

Calorie restriction and NAD⁺/sirtuin counteract the hallmarks of aging

Shaday Michan¹

¹*Instituto Nacional de Geriatria, Institutos Nacionales de Salud, D. F., Mexico*

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1. ABSTRACT

Among diverse environmental factors that modify aging, diet has a profound effect. Calorie restriction (CR), which entails reduced calorie consumption without malnutrition, is the only natural regimen shown to extend maximum and mean lifespan, as well as healthspan in a wide range of organisms. Although the knowledge about the biological mechanisms underlying CR is still incipient, various approaches in biogerontology research suggest that CR can ameliorate hallmarks of aging at the cellular level including telomere erosion, epigenetic alterations, stem cells depletion, cellular senescence, mitochondrial dysfunction, genomic instability, proteostasis imbalance, impaired nutrient sensing and abnormal intercellular communication. Currently, the NAD⁺/sirtuin pathway is one of the few mechanisms described to mediate CR effects and sirtuin-activating compounds (STACs) mimic many effects of CR. Herein, we discuss the effects of CR on healthspan with emphasis on neuroprotection, how CR counteracts cellular aging, how sirtuin pathways intertwine with CR, and the relevance of STACs in mimicking CR effects.

2. INTRODUCTION

It has been nearly eighty years since Clive M. McCay first demonstrated that a decrease in energy intake by 30 % increased lifespan in white rats (1). Since then a large body of experiments has confirmed that calorie restriction (CR), i.e., a reduction of calorie consumption to 60-90% of a normal balanced diet without malnutrition can increase longevity of a variety of species, from unicellular yeast to multicellular fungus and metazoans including fruit flies, worms, fish, rodents, non-human primates and probably humans as well (2–8). Houseflies are an exception to the rule, however (9). The findings in primates are less clear, with conflicting results in the only two large-scale primate studies reported thus far. While CR did not increase the average lifespan of the rhesus monkey cohort at the NIH, a cohort of similar monkeys housed in Wisconsin lived longer on a CR diet (10,11). Although both cohorts were fed a 30%-reduced calorie diet, factors such as differences in food composition, genetic background and experimental design may account for these discrepancies. Nonetheless, CR is the only non-genetic strategy known to extend not only mean and maximum

lifespan but also healthspan by delaying the onset of age-related disorders such as cancer, cardiovascular disease, diabetes, inflammation, arthritis, diverticulosis, neurodegeneration, and cognitive and motor impairment (10–13). Experiments in rodents and monkeys have shown that CR ameliorates several changes associated with aging including sarcopenia, high blood pressure, osteoporosis, endometriosis, body fat accumulation, and glucoregulation imbalance (5). CR decreases basal metabolic rate and energy expenditure but maintains higher physical activity in rhesus monkeys (14). Reduced body core temperature has been associated to a prolonged life (15), yet it is not surprising that by regulating energy homeostasis CR may induce hypothermia. In fact, similar to rodents and monkeys, humans fed a CR diet for 6 years sustained lower body temperature as compared with subjects who consumed a typical Western diet (16).

3. EFFECTS OF CR IN COGNITION AND NEUROPROTECTION

CR has been shown to protect against age-related cognitive decline and associated brain changes. For example, CR attenuates cognitive decline due to normal aging in rodents (17) and improves motor coordination, locomotor activity and maze learning (18). CR also enhances associative memory in the mouse and facilitates synaptic plasticity through mechanisms dependent on NMDA receptors (19). In rats, CR stabilizes age-related loss of synaptic proteins in the hippocampus as well as sustains hippocampal-dependent spatial learning at old age as revealed by the Morris water maze (20). In the senescence-accelerated mouse strain P8 (SAMP8), CR reduces performance defects in a passive avoidance task (21), while in humans, short-term CR facilitates memory performance (22).

CR ameliorates many markers of brain aging in non-human primates, such as the accumulation of non-heme iron, white matter atrophy, pathogenic amyloid-beta deposition and astrogliosis in non-human primates (23–25). It also delays motor neurons death due to aging in rats (26). Similarly, at the mouse skeletal neuromuscular junctions, CR reverses age-associated deterioration including pre- and postsynaptic abnormalities and loss of motor neurons and muscle fibers (27). Alternatively, CR exerts a neuroprotective role in the nervous system by stimulating astrocytes functioning including glutamate uptake and glutamine synthetase activity in the rat hippocampus (28). Also, CR increases resistance to seizures in rats (29).

A number of studies show that CR improves signs of neurodegeneration in diverse disease models. For instance, it sharply suppresses age-related paralysis in nematode models of both Huntington's disease (HD) and Alzheimer's disease (AD) in which a 35 polyglutamine track or human amyloid-beta 42, respectively, are expressed in the body wall. In addition, CR was able to extend median and maximum survival of the AD worm model (30). Similarly, in a genetic mouse model of AD, 3xTgAD, CR lowered the levels of pathogenic amyloid-beta peptides 40 and 42, and phospho-tau in the

hippocampus as well as improved age-related cognitive performance (31). Furthermore, a 4 month CR regimen benefitted mice with double knockout of presenilin-1 and presenilin-2 specific to the forebrain, which recapitulate many of the neurodegenerative phenotypes of AD, improving novel object recognition and contextual fear conditioning memory and attenuating ventricle enlargement, caspase-3 activation, astrogliosis and tau hyperphosphorylation (32). In a neurotoxin-induced model of Parkinson's disease in the rhesus monkey, CR was shown to mitigate the severity of neurochemical deficits and motor dysfunction, presumably by increasing the levels of glial cell line-derived neurotrophic factor, which may promote the survival of dopaminergic neurons (33).

4. CR COUNTERACTS HALLMARKS OF CELLULAR AGING

At present it is not well understood how CR impacts cellular processes nor which specific molecular mechanisms underlie its effects. Nonetheless, research is accumulating that reduced caloric intake may counteract cellular hallmarks of aging. This next section reviews how CR is known to impact the nine hallmarks of cellular aging as proposed by López-Otín and colleagues, which include: 1) genomic instability, 2) telomere erosion, 3) epigenetic alteration, 4) proteostasis imbalance, 5) impaired nutrient sensing, 6) mitochondrial dysfunction, 7) cellular senescence, 8) stem cells depletion and 9) abnormal intercellular communication (34).

4.1. Genomic instability is reduced by CR

CR has been shown to protect the genome from damage in organisms ranging from yeast to mammals. In yeast, CR decreases rDNA recombination and the formation of extrachromosomal DNA circles, where this accumulation is considered a cause of aging (35,36). In rodents, CR ameliorates DNA repair inefficiencies that tend to increase with age in many repair processes including non-homologous end joining (NHEJ), nucleotide excision repair (NER), and base excision repair (BER). For instance, CR improves NHEJ by increasing XRCC4, which forms a complex with ligase 4 to enhance joining activity (37). Also, CR increases the fidelity of both polymerases alpha and beta in aged animals (38) and decreases the age-dependent decline in NER across the genome (39). Reduced BER is detected in many aged animal tissues such as brain, liver, spleen and testes and is counteracted by CR through upregulation of the rate-limiting enzyme in the BER pathway, DNA polymerase beta, at the level of protein, mRNA and enzyme activity. Interestingly, CR also stimulates BER in young animals, possibly conferring an anti-tumor effect at early ages (40). In BER pathway as well, the apyrimidinic/apurinic endonuclease (APE) is a key protein for removing oxidative DNA lesions. CR counteracts the amelioration of APE activity with age in different areas of the brain, e.g., the frontal/parietal cortex, cerebellum, brainstem, midbrain and hypothalamus (41). CR does not however appear to reduce chromosomal aberrations related to aging in adult rhesus monkeys (42).

4.2. Telomere erosion is decreased by CR

The gradual loss of DNA at the ends of chromosomes, regions known as telomeres, is associated with a number of aging phenotypes and illnesses including dementia, diabetes mellitus, ulcerative colitis, cancer and metabolic syndrome. Telomeres deplete after approximately 50–70 cell divisions in humans, which eventually impairs cell reproduction leading to aging and death (43). Studies in mice show that CR attenuates age-associated telomere erosion in leukocytes and various tissues including lung, kidney-cortex and muscle fiber, and this effect synergizes with the increase in healthspan and longevity observed with overexpression of telomerase reverse transcriptase (TERT), a catalytic subunit of telomerase enzyme that elongates telomeres (44). Similarly, CR reduces telomere shortening in lens epithelial cells and leukocytes of brown Norway rats and humans, respectively. In contrast, however, no reversion of age-dependent telomere shortening was detected in skin or leukocytes in caloric-restricted rhesus monkeys (45,46).

4.3. Epigenetic alterations are counteracted by CR

CR modifies DNA methylation and histone remodeling, two major epigenetic pathways, which are commonly dysregulated with aging and lead to aberrant expression of key genes (47). CR influences activity of DNA methyltransferases (DNMT) to regulate TERT, p16 and p21 (48). In the mouse hippocampus, CR decreases immunoreactivity of DNMT3a, potentially impacting synaptic plasticity and cognition (49). In agreement, CR attenuates age-related changes in the levels of the epigenetic molecules 5-methylcytidine (5-mC) and 5-hydroxymethylcytosine (5-hmC) in the mouse hippocampus (50). Interestingly, 5mC and 5-hmC levels are significantly decreased in the hippocampus of AD patients and this negatively correlates with amyloid plaques and neurofibrillary tangle load (51).

As for histone remodeling, CR activates NAD⁺-dependent deacetylase SIRT1 and histone deacetylase HDAC1, inducing expression of master regulator genes, including HIF-1 α , HSF1, p53, PGC-1 α , Foxo, Ku70, as well as regulators of brain functions such as CLOCK, ADAM-10, BDNF, MAO-A, RAR β and Tau (52,53). Furthermore, CR may prevent age-related epigenetic changes induced by altered levels of HDAC2 in the mouse hippocampus (54). Thus, through modifications of epigenomic architecture, CR plausibly causes massive transcriptome changes. For instance, studies in *Drosophila* demonstrate that CR delays age-related loss of gene silencing (55). In addition, CR induced by intermittent fasting, upregulates 1708 genes involved in aging and stress response in *C. elegans* (56), while in mouse liver, CR significantly alters the expression of 2500 genes (57). Other DNA microarray analyses show that CR produces a shift towards a younger and healthier gene transcription profile in the skeletal muscle of mice, rats and humans (58,59). Also, the transcription profile of the presenilin double knockout mouse brain, revealed that besides mitigating various signs of AD, CR induces the expression of neurogenesis related proteins, while inhibiting transcription of genes involved in inflammation (32). In rhesus monkeys,

CR may reverse age-dependent microRNA expression to a younger profile in skeletal muscle (60). A recent study shows that the level of microRNA-80, which targets histone acetyltransferase CBP-1, is decreased by CR in *C. elegans*, leading to induction of CBP-1-dependent metabolic pathways such as the forkhead transcription factors daf-16/FOXO and heat shock factor-1 (HSF-1), which promote longevity and healthspan (61).

4.4. Proteostasis imbalance is modified by CR

The cellular proteome is vulnerable to environmental stress such as heat shock, oxidative damage and heavy metals. To maintain integrity under challenging conditions, cells mount a dynamic yet precise response, including changes in folding, trafficking, synthesis, breakdown and concentration of proteins. Thus, CR may confer protection against pathology and aging through counteracting protein imbalance at multiple regulatory levels (62).

A decrease in protein translation has a profound effect on aging (63), thus it is not surprising that the effects of CR are linked to mRNA translational changes. Inhibition of translational elongation machinery by eukaryotic elongation factor 2 kinase (eEF2K) promotes survival of cells and worms under nutrient deprivation (64). In addition, studies indicate that CR slows down translation rates by modulating the levels of critical components of mRNA translational machinery, e.g., 60S ribosomal subunit or the translational repressor 4E-BP (65,66). In fact, 4E-BP is required to extend fly lifespan under CR (66). The increase of 4E-BP by CR reduces global mRNA translation, while stimulating nuclear-encoded mitochondrial gene expression. Interestingly, 5'UTRs of upregulated mitochondrial products are shorter and have weaker secondary structures compared to the whole fly genome. Under this scenario CR seems to trigger differential regulation of protein translation within cells, favoring mitochondrial protein synthesis. Though the mechanisms of differential mRNA upregulation mediated by 4E-BP are not yet clear, this study suggests that it may be faster and require less energy than other forms of regulation, such as transcription or posttranslational modifications.

In line with the reduction in protein translation, a large-scale proteome study in mouse liver reveals that long term CR produces a massive decay in absolute protein synthesis and proteolytic rates. However, here it is suggested that rather than increasing mitochondrial translation, CR prolongs half-life specifically of mitochondrial proteins. It is important to highlight that in the context of this study, functionally related proteins displayed similar relative concentrations and replacement rates, suggesting a fine-tuned regulatory mechanism for coordinating the proteome turnover decay in CR (67). Whether CR stimulates protein synthesis, particularly in the mitochondria, remains controversial since several lines of evidence support the notion that CR stimulates protein biogenesis and degradation (68–70) instead of lessening protein turnover as discussed above. Additionally, no changes in protein levels have been detected in diverse rodent tissues under CR (71–73).

Autophagy regulates major clearance of long-lived proteins and cytoplasmic organelles including damaged mitochondria by sequestering deteriorated material into autophagosomes and delivering it to lysosomes for degradation (74). Studies show that CR enhances autophagy in several species including worms and mammals (75–77). In rats, CR increases the autophagy markers in the kidney and reverses age-dependent decay of a similar proteolytic pathway in liver cells (78,79). In addition, intact autophagy genes are essential to extend *C. elegans* lifespan in a genetic model of CR (80). Autophagy plays an important role in neuroprotection (81). Indeed, genetic specific ablation of the autophagy gene Atg5 in neurons causes progressive signs of neurodegeneration, including deficits in motor function and aggregation of cytoplasmic inclusion bodies in neurons (82). Similarly, lack of Atg7 specifically in the central nervous system causes massive neuronal loss, behavioral deficits and premature death in mice (83). Not unexpectedly given its large neuroprotective effect, CR causes a marked enhancement of autophagy in mouse neurons (84,85). In a mouse model of amyotrophic lateral sclerosis CR-induced autophagy stimulates degradation of mutant SOD1 aggregates early in the disease process, resulting in potential protective effects (86).

The proteasome is a proteolytic complex that targets proteins for degradation through the ubiquitin-proteasome system (UPS). Conflicting data show that either an increase or decrease in proteasome activity has been linked to aging (87,88). For instance, neurons of aging worms display reduced levels of UPS-mediated proteolysis (89) and also a depletion of UPS activity is linked to neurodegeneration (90,91). CR, however, enhances this pathway in yeast and rat brown adipose tissue (92–94). Accordingly, studies in worms indicate that proteins involved in the ubiquitination of substrates, including a E3 ubiquitin ligase (WWP-1) and a E2 ubiquitin conjugating enzyme (UBC-18), are indispensable for the prolongation of lifespan by CR (95). In agreement, recent large-scale approaches aimed at analyzing transcriptional pathways involved in CR reveal that intermittent fasting induces UPS-dependent protein degradation in worms (56). In contrast, CR improves cardiac functioning in rats by doing the opposite, i.e., by counteracting the age-associated increase of the UPS (96). This agrees with several other studies, which demonstrate that activity of proteasome system increases with aging in yeast, mole rats, and centenarians (97–99).

Aberrant protein folding and aggregation are linked to various age-related pathologies and neurodegenerative disorders such as Alzheimer's, Parkinson's, and Huntington's diseases. The heat shock response is a cytoprotective mechanism against proteotoxicity that plays a critical role in the maintenance of proteostasis. It involves the prompt and massive expression of molecular chaperones besides additional protective components that stabilize and fold nascent products, prevent misfolding and conformational changes to proteins by denaturing conditions and/or promoting recovery of damaged proteins (100). Experiments in *C.*

elegans demonstrate that CR synergizes with heat shock to induce the heat shock response (101). CR protects against proteotoxicity, although without clearing aggregation, in three different worm models of disease, including polyglutamine tracts, amyloid-beta 42 and an accumulation prone form of GFP. Instead, the activity of the heat shock transcription factor (HSF-1), a master regulator of heat shock response conserved from yeast to humans, is required for thermotolerance, protection against proteotoxicity and lifespan extension by CR (30). Interestingly, recent investigations suggest that heat shock response controls autophagy (102). Thus, it remains to be elucidated how the molecular mechanisms of protein homeostasis are intertwined in response to CR.

4.5. Mitochondrial dysfunction is relieved by CR

It is well accepted that CR protects mitochondrial integrity and function, though the mechanisms underlying these beneficial effects are still a matter of controversy. Experiments in diverse models from yeast to mammals demonstrate that CR prolongs lifespan by increasing respiration (103–105). In line with this, CR induces the expression of a number of genes encoding proteins involved in energy metabolism (106). Also, it raises mitochondrial membrane permeabilization, electron transport chain and ATP production, while reducing energy expenditure and the steady-state levels of reactive oxygen species (ROS), thus minimizing oxidative damage to DNA and proteins (73,107–110). The reduction of ROS by CR, in addition to bursting antioxidant defenses, may be explained by a decrease in mitochondrial proton leak (i.e., proton flux across the mitochondrial inner membrane independent of ATP formation) (109). Accordingly, it has been suggested that CR lowers the steady-state reduction degree of complex I, thus decreasing the release of ROS per unit electron flow, without changing oxygen consumption (111). Thus, it has been proposed that CR may induce lifespan extension and cellular protection by reducing the generation of ROS in the mitochondria (112). This premise agrees with the inverse correlation between H₂O₂ generation and maximum lifespan revealed by a study with several species including fly, mouse, rat, guinea pig, rabbit, pig, and cow (113), as well as with studies in yeast showing that disruption of mitochondrial superoxide dismutase (SOD2) significantly shortens lifespan by CR (114). Likewise, CR prevents neurodegeneration caused by increased oxidative damage in worms (115) and in the aging rodent nervous system (116–118). A decrease in oxidative stress by CR may be due to the induction of the peroxisome proliferation-activated receptor coactivator 1 alpha (PGC-1alpha), which is a master regulator of mitochondrial function that acts in response to specific metabolic demands to enhance bioenergetics (70).

In contrast, several lines of evidence indicate that CR favors the generation of damaging agents that induce *hormesis* (119). This concept was first adapted in a biogerontological context by Rathan (2001) and refers to the beneficial effects triggered by the exposure to a mild repeated stress, which activates cellular responses that protect against damage or that repair the damage once it occurs (119). Thus, mitochondrial *hormesis* strengthens

cells by keeping stress resistance machinery “awake” ready for coping with challenging conditions. Consistent with this concept, experiments show that CR increases ROS formation, catalase activity, oxidative stress resistance and survival rates in worms, and treatment with different antioxidants and vitamins no longer sustains lifespan extension (104). Inhibition of mitochondrial complex I, which is known to increase ROS, mimics CR effects in *C.elegans*, such as increase lifespan extension, activity and stress resistance. Animals treated with complex I inhibitors do not respond to lifespan extension by CR and neither do those with ablation of redox-sensing neurons, suggesting that redox signal is essential for mediating CR effects on longevity (120). CR also provides neuroprotection to the rat brain by preventing apoptosis through counteracting cytochrome c release from the mitochondria and decreasing caspase-2 activity, in an event mediated by ARC (apoptosis repressor with a caspase recruitment domain) (121). However, other studies have detected no changes in mitochondrial metabolic parameters by CR (122).

Whether CR exerts its protective effect by increasing the synthesis of mitochondrial material has also been a long-standing debate. A number of studies suggest that CR enhances mitochondrial biogenesis (68–70,123,124). For instance, the many mitochondrial proteins, including cytochrome c oxidase, citrate synthase, and mitofusin, increased in the brain, as do respiration rates, in a nitric oxide-dependent way to promote neuronal survival under CR conditions (125). Conversely, other data show that CR improves mitochondrial function without increasing mitochondrial content, by reducing protein translation as reviewed in the section above (72,73,105,114). In accordance, recent studies reveal that CR may confer protection against mitochondrial proteotoxicity through the upregulation of prohibitin levels (126). Prohibitins are evolutionary conserved proteins located in the inner mitochondrial membrane, which mediate mitochondrial protein folding, assembly of the electron transport chain, regulation of mitochondrial proteases, and maintenance of mitochondrial membrane and cristae structure (127). Lack of prohibitins promotes proteotoxicity and induces a mitochondrial unfolded protein response. Interestingly, CR counteracts proteotoxic stress in prohibitin mutants of yeast and worms through the reduction of cytoplasmic mRNA translation (114).

4.6. Cellular senescence is reversed by CR

Cellular or replicative senescence prevents cells from proliferating indefinitely. Telomere attrition, DNA damage and impaired mitogenic signals may cause a senescent phenotype that is characterized by: 1) growth arrest unresponsive to mitogens, 2) resistance to apoptosis, and 3) adoption of abnormal differentiated functions (128). Studies in the skin of baboons show that senescent cells increase exponentially with age (129). However, senescence does not appear to be restricted to dividing cells since postmitotic cells may also adopt a senescent phenotype. For instance, neurons of aging mice show multiple pro-inflammatory and pro-oxidant markers of senescence in response to DNA damage such as accumulation of reactive oxygen species, interleukin

secretion, activation of the lysosomal hydrolase beta-galactosidase (sen-beta-Gal) and heterochromatinization. The canonical senescence pathway, p21 (CDKN1A), mediates these effects in neurons (130).

CR protects many different types of cells from senescence. It delays immune senescence in nonhuman primates, which may contribute to extended lifespan by decreasing vulnerability to infections (131). Short-term CR in rats retards renal senescence (132), while in mice, a mild decrease in energy intake of about 28% for 3 months starting at 14 months of age, is sufficient to decrease not only the numbers of senescent cells in mouse mitotic tissues like intestine and liver, but also markers of senescence in Purkinje neurons (130).

4.7. Stem cells depletion is attenuated by CR

Research in this field has shown that CR has the influence of enhancing the capacity of stem cells to self-renew, proliferate, differentiate, and replace cells in several adult tissues as well as reprogramming stem-like cells (133). For instance, short-term CR promoted stem cells availability and activity in the muscle of young and old animals and this effect correlated with an increased amount of mitochondria and activation of metabolic and longevity regulators (124). Interestingly, CR not only improved endogenous muscle repair but also enhanced the contribution of donor cells to regenerating muscle after transplant in either donor or recipient animals (124). In the mouse hippocampus, long-term CR promotes survival of glial cells (134) and increases the number of divisions that neural stem and progenitor cells undergo in the aging brain (135). Experiments in rats reveal that CR enhances neurogenesis by reducing death of newly produced cells, instead of inducing cell proliferation. Interestingly, this effect was associated with an increase of brain-derived neurotrophic factor (BDNF) in hippocampal cells of animals under CR (136).

4.8. Impaired nutrient sensing is reprogrammed by CR

Only a few major evolutionary conserved pathways for sensing energy status have been identified as important regulators of healthspan and aging, namely, insulin/insulin growth factor I signaling (IIS) (137–141); serine/threonine kinases Sch9/S6K/Akt or mouse target of rapamycin (mTOR) (142–148); Ras/adenylate cyclase (AC)/cAMP-dependent protein kinase A (PKA) (148–150); adenosine monophosphate-activated protein kinase (AMPK) (151,152); and NAD⁺-dependent deacylases/ADP-ribosylases (sirtuins) (153). In several species including humans, CR counteracts the changes in nutrient-sensitive pathways that occur during aging and disease (154); for example, CR inhibits IGF-1/insulin, Ras/AC/cAMP, Sch9/S6K/Akt, and mTOR pathways while increasing AMPK and sirtuins (59,132,148,155–157). Also, transcription factors HSF-1 and SKN-1 as well as the eIF2 α kinase general control nonderepressible 2 (GCN-2) mediate CR-induced longevity (30, 158, 159) (Figure 1).

These master regulators integrate energy signals to impact a diverse array of cellular processes in response to CR. For instance, a decrease in mTOR activity may

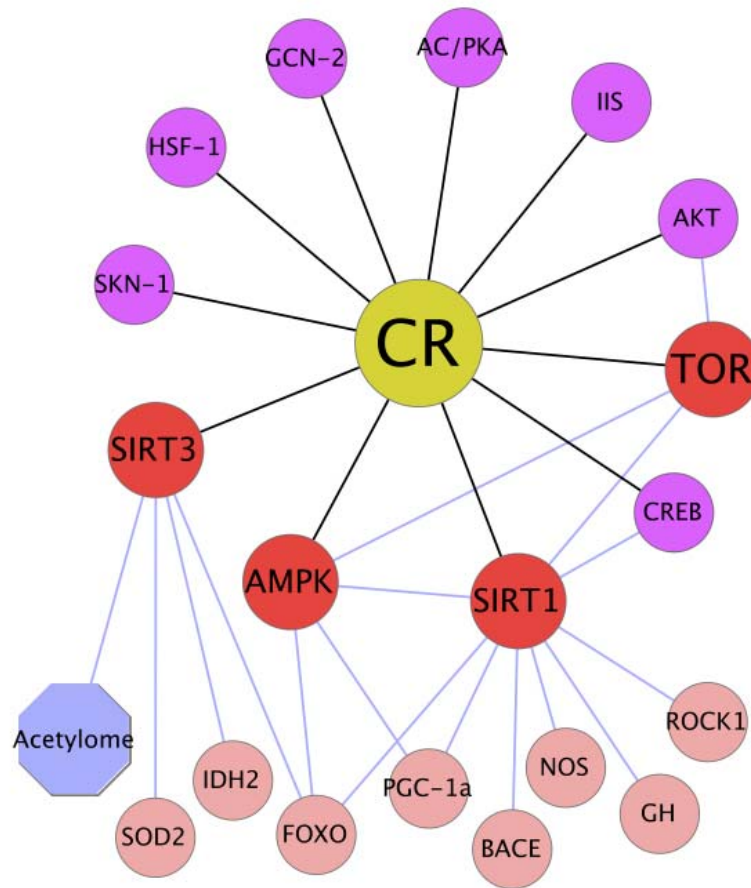


Figure 1. Molecular pathways that mediate the effects of calorie restriction. Scheme made with Cytoscape (239).

preserve stem cell function, decrease cellular senescence, mitigate inflammation, reduce protein synthesis and enhance mitochondrial metabolism (160). Also, a decrease in mTOR activity by CR is linked to a massive increase in autophagy (84). Recent studies show that mTOR regulates lysosomal ATP-sensitive two-pore Na (+) channels to couple cell energy levels to endolysosomal function during food restriction (161). Additionally, mTOR may regulate the expression of others key regulators including AMPK and Akt. Reduction of mTOR may also underlie diverse protective effects of CR in diseases such as cancer, progeria, polycystic kidney disease, obesity, macular degeneration, cardiac disease and neurodegeneration (160). Also noteworthy, studies in rodents suggest that both CR and mTOR inhibition display a positive effect in epilepsy by having anticonvulsant and antiepileptogenic effects (162–164).

AMPK participates in the induction of autophagy and decreases oxidative stress to protect cells from senescence under CR (132). It also regulates lipid oxidation. Interestingly, studies in worms suggest that

AMPK activity is required to promote lifespan extension under different CR regimens (165). AMPK also regulates mitochondrial fitness and stimulates mitochondrial biogenesis during food restriction through the activation of PGC-1alpha (166). It has been shown that AMPK suppresses cell growth and biosynthetic processes in response to energy stress, in part through inhibition of the mTOR pathway (167). Alternatively, AMPK may mediate the ability of CR to increase stress resistance by increasing the activity of FOXO transcription factors, similar to the effect seen by a decreased in IIS pathway (168,169).

In many species, ablation of components of the IIS pathway, including, ligands (insulin, IGF-1, IGF2), receptors (IGF-1R, INR, daf2), receptor substrates (CHICO, IRS1,2, IST-1), phosphoinositide-3-kinases (PI3K, AGE-1), PI3K antagonists (daf-18, PTEN), protein kinases (PDK, AKT), and downstream effectors such as the forkhead transcription factors (daf-16, FOXOs) increase lifespan and stress resistance to oxidative stress (170). Accordingly, CR induces physiological changes associated to the IIS pathway, e.g., it produces a decrease in growth

hormone, insulin, and IIS hormone axis and increase in insulin sensitivity (171). Mitigation of IIS signaling by CR has positive effects in diseases, for example, reduced levels of IGF-1 induced by CR significantly decreases tumor growth and metastasis in mice. Conversely, IGF-1 supplementation hampers the anti-tumorigenic effect of CR (172). Modifications of IIS pathway also have an important impact in protecting against proteotoxicity, a hallmark of many neurodegenerative diseases (140).

4.9. Altered intercellular communication is improved by CR

A number of studies in different models have shown that non-cell-autonomous signaling influences aging and age-related diseases. An example of this is the paracrine or endocrine function of insulin, insulin-like molecules and hormones of the IIS pathway, which may signal neighboring cells or those far from the tissue where it was produced. Accordingly, alteration of the IIS pathway either systemically or in a tissue specific manner including in fat cells, intestine, germ line or neurons, leads to lifespan extension (173). Consequently, CR modifies intercellular communication encompassing endocrine and paracrine function. Experiments demonstrate that worms rely on an endocrine mechanism mediated by two sensory neurons (ASIs) for achieving lifespan extension under food deprivation. In this system, CR induces the transcription factor SKN-1 in ASIs, which signal non-neuronal peripheral tissues to adjust metabolic status (158). At another site, CR enhances stem-cell function through inducing non-cell autonomous paracrine signaling in mammals mediated by mTOR. CR downregulates mTOR specifically in Paneth cells of the intestinal stem-cells niche, which stimulates the production of the paracrine factor, cyclic ADP ribose, to regulate stem-cell self-renewal (174).

In mammals, reduction of endocrine signals is a distinctive feature of CR, which decreases plasma concentrations of several different hormones such as insulin, triiodothyronine (T₃), thyroxine (T₄), growth hormone (GH), corticosterone and circadian rhythms of adenocorticotrophic hormone (ADCH) (175). Decreased secretion of pituitary, hypothalamic and target gland hormones influence a wide range of physiological processes and body functions, which in turn favor longevity and protect from age-related disorders.

5. THE NAD⁺/SIRTIIN PATHWAY

Sirtuins are a family of evolutionarily conserved proteins, which depend on the metabolic substrate NAD⁺ for the two types of enzymatic activities they display: mono ADP-ribosylation and deacylation. The latter enzymatic activity may include the removal of different acyl moieties of various carbon length, saturation, and chemical composition, such as acetyl, succinyl, malonyl, crotonyl, myristoyl, palmitoyl and lipoyl (176). Through these activities, sirtuins may target post-translationally either histones to regulate the epigenome or modify non-histone proteins to regulate the proteome. Seven members (SIRT1-SIRT7) form the human sirtuin family of proteins. All are

ubiquitously expressed in tissues, yet are specifically confined to cell compartments where they participate in cellular and physiological functions with specialized enzymatic activity (Table 1). For example, SIRT1, SIRT2 and SIRT3, are the most efficient in removing acetyl groups from lysine residues of proteins in the nucleus, cytoplasm and mitochondria, respectively. While SIRT4 has ADP-ribosyl transferase and SIRT5 acts as a robust desuccinylase and demalonylase in the mitochondria.

The first sirtuin identified, Sir2, was first described for extending lifespan in yeast (177), and later a similar effect was confirmed for its orthologs in worms (Sir2.1) (178) and in flies (dSIR2) (179). More recently, the role of Sir2 in regulating lifespan in simple metazoans has been challenged (180), but nevertheless, a growing body of evidence supports the notion that sirtuins influence the hallmarks of aging, delay the onset and improve the prognosis of age-related diseases, or even extend lifespan in mice. For instance, overexpression of SIRT1 specifically in the brain or ubiquitous overexpression of SIRT6 increases lifespan (181,182). SIRT1 is the most comprehensively studied sirtuin and as shown in Table 1 several protective functions have been described for it in the context of cancer, inflammation, cardiac hypertrophy and metabolic imbalance (52,183,184). In the brain, SIRT1 targets diverse pathways including circadian rhythms (CLOCK), anxiety (MAO-A), learning, memory and synaptic plasticity (YY1-CREB-BDNF and Insulin ERK1/2), neurogenesis and neuroendocrine function (53,185–190). Mouse strains engineered to overexpress SIRT1 in brain display protective effects when bred to disease models of neurodegeneration; including models of Alzheimer's, Huntington's, and Parkinson's (191–193). In contrast, genetic deletion of SIRT2 leads to neuroprotection in PD (194). Interesting, recent studies show that the pathogenic risk allele ApoE4 linked to AD reverses the SIRT1/SIRT2 protective ratio, suggesting that sirtuin dysregulation might account for ApoE4 malignancy (195). Studies have shown that sirtuins also impact major metabolic pathways through deacetylation and succinylation of the mitochondrial proteome by SIRT3 and SIRT5, respectively (196).

6. CR EFFECTS MEDIATED BY THE NAD⁺/SIRTIIN PATHWAY

The role of sirtuins on aging was described 15 years ago and ever since remarkable progress has been made in understanding their participation in modulating cellular adaptations triggered by CR. At present there is mounting evidence that sirtuin pathway underlie healthspan effects of CR in different settings including aging, cancer, inflammation, cardiopathy, metabolic imbalance and neurodegeneration. SIRT1 and SIRT3 are induced by CR in diverse tissues (132,197–199); for example, increased levels of SIRT1 induced by CR promote mammalian cell survival (200). Together with mTOR and AMPK, SIRT1 activation protects from kidney senescence (132). While sirtuin ablation hampers CR response, overexpression mimics phenotypes seen under CR (181,201). Studies in knockout mice show that SIRT1 is essential for lifespan

Table 1. Features of the seven human sirtuins

Sirtuin	Cellular localization	Enzymatic activities	Cellular functions	Physiological involvement	Targets
SIRT1	Nucleous (euchromatin) and shuffles to cytoplasm	Strong deacetylase Long fatty acid deacylase	DNA repair, genome instability, intercellular communication, epigenetic regulation, cellular communication, proteostasis, nutrient sensing, glucose and lipid metabolism, mitochondrial function, cellular senescence, stem cells differentiation and proliferation, hypoxia cellular response, apoptosis, autophagy	Neurodegeneration, retinopathy, learning and memory, anxiety, synaptic plasticity, dendritic branching, neurogenesis, immune response, cancer, cardiac hypertrophy, diabetes, circadian rhythms, aging	P53, p65, p73, FOXO3a, FOXO1, FOXO4, cortactin, Tau, RARbeta, MAO-A, HTT, Pax3, XPA-1, HIF-1alpha, STAT3, NF-1alpha, PGC-1alpha, beta-catenin, TSC2, TORC, TIP60, p300, CREB, HDAC1, APE1, FXR, H3, MeCP2, E2F1, BALM1, PER2, PLM, LXR, YY-1, IRS-2, SOD2, Necdin, Ku70, Atg5, Atg7, Atg8, Nrf2, HES1, HEY2, TAF4, Mcm10, USP22, SAGA, Tat/HIV-1
SIRT2	Cytoplasm and shuffles to the nucleus	Strong deacetylase Long fatty acid deacylase	Cell cycle regulation, stress tolerance, proteostasis, stem cell differentiation, programmed necrosis	Brain inflammation, myelination, neurodegeneration, tumorigenesis, adipocyte and oligodendroglia differentiation	Alpha-tubulin, p65, FOXO1, FOXO3a, H3, H4, HOXA10, 14-3-3beta/gamma, cortactin, APC, CDC-20, eIF5A, Par3, p65, TORC, CDK9, RIP1
SIRT3	Mitochondria	Strong deacetylase Long fatty acid deacylase	Mitochondrial function, ketone metabolism, oxidative stress, fatty acids and urea cycle	Tumorigenesis, cardiac hypertrophy, hearing loss, metabolism	Ciclofilina D, MDH, MRPL10, SOD2, HMGCs2, Idh2, LCAD, Ku70, CypD, FOXO3a, SDH, AceCS2, GDH, OTC, Skp2
SIRT4	Mitochondria	ADP-ribosyl transferase Weak deacetylase Long fatty acid deacylase	Mitochondrial function, fatty acids oxidation, energy homeostasis, apoptosis,	Tumorigenesis, insulin secretion, glutamine metabolism.	GDH, IDE, ANT2, ANT3, MCD
SIRT5	Mitochondria	Strong desuccinylase Strong demalonylase Weak deacetylase Long fatty acid deacylase	Urea cycle, apoptosis, oxidative stress	Metabolism	CPS1
SIRT6	Nucleus (heterochromatin)	ADP-ribosyl transferase Strong demyristoylase Weak deacetylase Long fatty acid deacylase	DNA repair, glucose homeostasis, genome stability, epigenetic regulation, cellular senescence, telomere maintenance,	Aging, tumorigenesis, cardiac hypertrophy, inflammation, obesity, liver function	CtIP, H3, GCN5, HIF1-alpha, c-Jun, H3
SIRT7	Nucleolus	Weak deacetylase	Apoptosis, epigenetic regulation, stress resistance	Inflammatory cardiomyopathy, fatty liver, tumorigenesis	p53, PAF53

extension by CR and that it regulates metabolic rate and the increase in physical activity in response to low-calorie intake (202,203).

Neuronal SIRT1 is required for CR-dependent reduction of somatotrophic signaling, which modulates GH and/or IGF-I synthesis and availability. In contrast to other tissues, reduced SIRT1 in hypothalamic neurons, rather than elevated levels, is linked to this phenomenon, which agrees with the positive regulation of GH by SIRT1 (204). An interplay between SIRT1 and cAMP responsive-element binding (CREB)-1 —both necessary for CR response— may regulate in part the effects of low-calorie diet in behavior, memory and synaptic plasticity. CREB induces SIRT1 expression, which targets PGC-1alpha and NO synthase (NOS) in neurons to initiate responses to enhance brain fitness (205). In worms, Sir2.1 is necessary for the protective effect of CR against neurodegeneration of dopaminergic neurons (206) and also mediates the

induction of heat shock response to counteract polyglutamine proteotoxicity (101). In mice, CR-dependent induction of SIRT1 reduces amyloid-beta toxicity thorough downregulation of the serine/threonine Rho kinase (ROCK1), which in turns increases the activity of the non-amyloidogenic alpha-secretase (207). Alternatively, in response to energy deficiency SIRT1 in cooperation with PGC-1alpha and PPARgamma (peroxisome proliferator-activated receptor gamma) reduces the expression of beta-secretase (BACE1) —the rate-limiting enzyme for amyloid-beta generation— in the rodent brain (208).

In the mitochondria both CR and SIRT3 play a large role in regulating protein acetylation and thereby cellular metabolism. SIRT3 is a major regulator of metabolic responses to CR through deacetylating proteins involved in mitochondrial function and metabolism (209). For instance, SIRT3 has been shown to mediate the protective effects of CR against oxidative damage by deacetylating and enhancing the activity of the

mitochondrial antioxidant enzyme superoxide dismutase 2 (SOD2) (210). Also under CR, SIRT3 targets and induces isocitrate dehydrogenase 2 (IDH2), which increases both NADPH levels and reduced glutathione in mitochondria. This molecular mechanism, which decreases oxidative stress, also underlies the protective effects of CR against age-associated hearing loss (211). SIRT3 upregulation in the liver by low-energy uptake activates fatty acid oxidation (199). In contrast to the beneficial effects of SIRT3 induction by CR, a lack of SIRT4 in the mitochondria mimics the increase in amino acid-stimulated insulin secretion observed under CR (212). Higher levels of SIRT5 protein have been detected in the brain of calorie-restricted rats compared to those fed *ad libitum*, but the physiological impact of elevated SIRT5 remains to be determined.

7. SIRTUIN-ACTIVATING COMPOUNDS MIMIC CR

The search for sirtuin activating compounds (STACs) led to the identification of resveratrol—a plant-derived polyphenol—as a potent SIRT1 inducer in a study that screened about 500 natural compounds (213). Resveratrol was found to extend lifespan in diverse species including yeast (213), worms (214), bees (215) and the fish *Nothobranchius furzeri* (216).

Likewise, resveratrol increases the survival and healthspan of mice fed a high-fat diet (60 % fat), reversing their physiological patterns to those observed with a normal diet. Resveratrol increases insulin sensitivity and reduces levels of IGF-1. It also increases the number of mitochondria and induces SIRT1 and the activity of the master regulators of energy metabolism AMPK and PGC-1 α (217,218). While resveratrol does not extend the lifespan of mice fed a normal diet, it does lead to changes in gene expression in multiple tissues similar to those observed with CR and delays symptoms of age-related deterioration, including inflammation, vascular endothelial apoptosis, albuminuria and cataract formation, and increases elasticity of the aorta, motor coordination and bone mineral density (219). In line with this, transcriptional shifts in heart, skeletal muscle and brain induced by resveratrol mimic those observed by CR. For instance, both CR and resveratrol downregulate gene pathways involved in cardiac and skeletal muscle aging, thus reducing age-related dysfunction in those tissues. Resveratrol parallels the effects of CR in insulin mediated glucose uptake in muscle (220). Strikingly, treatment with resveratrol for 30 days in obese humans also produces metabolic changes mimicking the effects of CR. Specifically, resveratrol-treated subjects slept less, had a lower resting metabolic rate, and their muscles showed elevated SIRT1 and PGC-1 α proteins, increased activity of AMPK and citrate synthase as well as increased mitochondrial respiration without changing mitochondrial content (221).

A large body of research has documented the effects of resveratrol in multiple pathologies and aging-related disorders (222). For example, it reduces vascular endothelial inflammation by increasing autophagy through

concerted activation of various components of the CR response, including cAMP-PKA-AMPK-SIRT1. Also, autophagy activation by resveratrol through AMPK-SIRT1 plays a neuroprotective role in cellular models of PD. Likewise, this polyphenol, protects from neurodegeneration in model organisms of diseases, such as multiple sclerosis (223), HD (224,225), PD (226) and AD (227). Noteworthy, by activating SIRT1, resveratrol alleviates the premature aging phenotype in a mouse model of progeria including adult stem cell exhaustion, body weight loss, trabecular bone structure and mineral density impairment. Consequently, progeroid mice live longer on a CR diet (228).

At present, other pharmacologically synthesized STACs have also been tested, e.g., SRT1720, SRT3657, and A3. For instance, SRT1720 promotes healthspan and survival in obese mice by alleviating metabolic disorders induced by a high fat diet (229,230). SRT365 has a neuroprotective effect against DNA damage in neurons (231) as well as in the mouse model of AD, CDK5-p25 (232). As for compound A3, it decreases infarct volume in a model of cerebral ischemia (233). In addition to regulating SIRT1 by an allosteric mechanism (234), STACs are also thought to act through parallel pathways including AMPK, S6 kinase, NF- κ B, interleukins, FOXO3 and ERK1/2 (222,235–238).

8. CONCLUDING REMARKS

CR has a large effect on aging and healthspan by counteracting the nine cellular hallmarks of aging: 1) genomic instability, 2) telomere erosion, 3) epigenetic alteration, 4) proteostasis imbalance, 5) impaired nutrient sensing, 6) mitochondrial dysfunction, 7) cellular senescence, 8) stem cells depletion and 9) abnormal intercellular communication. However, it not clear as yet to what extent CR impacts each of the cellular hallmarks. For example, it is not well understood how CR affects telomeres, genomic instability and stem cell function, whereas the mechanisms through which it prevents mitochondrial dysfunction and modifies protein proteostasis are matters of controversy. Neither is it understood the differential regulation of nutrient sensing pathways implicated in CR in the variety of mammalian tissues. Furthermore, there are still several unresolved questions about the molecular mechanisms underlying the effects of CR. At present only a few pathways have been identified as mediators of CR effects including mTOR, AMPK, IIS and sirtuins, and although a crosstalk among them is likely, it is not clear how these pathways coordinately produce the benefits of CR. The NAD⁺ sirtuin pathway, discussed in some detail here, is a molecular mechanism that deserves further exploration. Although SIRT1 has been the focus of studies thus far, other sirtuins are emerging as important regulators, for example SIRT3, which has a striking effect on metabolism through deacetylating a large fraction of mitochondrial proteome. Despite the potential relevance of SIRT3, it is unknown how this sirtuin affects brain function, or how it may be linked to CR-induced neuroprotection. Besides deacetylation activity, sirtuins also regulate proteins by

removing others acyl groups such as malonyl, succinyl and long fatty acids. It therefore remains to be elucidated how these various activities are altered under CR conditions and how they may regulate cellular processes in different tissues. STACs, which mainly activate SIRT1, display CR-mimetic effects, yet activators for the other sirtuins still remain to be discovered.

Whether CR extends lifespan in humans is still an open question, yet the diverse benefits of CR with respect to healthspan and longevity in many species including primates, indicate that future research in this field has important implications for humans. However, even if CR was unequivocally shown to promote healthy longevity in humans, the difficulties of adhering to a low calorie diet would limit the successful implementation of this diet. Thus, understanding the molecular mechanisms underlying the beneficial effects of CR is particularly important, and would allow for the exploration of novel avenues to mimic the metabolic and functional changes produced by CR.

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10. REFERENCES

1. C McCay, M Crowell, L Maynard: The effect of retarded growth upon the length of life span and upon the ultimate body size *J Nutr* 10, 63–79 (1935)
2. J Smith, JM McClure, M Matecic, JS Smith: Calorie restriction extends the chronological lifespan of *Saccharomyces cerevisiae* independently of the Sirtuins. *Aging Cell* 6, 649–662 (Blackwell Publishing Ltd, 2007)
3. AD van Diepeningen, MFPM Maas, DHEW Huberts, DJ Goedbloed, DJP Engelmoer, SM Slakhorst, AB Koopmanschap, F Krause, NA Dencher, CH Sellem, A Sainsard-Chanet, RF Hoekstra, AJM Debets: Calorie restriction causes healthy life span extension in the filamentous fungus *Podospora anserina*. *Mech Ageing Dev* 131, 60–68 (2010)
4. PYY Wang, N Neretti, R Whitaker, S Hosier, C Chang, D Lu, B Rogina, SL Helfand: Long-lived Indy and calorie restriction interact to extend life span. *Proc Natl Acad Sci U S A* 106, 9262–9267 (2009)
5. RM Anderson, D Shanmuganayagam, R Weindruch: Calorie Restriction and Aging: Studies in Mice and Monkeys *Toxicol Pathol* 37, 47–51 (2009)
6. LWS Finley, MC Haigis: The coordination of nuclear and mitochondrial communication during aging and calorie restriction. *Ageing Res Rev* 8, 173–188 (2009)
7. ED Smith, TL Kaeberlein, BT Lydum, J Sager, KL Welton, BK Kennedy, M Kaeberlein: Age- and calorie-independent life span extension from dietary restriction by bacterial deprivation in *Caenorhabditis elegans*. *BMC Dev Biol* 8, 49 (2008)
8. GS Roth, MA Lane, DK Ingram, JA Mattison, D Elahi, JD Tobin, D Muller, EJ Metter: Biomarkers of caloric restriction may predict longevity in humans. *Science* 297, 811 (2002)
9. TM Cooper, RJ Mockett, BH Sohal, RS Sohal, WC Orr: Effect of caloric restriction on life span of the housefly, *Musca domestica*. *FASEB J* 18, 1591–1593 (2004)
10. RJ Colman, RM Anderson, SC Johnson, EK Kastman, KJ Kosmatka, TM Beasley, DB Allison, C Cruzen, HA Simmons, JW Kemnitz, R Weindruch: Caloric Restriction Delays Disease Onset and Mortality in Rhesus Monkeys. *Science* 325(5937), 201–204 (2009)
11. JA Mattison, GS Roth, TM Beasley, EM Tilmont, AM Handy, RL Herbert, DL Longo, DB Allison, JE Young, M Bryant, D Barnard, WF Ward, W Qi, DK Ingram, R de Cabo: Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study. *Nature* 489, 318–321 (2012)
12. J Nikolich-Zugich, I Messaoudi: Mice and flies and monkeys too: caloric restriction rejuvenates the aging immune system of non-human primates. *Exp Gerontol* 40, 884–893 (2005)
13. R Weindruch, RL Walford, S Fligiel, D Guthrie: The retardation of aging in mice by dietary restriction: longevity, cancer, immunity and lifetime energy intake *J Nutr* 116, 641–654 (1986)
14. Y Yamada, RJ Colman, JW Kemnitz, ST Baum, RM Anderson, R Weindruch, DA Schoeller: Long-term calorie restriction decreases metabolic cost of movement and prevents decrease of physical activity during aging in rhesus monkeys. *Exp Gerontol* 48, 1226–1235 (2013)
15. B Conti, M Sanchez-Alavez, R Winsky-Sommerer, MC Morale, J Lucero, S Brownell, V Fabre, S Huitron-Resendiz, S Henriksen, EP Zorrilla, L de Lecea, T Bartfai: Transgenic mice with a reduced core body temperature have an increased life span. *Science* 314, 825–828 (2006)
16. A Soare, R Cangemi, D Omodei, JO Holloszy, L Fontana: Long-term calorie restriction, but not endurance exercise, lowers core body temperature in humans. *Aging (Albany NY)* 3, 374–379 (2011)
17. LW Means, JL Higgins, TJ Fernandez: Mid-life onset of dietary restriction extends life and prolongs cognitive functioning. *Physiol Behav* 54, 503–508 (1993)
18. DK Ingram, R Weindruch, EL Spangler, JR Freeman, RL Walford: Dietary restriction benefits learning and motor performance of aged mice. *J Gerontol* 42, 78–81 (1987)
19. A Fontán-Lozano, JL Sáez-Cassanelli, MC Inda, M de los Santos-Arteaga, SA Sierra-Domínguez, G López-Lluch, JM Delgado-García, AM Carrión: Caloric restriction

increases learning consolidation and facilitates synaptic plasticity through mechanisms dependent on NR2B subunits of the NMDA receptor. *J Neurosci* 27, 10185–10195 (2007)

20. MM Adams, L Shi, MC Linville, ME Forbes, AB Long, C Bennett, IG Newton, CS Carter, WE Sonntag, DR Riddle, JK Brunso-Bechtold: Caloric restriction and age affect synaptic proteins in hippocampal CA3 and spatial learning ability. *Exp Neurol* 211, 141–149 (2008)

21. T Komatsu, T Chiba, H Yamaza, K Yamashita, A Shimada, Y Hoshiyama, T Henmi, H Ohtani, Y Higami, R de Cabo, DK Ingram, I Shimokawa: Manipulation of caloric content but not diet composition, attenuates the deficit in learning and memory of senescence-accelerated mouse strain P8. *Exp Gerontol* 43, 339–346 (2008)

22. A V Witte, M Fobker, R Gellner, S Knecht, A Flöel: Caloric restriction improves memory in elderly humans. *Proc Natl Acad Sci U S A* 106, 1255–1260 (2009)

23. EK Kastman, AA Willette, CL Coe, BB Bendlin, KJ Kosmatka, DG McLaren, G Xu, E Canu, AS Field, AL Alexander, M Lou Voytko, TM Beasley, RJ Colman, RH Weindruch, SC Johnson: A calorie-restricted diet decreases brain iron accumulation and preserves motor performance in old rhesus monkeys. *J Neurosci* 32, 11897–11904 (2012)

24. W Qin, M Chachich, M Lane, G Roth, M Bryant, R de Cabo, MA Ottinger, J Mattison, D Ingram, S Gandy, GM Pasinetti: Calorie restriction attenuates Alzheimer's disease type brain amyloidosis in Squirrel monkeys (*Saimiri sciureus*). *J Alzheimers Dis* 10, 417–422 (2006)

25. A Sridharan, M Pehar, MS Salamat, TD Pugh, BB Bendlin, AA Willette, RM Anderson, JW Kemnitz, RJ Colman, RH Weindruch, L Puglielli, SC Johnson: Calorie restriction attenuates astrogliosis but not amyloid plaque load in aged rhesus macaques: a preliminary quantitative imaging study. *Brain Res* 1508, 1–8 (2013)

26. K Kanda: Effects of food restriction on motoneuronal loss with advancing age in the rat. *Microsc Res Tech* 59, 301–305 (2002)

27. G Valdez, JC Tapia, H Kang, GD Clemenson, FH Gage, JW Lichtman, JR Sanes: Attenuation of age-related changes in mouse neuromuscular synapses by caloric restriction and exercise. *Proc Natl Acad Sci USA* 107, 14863–14868 (2010)

28. LC Ribeiro, A Quincozes-Santos, MC Leite, RT Abib, J Kleinkauf-Rocha, R Biasibetti, LN Rotta, ST Wofchuk, MLS Perry, CA Gonçalves, C Gottfried: Caloric restriction increases hippocampal glutamate uptake and glutamine synthetase activity in Wistar rats. *Neurosci Res* 64, 330–334 (2009)

29. KJ Bough, R Valiyil, FT Han, DA Eagles: Seizure resistance is dependent upon age and calorie restriction in rats fed a ketogenic diet. *Epilepsy Res* 35, 21–28 (1999)

30. KA Steinkraus, ED Smith, C Davis, D Carr, WR Pendergrass, GL Sutphin, BK Kennedy, M Kaerberlein: Dietary restriction suppresses proteotoxicity and enhances longevity by an hsf-1-dependent mechanism in *Caenorhabditis elegans*. *Aging Cell* 7, 394–404 (2008)

31. VKM Halagappa, Z Guo, M Pearson, Y Matsuoka, RG Cutler, FM Laferla, MP Mattson: Intermittent fasting and caloric restriction ameliorate age-related behavioral deficits in the triple-transgenic mouse model of Alzheimer's disease. *Neurobiol Dis* 26, 212–220 (2007)

32. P Wu, Q Shen, S Dong, Z Xu, JZ Tsien, Y Hu: Calorie restriction ameliorates neurodegenerative phenotypes in forebrain-specific presenilin-1 and presenilin-2 double knockout mice. *Neurobiol Aging* 29, 1502–1511 (2008)

33. N Maswood, J Young, E Tilmont, Z Zhang, DM Gash, GA Gerhardt, R Grondin, GS Roth, J Mattison, MA Lane, RE Carson, RM Cohen, PR Mouton, C Quigley, MP Mattson, DK Ingram: Caloric restriction increases neurotrophic factor levels and attenuates neurochemical and behavioral deficits in a primate model of Parkinson's disease. *Proc Natl Acad Sci USA* 101, 18171–18176 (2004)

34. C López-Otin, MA Blasco, L Partridge, M Serrano, G Kroemer: The hallmarks of aging. *Cell* 153, 1194–1217 (2013)

35. D Sinclair, L Guarente: Extrachromosomal rDNA circles—a cause of aging in yeast. *Cell* 91, 1033–1042 (1997)

36. M Riesen, A Morgan: Calorie restriction reduces rDNA recombination independently of rDNA silencing. *Aging Cell* 8, 624–632 (2009)

37. JE Lee, JI Heo, SH Park, JH Kim, YJ Kho, HJ Kang, HY Chung, JL Yoon, JY Lee: Calorie restriction (CR) reduces age-dependent decline of non-homologous end joining (NHEJ) activity in rat tissues. *Exp Gerontol* 46, 891–896 (2011)

38. VK Srivastava, DL Busbee: Decreased fidelity of DNA polymerases and decreased DNA excision repair in aging mice: effects of caloric restriction. *Biochem Biophys Res Commun* 182, 712–721 (1992)

39. Z Guo, A Heydari, A Richardson: Nucleotide excision repair of actively transcribed versus nontranscribed DNA in rat hepatocytes: effect of age and dietary restriction. *Exp Cell Res* 245, 228–238 (1998)

40. DC Cabelof, S Yanamadala, JJ Raffoul, Z Guo, A Soofi, AR Heydari: Caloric restriction promotes genomic stability by induction of base excision repair and reversal of its age-related decline *DNA Repair (Amst)* 2, 295–307 (2003)

41. GE Kisby, SG Kohama, A Olivas, M Churchwell, D Doerge, E Spangler, R de Cabo, DK Ingram, B Imhof, G Bao, YW Kow: Effect of caloric restriction on base-excision repair (BER) in the aging rat brain. *Exp Gerontol* 45, 208–216 (2010)
42. CM Moore, BG Dunn, CA McMahan, MA Lane, GS Roth, DK Ingram, JA Mattison: Effects of calorie restriction on chromosomal stability in rhesus monkeys (*Macaca mulatta*). *Age (Dordr)* 29, 15–28 (2007)
43. M Armanios, EH Blackburn: The telomere syndromes. *Nat Rev Genet* 13, 693–704 (2012)
44. E Vera, B Bernardes de Jesus, M Foronda, JM Flores, MA Blasco: Telomerase reverse transcriptase synergizes with calorie restriction to increase health span and extend mouse longevity. *PLoS One* 8, e53760 (2013)
45. JD Kark, N Goldberger, M Kimura, R Sinnreich, A Aviv: Energy intake and leukocyte telomere length in young adults. *Am J Clin Nutr* 95, 479–487 (2012)
46. WR Pendergrass, PE Penn, J Li, NS Wolf: Age-related telomere shortening occurs in lens epithelium from old rats and is slowed by caloric restriction. *Exp Eye Res* 73, 221–228 (2001)
47. Y Li, M Daniel, TO Tollefsbol: Epigenetic regulation of caloric restriction in aging. *BMC Med* 9, 98 (2011)
48. AY So, JW Jung, S Lee, HS Kim, KS Kang: DNA methyltransferase controls stem cell aging by regulating BMI1 and EZH2 through microRNAs. *PLoS One* 6, e19503 (2011)
49. L Chouliaras, DLA van den Hove, G Kenis, J Dela Cruz, MAM Lemmens, J van Os, HWM Steinbusch, C Schmitz, BPF Rutten: Caloric restriction attenuates age-related changes of DNA methyltransferase 3a in mouse hippocampus. *Brain Behav Immun* 25, 616–623 (2011)
50. L Chouliaras, DLA van den Hove, G Kenis, S Keitel, PR Hof, J van Os, HWM Steinbusch, C Schmitz, BPF Rutten: Age-related increase in levels of 5-hydroxymethylcytosine in mouse hippocampus is prevented by caloric restriction. *Curr Alzheimer Res* 9, 536–544 (2012)
51. L Chouliaras, D Mastroeni, E Delvaux, A Grover, G Kenis, PR Hof, HWM Steinbusch, PD Coleman, BPF Rutten, DLA van den Hove: Consistent decrease in global DNA methylation and hydroxymethylation in the hippocampus of Alzheimer's disease patients. *Neurobiol Aging* 34, 2091–2099 (2013)
52. S Michan, D Sinclair: Sirtuins in mammals: insights into their biological function *Biochem J* 404, 1–13 (2007)
53. S Michan: Acetylome regulation by sirtuins in the brain: from normal physiology to aging and pathology. *Curr Pharm Des* 19, 6823–6838 (2013)
54. L Chouliaras, DLA van den Hove, G Kenis, M van Draanen, PR Hof, J van Os, HWM Steinbusch, C Schmitz, BPF Rutten: Histone deacetylase 2 in the mouse hippocampus: attenuation of age-related increase by caloric restriction. *Curr Alzheimer Res* 10, 868–876 (2013)
55. N Jiang, G Du, E Tobias, JG Wood, R Whitaker, N Neretti, SL Helfand: Dietary and genetic effects on age-related loss of gene silencing reveal epigenetic plasticity of chromatin repression during aging. *Aging (Albany NY)* 5, 813–24 (2013)
56. M Uno, S Honjoh, M Matsuda, H Hoshikawa, S Kishimoto, T Yamamoto, M Ebisuya, T Yamamoto, K Matsumoto, E Nishida: A fasting-responsive signaling pathway that extends life span in *C. elegans*. *Cell Rep* 3, 79–91 (2013)
57. WC Fok, A Bokov, J Gelfond, Z Yu, Y Zhang, M Doderer, Y Chen, M Javors, WH Wood, Y Zhang, KG Becker, A Richardson, VI Pérez: Combined treatment of rapamycin and dietary restriction has a larger effect on the transcriptome and metabolome of liver. *Aging Cell* 13, 311–319 (2014)
58. C Lee, R Klopp, R Weindruch, T Prolla: Gene expression profile of aging and its retardation by caloric restriction. *Science* 285, 1390–1393 (1999)
59. EM Mercken, SD Crosby, DW Lamming, L JeBailey, S Krzysik-Walker, DT Villareal, M Capri, C Franceschi, Y Zhang, K Becker, DM Sabatini, R de Cabo, L Fontana: Calorie restriction in humans inhibits the PI3K/AKT pathway and induces a younger transcription profile. *Aging Cell* 12, 645–651 (2013)
60. EM Mercken, E Majounie, J Ding, R Guo, J Kim, M Bernier, J Mattison, MR Cookson, M Gorospe, R de Cabo, K Abdelmohsen: Age-associated miRNA alterations in skeletal muscle from rhesus monkeys reversed by caloric restriction. *Aging (Albany NY)* 5, 692–703 (2013)
61. M Vora, M Shah, S Ostafi, B Onken, J Xue, JZ Ni, S Gu, M Driscoll: Deletion of microRNA-80 activates dietary restriction to extend *C. elegans* healthspan and lifespan. *PLoS Genet* 9, e1003737 (2013)
62. RC Taylor, A Dillin: Aging as an event of proteostasis collapse. *Cold Spring Harb Perspect Biol* 3, pii: a004440 (2011)
63. M Hansen, S Taubert, D Crawford, N Libina, SJ Lee, C Kenyon: Lifespan extension by conditions that inhibit translation in *Caenorhabditis elegans*. *Aging Cell* 6, 95–110 (2007)
64. G Leprivier, M Remke, B Rotblat, A Dubuc, ARF Mateo, M Kool, S Agnihotri, A El-Naggar, B Yu, SP

- Somasekharan, B Faubert, G Bridon, CE Tognon, J Mathers, R Thomas, A Li, A Barokas, B Kwok, M Bowden *et al.*: The eEF2 kinase confers resistance to nutrient deprivation by blocking translation elongation. *Cell* 153, 1064–1079 (2013)
65. KK Steffen, VL MacKay, EO Kerr, M Tsuchiya, D Hu, LA Fox, N Dang, ED Johnston, JA Oakes, BN Tchao, DN Pak, S Fields, BK Kennedy, M Kaerberlein: Yeast life span extension by depletion of 60s ribosomal subunits is mediated by Gcn4. *Cell* 133, 292–302 (2008)
66. BM Zid, AN Rogers, SD Katewa, MA Vargas, MC Kolipinski, TA Lu, S Benzer, P Kapahi: 4E-BP extends lifespan upon dietary restriction by enhancing mitochondrial activity in *Drosophila*. *Cell* 139, 149–160 (2009)
67. JC Price, CF Khambatta, KW Li, MD Bruss, M Shankaran, M Dalidd, NA Floreani, LS Roberts, SM Turner, WE Holmes, MK Hellerstein: The effect of long term calorie restriction on *in vivo* hepatic proteostasis: a novel combination of dynamic and quantitative proteomics. *Mol Cell Proteomics* 11, 1801–1814 (2012)
68. E Nisoli, C Tonello, A Cardile, V Cozzi, R Bracale, L Tedesco, S Falcone, A Valerio, O Cantoni, E Clementi, S Moncada, MO Carruba: Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. *Science* 310, 314–317 (2005)
69. AE Civitarese, S Carling, LK Heilbronn, MH Hulver, B Ukropcova, WA Deutsch, SR Smith, E Ravussin: Calorie Restriction Increases Muscle Mitochondrial Biogenesis in Healthy Humans *PLoS Med* 4, e76 (2007)
70. G López-Lluch, N Hunt, B Jones, M Zhu, H Jamieson, S Hilmer, M V Cascajo, J Allard, DK Ingram, P Navas, R de Cabo: Calorie restriction induces mitochondrial biogenesis and bioenergetic efficiency. *Proc Natl Acad Sci USA* 103, 1768–1773 (2006)
71. BF Miller, MM Robinson, DJ Reuland, JC Drake, FF Peelor, MD Bruss, MK Hellerstein, KL Hamilton: Calorie restriction does not increase short-term or long-term protein synthesis. *J Gerontol A Biol Sci Med Sci* 68, 530–538 (2013)
72. BF Miller, MM Robinson, MD Bruss, M Hellerstein, KL Hamilton: A comprehensive assessment of mitochondrial protein synthesis and cellular proliferation with age and calorie restriction. *Aging Cell* 11, 150–161 (2012)
73. IR Lanza, P Zabielski, KA Klaus, DM Morse, CJ Heppelmann, HR Bergen, S Dasari, S Walrand, KR Short, ML Johnson, MM Robinson, JM Schimke, DR Jakaitis, YW Asmann, Z Sun, KS Nair: Chronic caloric restriction preserves mitochondrial function in senescence without increasing mitochondrial biogenesis. *Cell Metab* 16, 777–788 (2012)
74. B Levine, DJ Klionsky: Development by self-digestion: molecular mechanisms and biological functions of autophagy. *Dev Cell* 6, 463–477 (2004)
75. C Mörck, M Pilon: *C. elegans* feeding defective mutants have shorter body lengths and increased autophagy. *BMC Dev Biol* 6, 39 (2006)
76. C Mörck, M Pilon: Caloric restriction and autophagy in *Caenorhabditis elegans*. *Autophagy* 3, 51–53 (2007)
77. E Bergamini, G Cavallini, A Donati, Z Gori: The role of autophagy in aging: its essential part in the anti-aging mechanism of calorie restriction. *Ann N Y Acad Sci* 1114, 69–78 (2007)
78. A Donati, G Cavallini, C Paradiso, S Vittorini, M Pollera, Z Gori, E Bergamini: Age-related changes in the autophagic proteolysis of rat isolated liver cells: effects of antiaging dietary restrictions. *J Gerontol A Biol Sci Med Sci* 56, B375–83 (2001)
79. G Cavallini, A Donati, Z Gori, M Pollera, E Bergamini: The protection of rat liver autophagic proteolysis from the age-related decline co-varies with the duration of anti-ageing food restriction. *Exp Gerontol* 36, 497–506 (2001)
80. K Jia, B Levine: Autophagy is required for dietary restriction-mediated life span extension in *C. elegans*. *Autophagy* 3, 597–599 (2007)
81. G Mariño, F Madeo, G Kroemer: Autophagy for tissue homeostasis and neuroprotection. *Curr Opin Cell Biol* 23, 198–206 (2011)
82. T Hara, K Nakamura, M Matsui, A Yamamoto, Y Nakahara, R Suzuki-Migishima, M Yokoyama, K Mishima, I Saito, H Okano, N Mizushima: Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. *Nature* 441, 885–889 (2006)
83. M Komatsu, S Waguri, T Chiba, S Murata, J ichi Iwata, I Tanida, T Ueno, M Koike, Y Uchiyama, E Kominami, K Tanaka: Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature* 441, 880–884 (2006)
84. M Alirezaei, CC Kemball, CT Flynn, MR Wood, JL Whitton, WB Kiosses: Short-term fasting induces profound neuronal autophagy. *Autophagy* 6, 702–710 (2010)
85. JE Young, RA Martinez, AR La Spada: Nutrient deprivation induces neuronal autophagy and implicates reduced insulin signaling in neuroprotective autophagy activation. *J Biol Chem* 284, 2363–2373 (2009)
86. K Zhang, P Shi, T An, Q Wang, J Wang, Z Li, W Duan, C Li, Y Guo: Food restriction-induced autophagy modulates degradation of mutant SOD1 in an amyotrophic lateral sclerosis mouse model. *Brain Res* 1519, 112–119 (2013)

87. VA Vernace, L Arnaud, T Schmidt-Glenewinkel, ME Figueiredo-Pereira: Aging perturbs 26S proteasome assembly in *Drosophila melanogaster*. *FASEB J* 21, 2672–2682 (2007)
88. G Carrard, AL Bulteau, I Petropoulos, B Friguet: Impairment of proteasome structure and function in aging. *Int J Biochem Cell Biol* 34, 1461–1474 (2002)
89. G Hamer, O Matilainen, CI Holmberg: A photoconvertible reporter of the ubiquitin-proteasome system *in vivo*. *Nat Methods* 7, 473–478 (2010)
90. L Bedford, D Hay, A Devoy, S Paine, DG Powe, R Seth, T Gray, I Topham, K Fone, N Rezvani, M Mee, T Soane, R Layfield, PW Sheppard, T Ebendal, D Usoskin, J Lowe, RJ Mayer: Depletion of 26S proteasomes in mouse brain neurons causes neurodegeneration and Lewy-like inclusions resembling human pale bodies. *J Neurosci* 28, 8189–8198 (2008)
91. R Romero-Granados, Á Fontán-Lozano, FJ Aguilar-Montilla, ÁM Carrión: Postnatal proteasome inhibition induces neurodegeneration and cognitive deficiencies in adult mice: a new model of neurodevelopment syndrome. *PLoS One* 6, e28927 (2011)
92. FM da Cunha, M Demasi, AJ Kowaltowski: Aging and calorie restriction modulate yeast redox state, oxidized protein removal, and the ubiquitin–proteasome system *Free Radic Biol Med* 51, 664–670 (2011)
93. P Razeghi, KK Baskin, S Sharma, ME Young, S Stepkowski, MF Essop, H Taegtmeyer: Atrophy, hypertrophy, and hypoxemia induce transcriptional regulators of the ubiquitin proteasome system in the rat heart. *Biochem Biophys Res Commun* 342, 361–364 (2006)
94. Y Nakai, H Hashida, K Kadota, M Minami, K Shimizu, I Matsumoto, H Kato, K Abe: Up-regulation of genes related to the ubiquitin-proteasome system in the brown adipose tissue of 24-h-fasted rats. *Biosci Biotechnol Biochem* 72, 139–148 (2008)
95. AC Carrano, Z Liu, A Dillin, T Hunter: A conserved ubiquitination pathway determines longevity in response to diet restriction. *Nature* 460, 396–399 (2009)
96. F Li, L Zhang, J Craddock, AJ Bruce-Keller, K Dasuri, A Nguyen, JN Keller: Aging and dietary restriction effects on ubiquitination, sumoylation, and the proteasome in the heart *Mech Ageing Dev* 129, 515–521 (2008)
97. U Kruegel, B Robison, T Dange, G Kahlert, JR Delaney, S Kotireddy, M Tsuchiya, S Tsuchiyama, CJ Murakami, J Schleit, G Sutphin, D Carr, K Tar, G Dittmar, M Kaerberlein, BK Kennedy, M Schmidt: Elevated proteasome capacity extends replicative lifespan in *Saccharomyces cerevisiae*. *PLoS Genet* 7, e1002253 (2011)
98. VI Pérez, R Buffenstein, V Masamsetti, S Leonard, AB Salmon, J Mele, B Andziak, T Yang, Y Edrey, B Friguet, W Ward, A Richardson, A Chaudhuri: Protein stability and resistance to oxidative stress are determinants of longevity in the longest-living rodent, the naked mole-rat. *Proc Natl Acad Sci USA* 106, 3059–3064 (2009)
99. N Chondrogianni, I Petropoulos, C Franceschi, B Friguet, ES Gonos: Fibroblast cultures from healthy centenarians have an active proteasome. *Exp Gerontol* 35, 721–728 (2000)
100. RI Morimoto: The heat shock response: systems biology of proteotoxic stress in aging and disease. *Cold Spring Harb Symp Quant Biol* 76, 91–99 (2011)
101. R Raynes, BD Leckey, K Nguyen, SD Westerheide: Heat shock and caloric restriction have a synergistic effect on the heat shock response in a sir2.1-dependent manner in *Caenorhabditis elegans*. *J Biol Chem* 287, 29045–29053 (2012)
102. K Dokladny, MN Zuhl, M Mandell, D Bhattacharya, S Schneider, V Deretic, PL Moseley: Regulatory coordination between two major intracellular homeostatic systems: heat shock response and autophagy. *J Biol Chem* 288, 14959–14972 (2013)
103. SJ Lin, M Kaerberlein, AA Andalis, LA Sturtz, PA Defossez, VC Culotta, GR Fink, L Guarente: Calorie restriction extends *Saccharomyces cerevisiae* lifespan by increasing respiration. *Nature* 418, 344–348 (2002)
104. TJ Schulz, K Zarse, A Voigt, N Urban, M Birringer, M Ristow: Glucose restriction extends *Caenorhabditis elegans* life span by inducing mitochondrial respiration and increasing oxidative stress. *Cell Metab* 6, 280–293 (2007)
105. S Hempenstall, MM Page, KR Wallen, C Selman: Dietary restriction increases skeletal muscle mitochondrial respiration but not mitochondrial content in C57BL/6 mice. *Mech Ageing Dev* 133, 37–45 (2012)
106. Y Higami, TD Pugh, GP Page, DB Allison, TA Prolla, R Weindruch: Adipose tissue energy metabolism: altered gene expression profile of mice subjected to long-term caloric restriction. *FASEB J* 18, 415–417 (2004)
107. KM Choi, HL Lee, YY Kwon, MS Kang, SK Lee, CK Lee: Enhancement of mitochondrial function correlates with the extension of lifespan by caloric restriction and caloric restriction mimetics in yeast. *Biochem Biophys Res Commun* 441, 236–242 (2013)
108. JS Choi, KM Choi, CK Lee: Caloric restriction improves efficiency and capacity of the mitochondrial electron transport chain in *Saccharomyces cerevisiae*. *Biochem Biophys Res Commun* 409, 308–314 (2011)
109. K Hagopian, ME Harper, JJ Ram, SJ Humble, R Weindruch, JJ Ramsey: Long-term caloric restriction reduces proton leak and hydrogen peroxide production in liver mitochondria. *Am J Physiol Endocrinol Metab* 288, E674–84 (2005)

110. JP DeLany, BC Hansen, NL Bodkin, J Hannah, GA Bray: Long-Term Calorie Restriction Reduces Energy Expenditure in Aging Monkeys. *J Gerontol A Biol Sci Med Sci* 54, B5-11; discussion B12-3 (1999)
111. M López-Torres, R Gredilla, A Sanz, G Barja: Influence of aging and long-term caloric restriction on oxygen radical generation and oxidative DNA damage in rat liver mitochondria. *Free Radic Biol Med* 32, 882–889 (2002)
112. RS Sohal, R Weindruch: Oxidative Stress, Caloric Restriction, and Aging. *Science* 273, 59–63 (1996)
113. RS Sohal, I Svensson, UT Brunk: Hydrogen peroxide production by liver mitochondria in different species. *Mech Ageing Dev* 53, 209–215 (1990)
114. J Schleit, SC Johnson, CF Bennett, M Simko, N Trongtham, A Castanza, EJ Hsieh, RM Moller, BM Wasko, JR Delaney, GL Sutphin, D Carr, CJ Murakami, A Tocchi, B Xian, W Chen, T Yu, S Goswami, S Higgins *et al.*: Molecular mechanisms underlying genotype-dependent responses to dietary restriction. *Aging Cell* 12, 1050–1061 (2013)
115. A Calixto, JS Jara, FA Court: Diapause formation and downregulation of insulin-like signaling via DAF-16/FOXO delays axonal degeneration and neuronal loss. *PLoS Genet* 8, e1003141 (2012)
116. C Thrasivoulou, V Soubeyre, H Ridha, D Giuliani, C Giaroni, GJ Michael, MJ Saffrey, T Cowen: Reactive oxygen species, dietary restriction and neurotrophic factors in age-related loss of myenteric neurons. *Aging Cell* 5, 247–257 (2006)
117. K Opalach, S Rangaraju, I Madorsky, C Leeuwenburgh, L Notterpek: Lifelong calorie restriction alleviates age-related oxidative damage in peripheral nerves. *Rejuvenation Res* 13, 65–74 (2010)
118. S Sharma, R Singh, M Kaur, G Kaur: Late-onset dietary restriction compensates for age-related increase in oxidative stress and alterations of HSP 70 and synapsin 1 protein levels in male Wistar rats. *Biogerontology* 11, 197–209 (2010)
119. EJ Masoro: Role of hormesis in life extension by caloric restriction. *Dose Response* 5, 163–173 (2007)
120. S Schmeisser, S Priebe, M Groth, S Monajembashi, P Hemmerich, R Guthke, M Platzer, M Ristow: Neuronal ROS signaling rather than AMPK/sirtuin-mediated energy sensing links dietary restriction to lifespan extension. *Mol Metab* 2, 92–102 (2013)
121. RRJ Shelke, C Leeuwenburgh: Lifelong caloric restriction increases expression of apoptosis repressor with a caspase recruitment domain (ARC) in the brain. *FASEB J* 17, 494–496 (2003)
122. AJ Lambert, BJ Merry: Lack of effect of caloric restriction on bioenergetics and reactive oxygen species production in intact rat hepatocytes. *J Gerontol A Biol Sci Med Sci* 60, 175–180 (2005)
123. A Valle, R Guevara, FJ García-Palmer, P Roca, J Oliver: Caloric restriction retards the age-related decline in mitochondrial function of brown adipose tissue. *Rejuvenation Res* 11, 597–604 (2008)
124. M Cerletti, YC Jang, LWS Finley, MC Haigis, AJ Wagers: Short-term calorie restriction enhances skeletal muscle stem cell function. *Cell Stem Cell* 10, 515–519 (2012)
125. FM Cerqueira, FM Cunha, FRM Laurindo, AJ Kowaltowski: Calorie restriction increases cerebral mitochondrial respiratory capacity in a NO•-mediated mechanism: impact on neuronal survival. *Free Radic Biol Med* 52, 1236–1241 (2012)
126. M Artal-Sanz, N Tavernarakis: Prohibitin couples diapause signalling to mitochondrial metabolism during ageing in *C. elegans*. *Nature* 461, 793–797 (2009)
127. M Artal-Sanz, N Tavernarakis: Prohibitin and mitochondrial biology. *Trends Endocrinol Metab* 20, 394–401 (2009)
128. J Campisi: Cellular senescence: putting the paradoxes in perspective. *Curr Opin Genet Dev* 21, 107–112 (2011)
129. U Herbig, M Ferreira, L Condell, D Carey, JM Sedivy: Cellular senescence in aging primates. *Science* 311, 1257 (2006)
130. D Jurk, C Wang, S Miwa, M Maddick, V Korolchuk, A Tsolou, ES Gonos, C Thrasivoulou, MJ Saffrey, K Cameron, T von Zglinicki: Postmitotic neurons develop a p21-dependent senescence-like phenotype driven by a DNA damage response. *Aging Cell* 11, 996–1004 (2012)
131. I Messaoudi, J Warner, M Fischer, B Park, B Hill, J Mattison, MA Lane, GS Roth, DK Ingram, LJ Picker, DC Douek, M Mori, J Nikolich-Zugich: Delay of T cell senescence by caloric restriction in aged long-lived nonhuman primates. *Proc Natl Acad Sci U S A* 103, 19448–19453 (2006)
132. YC Ning, GY Cai, L Zhuo, JJ Gao, D Dong, S Cui, Z Feng, SZ Shi, XY Bai, XF Sun, XM Chen: Short-term calorie restriction protects against renal senescence of aged rats by increasing autophagic activity and reducing oxidative damage. *Mech Ageing Dev* (2013)
133. G Mazzocchi, MF Tevy, M Borghesan, MRD Vergini, M Vinciguerra: Caloric restriction and aging stem cells: The stick and the carrot? *Exp Gerontol* 50, 137–48 (2013)
134. L Bondolfi, F Ermini, JM Long, DK Ingram, M Jucker: Impact of age and caloric restriction on

neurogenesis in the dentate gyrus of C57BL/6 mice. *Neurobiol Aging* 25, 333–340 (2004)

135. JH Park, Z Glass, K Sayed, T V Michurina, A Lazutkin, O Mineyeva, D Velmeshev, WF Ward, A Richardson, G Enikolopov: Calorie restriction alleviates the age-related decrease in neural progenitor cell division in the aging brain. *Eur J Neurosci* 37, 1987–1993 (2013)

136. J Lee, W Duan, JM Long, DK Ingram, MP Mattson: Dietary restriction increases the number of newly generated neural cells, and induces BDNF expression, in the dentate gyrus of rats. *J Mol Neurosci* 15, 99–108 (2000)

137. C Kenyon, J Chang, E Gensch, A Rudner, R Tabtiang: A *C. elegans* mutant that lives twice as long as wild type. *Nature* 366, 461–464 (1993)

138. C Kenyon: The first long-lived mutants: discovery of the insulin/IGF-1 pathway for ageing. *Philos Trans R Soc Lond B Biol Sci* 366, 9–16 (2011)

139. M Holzenberger, J Dupont, B Ducos, P Leneuve, A Gélou, PC Even, P Cervera, Y Le Bouc: IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature* 421, 182–187 (2003)

140. E Cohen, JF Paulsson, P Blinder, T Burstyn-Cohen, D Du, G Estepa, A Adame, HM Pham, M Holzenberger, JW Kelly, E Masliah, A Dillin: Reduced IGF-1 Signaling Delays Age-Associated Proteotoxicity in Mice. *Cell* 139, 1157–1169 (2009)

141. D van Heemst, M Beekman, SP Mooijaart, BT Heijmans, BW Brandt, BJ Zwaan, PE Slagboom, RGJ Westendorp: Reduced insulin/IGF-1 signalling and human longevity. *Aging Cell* 4, 79–85 (2005)

142. SC Johnson, PS Rabinovitch, M Kaeberlein: mTOR is a key modulator of ageing and age-related disease. *Nature* 493, 338–345 (2013)

143. P Spilman, Inhibition of mTOR by Rapamycin Abolishes Cognitive Deficits and Reduces Amyloid- β N Podlaskaya, MJ Hart, J Debnath, O Gorostiza, D Bredesen, A Richardson, R Strong, V Galvan: Levels in a Mouse Model of Alzheimer's Disease. *PLoS One* 5, e9979 (2010)

144. DW Lamming, DM Sabatini: A radical role for TOR in longevity. *Cell Metab* 13, 617–618 (2011)

145. DW Lamming, L Ye, P Katajisto, MD Goncalves, M Saitoh, DM Stevens, JG Davis, AB Salmon, A Richardson, RS Ahima, DA Guertin, DM Sabatini, JA Baur: Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. *Science* 335, 1638–1643 (2012)

146. JJ Wu, J Liu, EB Chen, JJ Wang, L Cao, N Narayan, MM Fergusson, II Rovira, M Allen, DA Springer, CU Lago, S Zhang, W DuBois, T Ward, R deCabo, O

Gavrilova, B Mock, T Finkel: Increased mammalian lifespan and a segmental and tissue-specific slowing of aging after genetic reduction of mTOR expression. *Cell Rep* 4, 913–920 (2013)

147. M Kaeberlein, RW Powers, KK Steffen, EA Westman, D Hu, N Dang, EO Kerr, KT Kirkland, S Fields, BK Kennedy: Regulation of Yeast Replicative Life Span by TOR and Sch9 in Response to Nutrients. *Science* 310, 1193–1196 (2005)

148. M Wei, P Fabrizio, J Hu, H Ge, C Cheng, L Li, VD Longo: Life span extension by calorie restriction depends on Rim15 and transcription factors downstream of Ras/PKA, Tor, and Sch9. *PLoS Genet* 4, e13 (2008)

149. L Yan, DE Vatner, JP O'Connor, A Ivessa, H Ge, W Chen, S Hirotani, Y Ishikawa, J Sadoshima, SF Vatner: Type 5 adenylyl cyclase disruption increases longevity and protects against stress. *Cell* 130, 247–258 (2007)

150. MG Mirisola, VD Longo: Conserved role of Ras-GEFs in promoting aging: from yeast to mice. *Aging (Albany NY)* 3, 340–343 (2011)

151. D Stenlesen, JM Suh, J Seo, K Yu, KS Lee, JS Kim, KJ Min, JM Graff: Adenosine nucleotide biosynthesis and AMPK regulate adult life span and mediate the longevity benefit of caloric restriction in flies. *Cell Metab* 17, 101–112 (2013)

152. JY Lu, YY Lin, JC Sheu, JT Wu, FJ Lee, Y Chen, MI Lin, FT Chiang, TY Tai, SL Berger, Y Zhao, KS Tsai, H Zhu, LM Chuang, JD Boeke: Acetylation of Yeast AMPK Controls Intrinsic Aging Independently of Caloric Restriction. *Cell* 146, 969–979 (2011)

153. A Chalkiadaki, L Guarente: Sirtuins mediate mammalian metabolic responses to nutrient availability. *Nat Rev Endocrinol* 8, 287–296 (2012)

154. PW Estep, JB Warner, ML Bulyk: Short-term calorie restriction in male mice feminizes gene expression and alters key regulators of conserved aging regulatory pathways. *PLoS One* 4, e5242 (2009)

155. RW Powers, M Kaeberlein, SD Caldwell, BK Kennedy, S Fields: Extension of chronological life span in yeast by decreased TOR pathway signaling. *Genes Dev* 20, 174–184 (2006)

156. M Wei, P Fabrizio, F Madia, J Hu, H Ge, LM Li, VD Longo: Tor1/Sch9-regulated carbon source substitution is as effective as calorie restriction in life span extension. *PLoS Genet* 5, e1000467 (2009)

157. OM Palacios, JJ Carmona, S Michan, KYY Chen, Y Manabe, JLL Ward, LJ Goodyear, Q Tong: Diet and exercise signals regulate SIRT3 and activate AMPK and PGC-1 α in skeletal muscle. *Aging (Albany NY)* 1, 771–783 (2009)

158. NA Bishop, L Guarente: Two neurons mediate diet-restriction-induced longevity in *C. elegans*. *Nature* 447, 545–549 (2007)
159. J Gallinetti, E Harputlugil, JR Mitchell: Amino acid sensing in dietary-restriction-mediated longevity: roles of signal-transducing kinases GCN2 and TOR. *Biochem J* 449, 1–10 (2013)
160. M Kaeberlein: mTOR Inhibition: From Aging to Autism and Beyond. *Scientifica* (Cairo) 2013, 849186. Epub 2013 Nov 26.
161. C Cang, Y Zhou, B Navarro, YJ Seo, K Aranda, L Shi, S Battaglia-Hsu, I Nissim, DE Clapham, D Ren: mTOR regulates lysosomal ATP-sensitive two-pore Na(+) channels to adapt to metabolic state. *Cell* 152, 778–790 (2013)
162. A Azarbar, DC McIntyre, KL Gilby: Caloric restriction alters seizure disposition and behavioral profiles in seizure-prone (fast) versus seizure-resistant (slow) rats. *Behav Neurosci* 124, 106–114 (2010)
163. E Russo, R Citraro, A Constanti, G De Sarro: The mTOR signaling pathway in the brain: focus on epilepsy and epileptogenesis. *Mol Neurobiol* 46, 662–681 (2012)
164. P Curatolo, R Moavero: mTOR inhibitors as a new therapeutic option for epilepsy. *Expert Rev Neurother* 13, 627–638 (2013)
165. C Cantó, J Auwerx: Calorie restriction: is AMPK a key sensor and effector? *Physiology (Bethesda)* 26, 214–224 (2011)
166. C Cantó, Z Gerhart-Hines, JN Feige, M Lagouge, L Noriega, JC Milne, PJ Elliott, P Puigserver, J Auwerx: AMPK regulates energy expenditure by modulating NAD⁺ metabolism and SIRT1 activity. *Nature* 458, 1056–1060 (2009)
167. DM Gwinn, DB Shackelford, DF Egan, MM Mihaylova, A Mery, DS Vasquez, BE Turk, RJ Shaw: AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell* 30, 214–226 (2008)
168. S Ogg, S Paradis, S Gottlieb, GI Patterson, L Lee, HA Tissenbaum, G Ruvkun: The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. *Nature* 389, 994–999 (1997)
169. EL Greer, D Dowlatabadi, MR Banko, J Villen, K Hoang, D Blanchard, SP Gygi, A Brunet: An AMPK-FOXO Pathway Mediates Longevity Induced by a Novel Method of Dietary Restriction in *C. elegans*. *Curr Biol* 17, 1646–1656 (2007)
170. MDW Piper, C Selman, JJ McElwee, L Partridge: Separating cause from effect: how does insulin/IGF signalling control lifespan in worms, flies and mice? *J Intern Med* 263, 179–191 (2008)
171. T Parr: Insulin exposure controls the rate of mammalian aging. *Mech Ageing Dev* 88, 75–82 (1996)
172. SE Dunn, FW Kari, J French, JR Leininger, G Travlos, R Wilson, JC Barrett: Dietary restriction reduces insulin-like growth factor I levels, which modulates apoptosis, cell proliferation, and tumor progression in p53-deficient mice. *Cancer Res* 57, 4667–4672 (1997)
173. R Kaletsky, CT Murphy: The role of insulin/IGF-like signaling in *C. elegans* longevity and aging. *Dis Model Mech* 3, 415–419
174. ÖH Yilmaz, P Katajisto, DW Lamming, Y Gültekin, KE Bauer-Rowe, S Sengupta, K Birsoy, A Dursun, VO Yilmaz, M Selig, GP Nielsen, M Mino-Kenudson, LR Zukerberg, AK Bhan, V Deshpande, DM Sabatini: mTORC1 in the Paneth cell niche couples intestinal stem-cell function to calorie intake. *Nature* 486, 490–495 (2012)
175. A Armario, JL Montero, T Jolin: Chronic Food Restriction and the Circadian Rhythms of Pituitary-Adrenal Hormones, Growth Hormone and Thyroid-Stimulating Hormone. *Ann Nutr Metab* 31, 81–87 (1987)
176. JL Feldman, J Baeza, JM Denu: Activation of the Protein Deacetylase SIRT6 by Long-chain Fatty Acids and Widespread Deacylation by Mammalian Sirtuins. *J Biol Chem* 288, 31350–31356 (2013)
177. M Kaeberlein, M McVey, L Guarente: The SIR2/3/4 complex and SIR2 alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms. *Genes Dev* 13, 2570–2580 (1999)
178. HA Tissenbaum, L Guarente: Increased dosage of a sir-2 gene extends lifespan in *Caenorhabditis elegans*. *Nature* 410, 227–230 (2001)
179. B Rogina, SL Helfand: Sir2 mediates longevity in the fly through a pathway related to calorie restriction. *Proc Natl Acad Sci USA* 101, 15998–16003 (2004)
180. C Burnett, S Valentini, F Cabreiro, M Goss, M Somogyvári, MD Piper, M Hoddinott, GL Sutphin, V Leko, JJ McElwee, RP Vazquez-Manrique, AM Orfila, D Ackerman, C Au, G Vinti, M Riesen, K Howard, C Neri, A Bedalov *et al.*: Absence of effects of Sir2 overexpression on lifespan in *C. elegans* and *Drosophila*. *Nature* 477, 482–485 (2011)
181. A Satoh, CS Brace, N Rensing, P Clifton, DF Wozniak, ED Herzog, KA Yamada, SI Imai: Sirt1 extends life span and delays aging in mice through the regulation of Nk2 homeobox 1 in the DMH and LH. *Cell Metab* 18, 416–430 (2013)
182. Y Kanfi, S Naiman, G Amir, V Peshti, G Zinman, L Nahum, Z Bar-Joseph, HY Cohen: The sirtuin SIRT6 regulates lifespan in male mice. *Nature* 483, 218–221 (2012)

183. P Oberdoerffer, S Michan, M McVay, R Mostoslavsky, A Hartledore, J Stegmuller, J Vann, SK Park, A Hafner, P Loerch, SM Wright, KD Mills, A Bonni, BA Yankner, R Scully, TA Prolla, FW Alt, DA Sinclair: SIRT1 redistribution on chromatin promotes genomic stability but alters gene expression during aging. *Cell* 135, 907-918 (2008)
184. R Firestein, G Blander, S Michan, P Oberdoerffer, S Ogino, J Campbell, A Bhimavarapu, S Luikenhuis, R de Cabo, C Fuchs, WC Hahn, LP Guarente, D a Sinclair: The SIRT1 deacetylase suppresses intestinal tumorigenesis and colon cancer growth. *PLoS One* 3, e2020 (2008)
185. S Michan, Y Li, MMH Chou, E Parrella, H Ge, JM Long, JS Allard, K Lewis, M Miller, W Xu, RF Mervis, J Chen, KI Guerin, LEH Smith, MW McBurney, DA Sinclair, M Baudry, R de Cabo, VD Longo: SIRT1 is essential for normal cognitive function and synaptic plasticity. *J Neurosci* 30, 9695–9707 (2010)
186. J Gao, WY Wang, YW Mao, J Graff, JS Guan, L Pan, G Mak, D Kim, SC Su, LH Tsai: A novel pathway regulates memory and plasticity via SIRT1 and miR-134. *Nature* 466, 1105–1109 (2010)
187. S Libert, K Pointer, EL Bell, A Das, DE Cohen, JM Asara, K Kapur, S Bergmann, M Preisig, T Otowa, KS Kendler, X Chen, JM Hettema, EJ van den Oord, JP Rubio, L Guarente: SIRT1 activates MAO-A in the brain to mediate anxiety and exploratory drive. *Cell* 147, 1459–1472 (2011)
188. Y Nakahata, M Kaluzova, B Grimaldi, S Sahar, J Hirayama, D Chen, LP Guarente, P Sassone-Corsi: The NAD⁺-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. *Cell* 134, 329–340 (2008)
189. CR Ramadori G, Fujikawa T, Anderson J, Berglund ED, Frazao R, Michán S, Vianna CR, Sinclair DA, Elias CF: SIRT1 deacetylase in SF1 neurons protects against metabolic imbalance. *Cell Metab* 14, 301–312 (2011)
190. T Prozorovski, U Schulze-Topphoff, R Glumm, J Baumgart, F Schroter, O Ninnemann, E Siegert, I Bendix, O Brustle, R Nitsch, F Zipp, O Aktas: Sirt1 contributes critically to the redox-dependent fate of neural progenitors. *Nat Cell Biol* 10, 385–394 (2008)
191. H Jeong, DE Cohen, L Cui, A Supinski, JN Savas, JR Mazzulli, JR Yates, L Bordone, L Guarente, D Krainc: Sirt1 mediates neuroprotection from mutant huntingtin by activation of the TORC1 and CREB transcriptional pathway. *Nat Med* 18, 159–165 (2012)
192. G Donmez, D Wang, DE Cohen, L Guarente: SIRT1 Suppresses 2-Amyloid Production by Activating the \pm -Secretase Gene ADAM10. *Cell* 142, 320–332 (2010)
193. G Donmez, A Arun, CY Chung, PJ McLean, S Lindquist, L Guarente: SIRT1 Protects against α -Synuclein Aggregation by Activating Molecular Chaperones *J Neurosci* 32, 124–132 (2012)
194. L Liu, A Arun, L Ellis, C Peritore, G Donmez: Sirtuin 2 (SIRT2) Enhances 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Nigrostriatal Damage via Deacetylating Forkhead Box O3a (Foxo3a) and Activating Bim Protein. *J Biol Chem* 287, 32307–32311 (2012)
195. V Theendakara, A Patent, CA Peters Libeu, B Philpot, S Flores, O Descamps, KS Poksay, Q Zhang, G Cailing, M Hart, V John, R V Rao, DE Bredesen: Neuroprotective Sirtuin ratio reversed by ApoE4. *Proc Natl Acad Sci USA* (2013)
196. MJ Rardin, JC Newman, JM Held, MP Cusack, DJ Sorensen, B Li, B Schilling, SD Mooney, CR Kahn, E Verdin, BW Gibson: Label-free quantitative proteomics of the lysine acetylome in mitochondria identifies substrates of SIRT3 in metabolic pathways. *Proc Natl Acad Sci USA* 110, 6601–6606 (2013)
197. OM Palacios, JJ Carmona, S Michan, KY Chen, Manabe Y, Ward JL 3rd., Goodyear LJ, Tong Q: Diet and exercise signals regulate SIRT3 and activate AMPK and PGC-1 α in skeletal muscle. *Aging* (Albany NY) 1, 771-783 (2009)
198. D Chen, J Bruno, E Easlon, SJ Lin, HL Cheng, FW Alt, L Guarente: Tissue-specific regulation of SIRT1 by calorie restriction. *Genes Dev* 22, 1753–1757 (2008)
199. MD Hirschey, T Shimazu, E Goetzman, E Jing, B Schwer, DB Lombard, CA Grueter, C Harris, S Biddinger, OR Ilkayeva, RD Stevens, Y Li, AK Saha, NB Ruderman, JR Bain, CB Newgard, R V Farese, FW Alt, CR Kahn *et al.*: SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation. *Nature* 464, 121–125 (2010)
200. HY Cohen, C Miller, KJ Bitterman, NR Wall, B Hekking, B Kessler, KT Howitz, M Gorospe, R de Cabo, DA Sinclair: Calorie Restriction Promotes Mammalian Cell Survival by Inducing the SIRT1 Deacetylase. *Science* (305, 390–392 (2004)
201. G Ramadori, T Fujikawa, J Anderson, ED Berglund, R Frazao, S Michán, CR Vianna, DA Sinclair, CF Elias, R Coppari: SIRT1 Deacetylase in SF1 Neurons Protects against Metabolic Imbalance *Cell Metab* 14, 301–312 (2011)
202. EM Mercken, J Hu, S Krzysik-Walker, M Wei, Y Li, MW McBurney, R de Cabo, VD Longo: SIRT1 but not its increased expression is essential for lifespan extension in caloric restricted mice. *Aging Cell* (2013)
202. EM Mercken, J Hu, S Krzysik-Walker, M Wei, Y Li, MW McBurney, R de Cabo, VD Longo: SIRT1 but not its increased expression is essential for lifespan extension in caloric restricted mice. *Aging Cell* 1, 193-196 (2014)
203. G Boily, EL Seifert, L Bevilacqua, XH He, G Sabourin, C Estey, C Moffat, S Crawford, S Saliba, K

- Jardine, J Xuan, M Evans, ME Harper, MW McBurney: SirT1 Regulates Energy Metabolism and Response to Caloric Restriction in Mice. *PLoS One* 3, e1759 (2008)
204. D Cohen, A Supinski, M Bonkowski, G Donmez, L Guarente: Neuronal SIRT1 regulates endocrine and behavioral responses to calorie restriction. *Genes Dev* 23, 2812–2817 (2009)
205. S Fusco, C Ripoli, MV Podda, SC Ranieri, L Leone, G Toietta, MW McBurney, G Schütz, A Riccio, C Grassi, T Galeotti, G Pani: A role for neuronal cAMP responsive-element binding (CREB)-1 in brain responses to calorie restriction. *Proc Natl Acad Sci USA* 109, 621–626 (2012)
206. P Jadiya, M Chatterjee, SR Sammi, S Kaur, G Palit, A Nazir: Sir-2.1. modulates ‘calorie-restriction-mediated’ prevention of neurodegeneration in *Caenorhabditis elegans*: implications for Parkinson’s disease. *Biochem Biophys Res Commun* 413, 306–310 (2011)
207. W Qin, T Yang, L Ho, Z Zhao, J Wang, L Chen, W Zhao, M Thiagarajan, D MacGrogan, JT Rodgers, P Puigserver, J Sadoshima, H Deng, S Pedrini, S Gandy, AA Sauve, GM Pasinetti: Neuronal SIRT1 activation as a novel mechanism underlying the prevention of Alzheimer disease amyloid neuropathology by calorie restriction. *J Biol Chem* 281, 21745–21754 (2006)
208. R Wang, JJ Li, S Diao, YD Kwak, L Liu, L Zhi, H Büeler, NR Bhat, RW Williams, EA Park, FF Liao: Metabolic stress modulates Alzheimer’s β -secretase gene transcription via SIRT1-PPAR γ -PGC-1 in neurons. *Cell Metab* 17, 685–694 (2013)
209. AS Hebert, KE Dittenhafer-Reed, W Yu, DJ Bailey, ES Selen, MD Boersma, JJ Carson, M Tonelli, AJ Balloon, AJ Higbee, MS Westphall, DJ Pagliarini, TA Prolla, F Assadi-Porter, S Roy, JM Denu, JJ Coon: Calorie restriction and SIRT3 trigger global reprogramming of the mitochondrial protein acetylome. *Mol Cell* 49, 186–199 (2013)
210. X Qiu, K Brown, MD Hirschey, E Verdin, D Chen: Calorie restriction reduces oxidative stress by SIRT3-mediated SOD2 activation. *Cell Metab* 12, 662–667 (2010)
211. S Someya, W Yu, WC Hallows, J Xu, JM Vann, C Leeuwenburgh, M Tanokura, JM Denu, TA Prolla: Sirt3 mediates reduction of oxidative damage and prevention of age-related hearing loss under caloric restriction. *Cell* 143, 802–812 (2010)
212. MC Haigis, R Mostoslavsky, KM Haigis, K Fahie, DC Christodoulou, AJ Murphy, DM Valenzuela, GD Yancopoulos, M Karow, G Blander, C Wolberger, TA Prolla, R Weindruch, FW Alt, L Guarente: SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic beta cells. *Cell* 126, 941–954 (2006)
213. KT Howitz, KJ Bitterman, HY Cohen, DW Lamming, S Lavu, JG Wood, RE Zipkin, P Chung, A Kisielewski, LL Zhang, B Scherer, DA Sinclair: Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan *Nature* 425, 191–196 (2003)
214. K Zarse, S Schmeisser, M Birringer, E Falk, D Schmoll, M Ristow: Differential effects of resveratrol and SRT1720 on lifespan of adult *Caenorhabditis elegans*. *Horm Metab Res* 42, 837–839 (2010)
215. B Rascon, BP Hubbard, DA Sinclair, G V Amdam: The lifespan extension effects of resveratrol are conserved in the honey bee and may be driven by a mechanism related to caloric restriction *Aging (Albany NY)* 4, 499–508 (2012)
216. DR Valenzano, E Terzibasi, T Genade, A Cattaneo, L Domenici, A Cellerino: Resveratrol prolongs lifespan and retards the onset of age-related markers in a short-lived vertebrate. *Curr Biol* 16, 296–300 (2006)
217. JA Baur, KJ Pearson, NL Price, HA Jamieson, C Lerin, A Kalra, V V Prabhu, JS Allard, G Lopez-Lluch, K Lewis, PJ Pistell, S Poosala, KG Becker, O Boss, D Gwinn, M Wang, S Ramaswamy, KW Fishbein, RG Spencer *et al.*: Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* 444, 337–342 (2006)
218. M Lagouge, C Argmann, Z Gerhart-Hines, H Meziane, C Lerin, F Daussin, N Messadeq, J Milne, P Lambert, P Elliott, B Geny, M Laakso, P Puigserver, J Auwerx: Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1 α . *Cell* 127, 1109–1122 (2006)
219. KJ Pearson, JA Baur, KN Lewis, L Peshkin, NL Price, N Labinskyy, WR Swindell, D Kamara, RK Minor, E Perez, HA Jamieson, Y Zhang, SR Dunn, K Sharma, N Pleshko, LA Woollett, A Csiszar, Y Ikeno, D Le Couteur *et al.*: Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span. *Cell Metab* 8, 157–168 (2008)
220. JL Barger, T Kayo, JM Vann, EB Arias, J Wang, TA Hacker, Y Wang, D Raederstorff, JD Morrow, C Leeuwenburgh, DB Allison, KW Saupe, GD Cartee, R Weindruch, TA Prolla: A low dose of dietary resveratrol partially mimics caloric restriction and retards aging parameters in mice. *PLoS One* 3, e2264 (2008)
221. S Timmers, E Konings, L Bilet, RH Houtkooper, T van de Weijer, GH Goossens, J Hoeks, S van der Krieken, D Ryu, S Kersten, E Moonen-Kornips, MKC Hesselink, I Kunz, VB Schrauwen-Hinderling, EE Blaak, J Auwerx, P Schrauwen: Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. *Cell Metab* 14, 612–622 (2011)
222. JA Baur, DA Sinclair: Therapeutic potential of resveratrol: the in vivo evidence. *Nat Rev Drug Discov* 5, 493–506 (2006)
223. Z Fonseca-Kelly, M Nassrallah, J Uribe, RS Khan, K Dine, M Dutt, KS Shindler: Resveratrol neuroprotection in

a chronic mouse model of multiple sclerosis. *Front Neurol* 3, 84 (2012)

224. DJ Ho, NY Calingasan, E Wille, M Dumont, MF Beal: Resveratrol protects against peripheral deficits in a mouse model of Huntington's disease. *Exp Neurol* 225, 74–84 (2010)

225. JA Parker, M Arango, S Abderrahmane, E Lambert, C Tourette, H Catoire, C Néri: Resveratrol rescues mutant polyglutamine cytotoxicity in nematode and mammalian neurons. *Nat Genet* 37, 349–350 (2005)

226. DD Lofrumento, G Nicolardi, A Cianciulli, F De Nuccio, V La Pesa, V Carofiglio, T Dragone, R Calvello, MA Panaro: Neuroprotective effects of resveratrol in an MPTP mouse model of Parkinson's-like disease: Possible role of SOCS-1 in reducing pro-inflammatory responses. *Innate Immun* [Epub ahead of print] (2013)

227. D Kim, MDD Nguyen, MM Dobbin, A Fischer, F Sananbenesi, JT Rodgers, I Delalle, JA Baur, G Sui, SM Armour, P Puigserver, DA Sinclair, LHH Tsai: SIRT1 deacetylase protects against neurodegeneration in models for Alzheimer's disease and amyotrophic lateral sclerosis. *EMBO J* 26, 3169–3179 (2007)

228. B Liu, S Ghosh, X Yang, H Zheng, X Liu, Z Wang, G Jin, B Zheng, BK Kennedy, Y Suh, M Kaerberlein, K Tryggvason, Z Zhou: Resveratrol rescues SIRT1-dependent adult stem cell decline and alleviates progeroid features in laminopathy-based progeria. *Cell Metab* 16, 738–750 (2012)

229. RK Minor, JA Baur, AP Gomes, TM Ward, A Csiszar, EM Mercken, K Abdelmohsen, YK Shin, C Canto, M Scheibye-Knudsen, M Krawczyk, PM Irusta, A Martín-Montalvo, BP Hubbard, Y Zhang, E Lehmann, AA White, NL Price, WR Swindell *et al.*: SIRT1720 improves survival and healthspan of obese mice. *Sci Rep* 1, 70 (2011)

230. JN Feige, M Lagouge, C Canto, A Strehle, SM Houten, JC Milne, PD Lambert, C Matak, PJ Elliott, J Auwerx: Specific SIRT1 activation mimics low energy levels and protects against diet-induced metabolic disorders by enhancing fat oxidation. *Cell Metab* 8, 347–358 (2008)

231. MM Dobbin, R Madabhushi, L Pan, Y Chen, D Kim, J Gao, B Ahanonu, PC Pao, Y Qiu, Y Zhao, LH Tsai: SIRT1 collaborates with ATM and HDAC1 to maintain genomic stability in neurons. *Nat Neurosci* 16, 1008–1015 (2013)

232. J Gräff, M Kahn, A Samiei, J Gao, KT Ota, D Rei, LH Tsai: A dietary regimen of caloric restriction or pharmacological activation of SIRT1 to delay the onset of neurodegeneration. *J Neurosci* 33, 8951–8960 (2013)

233. M Hernández-Jiménez, O Hurtado, MI Cuartero, I Ballesteros, A Moraga, JM Pradillo, MW McBurney, I Lizasoain, MA Moro: Silent information regulator 1 protects the brain against cerebral ischemic damage. *Stroke* 44, 2333–2337 (2013)

234. BP Hubbard, AP Gomes, H Dai, J Li, AW Case, T Considine, T V Riera, JE Lee, SY E, DW Lamming, BL Pentelute, ER Schuman, LA Stevens, AJY Ling, SM Armour, S Michan, H Zhao, Y Jiang, SM Sweitzer *et al.*: Evidence for a common mechanism of SIRT1 regulation by allosteric activators. *Science* 339, 1216–1219 (2013)

235. SM Armour, JA Baur, SN Hsieh, A Land-Bracha, SM Thomas, DA Sinclair: Inhibition of mammalian S6 kinase by resveratrol suppresses autophagy. *Aging (Albany NY)* 1, 515–528 (2009)

236. NL Price, AP Gomes, AJY Ling, F V Duarte, A Martín-Montalvo, BJ North, B Agarwal, L Ye, G Ramadori, JS Teodoro, BP Hubbard, AT Varela, JG Davis, B Varamini, A Hafner, R Moaddel, AP Rolo, R Coppari, CM Palmeira *et al.*: SIRT1 is required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function. *Cell Metab* 15, 675–690 (2012)

237. S Santos Franco, L De Falco, S Ghaffari, C Brugnara, DA Sinclair, A Mattè, A Iolascon, N Mohandas, M Bertoldi, X An, A Siciliano, P Rimmelé, MD Cappellini, S Michan, E Zoratti, A Janin, L De Franceschi: Resveratrol accelerates erythroid maturation by activation of FOXO3 and ameliorates anemia in beta-thalassemic mice. *Haematologica* 2, 267–275 (2014)

238. J Hua, KI Guerin, J Chen, S Michán, A Stahl, NM Krah, MR Seaward, RJ Dennison, AM Juan, CJ Hatton, P Sapieha, DA Sinclair, LE Smith: Resveratrol inhibits pathologic retinal neovascularization in Vldlr(-/-) mice. *Invest Ophthalmol Vis Sci* 52, 2809–2816 (2011)

239. M Smoot, K Ono, J Ruscchinski, P-L Wang, T Ideker: Cytoscape 2.8: new features for data integration and network visualization. *Bioinformatics* 27, 431–432 (2011)

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Send correspondence to: Shaday Michan, Instituto Nacional de Geriatria, Blvd, Adolfo Ruiz Cortinez No, 2767, Col, San Jeronimo Lidice, Del, Magdalena Contreras, C.P. 10200 Mexico, D.F., Tel: 5255 55738686, E-mail: michan.sh@gmail.com