stability of E2A–PBX1 in acute lymphoblastic leukaemia	• Unknown	189
CPTH2 (synthetic)	HATs	• Inhibition of GCN5 family of HATs	• Unknown	189,190
C646 (synthetic)	HATs	• Inhibition of EP300 by competing with AcCoA for binding to the active sites	• Immunostimulation by inhibition of T_{Reg} cells in mice	13,82
Gallic acid (from different plants)	HATs	• Nonspecific acetyl transferase inhibition • Anti-proliferative effects on different cancer cell lines	• Anti-proliferative effects on xenografted mouse tumours in mice • Reduction in infiltration of the tumour by T_{Reg} cells in mice • Reduced weight gain in rats fed a high-fat diet • Suppression of amyloid-β neurotoxicity	80,191–193
Resveratrol (from different plants and red wine)	SIRT1	• Indirect or direct (allosteric) SIRT1 activation • Indirect SIRT1 activation, possibly achieved by inhibition of phosphodiesterase 4 and subsequent AMPK activation	• Extension of lifespan of mice on a high-fat diet • SIRT1-dependent autophagy induction in mammals and nematodes • Amelioration of metabolic syndrome and inflammation in rhesus monkeys fed an obesogenic diet • Amelioration of metabolic parameters in obese men • Induction of caloric restriction-like transcriptional signature and improvement of age-related parameters • Chemopreventative effect on colon cancer in the $Apc^{Min/+}$ mouse model • Prevention of β-cell dedifferentiation under high-fat and high-sugar conditions in non-human primates and isolated human islet cells • Improvement of insulin sensitivity and glucose control in patients with diabetes	97,98,101, 102,104,108, 118,194
Nicotinamide riboside and nicotinamide mononucleotide (from different foods, including milk and beer)	SIRT1	• SIRT1 activation related to an increase in the NAD/NADH ratio owing to a rise in SIRT1 substrate levels	• Protection against high-fat-diet-induced abnormalities • Lifespan extension in *Saccharomyces cerevisiae* • Amelioration of diet- and age-dependent diabetic pathophysiology	78,195,196
SRT1720 (synthetic)	SIRT1	• Allosteric activation of SIRT1	• Amelioration of metabolic syndrome in obese Zucker rats • Lifespan extension and amelioration of metabolic syndrome in high-fat diet-fed mice • Lifespan extension and improved insulin sensitivity in mice fed a standard diet	107–109
Quercetin (from different plants, red wine and green tea)	SIRT1	• Anti-proliferative activity in various cancer cell lines	• Increased mitochondrial biogenesis in brain and muscle with augmented exercise tolerance • Reduction in inflammation, weight gain and metabolic syndrome in mice • Tumour growth reduction in an orthotopic model of pancreatic cancer	197–199
Piceatannol (from *Picea abies* and red wine)	SIRT1	• Indirect or direct SIRT1 activation (piceatannol is an analogue of resveratrol) • Protection against amyloid-β-induced neuronal cell death	• Improvement of metabolic syndrome in *db/db* obese mice	200–203

AcCoA, acetyl coenzyme A; ACLY, ATP citrate lyase; ALS, amyotrophic lateral sclerosis; AMPK, AMP-activated protein kinase; Apc^{Min}, mutation in murine adenopolyposis coli gene that predisposes mice to tumours; CBP, CREB binding protein; COX2, cyclooxygenase 2; CPT1, carnitine *O*-palmitoyl transferase 1; CRM, caloric restriction mimetic; CTP, citrate transport protein; *db*, mutation in the leptin receptor gene that predisposes mice to diabetes; EP300, E1A-binding protein p300; GCN5, histone acetyltransferase 5 (also known as KAT2A); HAT, histone acetyl transferase; MPC, mitochondrial pyruvate carrier; NF-κB, nuclear factor-κB; PBX1, pre-B cell leukaemia transcription factor 1; PDH, pyruvate dehydrogenase; ROS, reactive oxygen species; SIRT1, sirtuin 1; STAT, signal transducer and activator of transcription; TIP60; histone acetyltransferase TIP60 (also known as KAT5); T_{Reg}, regulatory T.

Knocking down the gene encoding ACLY was shown to induce cytoprotective autophagy that delayed the toxicity caused by the expression of a mutant form of Huntingtin in cultured human cells, and knocking down the gene encoding AcCoA synthetase extended the lifespan of *D. melanogaster*[13,16]. Moreover, SIRT1-transgenic mice share features with mice that have been subjected to a calorically restricted diet, including improved metabolic parameters and motor capabilities[114,115], reduced DNA damage during ageing — which has oncosuppressive effects[116] — and ameliorated angiotensin II-induced vascular pathology[117]. Further evidence that SIRT1-activating compounds can be used to mimic caloric restriction comes from a randomized double-blind study of 11 obese but otherwise healthy men, in whom daily administration of 150 mg of resveratrol could efficiently reproduce the positive effects elicited by caloric restriction (such as reduced metabolic rate while sleeping, decreased arterial blood pressure and reduced intrahepatic lipids)[118]; these effects correlated with improved mitochondrial fitness and an increase in respiration rate, which may depend on SIRT1-mediated PGC1α activation[119]. It is tempting to speculate that resveratrol will be replaced by more specific sirtuin activators such as SRT1720 (see above) or other compounds that are being developed[120].

The knockout of genes encoding acetyl transferases (particularly HATs) is often embryonically lethal, which reflects the crucial role of these enzymes in several cellular processes and in early development[121]. Nevertheless, there is some genetic evidence that modulating acetyl transferase activity can be an efficient way to mimic dietary restriction. The KAT3 family of acetyl transferases (including CREB-binding protein (CBP) and EP300) is a particularly crucial hub that translates nutrient availability into transient or epigenetic modifications of effector proteins. Interestingly, genetic ablation of the cysteine histidine-rich 1 (CH1) domain of EP300 or of CBP, which is responsible for insulin responsiveness, mimicked the beneficial effect of caloric restriction as it increased insulin sensitivity and glucose tolerance, and also reduced body weight and white adipose tissue when these mice were fed either normal or high-fat diets[122]. EP300 consequently seems to be a reasonable target for mimicking caloric restriction, and the putative health-promoting effects of nonspecific acetyl transferase inhibitors (such as curcumin, gallic acid or EGCG) could indeed be linked to their ability to block EP300 activity[123]. On the basis of these considerations, it is particularly important to evaluate the therapeutic and toxicological profiles of more selective EP300 inhibitors (such as C464) in preclinical studies. It is reasonable to expect — but remains to be shown — that truly specific EP300 inhibitors might combine maximum efficacy with minimum toxicity. In favour of this possibility, small-molecule inhibitors of EP300 lack the toxicity of their natural template garcinol when added to T cells *in vitro*[124]. Similarly, analogues of anarcadic acid have been developed with the aim of generating agents that kill malignant cells but that spare normal cells[125]. These findings highlight the potential therapeutic value of more specific EP300 inhibitors.

CRMs, autophagy and ageing

Autophagy has preponderantly health-promoting effects, presumably because it constitutes a mechanism through which cells can rid their cytoplasm of unfolded proteins and deficient organelles, including dysfunctional mitochondria[126,127] (BOX 1). Normal and pathological ageing are reportedly associated with a diminished autophagic potential in multiple organ systems, which correlates with the depletion of pro-autophagic metabolites (such as NAD and spermidine) and proteins (such as sirtuins), as well as with the accumulation of autophagic cargo, including ubiquitylated proteins and uncoupled mitochondria. Genetic, pharmacological or nutritional manipulations that increase lifespan in model organisms usually stimulate autophagy, and its suppression compromises the longevity-extending effects of caloric restriction and all CRMs investigated in this respect[2]. Intriguingly, autophagy apparently can also participate in the prevention of obesity. Thus, mice that are partially resistant to autophagy as they are haploinsufficient for the expression of beclin 1 (an essential autophagy gene) or lack expression of the pro-autophagic protease ATG4B fail to lose weight upon starvation or treatment with the CRM hydroxycitrate[13]. Moreover, compared with wild-type controls, *Atg4b*$^{-/-}$ mice are hypersensitive to the induction of adiposity, hepatosteatosis and diabetes by sucrose (G.K., unpublished observations). This may be linked to the well-studied effects of autophagy on adipocyte differentiation, central appetite control, hepatic lipolysis (also known as lipophagy), inflammation and muscle metabolism. Notwithstanding these insights, little is known about the mechanisms through which the induction of autophagy may promote healthy ageing[128].

The detailed molecular mechanisms through which caloric restriction and CRMs induce autophagy are only partially understood. However, nutrient depletion and the CRMs spermidine and resveratrol cause the deacetylation of hundreds of proteins[13,55], as determined by mass spectrometry, and such deacetylated proteins are frequently connected to autophagy regulation. This suggests that CRMs induce autophagy through multiple effects on protein deacetylation rather than by modifying the function of just one or a few proteins[129]. Specifically, EP300 can mediate the inhibitory acetylation of several core components of the autophagic machinery (ATG5, ATG7, ATG8 and ATG12)[69] as well as that of the pro-autophagic transcription factor FOXO1 (REF. 70). EP300 can catalyse an inhibitory self-acetylation, and tissues from starved mice show a reduction of acetylated EP300, which can be detected using an antibody recognizing EP300 that is acetylated on Lys1499 (REF. 13). In addition to FOXO1, EP300 acetylates multiple transcription factors, thereby affecting gene transcription[130]. HDAC1 can deacetylate p53, and this reaction may promote autophagy through transcriptional effects[131]. Starvation (and rapamycin) can induce the downregulation of the histone acetyl transferase MOF (also known as KAT8), thereby reducing the acetylation of Lys16 of histone H4 (REF. 132). However, this modification does not induce autophagy, but rather reduces the expression of several autophagy-relevant genes as part of a negative feedback loop that avoids excessive, lethal autophagy[132].

Autophagy induction by caloric restriction or CRMs is coupled to the inhibition of mTORC1, which results in dephosphorylation of the principal mTORC1 substrates, p70 ribosomal S6 kinase-α (p70S6K; also known as RPS6KB1) and eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1)[13,133]. These data suggest that mTORC1 inhibition constitutes one of the principal mechanisms through which CRMs induce autophagy. Indeed, rapamycin, which is a highly specific mTORC1 inhibitor, is a potent inducer of autophagy that extends longevity in several species. This is thought to occur through autophagy induction, as has been documented at least for *D. melanogaster*[45]. Rapamycin is the sole US Food and Drug Administration (FDA)-approved drug that can increase the lifespan of middle-aged genetically heterogeneous mice (irrespective of sex) when given intermittently[66,134,135] and of inbred C57BL/6J male mice when applied continuously[136]. Rapamycin has potent immunosuppressive

side effects (which enabled its FDA approval as an agent that can prevent transplant rejection) and increases the incidence of diabetes, presumably through the inhibition of mTORC2 (REF. 137). In mice, continuous rapamycin treatment also raises the incidence of testicular degeneration and cataracts[138]. Future studies must determine whether the replacement of rapamycin by truly mTORC1-specific inhibitors and/or intermittent application regimens will reduce its side effects and hence facilitate its clinical application as a CRM.

Perspectives

The hypothesis that optimal CRMs are pharmacological agents that induce autophagy via protein deacetylation has several practical implications that warrant further investigation.

First, autophagy-regulatory deacetylation reactions might be used as biomarkers to measure the *in vitro* and *in vivo* effects of CRMs (for example, in blood cells). As an example, antibodies recognizing proteins carrying acetylated lysine residues (irrespective of the context) or — more specifically — antibodies recognizing EP300 only when it is acetylated on Lys1499 (REF. 139) can be used to detect the effect of starvation or CRMs on distinct tissues and cell types *in vivo* and *in vitro*[13]. Similarly, the deacetylation of specific sirtuin substrates can be determined as a proxy of sirtuin activity[140]. It remains to be determined which deacetylation reactions would be optimally suitable as caloric restriction- or CRM-relevant biomarkers.

Second, if the detection of deacetylation and the induction of autophagy constituted accurate biomarkers for predicting the efficacy of CRMs, such biomarkers might in turn facilitate pharmacological screens for the identification of new CRMs. In such screens, cultured cells would be exposed to candidate CRMs and autophagy would be detected (for example, by monitoring the redistribution of green fluorescent protein (GFP)–LC3 to autophagosomes)[141]. General protein deacetylation[25] or deacetylation of specific proteins[140] could also be measured.

Third, if deacetylation constituted the point at which autophagy is regulated by AcCoA, acetyl transferases and deacetylases, it seems obvious that particular combinations of autophagy inducers should interact in a synergistic manner. In the same way as a kinase inhibitor may be expected to synergize with an activator of a phosphatase acting on the same substrate, acetyl transferase inhibitors should synergize with deacetylase activators. As a proof of principle, spermidine was shown to synergize with resveratrol to induce autophagy[55]. It will be important to investigate such interactions in more detail to gain insights into their putative beneficial and collateral effects *in vivo*.

Fourth, it will be important to evaluate novel combination regimens in addition to CRMs. For example, mice expressing a mutant form of growth hormone receptor may mimic caloric restriction, as they are long-lived and show reduced levels of IGF1, and their lifespans are not extended further by caloric restriction or intermittent fasting[142–144]. Inhibition of IGF1 receptors activates autophagy via the inhibition of both AKT (also known as PKB) and TORC1 (REFS 145,146) — these molecules are well-known, phylogenetically conserved longevity regulators[147–149]. As cytosolic AcCoA and EP300 repress mTORC1 activity[13] and several CRMs extend lifespan by inhibiting the TOR pathway[44], it seems that the CRM-modulated and IGF1-, AKT-, and TORC1-dependent pathways functionally intersect. Thus, it will be interesting to investigate whether the combination of CRMs with inhibitors of IGF1, AKT or TORC1 is more effective than these agents alone.

Finally, the evaluation of current and future CRMs in clinical trials for the treatment of diabetes, metabolic syndrome and other diseases should benefit from the monitoring of suitable biomarkers. Beyond symptomatic clues such as weight loss and reduction of glycated haemoglobulin or hyperinsulinaemia, it will be interesting to define mechanism-based biomarkers, including the quantification of AcCoA, protein acetylation and autophagy in peripheral blood mononuclear cells.

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Competing interests statement
The authors declare no competing interests.

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