Calorie restriction: is AMPK as a key sensor and effector?

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Summary

Dietary restriction can extend lifespan in most organisms tested to date, suggesting that
mechanisms sensing nutrient and energy availability might regulate longevity. The AMP-activated
protein kinase (AMPK) has emerged as a key energy sensor with the ability to transcriptionally
reprogram the cell and metabolically adapt to external cues. In this review we will discuss the
possible role of AMPK in the beneficial effects of calorie restriction on health and lifespan.

Introduction

It is fascinating how a simple intervention like caloric restriction (CR) is the most consistent
intervention increasing lifespan, protecting against the deterioration of biological functions
and reducing the risk factor for the apparition of diabetes cardiovascular disease and cancer
(27). CR is usually defined as a moderate reduction, generally around 20-40%, in caloric
intake compared with ad libitum feeding, without compromising the basic nutritional needs.
The beneficial effects of CR on lifespan stretch all along the evolutionary scale (27). In
Rhesus monkeys CR lowered the incidence of aging-related death and reduced the incidence
of diabetes, cancer, cardiovascular disease and brain atrophy (18). Despite that we only have
preliminary evidence based on surrogate measures on how CR might impact on human
longevity, most available data sets indicate that CR exerts similar adaptative responses in
humans as in laboratory animals, and prevents the development of age-associated health
complications (47). In light of these attractive properties, many efforts have been dedicated
to finding out how CR acts. At first, it would be intuitive to think that the adaptive response
to low nutrient availability should be triggered by a mechanism with the ability to respond to
nutrient availability via either humoral or intracellular metabolites. Importantly, the
longevity of unicellular organisms, such as yeast, is modulated by CR. This indicates that
autocrine signals and intracellular nutrient sensors can fully account for such effects.

The AMP-activated protein kinase (AMPK) has emerged as a key nutrient sensor with the
ability to regulate whole-body metabolism. AMPK is activated upon an increase in the
AMP/ATP ratio, which reflects the energy status of the cell. A recent report combining
structural and biochemical approaches revealed that, apart from AMP, also ADP binding can
also crucially contribute to AMPK activation . Upon activation, AMPK turns on catabolic
pathways to restore ATP levels, both in a short time frame, by promoting glycolysis and
fatty acid oxidation, and in a long time frame, by increasing mitochondrial content and the
use of mitochondrial substrates as an energy source (12). Precisely, mitochondrial fitness is
emerging as a particularly interesting topic in the ageing field as many reports indicate that
the effective control of mitochondrial biogenesis, metabolism and turnover is crucial for a
healthy cellular and whole-body ageing (reviewed in (78)). The role of AMPK to sense

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energy stresses and act as a master regulator of mitochondrial biogenesis and metabolism, therefore, has led to the speculation that AMPK might mediate the beneficial effects of CR. While many reports in lower eukaryotic organism, like worms, clearly support such notion, the translation of such observations on other organisms remains largely unexplored. In this review we aim to discuss a number of observations that either direct or indirectly indicate that AMPK might act as an important agent on the regulation of lifespan and a mediator of the beneficial effects of CR, while also pointing out the diverse caveats present in such hypothesis.

AMPK enzymatic regulation and actions

AMPK is a heterotrimeric Ser/Thr kinase composed of an α, β and γ subunit (40). There are two different forms of α ($α_1$ and $α_2$) and β ($β_1$ and $β_2$) subunits, while three different γ isoforms ($γ_1$, $γ_2$ and $γ_3$) exist (40). The α subunits are the catalytic subunits of the functional heterotrimer and contains the Thr^{172} residue, whose phosphorylation is required for full enzymatic activity (43). The β subunit contains an evolutionary conserved carbohydrate binding domain, which allows AMPK to interact with glycogen particles, resulting in inhibition of AMPK when glycogen stores are high (51). The γ subunits contains four tandem repeats known as cystathionine β-synthase (CBS) motifs, which form an interface for interaction with two AMP or ATP molecules in a mutually exclusive way and a third AMP molecule in a non-exchangeable fashion (110). In basal conditions, the binding of ATP keeps the activity of the enzyme low. AMPK is only fully active after phosphorylation of Thr^{172} within the activation loop of the α subunit catalytic domain. The main upstream kinase in most cell types is the LKB1/STRAD/MO25 complex. This complex seems to be constitutively active and phosphorylates AMPK continuously, but, in basal conditions, the phosphate is immediately removed by protein phosphatises. However, upon energy stress, AMP concentrations increase and bind to the AMPKγ subunits, promoting a conformational change that renders AMPK a poorer substrate for dephosphorylation. Then, the increased phosphorylation levels of the Thr^{172} residue results in the full activation of the enzyme (96).

AMPK activation is generally linked to the stimulation of metabolic responses in order to prevent metabolic and energetic crisis in situations where ATP synthesis is compromised, such as in hypoxia, ischemia, low nutrient availability, or ATP consumption is accelerated, such as during exercise or fasting. Consequently, AMPK activation stimulates catabolic processes to generate ATP and inhibits ATP-consuming anabolic processes that are not required for the immediate survival of the cell. For the purpose of this review it is important to point out that AMPK acts as a master controller of mitochondrial metabolism, by promoting mitochondrial oxidation of lipid substrates and mitochondrial biogenesis through transcriptional means (12). For a detailed review of the metabolic and transcriptional actions of AMPK, we refer the reader to other recently published reviews (12, 40)

AMPK as a mediator of CR effects (I): Evidence from genetic models

In the absence of glucose, the sucrose non-fermenting 1 kinase (SNF1), the yeast homolog of AMPK, translocates to the nucleus, where it transcriptionally potentiates the respiratory metabolism of non-fermentable carbon sources (107). Interestingly, the beneficial effects of glucose restriction in yeast are related to increases in respiratory rates that occur when glucose levels in the media are lower. Several models (76, 86), albeit not all (60), indentify the capability to promote respiration with the ability to increase replicative and/or chronological lifespan. Hence, it would logical to postulate that if Snf1 is responsible in majority for derepressing genes linked to the use of non-fermentable carbon sources upon low glucose availability, it should be key for the effects of CR. In line with this hypothesis,

pioneer work by Ashrafi and colleagues demonstrated that Snf1 deletion results in loss of cellular fitness and decreased lifespan (5). The reasons for this decreased longevity, however, did not seem to be related to accelerated ageing, but, rather, to metabolic malfunctions (5). The role of Snf1 in yeast longevity seems, however, to be complex as both disruption and forced overactivation of Snf1 lead to reduced lifespan (5, 77, 79). It is likely that Snf1 activity must be tightly regulated upon CR in order to properly adapt to low nutrient availability without compromising lifespan.

While the evidence supporting a role for AMPK homologs in yeast for the adaptations to CR is somehow poor, it gains strength when moving to the *C. Elegans* model. Arguably, most of the genetic evidence supporting the role of AMPK as a mediator of the effects of CR on longevity has been raised in worms. In 2004, Apfeld and colleagues reported that the genetic deletion of the worm AMPKα homolog (aak-2) decreases worm lifespan by 12 % due to accelerated ageing (4). Conversely, worms overexpressing aak-2 lived 13% longer (4). Subsequent research elucidated that glucose restriction increases aak-2 activity in nemadotes and that aak-2 is required for the shift to respiratory metabolism in glucose-restricted conditions (97). Finally, extensive work (34, 35, 97) has determined that several CR regimes, albeit not all (34, 80), require aak-2 to promote lifespan extension.

Global deletion of the drosophila AMPK is lethal (70), forcing alternative strategies to study its influence on the responses to CR and impact on longevity. Using time- and tissue-specific RNAi systems in Drosophila, it was recently reported that inhibition of AMPK in muscle is enough to decrease the lifespan of flies (106). A parallel study indicates that reduced global AMPK activity decreases lifespan in drosophila and reduces lifespan extension by starvation (58). Interestingly, another recent report indicates that overexpression of LKB1, the upstream kinase for AMPK, promotes lifespan on Drosophila (31). However, whether AMPK participates in the lifespan promoting properties of CR in Drosophila or any mammalian organism, has not yet been tested.

AMPK as a mediator of CR effects (II): Evidence based molecular actions

Regulation of mitochondrial biogenesis

Initial clues linking AMPK with CR and the ageing process were provided by studies indicating that the capacity to pharmacologically or physiologically activate AMPK is lower in aged rodents than in young ones (92). While the mechanisms underlying such observation are still elusive, it might provide indications on why ageing is linked with defective mitochondrial and dysregulated lipid homeostasis, and why the effects of CR are generally linked to mitochondrial fitness.

Defective mitochondrial function seems to be the signature of mammalian ageing. Using NMR spectroscopy it was shown how ageing was associated with increased fat accumulation in muscle and liver, probably due a notable 40% reduction in mitochondrial oxidative phosphoryaltion activity (89). Mitochondria constitute a very attractive link to the physiological decay observed during ageing, as defective mitochondrial function and energy metabolism would explain the constellation of defects observed during ageing, such as oxidative stress, excessive inflammation, defective protein and organelle turnover and accumulation of covalent protein modifications emerging from metabolism side-products.

The fact that AMPK is a master regulator of mitochondrial biogenesis and lipid metabolism makes it a likely candidate to influence lifespan. The mechanisms by which AMPK regulates mitochondrial biogenesis are beginning to be elucidated. Transgenic animals have helped identifying PGC-1α as a crucial mediator of AMPK action on mitochondrial genes in mouse skeletal muscle (54, 73). PGC-1α is a transcriptional coregulator that orchestrates the

mitochondrial biogenesis program by coactivating a number of nuclear transcription factors that control genes involved in mitochondrial function and lipid oxidation (14). The activity of PGC-1α is critically controlled by its acetylation status (56). Generally, the basal activity of PGC-1α is rather low, as it is highly acetylated (74). In order to fully achieve its coactivating potential, PGC-1α requires deacetylation by the NAD^+-dependent deacetylase SIRT1 (93). The mechanism by which AMPK impacts on PGC-1α has been recently deciphered. Upon activation, AMPK directly phosphorylates PGC-1α at Thr^{177} and Ser^{538}. The phosphorylation of these residues enables deacetylation by SIRT1 and, therefore, activation (15). In addition to PGC-1α, AMPK can also influence the activity of a number of transcriptional factors related to mitochondrial biogenesis and lipid oxidation, such as MEF2, PPARα and PPARδ [for extensive review, see (12)].

Regulation of known CR regulators of lifespan

The cooperative nature of AMPK and SIRT1 expands beyond PGC-1α. Work from Vittorio Sartorelli's and our lab demonstrated that AMPK and SIRT1 activities are positively linked (15, 30). The mechanism by which AMPK impacts on SIRT1 activity does not rely on direct interaction or phosphorylation events, but AMPK rather promotes an intracellular increase in NAD^+ levels, the rate-limiting substrate for the deacetylase activity of SIRT1 (15, 30). By increasing NAD^+ levels, AMPK allows higher levels of SIRT1 activity. By phosphorylating PGC-1α, AMPK promotes specificity in SIRT1 action. This exemplifies how the cross-talk between different post-translational modifications (i.e: phosphorylation and acetylation) is of utmost importance to understand how similar transcriptional regulators can elicit specific actions depending on the context of its activation.

Furthermore, the link between AMPK and SIRT1 has important consequences on the CR and ageing fields. In lower eukaryotes manipulations of the SIRT1 gene or of its homologs has a strong influence in lifespan (13). In yeast, overexpression of Sir2, the SIRT1 homolog, is enough to enhance replicative lifespan (75). Furthermore, mimicking CR in yeast by reducing glucose in the medium from 2% to 0.5% was unable to extend lifespan in yeast lacking the Sir2 gene or models aimed to decrease Sir2 function (1, 75), suggesting that Sir2 is a crucial mediator of the effects of CR on lifespan in yeast. Further reinforcing such concept, overexpression of the SIRT1 homolog in worms and Drosophila also enhanced lifespan (104). Furthermore, the lack of Sir2 in Drosophila is enough to prevent the effects of CR on lifespan (94). While there are no reports so far supporting that SIRT1 enhances lifespan in mammals, the evidence obtained to date support that it improves healthy aging (6, 46, 90). Consequently, if AMPK promotes SIRT1 activity and expression, it would be likely that it could act as a lifespan/healthspan regulator.

The FOXO family of transcription factors provide a second molecular link between AMPK, CR and enhanced lifespan. Genetic studies in many organisms have provide substantial evidence that the FOXO transcription factors have conserved the ability to promote longevity (27). The activity of FOXOs is linked to the promotion of lipid metabolism, resistance to oxidative stress and pathogens, protection of protein structure and authophagy (for review, see (37)). These finding suggest that FOXOs enhance lifespan by protecting the cell from various stresses, including nutritional stress. The relation of AMPK with FOXOs was brought into the spotlight when FOXOs were reported as possible mediators of the effects of AMPK on autophagy (83). Furthermore, AMPK can directly phosphorylate different members of the FOXO family of transcription factors (36). Amongst them, FOXO3 is phosphorylated by AMPK in up to 6 residues (36). Mutation of these residues impaired the ability of AMPK to promote key transcriptional responses during glucose-deprivation, including the transcriptional activation of oxidative protection genes (36). FOXO phosphorylation by AMPK does not influence FOXO subcellular localization, but rather its activity (36). As for PGC-1α, FOXO activity is also critically controlled through

acetylation/deacetylation, which is altered by SIRT1 (10, 29, 82). It is tempting to speculate that AMPK phosphorylation of FOXO could also serve as a signal for its deacetylation, which, in turn, seems to provide FOXO with specificity towards the regulation of oxidative stress genes (10), suggesting that, like for PGC-1α, the modifications of FOXO by AMPK and SIRT1 might be interconnected.

Regulation of mTOR signalling

The mammalian target of rapamycin (mTOR) kinase provides another riveting link between AMPK, CR and longevity. mTOR is as a central regulator of eukaryotic growth and cell division in response to nutrient and growth factor cues. TOR proteins are highly conserved from yeast to humans (100). The identification and name of mTOR derives from studies of the growth inhibiting properties of the anti-fungal compound rapamycin (45). mTOR is generally activated by growth hormones (e.g. insulin) and promotes anabolism, cell growth and division (100). The role of mTOR, or its homologs, as an important longevity pathway has been firmly established by studies on many different eukaryotic models, indicating that a reduction in the activity of the mTOR complex 1 (mTORC1) is sufficient to increase lifespan significantly [for review, see (48, 103)]. In mammals it has been shown that CR decreases mTOR signalling (103). Feeding mice with rapamycin extends median and maximal lifespan of different mouse strains (42). Further supporting the role of mTOR on longevity, deletion of ribosomal S6 protein kinase 1 (S6K1), a downstream component of the mTOR signaling pathway, led to increased life span and resistance to age-related pathologies, such as bone, immune, and motor dysfunction and loss of insulin sensitivity (98). Importantly, deletion of S6K1 induced gene expression patterns similar to those seen in CR (98). From a metabolic perspective, mice lacking S6K are prevented against obesity and metabolic disease, linked to a higher mitochondrial biogenesis (109). It is also interesting to note that in a genome-wide array for genes changed upon dietary restriction in Drosophila, it became evident that 4E-BP, another downstream target of mTOR, might be key for the effects of CR (115). 4E-BP (eukaryotic translation initiation factor 4E binding protein) is a translational repressor, which is inhibited upon mTOR activation (32). 4E-BP is induced upon CR in flies, and mediates the effects of CR on mitochondrial biogenesis and longevity (115). These findings further support that attenuation of mTOR signalling might be a key step by which CR impacts on lifespan extension. Interestingly, AMPK activation is the best-described intracellular trigger for mTOR inhibition. By phosphorylating both Raptor (a component of the mTORC1 complex) (38) and TSC2 (an upstream regulator of mTOR) (53), AMPK inhibits mTOR, and, therefore, of S6K1 and leads to the reduction of many anabolic processes. As the downregulation of mTOR has been shown to promote CR-like effects on lifespan in several organisms (64), the regulation of mTOR by AMPK provides a very likely mechanism by which AMPK could influence lifespan.

Regulation of autophagy

The possible influence of autophagy on longevity is raising major attention (66). Autophagy is an evolutionarily conserved process in which portions of the cytoplasm, including superfluous or damaged organelles and misfolded or aggregated proteins, are engulfed in double-membrane vesicles called autophagosomes for degradation and recycling (66). It has been repeatedly reported that the autophagic activity of living cells decreases with age, probably contributing to the accumulation of damaged macromolecules and organelles during aging. Moreover, autophagy modulation in different model organisms has yielded results suggesting that the maintenance of a proper autophagic activity is a key adaptation by which CR enhances longevity [for review, see (66)]. Autophagy is controlled through a tight molecular network which includes most of the above-described players: AMPK, SIRT1, FOXO and mTOR. While nutrient scarcity promotes autophagy, the mechanisms by which this happens are only beginning to be elucidated. Under low nutrient conditions, AMPK

activation promotes authophagy by activating Ulk1 through direct phosphorylation (25, 65, 71). Ulk1 is a critical kinase that governs the cascade of events triggering autophagy (81). Elegant studies by two different groups identified multiple, yet different, key AMPK sites on Ulk1 (25, 65). Therefore, while phosphorylation of Ulk1 by AMPK is convincingly demonstrated, the mechanistic implications of the different phosphorylable residues are not yet clear. Upon nutrient and growth factor abundance, mTOR gets active and phosphorylates Ulk1 at Ser^{757}, which disrupts the interaction between AMPK and Ulk1, therefore, inactivating Ulk1 and downregulating autophagy rates (65). AMPK can also impact on autophagy through the modulation of SIRT1 (69). SIRT1 forms molecular complexes with several components of the autophagy machinery, including Atg5, Atg7 and Atg8, which act as critical regulators of the autophagosome formation. SIRT1 deacetylates these proteins in an NAD^+-dependent manner, even though the substrate residues and the mechanistic consequences of this deacetylation have not yet been elucidated (69). The absence of SIRT1 considerably increased the acetylation level of these autophagy proteins. Consistent with these observations, autophagy during starvation is impeded in embryonic fibroblasts of $SIRT1^{-/-}$ mice (69). The lack of SIRT1 led to the accumulation of damaged organelles, especially mitochondria (69), which might explain why defective SIRT1 activity systematically correlates with deficiencies in energy metabolism. This way, the impact of AMPK on autophagy could be mediated not only by the direct activation of Ulk1, but also through the indirect modulation of the acetylation status of key autophagosome formation proteins by SIRT1.

Finally, the FOXO family of transcription factors has also been linked to autophagic processes, especially in cardiac and skeletal muscle. During nutrient scarcity, FOXO1 and FOXO3 move to the nucleus and occupy the promoters of genes related to autophagy (99, 112, 113). By blocking FOXO activity, the autophagic capacity of the tissues is greatly compromised. This is evidenced, for example, by the fact that cardiomyocyte cell size is reduced by autophagic procedures upon starvation, an effect that its blocked when FOXO activity is prevented (99). Interestingly, there is evidence indicating that the deacetylation of FOXO by SIRT1 is required for FOXO-induced autophagy-related gene expression (41). This further illustrates the networking interaction between AMPK, FOXO and SIRT1 in the regulation of many cellular processes involved in the adaptations to calorie restriction and promotion of longevity, opposing the action of mTOR (Figure 1).

AMPK as a mediator of CR effects (III): Pharmacological evidence

The probable influence of AMPK on the adapations to CR and the promotion of healthy longevity is also supported by a number of observations derived from pharmacological approaches. Given the numerous beneficial effects of CR but the unlikeliness that people would adapt such a rather "severe" diet, there has been a strong interest in developing calorie restriction mimetic compounds that could be used pharmacologically. Probably, the first approach to achieve such a goal was the use of 2-deoxyglucose (2DG), a glycolysis inhibitor. This simple approach recapitulated many features of calorie restriction, like an increase in insulin sensitivity and a decrease in core body temperature (52). Of note, treatment with 2DG at therapeutic doses leads to energy stress and potently activates AMPK (44). The strong toxicity of 2DG use at the therapeutic doses, however, discouraged the use of such compound and strategy as a mimic for CR studies.

The compound that has probably epitomized the concept of "CR mimetic" might be resveratrol (Rsv). Rsv is a small polyphenol present in, amongst others, red grapes and was initially described to have cancer chemopreventive activity (55). A decade ago, SIRT1 emerged as candidate mediator of CR-induced replicative life extension in yeast. In an attempt to find small-molecule SIRT1 activators, Rsv was identified as a direct SIRT1

activator in an in vitro screen (49). Subsequently, Rsv was shown to extend lifespan in numerous studies in yeast, worms and flies in a sirtuin dependent manner (13). These observations indicated that Rsv treatment could by itself be enough to mimic many features of CR. This CR-mimetic effects were also observed in mammals from a metabolic perspective, as Rsv protects against insulin resistance, enhances mitochondrial biogenesis and recapitulates metabolic transcriptional profiles that resemble those of animals on CR (8, 67). While there are some differences between the effects of Rsv and CR in mammals [summarized in (7)], its characteristics as a CR mimetic are arguably the most extensively documented to date. However, a couple of studies in 2005 alerted about the possibility that Rsv may not be a direct SIRT1 activator and that the results on the in vitro screen were an artifact derived from the use of a fluorescent substrate (9, 62). The SIRT1-dependency of many Rsv effects clearly argued that SIRT1 was being activated upon Rsv treatment (67), but as an indirect downstream event of some mechanism that was not yet clear. Some light was brought into this issue when our lab identified SIRT1 as a downstream metabolic mediator of AMPK actions (15). Indeed, by early 2007 several labs reported that Rsv had the ability to activate AMPK (8, 20, 111). In our hands Rsv can activate AMPK in C2C12 myotubes in less than 5 minutes after treatment, while activation of SIRT1 takes hours (Canto C and Auwerx J, unpublished observations). To date, the activation of AMPK is the earliest signalling event described for Rsv. The mechanism by which Rsv activates AMPK has also been exquisitely unveiled recently. In early reports on Rsv, this polyphenol was described to compromise ATP synthesis by directly inhibiting ATP synthase in the mitochondrial respiratory chain (114). Using AMPK mutants that are insensitive to changes in AMP/ATP ratio, it was demonstrated that Rsv activates AMPK as a consequence of decreased ATP production (44). The question was then, do the metabolic effects of Rsv arise from AMPK activation? The answer so far might be notoriously affirmative. Using the AMPKγ_3 knock-out mouse model we demonstrated that defective AMPK prevents the activation of SIRT1 upon Rsv treatment (16). Consequently, PGC-1α acetylation levels and, therefore, activity were unresponsive to Rsv treatment when AMPK activation is compromised (16). A parallel study by Um et al, using either AMPKα_1 or AMPKα_2 knock-out, showed that AMPK was required by resveratrol to induce CR-like effects, like increasing insulin sensitivity, glucose tolerance and enhancing mitochondrial biogenesis (108). A third study in worms indicates that resveratrol requires intact AMPK activity to enhance lifespan (34). In combination, these studies unequivocally prove that AMPK is a central target for the CR-like metabolic effects of resveratrol.

Other pharmacological evidence supporting the role of AMPK as a key effector of CR would come from the effects of metfomin on longevity. Metfomin is a biguanide commonly used in the treatment of type 2 diabetes, due to its ability to suppress glucose production in liver. Strikingly, metformin promotes CR-like transcriptional changes (22). Similarly, other CR effects are also observed upon metformin treatment, as for example, the prevention of tumor development (2, 3). In addition, reduced all-cause mortality has been associated with metformin treatment in both diabetic and cardiovascular disease patients (95). As with resveratrol, many effects of metformin derive from its capacity to inhibit complex I of the mitochondrial respiratory chain, decrease ATP production and activate AMPK (44). While the real burden of metformin effects that can be attributed to AMPK is still a matter of debate (28), this observation also contributes to the notion that AMPK might be a convergent point for these compounds to promote CR mimetic effects.

Evidence against AMPK as a mediator of calorie restriction effects

Despite the amount of evidence supporting that AMPK could be a good candidate mediator of the adaptations to CR, this hypothesis has also some caveats (summarized in Figure 2). The fact that AMPK triggers adaptations resembling those seen with CR does not imply that

this is the mechanism by which CR acts. Indeed, this might be the case, as a major concern is whether the stress promoted by CR is robust enough to activate AMPK, at least in mammals. There are conflictive observations in this sense. Early studies indicated that heart, muscle and liver from mice that have been on CR for 4 months did not show any change in AMPK activity compared to ad libitum fed mice (33). Another report supports these observations by showing no changes or even decreases in AMPK activity upon CR (105). In some sense, this is not surprising, as it is also not evident to see AMPK activation in response to even more drastic nutritional challenges such as fasting (see (21) for review). These publications, however, are at odds with other studies indicating that CR leads to AMPK activation in rat heart and liver, as well as mouse skeletal muscle (24, 57, 87, 101). Therefore, whether AMPK is activated or not upon CR is still a largely unresolved issue. Similarly, whether AMPK activation in the latter studies constitutes the cause or the consequence of the beneficial effects of CR is also not clear. Genetic studies in yeast (5), indicate that constitutive activation of AMPK might actually be deleterious for lifespan. Similarly, higher basal AMPK activity can be deleterious in some mammalian tissues, as reported in the Wolf-Parkinson-White syndrome, where mutations that activate AMPK predispose to the development of cardiomyopathy (11). These observations cast some doubt about whether higher AMPK activity could be the way by which CR acts. While it is unlikely that AMPK is constitutively and continuously active upon CR or that CR per se is an energy stress intense enough to increase AMPK activity, this does not rule out, however, that AMPK might be activated in a more subtle temporally- or spatially-restricted fashion upon CR. Similarly, it is likely that CR prevents the decrease in AMPK sensitivity for activation observed upon ageing (92), allowing the maintenance of its proper function on metabolic adaptations and mitochondrial gene expression modulation. The question is, if AMPK activation upon CR is not clear, why is there such abundant genetic evidence indicating that AMPK is required for many responses to CR? One explanation might be that AMPK defective models are known to have lower basal mitochondrial gene expression (59). Hence, the abnormal response to CR in AMPK-deficient models might not stem from AMPK activation *per se*, but from the deficient mitochondrial function.

A second argument that complicates the vision of AMPK as a crucial mediator of CR-induced effects is that the AMPK-dependency seems to be contingent on the particularities of the regime/diet and organism used for the experiment. It is evident that CR can promote, under certain conditions, lifespan extension in the absence of AMPK (34, 80). While it is generally assumed that AMPK might contribute to the lifespan extension effects of CR by promoting mitochondrial fitness, this hypothesis also raises some concerns. These concerns can be summarized as follows: 1./ the fact that CR promotes mitochondrial biogenesis and activity in mammals is still contested (17, 39, 85, 91, 102); 2./ CR can also promote longevity in respiratory-deficient yeast models (60); and 3./ there are numerous models indicating that, actually, decreased mitochondrial respiration can also provide CR-like effects on worms lifespan (23, 26, 72). A similar argument could be made for the hypothesis that AMPK might mediate CR-induced lifespan extension through SIRT1, as activation of SIRT1 upon CR has not been observed in some organisms (60, 63) or mammalian tissues (19), and there is also evidence indicating that SIRT1 can be dispensable in lower eukaryote models for the effects of CR on replicative lifespan (61, 80, 97).

A third weakness relates to the current pharmacological evidence obtained in mammals. A key feature of CR is that it can enhance healthspan and lifespan both in control and disease models. Rsv, which has been proposed as a CR mimetic, only extends mammalian lifespan when mice are fed a high-fat diet, but not in mice fed a regular chow diet, despite the clear metabolic effects that Rsv has on chow-fed mice (8, 88). Given that most of the metabolic effects of Rsv are attributed to AMPK activation (16, 108), this observation argues against the possibility that activation of AMPK per se could be enough to promote all CR-induced

effects. Similarly, while metformin improves healthspan in situations of disease, there is no report to date indicating that metformin can promote CR-like effects in otherwise healthy mammalian models. In contrast, the effects of the mTORC1 inhibitor, rapamycin, can be observed in regular rodents, suggesting that many of the effects from AMPK activation might be a secondary consequence from mTOR inhibition.

Conclusions and future directions

Genetic evidence in lower eukaryotes indicated that AMPK is required for many of the adaptations triggered by CR, including lifespan extension. Similarly, AMPK activation impacts on mitochondrial metabolism and on the activity of the FOXO, the sirtuin and mTOR signalling pathways, all of which have been tightly linked to CR and the promotion of a healthy longevity. Combined these arguments indicate that AMPK might be an important link to sense and adapt to CR. However, a number of caveats also indicate that the fact that AMPK can mimic certain aspects of CR does not necessarily mean that AMPK is the natural effector of the effects of CR. The clarification of these controversies will require future attention. The available AMPK deficient mouse models and the standardization of mammalian CR protocols will contribute to shed light into this issue. In any of these cases, the evidence reviewed here argues that AMPK activation can be a useful pharmacological tool to achieve a major number of the beneficial effects of CR. Culminating evidence clearly indicates that chronic feeding of rodents with AMPK agonists improves muscle endurance (67, 84), prevents against metabolic disease (67), allows proper circadian regulation (68) and suppresses tumorigenesis (50). For these reasons, it is likely that the unwillingness of mankind to engage in drastic lifestyle interventions, such as CR, will further strengthen AMPK's position as a main beacon of hope for the prevention and/or treatment of the current epidemic of metabolic and age-related diseases.

Acknowledgments

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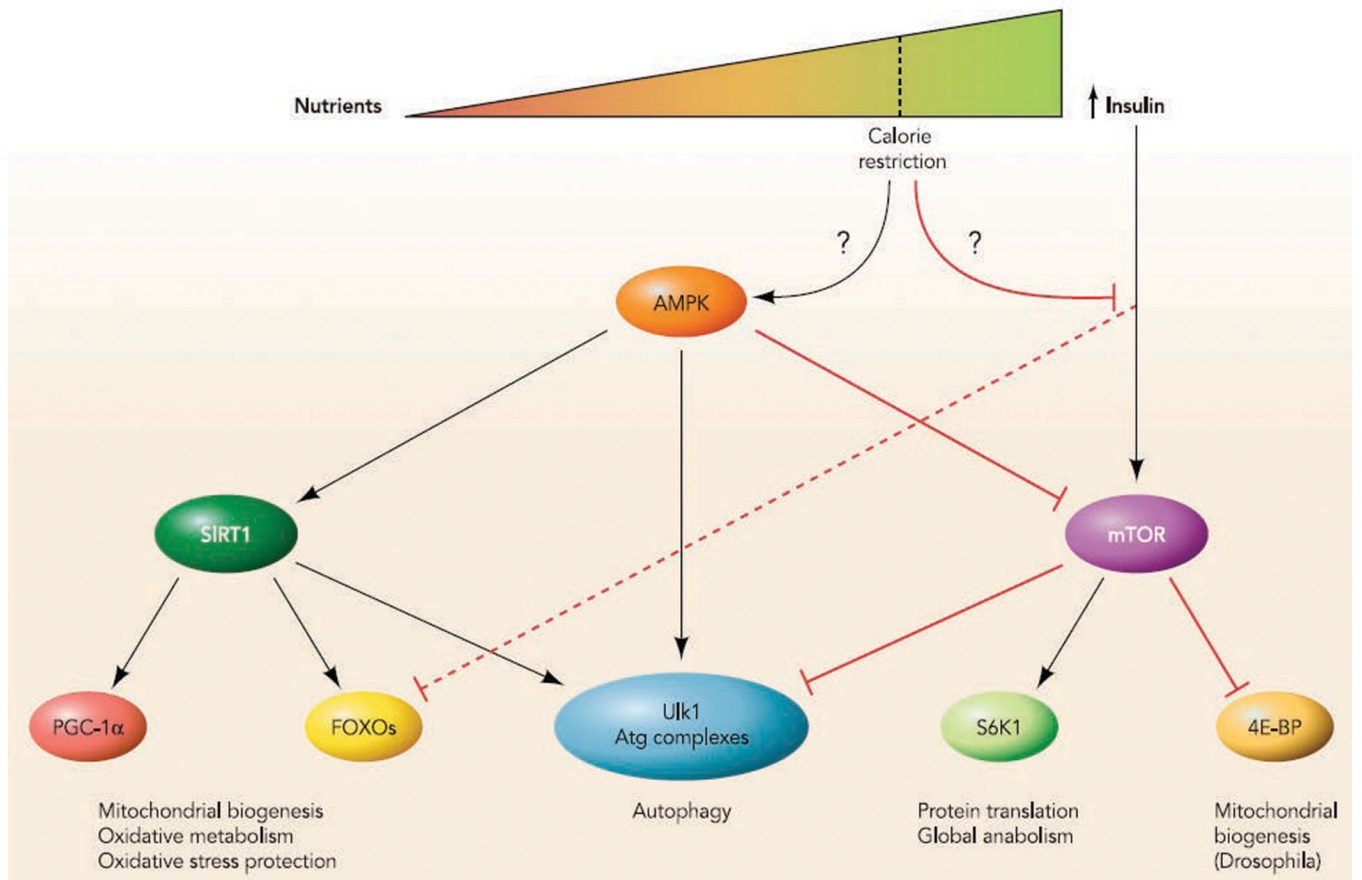
Figure 1.

	Yeast	Worms	Flies	Rodents
Evidence supporting a role for AMPK during CR	• Lack of AMPK (Snf1p) reduces lifespan • Snf1 is required for the use of nonfermentable substrates upon glucose restriction	• AMPK (aak-2) is required for most of the effects of CR and on lifespan • Overexpression of aak-2 increases lifespan • Glucose restriction activates aak-2	• Whole body and muscle-specific knockdown of AMPK is enough to decrease lifespan. • Loss of AMPK prevents lifespan extension upon CR	• Most CR-mimetics activate AMPK • AMPK interacts with virtually all pathways involved in longevity • AMPK agonists prevent the incidence of age-related diseases
Evidence against a role for AMPK during CR	• Forced overexpression of Snf1 reduces lifespan	• Aak-2 requirement for lifespan extension is regime dependent. • Some CR protocols do not require Aak-2 to enhance lifespan	• Models lacking AMPK have major phenotypes, complicating the evaluation of its role on lifespan.	• No conclusive genetic evidence available • AMPK activation upon CR is controversial • CR-mimetics failed to increase lifespan

Figure 2.