Caloric Restriction

Subjects: Cell & Developmental Biology

Submitted by: Kalina Duszka

Definition

Caloric restriction (CR) is a traditional but scientifically verified approach to promoting health and increasing lifespan. CR exerts its effects through multiple molecular pathways that trigger major metabolic adaptations. It influences key nutrient and energy-sensing pathways including mammalian target of rapamycin, Sirtuin 1, AMP-activated protein kinase, and insulin signaling, ultimately resulting in reductions in basic metabolic rate, inflammation, and oxidative stress, as well as increased autophagy and mitochondrial efficiency.

1. Introduction

Caloric restriction (CR) is one of the primary interventions for weight loss and health maintenance. As early as the 16th century, Luigi Cornaro (1484–1566) described the beneficial effects of this approach in his “Discorsa della vita sobria.” Later, at the beginning of the 20th century, the first experimental evidence emerged when Osborne *et al.* reported that CR slowed the growth of rats but prolonged their lifespan [1]. In rats, a CR of 40% applied from weaning onward has been linked to a lifespan extension of almost two fold [2]. In fact, CR has been associated with increases in mean and maximum life span, regardless of sex, in multiple species, including various rat and mouse strains, yeasts, worms, fruit flies, fishes, hamsters, dogs, cows, and owls [3]. The effects of CR in these organisms include reduced neurodegenerative disease incidence, diminished rates of age-specific mortality, and a lower incidence of cancer, diabetes, atherosclerosis, and cardiovascular disease. CR also is linked to delayed onset of age-related processes, such as immunosenescence, sarcopenia, and atrophy of the brain grey matter [3-7]. In monkeys, CR leads to diabetes suppression and a reduced incidence of neoplasia and cardiovascular diseases by up to 50% [6]. These effects have been attributed to a reduction in major risk factors, including cholesterol, C-reactive protein, blood pressure, and intima–media thickness of the carotid arteries [7-9]. Beneficial outcomes of CR have been consistently reported, which supports this approach considering that distinct CR protocols are used in different publications. CR applied in diverse studies ranges from 10% to up to 50% of daily caloric intake. Furthermore, the length of CR varies from a few weeks to life-long treatment. Additionally, some protocols restrict all nutrients, whereas others limit macronutrients only and supplement micronutrients in order to investigate selectively the impact of calorie reduction and prevent malnutrition making a distinction between “dietary restriction” and “energy restriction” [10]. As expected, the type of CR protocol influences the magnitude of outcomes [11,12]. Moreover, the results obtained for experimental models cannot be directly translated to humans [13]. Therefore, it is important to compile the results of multiple studies to identify common patterns of responses regardless of the type of CR. A comparison of the responses from different species may help to draw a more comprehensive picture of the outcomes of CR.

CR has been tied to a complex network of pathways implicating insulin-like growth factor 1 (IGF-1), sirtuins (SIRTs), adenosine monophosphate (AMP) activated protein kinase (AMPK), and target of rapamycin (TOR). The sympathetic and neuroendocrine systems, as well as thyroid hormones, adipokines, and ghrelin, also have been associated with the beneficial outcomes of CR [4]. This ensemble of processes associated with CR affects the whole body, manifesting in reduced inflammation, body fat mass, resting metabolic rate, and body temperature and improved insulin sensitivity [14].As a result of the variety of outcomes related to CR and the complexity of the contributing pathways, the exact mechanisms underlying these health benefits are still not well understood.

2. Major pathways affected by CR

2.1. mTOR

The mammalian (m)TOR pathway is a major nutrient sensor signaling pathway known to regulate longevity. TOR is a well-conserved Ser/Thr protein kinase that belongs to the family of phosphatidylinositol 3 (PI3) kinase–related kinases [15,16]. It functions as an essential part of two complexes, mTORC1 and mTORC2, which have some proteins in common and some different proteins between them [15]. mTORC1 comprises the following core subunits: mTOR, mLST8 (mammalian lethal with sec-13 or GβL), DEPTOR (DEP domain-containing mTOR-interacting protein Tti1/Tel2 complex), PRAS40 (proline-rich Akt substrate of 40 kDa), and Raptor (regulatory-associated protein of mammalian target of rapamycin). mTORC2 is composed of mTOR, mLST8, DEPTOR, the Tti1/Tel2 complex, Rictor (rapamycin-insensitive companion of mTOR), mSin1 (mammalian stress-activated MAP kinase–interacting protein 1 or MAPKAP1), and protor1/2 (protein observed with Rictor 1 and 2) [17-20]. The configuration of each of these two complexes is conserved from yeast to mammals [21]. mTORC1 is sensitive to inhibition by rapamycin and plays essential roles in the regulation of mRNA translation and autophagy. Cellular energy and nutrient status regulate it directly, whereas mTORC2, which is not rapamycin sensitive, functions mainly as an important regulator of the cellular actin cytoskeleton [22,23].

Rheb (Ras homolog enriched in the brain) is a GTPase that in its GTP-bound form directly binds to and activates mTOR [24-27]. Rheb activity is inhibited by the heterodimer complex of tuberous sclerosis proteins 1 and 2 (TSC1 and TSC2) [28-33]. TSC1/2 mediates for mTORC1 many of the upstream signals from growth factors, such as insulin and IGF-1, which stimulate the PI3K and Ras pathways. The effector kinases of these pathways, including Akt (or protein kinase B or PKB), extracellular-signal-regulated kinase 1/2 (ERK1/2), and S6K1, directly phosphorylate and inactivate the TSC1/TSC2 complex, leading to activation of mTORC1 [27,30,34-40]. Rheb also can transmit upstream signals from the p38β–PRAK pathway, which is activated upon glucose starvation [41]. Finally, as a core component of mTORC2, mTOR functions as a tyrosine-protein kinase that promotes activation of the insulin receptor and IGF-1 receptors [42]. These interactions illustrate the tightly interconnected signaling between mTOR and insulin.

The mTOR pathway integrates inputs from major intracellular and extracellular physiological stimuli (growth factors, stress, energy balance, oxygen, amino acids) and controls many major downstream processes, including macromolecule synthesis, autophagy, cell cycle, growth, and metabolism [15,16,43]. For example, the canonical Wnt pathway, AMPK, some proinflammatory cytokines such as tumor necrosis factor-α (TNFα), and the hypoxia-inducible proteins REDD1 and REDD2 modulate mTORC1 activity via TSC1/2 [44-49]. In addition to phosphorylating TSC1/2, AMPK phosphorylates Raptor, leading to the allosteric inhibition of mTOR [50]. mTORC1 activity is further regulated by lipid-derived signaling molecules (phosphatidic acid) [51], the redox status of the cell [52], and amino acids, particularly leucine and arginine [53,54]. DNA damage also signals to mTORC1 through multiple mechanisms, all of which require p53-dependent transcription, induction of the expression of TSC2 and phosphatase and tensin homolog deleted on chromosome 10 (PTEN), and AMPK activation [55-57].

Downstream signaling of mTORC1 controls autophagy and energy metabolism, including the glycolytic flux, lipid synthesis [58-61], and cholesterol synthesis via activation of sterol regulatory element-binding protein (SREBP) 1/2 [58,62,63]. mTORC1 also promotes anabolism in the fed state by controlling lipid metabolism in the liver through modulation of *Srebp1c* expression, a regulator of lipogenesis and lipid storage [64,65].

Under mTORC1 regulation, mitochondrial DNA content and the expression of genes involved in oxidative metabolism increase. mTORC1 exerts this effect in part by mediating the nuclear association between PPARγ coactivator 1α (PGC-1α) and the transcription factor Yin-Yang 1, which positively regulate mitochondrial biogenesis and oxidative function [66].

Activation of mTOR also leads to the phosphorylation of many target proteins related to the translational machinery and ribosome biogenesis, such as p70 ribosomal S6 kinase (S6K) and eukaryotic initiation factor 4E-binding protein (4E-BP) [43,67-72]. Regulation of protein metabolism also is a much-recognized function

of mTOR. Amino acid activation of mTORC1 promotes protein synthesis via activation of S6K and/or inhibition of 4E-BP, whereas inactivation of mTORC1 promotes degradation of damaged proteins and intracellular organelles via autophagy [73,74].

mTORC2 functions mainly as an important regulator of the actin cytoskeleton through its stimulation of F-actin stress fibers,paxillin, RhoA, Rac1, Cdc42, and protein kinase C (PKC) α [19]. mTORC2 phosphorylates Akt [75,76] and thus affects metabolism and cell survival. mTORC2 also directly activates SGK1, a kinase controlling ion transport and growth [77]. Both Akt and SGK1 phosphorylate FoxO1/3a [78-80].

Because of its role as an amino acid sensor, the TOR pathway has been proposed as a mediator of CR. High activity of mTORC1 is a major driving force of aging, whereas suppression of mTOR is tied to many of the benefits associated with CR, including lifespan extension [81-84], as has been demonstrated in yeast [81,85], worms [82], and flies [83]. Rapamycin treatment slightly extends lifespan in flies subjected to CR [86]. In yeast, CR does not further extend the lifespan in the absence of *TOR1,* one of the two *TOR* genes in yeast, suggesting that TOR inhibition and CR promote lifespan via a common mechanism [81]. Similarly, in *C. elegans*, using RNA interference against TOR or autophagy genes in *eat-2* mutant worms, which have impaired feeding behavior and are used as a genetic model for CR, does not extend the lifespan [87,88]. Furthermore, inhibition of one of the principal targets of TOR signaling, S6K, extends the lifespan of *eat-2 C. elegans* [89]. Of note, mTOR activation in the rat's brain results in reduced food intake by promoting expression of the orexigenic neuropeptide Y and agouti-related peptide in the hypothalamus [90,91]. These data suggest that CR and TOR inhibition promote lifespan via overlapping pathways.

2.2. AMPK

CR decreases energy input, which leads to activation of a signaling cascade to generate fuel and increase longevity. Decreased glucose intake reduces carbon flow through the glycolytic pathway and slows the conversion of ADP to ATP. As a principal cellular energy sensor, AMPK monitors the AMP:ATP and ADP:ATP ratios. Functionally, AMPK is a serine/threonine kinase comprising one catalytic subunit, α, and two regulatory subunits, β and γ. Each of the subunits occurs as different isoforms (α1, α2, β1, β2, γ1, γ2, γ3) allowing for different versions of AMPK in various tissues [92,93]. From nematodes to humans, the kinase activity of AMPK is rapidly increased by the binding of AMP or ADP to the AMPKγ subunit [94]. This binding promotes allosteric activation and phosphorylation of AMPK by the upstream AMPK kinase and thus also inhibits its dephosphorylation [95]. An alternative activating pathway triggers AMPK in response to increases in cellular Ca^{2+} and involves the Ca^{2+}/calmodulin-dependent protein kinase kinase β [96]. Once activated, AMPK promotes ATP preservation by repressing energy-consuming biosynthetic pathways while enhancing the expression or activity of proteins involved in catabolism. This process results in the mobilization of deposited energy to restore the ATP supply[97]. Several downstream factors including CREB-regulated transcriptional coactivator-2 (CRTC2) [98], TBC1D1/AS160 [99,100], PGC-1α [101], and histone deacetylase (HDAC) 5 [102] mediate the impact of AMPK on metabolism. Functionally, AMPK phosphorylates acetyl-CoA carboxylase 1 (ACC1) and ACC2 [103,104], SREBP1c [105], glycerol phosphate acyl-transferase, [106] and HMG-CoA reductase [107], resulting in inhibition of FA, cholesterol, and TG synthesis while activating FA uptake and β-oxidation. Additionally, AMPK prevents protein biosynthesis by inhibiting mTOR and TIF-IA/RRN3, a transcription factor for RNA polymerase I that is responsible for ribosomal RNA synthesis [108]. AMPK also influences glucose metabolism by stimulating both nutrient-induced insulin secretion from pancreatic β-cells [109] and glucose uptake by phosphorylating Rab-GTPase-activating protein TBC1D1, which ultimately induces fusion of glucose transporter (GLUT)4 vesicles with the plasma membrane in skeletal muscle [110]. AMPK stimulates glycolysis by phosphorylation of 6-phosphofructo-2-kinase (fructose-2,6-bisphosphatase 2) [111] and, in parallel, inhibits glycogen synthesis through phosphorylation of glycogen synthase [112]. In the liver, AMPK inhibits gluconeogenesis by inhibiting transcription factors including hepatocyte nuclear factor 4 and CRTC2 [113-115]. AMPK also affects the energy balance by regulating circadian metabolic activities and promoting feeding through its action in the hypothalamus [116,117]. It promotes mitochondrial biogenesis via PGC-1α [101] and activates antioxidant defenses. AMPK plays a major role in metabolism but is also involved in inflammation,

cell growth, autophagy, and apoptosis [118]. Therefore, reducing AMPK signaling exerts a cytostatic and tumor-suppressing effect [119,120].

In *C. elegans*, the lifespan extension effect of CR depends on AMPK [121,122]. Similarly, in *Drosophila*, pathways mediating increased lifespan include AMPK activation [123]. In addition, tissue-specific overexpression of AMPK in muscle and body fat extends lifespan in *Drosophila*, whereas AMPK RNA interference shortens the lifespan [124].

2.3. Insulin signaling

Increased glucose levels in serum after food intake promote insulin secretion from pancreatic β-cells, which in turn activates insulin receptors on the surface of target cells. The tyrosine kinase activity of the insulin receptor triggers a signaling cascade starting with the activation of insulin receptor substrates (IRS 1–4) followed by phosphorylation of PI3K, which is responsible for metabolic actions including PDK1 and Akt activation. Akt occurs in three isoforms (1-3) with Akt2 being essential for glucose homeostasis, whereas Akt1 is important for growth and Akt3 for brain development [125]. The Akt-driven inhibition of AS160 phosphorylation induces GLUT4 to translocate to the cell membrane, which promotes glucose transport into the intracellular compartment. Akt also phosphorylates and deactivates glycogen synthase (GS) kinase 3 (GSK3) which stimulates GS and glycogen production. In parallel, it disrupts the CBP/Torc2/CREB complex and consequently inhibits gluconeogenesis. Moreover, Akt activates mTOR, which facilitates protein synthesis, whereas mTORC2 is a critical regulator of Akt [126]. Another Akt regulator, tumor suppressor PTEN, previously mentioned in the context of mTOR, prevents Akt activation, and reduces mTOR activity. In line with the above, inhibition of IGF-1/PI3K/Akt signaling participates in the anti-cancer and DNA-repair activity of CR [127-129]. Further, Akt activation leads to inhibitory phosphorylation of FOXO1 resulting in its nuclear exclusion [130]. Therefore, Akt functions at the crossroads of several pathways responding to CR.

Among other pathways affected by insulin signaling, the most important include mitogen-activated protein kinase (MAPK), which regulates growth; SREBP-1, which promotes lipid and cholesterol synthesis; and the family of FoxO transcriptional regulators, which regulate metabolism and autophagy. In general, insulin signals an abundance of fuels and thus promotes storage and prevents further production of energy molecules [131-134].

The beneficial effects of CR have been associated with changes in metabolism, modification of the activity of the insulin/IGF-1 pathways, reduction in fat mass, and increased stress resistance because of FoxO activation [135-137]. Insulin release and insulin actionseem to play a major role in the control of aging. Modulation of longevity by insulin signaling is supported by the extended lifespan associated with mutations in the insulin/IRS/growth hormone (GH)/IGF-1/FOXO signaling pathways in humans, mice, *C. elegans*, and *Drosophila* [138-144]. Female, but not male, Igf1r+/- mice live on average 33% longer than their wild-type counterparts [142], and fat-specific deletion of Igf1r results in an 18% increased longevity in both sexes [138]. Accordingly, GH receptor/binding protein knockout (GHR/BP-KO) mice are characterized by markedly extended lifespan and show severely reduced plasma IGF-1 and insulin levels, as well as low glucose levels [145,146]. Transgenic Klotho mice, which also have an increased lifespan, are insulin resistant. These findings collectively suggest that aging can be delayed by reducing insulin signaling [147]. It has even been hypothesized that insulin resistance is a physiological protective mechanism against aging and age-related disorders [148].

2.4. Sirtuins

A CR-related decrease in energy levels leads to activation of several signaling cascades. Decreased glucose intake reduces the flow of carbon through the glycolytic pathway and regeneration of ATP from ADP, which eventually alters the NAD+:NADH ratio. This shift activates SIRTs, which serve as both energy sensors and transcriptional effectors by acting as NAD+-dependent HDACs. In addition to CR and fasting, exercise activates SIRTs [149,150], which are remarkably conserved and can even be found in archaebacteria [151]. Originally categorized as class III HDACs, SIRTs are involved in the proper functioning

of nucleic acids including DNA repair, homologous recombination, and DNA deacetylation, and promote transcriptional gene silencing [152,153].

The seven subtypes of SIRTs (SIRT1–7) in mice and humans vary in their cellular distribution and function. SIRT1–SIRT3, SIRT5, and SIRT6 catalyze deacetylation, whereas SIRT4 and SIRT6 have ADP-ribosylation capacity. In addition to histones, SIRT substrates include several transcriptional regulators, such as the nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB), p53, FOXO, and PGC-1α, but also enzymes, including acetyl coenzyme A (CoA) synthetase 2 (AceCS2), long-chain acyl-coenzyme A dehydrogenase (LCAD), HMGCS2, superoxide dismutase 2, and structural proteins, such as α-tubulin [154-158]. Therefore, SIRTs influence a wide range of cellular processes including circadian clocks, cell cycle, mitochondrial biogenesis, and energy homeostasis, and on the whole-body level regulate aging, apoptosis, inflammation, and stress resistance [159,160].

SIRT1 is the most thoroughly investigated mammalian SIRT and is closely involved in metabolism. Studies in *S. cerevisiae* have shown that an extra copy of the Sir2 gene, a yeast homolog of mammalian Sirt1, increases lifespan in a dose-dependent manner [161,162]and deletion of this gene shortens lifespan [161]. In yeast and *Drosophila*, lack of Sir2 and dSir2, respectively, prevents CR-associated life extension [163-165]. SIR2, a yeast analog of Sirt1, assists in DNA repair and regulates genes that change expression with age [166].

The most important metabolic regulator affected by SIRT1 is PGC-1α, which is activated by SIRT1-mediated deacetylation[167,168]. Deacetylated PGC-1α increases hepatic gluconeogenic activity [167], whereas in muscle and BAT, PGC-1α enhances mitochondrial activity. The activity of PGC-1α translates into increased exercise capacity and thermogenesis, leading to protection against the onset of obesity and associated metabolic dysfunction [169]. Deacetylation of PGC-1α by SIRT1 depends on cellular NAD^+levels, so the status of cellular energy affects PGC-1α activity, which adapts cellular energy production through mitochondrial biogenesis and function. Furthermore, among the SIRT1 substrates are factors that control cell proliferation and apoptosis, including the tumor suppressor protein p53 [170]. Overexpression of SIRT1 hinders p53 transcriptional activity and p53-dependent apoptosis triggered by DNA damage and oxidative stress, whereas overexpression of dominant-negative SIRT1 can enhance cellular stress responses [170,171].

SIRTs also regulate the activity of the FOXO family of transcription factors [172,173], which affects cell differentiation, transformation, and metabolism as well as play an important role in CR and longevity regulation [174-176]. SIRT1-mediated deacetylation FOXO1 affects its shuttling between the nucleus and cytoplasm, influencing the expression of FOXO1 target genes and promoting gluconeogenesis and glucose release from hepatocytes [177]. Deacetylation of FOXO3a by SIRT1 increases its translocation from the cytoplasm to the nucleus [178] and its DNA-binding activity. In the nucleus, SIRT1 and FOXO3a form a complex that induces cell-cycle arrest and resistance to oxidative stress, also inhibiting the ability of FOXO3a to induce apoptosis [177]. SIRT1 directly suppresses expression of UCP2, leading to improved coupling of mitochondrial respiration and ATP synthesis, which induces insulin secretion in β-cells [179]. Confirming the role of SIRT1 in the pancreas, $SIRT1^{-/-}$ mice are characterized by impaired insulin secretion in response to glucose compared with wild-type littermates [179,180]. Conversely, β-cell–specific SIRT1-overexpressing mice exhibit improved glucose tolerance and an enhanced glucose-stimulated insulin secretion [180]. In contrast, SIRT4 has an inhibitory effect on amino acid–stimulated insulin secretion. It represses the activity of glutamate dehydrogenase, reducing α-ketoglutarate production and ATP generation, which are known to activate insulin secretion in pancreatic β-cells [181]. To avoid amino acid–stimulated insulin secretion during CR, when amino acid turnover increases, CR decreases SIRT4 activity, which is opposite to the induction of SIRT1 activity during CR [181]. Considering that NAD^+ controls the activities of both SIRT4 and SIRT1, their opposing effects on insulin secretion are surprising, and the full implications remain to be understood.

The role of other SIRT family members has been less investigated, and their function thus is less well

known. SIRT2 is localized mainly in the cytoplasm, where it deacetylates tubulin filaments, HOXA10, and FOXO [182-185]. It takes part in multiple processes including cell cycle regulation [186], lifespan extension [161,187], and glucose and lipid metabolism [155,188]. SIRT3 plays an important role in mitochondria maintenance by acting as a deacetylase for a number of mitochondrial matrix proteins [189,190]. During a prolonged fast, SIRT3 activates FA breakdown by deacetylation of LCAD [157] and stimulates the production of ketone bodies by activating HMGCS2 [156]. Of note, SIRT3 is genetically linked to lifespan in the elderly [191].

SIRT4 has ADP-ribosylation activity and in addition to blocking amino acid–induced insulin secretion [181], it regulates FA oxidation in hepatocytes and myocytes [192]. Both SIRT4 and SIRT5 show mitochondrial localization [181,193]. SIRT6 resides in the nucleus and is involved in genomic DNA stability and promotes the repair of DNA double-strand breaks [194]. SIRT6-deficient mice present a shortened lifespan and a degenerative aging-like phenotype [195]. In contrast, transgenic male mice overexpressing SIRT6 display lower serum levels of IGF-1, higher levels of IGF-1–binding protein, and modified phosphorylation patterns of different components of the IGF-1 signaling pathway, possibly contributing to about a 15% increase in lifespan when compared to wild-type animals [196].

SIRT1 and SIRT6 are both connected with CR-triggered extension of ovarian lifespan, mediated by inhibition of the transition from primordial to developing follicles and by a delay in the growth phase of follicles to preserve the supply of germ cells [197]. SIRT7 is associated with nucleoli and is implicated in activation of transcription by RNA polymerase I [198] as well as repair of double-strand breaks by non-homologous end-joining [199]. SIRT7 knockout mice display features of premature aging [199]. SIRT1, SIRT6, and SIRT7 facilitate DNA repair, and this repair slows the aging process. During CR, except for SIRT4, the expression and activity of SIRTs are increased in many tissues, including adipose and brain [200-202], heart [203,204], and liver [205]. SIRT1 mediates a broad array of physiological effects of CR. The overexpression of SIRT in worms and flies increases their lifespan [164,165], and accordingly, mutants of SIRT do not show lifespan extension by CR [163,206]. Moreover, transgenic mice overexpressing SIRT1 show phenotypes similar to those of CR mice [207]. The previously mentioned role of Sir2 in lifespan is particularly critical in the context of CR.

Resveratrol, a polyphenolic compound present in, for example, red grapes and wine, stimulates SIRT1 expression, resulting in extended lifespan and health span in treated animals [208]. SIRT1 activation by resveratrol mimics CR and delays aging in a wide range of organisms, from *S. cerevisiae* [209] to *C. elegans* to *Drosophila* [210] and mice [211]. Resveratrol is considered one of the mimetics not only of CR but also of exercise [208,212]. In mice, resveratrol inhibits gene expression profiles associated with muscle aging and age-related cardiac dysfunction [213]. The compound protects mice against diet-induced obesity and the associated insulin resistance through enhanced mitochondrial function mediated by PGC-1α [169].

3. Major outcomes of CR

3.1. Oxidative stress reduction

ROS are generated as a byproduct of cellular respiration, contributing to the accumulation of oxidative damage and the formation of a range of oxidation products of different macromolecules including lipids, proteins, and nucleic acids [214]. A small amount of ROS is normally beneficial because it plays an important role in cellular processes such as cell cycle progression, regulation of signaling pathways in response to intra- and extracellular stimuli, and inflammation [215]. However, high uncontrolled levels of ROS are detrimental.

During oxidative stress, the sustained production of ROS and reactive nitrogen species leads to a perturbed equilibrium between pro-oxidants and antioxidants. Consequently, macromolecules, organelles, and cells are altered, and if much damage accumulates, necrotic or apoptotic cell death occurs. The “free radical theory” of aging [216] proposes that the generation of oxidative stress is a major factor contributing to the onset of the aging process and age-related diseases. Therefore, the mammalian life

span is reduced in relation to the mitochondrial production of oxidizing free radicals [215]. CR likely exerts its diverse benefits through reducing ROS levels and suppressing age-related oxidative stress while supporting the antioxidant defense system [217-219]. CR diminishes the impact of ROS through three processes: reduction of oxygen free-radical generation by slowing metabolism, acceleration of ROS neutralization, and stimulation of the repair of ROS-damaged molecules [220-224].

3.2. Mitochondrial function

One of the several theories tightly connected with the effects of ROS is the “mitochondrial theory of aging”, which proposes that mitochondria are the critical component in the aging process. In fact, mitochondrial DNA damage and dysfunction increase with aging and are associated with a vast number of pathologies. Defective mitochondria determine the turnover not only of the organelles themselves but also whole cells, resulting in the acceleration of aging [215,225,226]. Aging has been linked to a reduced capacity for oxidative phosphorylation in the muscle and heart, most likely because of a decline in mitochondrial content and/or function [227-229]. Accordingly, young individuals have higher respiratory function compared to the elderly [230-232]. Disturbed mitochondrial electron transfer increases the likelihood of electron leakage and ROS production. Consequently, components of the electron transport chain and mitochondrial DNA become damaged, leading to further increases in intracellular ROS levels and a decline in mitochondrial function.Because mitochondrial DNA is spatially close to the source of ROS production, it is thought to be particularly vulnerable to ROS-mediated lesions [216,233].

An interesting feature of CR, one associated with ROS and changes in metabolism, is mitochondria biogenesis, which is relatively high in various tissues such as in the brain, heart, liver, and particularly the BAT of mice [202,234]. It is associated with activation of the master regulator of mitochondrial biogenesis, PGC-1α [235-237]. PGC-1α is expressed at a high level in BAT, heart, skeletal muscle, brain, and kidney, whereas its expression is low in the liver and very low in WAT [238]. Various physiological stimuli highly induce PGC-1α in different organs. It is increased in BAT by cold exposure and in skeletal muscle by exercise and decreased ATP level, whereas in the liver, it is mostly affected by CR [239]. When ectopically expressed in fat or muscle cells, PGC-1α strongly increases mitochondrial biogenesis and oxidative metabolism, which correlates with an increase in mitochondrial DNA and the expression of multiple mitochondrial genes [239,240]. To prevent a mitochondrial biogenesis–associated increase in ROS levels, PGC-1α also induces expression of the antioxidant genes *GPx1* and *MnSOD* [241]. One hypothesis regarding the beneficial outcomes of CR proposes is that CR preserves mitochondrial function by maintaining protein and DNA integrity through decreasing mitochondrial oxidant emission and increasing endogenous antioxidant activity [242,243]. Its impact on mitochondria biogenesis remains a matter of discussion [244,245].

In addition to affecting mitochondria biogenesis, PGC-1α also influences metabolism. It mediates a fasting-induced increase in FA metabolism and downregulation of pyruvate dehydrogenase, which is part of the mitochondrial pyruvate dehydrogenase complex that catalyzes the reaction representing pyruvate entry into the tricarboxylic acid cycle. In PGC-1α knockout mice, pyruvate dehydrogenase fails to adapt to CR, and the ability of the mice to endure prolonged starvation is decreased [246]. PGC-1α knockout mice also show a reduced content of mitochondrial electron transport chain proteins in skeletal muscle [247,248]. The activity of PGC-1α is directly regulated by the energy sensors SIRT1 and AMPK [101,167]. Functionally, the transcriptional activity of PGC-1α relies on its interactions with transcriptional factors for controlling FA metabolism. Of note, all three PPAR isotypes are subject to transcriptional coactivation by PGC-1α and are major executors of PGC-1α–induced regulation [238,249-251].

3.3. Reduction of inflammation

The “inflammation hypothesis of aging” posits a molecular mechanism of aging based on inflammation. Inflammation is a complex defense reaction to insult and both physiological and nonphysiological stress, induced by agents such as chemicals, drugs, or microbial entities. Inflammation responses are activated by well-coordinated, sequential events controlled by humoral and cellular reactions. Elevated tissue levels of

TNFα, IL-1, and IL-6, among other proinflammatory mediators, have been observed in experimental animal models of inflammation. With aging, inflammatory responses may be overactive or even cause damage, resulting in pathological conditions [14].

During aging, a shift occurs in the ratio of naive to memory T cells, with associated changes in the cytokine profile in favor of inflammatory cytokines such as TNFα, IL-1, IL-6, INFγ, and transforming growth factor β [252-255]. There is also a progressively higher dysregulation of immune cells and proinflammatory responses. Macrophages from old mice produce more prostaglandin E2 than those from young mice because of higher COX-2 activity [256]. One major causative factor in tissue inflammation is the uncontrolled overproduction ROS/reactive nitrogen species. The transcriptional regulator NF-κB is an inflammatory reaction factor of major importance that is extremely sensitive to oxidants [257-262]. Enhanced IL-6 production by activated NF-κB has been implicated in many pathophysiological dysfunctions of aging ranging, from Alzheimer's disease to atherosclerosis [263]. CR exhibits a broad and effective anti-inflammatory effect. It blunts age-triggered increases in COX-2 levels and activity through the modulation of NF-κB and IκB, in which COX-2–derived ROS generation decreases. Also, the production of iNOS, IL-β, IL-6, TNFα, and prostanoids such as TXA 2, prostacyclin 2, and prostaglandin E2 is suppressed [14,219]. The prevention of the age-related decline triggered by CR correlates with dampening the reduction of PPAR expression and activity seen during aging. Therefore, under CR conditions, higher PPAR expression may play a role in the suppression of the age-induced increase in inflammation [264]. PPARs are implicated in inflammation at the transcriptional level by interfering with proinflammatory mediators such as NF-κB, STAT-1, and activating protein-1, leading to downregulation of the gene targets of these factors [265-268]. In this way, PPARα and PPARγ inhibit the expression of inflammatory genes, such as COX-2, iNOS, cytokines, metalloproteases, and acute-phase proteins [266,269]. Inflammatory eicosanoids serve as ligands for PPARs, and levels of these signaling molecules, including prostaglandins and leukotrienes, increase with age [270].

3.3.1. Metabolic adaptation

The shortage of energy during CR leads to a sequence of metabolic changes. Following depletion of dietary glucose, glycogen is mobilized as an energy supply and, upon prolonged CR, hepatic metabolism shifts to gluconeogenesis to prevent hypoglycemia. Further energy restriction, carbohydrate depletion triggers a shift to fat recruitment and ketone body production.

3.3.2.Physical exercise

Exercise, like CR, yields multiple beneficial effects. Research outcomes point towards the effectiveness of regular moderate exercise in preventing and delaying several metabolic disorders, chronic diseases, and premature death. Increased physical activity reduces mortality risk from many age-related diseases, including cardiovascular disease, stroke, T2D, certain cancers, hypertension, obesity, depression, and osteoporosis [271-275]. However, in rodents, exercise improves the mean lifespan without increasing maximum longevity [276,277]. Similarly, high physical activity fails to extend maximum lifespan in humans [278]. Compared to exercise, long-term CR in humans improves several biomarkers related to aging [279,280]. Accordingly, exercise has been deemed as unable to fully mimic the beneficial hormonal and/or metabolic changes associated with CR [281]. Therefore, despite a mutual influence with CR on similar molecular pathways and providing multiple advantages, physical activity has been recognized as yielding inferior benefits compared to CR.

3.4. Longevity and aging

Both genetic and environmental factors control the progression of aging. Aging is associated with immunosenescence, increased oxidative stress, decreased hormonal secretion, changes in metabolic rate, mitochondrial function, insulin resistance, and dysregulated lipid metabolism [282-284]. Preservation of insulin sensitivity by reducing levels of blood glucose and insulin without compromising glucose fuel may prevent age-related metabolic phenotype [140]. Glucose metabolism maintenance is a key feature of the anti-aging actions of CR [3]. In fact, genes connected with the insulin/IGF-1 signaling pathway have been

proposed as longevity candidate markers [143,144,285]. Paradoxically, impaired insulin signaling through the insulin receptor or its substrates increases rather than decreases lifespan in a number of mouse models [138,143,144].

References

1. Osborne, T.B.; Mendel, L.B.; Ferry, E.L. The Effect of Retardation of Growth Upon the Breeding Period and Duration of Life of Rats. *Science* **1917**, *45*, 294-295, doi:10.1126/science.45.1160.294.
2. McCay, C.M.; Crowell, M.F.; Maynard, L.A. The effect of retarded growth upon the length of life span and upon the ultimate body size. 1935. *Nutrition* **1989**, *5*, 155-171; discussion 172.
3. Masoro, E.J. Overview of caloric restriction and ageing. *Mech Ageing Dev* **2005**, *126*, 913-922, doi:10.1016/j.mad.2005.03.012.
4. Speakman, J.R.; Mitchell, S.E. Caloric restriction. *Mol Aspects Med* **2011**, *32*, 159-221, doi:10.1016/j.mam.2011.07.001.
5. Weindruch, R.; Walford, R.L.; Fligiel, S.; Guthrie, D. The retardation of aging in mice by dietary restriction: longevity, cancer, immunity and lifetime energy intake. *J Nutr* **1986**, *116*, 641-654, doi:10.1093/jn/116.4.641.
6. Colman, R.J.; Anderson, R.M.; Johnson, S.C.; Kastman, E.K.; Kosmatka, K.J.; Beasley, T.M.; Allison, D.B.; Cruzen, C.; Simmons, H.A.; Kemnitz, J.W., et al. Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science* **2009**, *325*, 201-204, doi:10.1126/science.1173635.
7. Anderson, R.M.; Shanmuganayagam, D.; Weindruch, R. Caloric restriction and aging: studies in mice and monkeys. *Toxicol Pathol* **2009**, *37*, 47-51, doi:10.1177/0192623308329476.
8. Fontana, L.; Meyer, T.E.; Klein, S.; Holloszy, J.O. Long-term calorie restriction is highly effective in reducing the risk for atherosclerosis in humans. *Proc Natl Acad Sci U S A* **2004**, *101*, 6659-6663, doi:10.1073/pnas.0308291101.
9. Fontana, L.; Klein, S. Aging, adiposity, and calorie restriction. *JAMA* **2007**, *297*, 986-994, doi:10.1001/jama.297.9.986.
10. Cerqueira, F.M.; Kowaltowski, A.J. Commonly adopted caloric restriction protocols often involve malnutrition. *Ageing Res Rev* **2010**, *9*, 424-430, doi:10.1016/j.arr.2010.05.002.
11. Dogan, S.; Ray, A.; Cleary, M.P. The influence of different calorie restriction protocols on serum pro-inflammatory cytokines, adipokines and IGF-I levels in female C57BL6 mice: short term and long term diet effects. *Meta Gene* **2017**, *12*, 22-32, doi:10.1016/j.mgene.2016.12.013.
12. Dogan, S.; Rogozina, O.P.; Lokshin, A.E.; Grande, J.P.; Cleary, M.P. Effects of chronic vs. intermittent calorie restriction on mammary tumor incidence and serum adiponectin and leptin levels in MMTV-TGF-alpha mice at different ages. *Oncol Lett* **2010**, *1*, 167-176, doi:10.3892/ol_00000031.
13. Phelan, J.P.; Rose, M.R. Why dietary restriction substantially increases longevity in animal models but won't in humans. *Ageing Res Rev* **2005**, *4*, 339-350, doi:10.1016/j.arr.2005.06.001.
14. Chung, H.Y.; Kim, H.J.; Kim, J.W.; Yu, B.P. The inflammation hypothesis of aging: molecular modulation by calorie restriction. *Ann N Y Acad Sci* **2001**, *928*, 327-335.
15. Bhaskar, P.T.; Hay, N. The two TORCs and Akt. *Dev Cell* **2007**, *12*, 487-502, doi:10.1016/j.devcel.2007.03.020.
16. Wullschleger, S.; Loewith, R.; Hall, M.N. TOR signaling in growth and metabolism. *Cell* **2006**, *124*, 471-484, doi:10.1016/j.cell.2006.01.016.
17. Jacinto, E.; Facchinetti, V.; Liu, D.; Soto, N.; Wei, S.; Jung, S.Y.; Huang, Q.; Qin, J.; Su, B. SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. *Cell* **2006**, *127*, 125-137, doi:10.1016/j.cell.2006.08.033.
18. Pearce, L.R.; Huang, X.; Boudeau, J.; Pawlowski, R.; Wullschleger, S.; Deak, M.; Ibrahim, A.F.; Gourlay, R.; Magnuson, M.A.; Alessi, D.R. Identification of Protor as a novel Rictor-binding component of mTOR complex-2. *Biochem J* **2007**, *405*, 513-522, doi:10.1042/BJ20070540.
19. Sarbassov, D.D.; Ali, S.M.; Kim, D.H.; Guertin, D.A.; Latek, R.R.; Erdjument-Bromage, H.; Tempst, P.; Sabatini, D.M. Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr Biol* **2004**, *14*, 1296-1302,

doi:10.1016/j.cub.2004.06.054.
20. Zheng, X.; Liang, Y.; He, Q.; Yao, R.; Bao, W.; Bao, L.; Wang, Y.; Wang, Z. Current models of mammalian target of rapamycin complex 1 (mTORC1) activation by growth factors and amino acids. *Int J Mol Sci* **2014**, *15*, 20753-20769, doi:10.3390/ijms151120753.
21. Tatebe, H.; Shiozaki, K. Evolutionary Conservation of the Components in the TOR Signaling Pathways. *Biomolecules* **2017**, *7*, doi:10.3390/biom7040077.
22. Loewith, R.; Jacinto, E.; Wullschleger, S.; Lorberg, A.; Crespo, J.L.; Bonenfant, D.; Oppliger, W.; Jenoe, P.; Hall, M.N. Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. *Mol Cell* **2002**, *10*, 457-468.
23. Jacinto, E.; Loewith, R.; Schmidt, A.; Lin, S.; Ruegg, M.A.; Hall, A.; Hall, M.N. Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat Cell Biol* **2004**, *6*, 1122-1128, doi:10.1038/ncb1183.
24. Yamagata, K.; Sanders, L.K.; Kaufmann, W.E.; Yee, W.; Barnes, C.A.; Nathans, D.; Worley, P.F. rheb, a growth factor- and synaptic activity-regulated gene, encodes a novel Ras-related protein. *J Biol Chem* **1994**, *269*, 16333-16339.
25. Long, X.; Lin, Y.; Ortiz-Vega, S.; Yonezawa, K.; Avruch, J. Rheb binds and regulates the mTOR kinase. *Curr Biol* **2005**, *15*, 702-713, doi:10.1016/j.cub.2005.02.053.
26. Sato, T.; Nakashima, A.; Guo, L.; Tamanoi, F. Specific activation of mTORC1 by Rheb G-protein in vitro involves enhanced recruitment of its substrate protein. *J Biol Chem* **2009**, *284*, 12783-12791, doi:10.1074/jbc.M809207200.
27. Inoki, K.; Li, Y.; Xu, T.; Guan, K.L. Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes Dev* **2003**, *17*, 1829-1834, doi:10.1101/gad.1110003.
28. Garami, A.; Zwartkruis, F.J.; Nobukuni, T.; Joaquin, M.; Roccio, M.; Stocker, H.; Kozma, S.C.; Hafen, E.; Bos, J.L.; Thomas, G. Insulin activation of Rheb, a mediator of mTOR/S6K/4E-BP signaling, is inhibited by TSC1 and 2. *Mol Cell* **2003**, *11*, 1457-1466.
29. Wang, X.; Proud, C.G. The mTOR pathway in the control of protein synthesis. *Physiology (Bethesda)* **2006**, *21*, 362-369, doi:10.1152/physiol.00024.2006.
30. Tee, A.R.; Manning, B.D.; Roux, P.P.; Cantley, L.C.; Blenis, J. Tuberous sclerosis complex gene products, Tuberin and Hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb. *Curr Biol* **2003**, *13*, 1259-1268.
31. Zhang, Y.; Gao, X.; Saucedo, L.J.; Ru, B.; Edgar, B.A.; Pan, D. Rheb is a direct target of the tuberous sclerosis tumour suppressor proteins. *Nat Cell Biol* **2003**, *5*, 578-581, doi:10.1038/ncb999.
32. Kwiatkowski, D.J. Tuberous sclerosis: from tubers to mTOR. *Ann Hum Genet* **2003**, *67*, 87-96.
33. Miyazaki, M.; McCarthy, J.J.; Esser, K.A. Insulin like growth factor-1-induced phosphorylation and altered distribution of tuberous sclerosis complex (TSC)1/TSC2 in C2C12 myotubes. *FEBS J* **2010**, *277*, 2180-2191, doi:10.1111/j.1742-4658.2010.07635.x.
34. Dan, H.C.; Sun, M.; Yang, L.; Feldman, R.I.; Sui, X.M.; Ou, C.C.; Nellist, M.; Yeung, R.S.; Halley, D.J.; Nicosia, S.V., et al. Phosphatidylinositol 3-kinase/Akt pathway regulates tuberous sclerosis tumor suppressor complex by phosphorylation of tuberin. *J Biol Chem* **2002**, *277*, 35364-35370, doi:10.1074/jbc.M205838200.
35. Manning, B.D.; Tee, A.R.; Logsdon, M.N.; Blenis, J.; Cantley, L.C. Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/akt pathway. *Mol Cell* **2002**, *10*, 151-162.
36. Inoki, K.; Li, Y.; Zhu, T.; Wu, J.; Guan, K.L. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nat Cell Biol* **2002**, *4*, 648-657, doi:10.1038/ncb839.
37. Ma, L.; Chen, Z.; Erdjument-Bromage, H.; Tempst, P.; Pandolfi, P.P. Phosphorylation and functional inactivation of TSC2 by Erk implications for tuberous sclerosis and cancer pathogenesis. *Cell* **2005**, *121*, 179-193, doi:10.1016/j.cell.2005.02.031.
38. Roux, P.P.; Ballif, B.A.; Anjum, R.; Gygi, S.P.; Blenis, J. Tumor-promoting phorbol esters and activated Ras inactivate the tuberous sclerosis tumor suppressor complex via p90 ribosomal S6 kinase. *Proc Natl Acad Sci U S A* **2004**, *101*, 13489-13494, doi:10.1073/pnas.0405659101.
39. Potter, C.J.; Pedraza, L.G.; Xu, T. Akt regulates growth by directly phosphorylating Tsc2. *Nat Cell Biol*

2002, *4*, 658-665, doi:10.1038/ncb840.
40. Vander Haar, E.; Lee, S.I.; Bandhakavi, S.; Griffin, T.J.; Kim, D.H. Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40. *Nat Cell Biol* **2007**, *9*, 316-323, doi:10.1038/ncb1547.
41. Zheng, M.; Wang, Y.H.; Wu, X.N.; Wu, S.Q.; Lu, B.J.; Dong, M.Q.; Zhang, H.; Sun, P.; Lin, S.C.; Guan, K.L., et al. Inactivation of Rheb by PRAK-mediated phosphorylation is essential for energy-depletion-induced suppression of mTORC1. *Nat Cell Biol* **2011**, *13*, 263-272, doi:10.1038/ncb2168.
42. Yin, Y.; Hua, H.; Li, M.; Liu, S.; Kong, Q.; Shao, T.; Wang, J.; Luo, Y.; Wang, Q.; Luo, T., et al. mTORC2 promotes type I insulin-like growth factor receptor and insulin receptor activation through the tyrosine kinase activity of mTOR. *Cell Res* **2016**, *26*, 46-65, doi:10.1038/cr.2015.133.
43. Arsham, A.M.; Neufeld, T.P. Thinking globally and acting locally with TOR. *Curr Opin Cell Biol* **2006**, *18*, 589-597, doi:10.1016/j.ceb.2006.09.005.
44. Lee, D.F.; Kuo, H.P.; Chen, C.T.; Hsu, J.M.; Chou, C.K.; Wei, Y.; Sun, H.L.; Li, L.Y.; Ping, B.; Huang, W.C., et al. IKK beta suppression of TSC1 links inflammation and tumor angiogenesis via the mTOR pathway. *Cell* **2007**, *130*, 440-455, doi:10.1016/j.cell.2007.05.058.
45. Inoki, K.; Ouyang, H.; Zhu, T.; Lindvall, C.; Wang, Y.; Zhang, X.; Yang, Q.; Bennett, C.; Harada, Y.; Stankunas, K., et al. TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth. *Cell* **2006**, *126*, 955-968, doi:10.1016/j.cell.2006.06.055.
46. Inoki, K.; Zhu, T.; Guan, K.L. TSC2 mediates cellular energy response to control cell growth and survival. *Cell* **2003**, *115*, 577-590.
47. Brugarolas, J.; Lei, K.; Hurley, R.L.; Manning, B.D.; Reiling, J.H.; Hafen, E.; Witters, L.A.; Ellisen, L.W.; Kaelin, W.G., Jr. Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. *Genes Dev* **2004**, *18*, 2893-2904, doi:10.1101/gad.1256804.
48. DeYoung, M.P.; Horak, P.; Sofer, A.; Sgroi, D.; Ellisen, L.W. Hypoxia regulates TSC1/2-mTOR signaling and tumor suppression through REDD1-mediated 14-3-3 shuttling. *Genes Dev* **2008**, *22*, 239-251, doi:10.1101/gad.1617608.
49. Reiling, J.H.; Hafen, E. The hypoxia-induced paralogs Scylla and Charybdis inhibit growth by down-regulating S6K activity upstream of TSC in Drosophila. *Genes Dev* **2004**, *18*, 2879-2892, doi:10.1101/gad.322704.
50. Gwinn, D.M.; Shackelford, D.B.; Egan, D.F.; Mihaylova, M.M.; Mery, A.; Vasquez, D.S.; Turk, B.E.; Shaw, R.J. AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell* **2008**, *30*, 214-226, doi:10.1016/j.molcel.2008.03.003.
51. Fang, Y.; Vilella-Bach, M.; Bachmann, R.; Flanigan, A.; Chen, J. Phosphatidic acid-mediated mitogenic activation of mTOR signaling. *Science* **2001**, *294*, 1942-1945, doi:10.1126/science.1066015.
52. Sarbassov, D.D.; Sabatini, D.M. Redox regulation of the nutrient-sensitive raptor-mTOR pathway and complex. *J Biol Chem* **2005**, *280*, 39505-39509, doi:10.1074/jbc.M506096200.
53. Proud, C.G. Signalling to translation: how signal transduction pathways control the protein synthetic machinery. *Biochem J* **2007**, *403*, 217-234, doi:10.1042/BJ20070024.
54. Dann, S.G.; Thomas, G. The amino acid sensitive TOR pathway from yeast to mammals. *FEBS Lett* **2006**, *580*, 2821-2829, doi:10.1016/j.febslet.2006.04.068.
55. Feng, Z.; Zhang, H.; Levine, A.J.; Jin, S. The coordinate regulation of the p53 and mTOR pathways in cells. *Proc Natl Acad Sci U S A* **2005**, *102*, 8204-8209, doi:10.1073/pnas.0502857102.
56. Stambolic, V.; MacPherson, D.; Sas, D.; Lin, Y.; Snow, B.; Jang, Y.; Benchimol, S.; Mak, T.W. Regulation of PTEN transcription by p53. *Mol Cell* **2001**, *8*, 317-325.
57. Budanov, A.V.; Karin, M. p53 target genes sestrin1 and sestrin2 connect genotoxic stress and mTOR signaling. *Cell* **2008**, *134*, 451-460, doi:10.1016/j.cell.2008.06.028.
58. Duvel, K.; Yecies, J.L.; Menon, S.; Raman, P.; Lipovsky, A.I.; Souza, A.L.; Triantafellow, E.; Ma, Q.; Gorski, R.; Cleaver, S., et al. Activation of a metabolic gene regulatory network downstream of mTOR complex 1. *Mol Cell* **2010**, *39*, 171-183, doi:10.1016/j.molcel.2010.06.022.
59. Hudson, C.C.; Liu, M.; Chiang, G.G.; Otterness, D.M.; Loomis, D.C.; Kaper, F.; Giaccia, A.J.; Abraham, R.T. Regulation of hypoxia-inducible factor 1alpha expression and function by the mammalian target of rapamycin. *Mol Cell Biol* **2002**, *22*, 7004-7014.
60. Kim, J.E.; Chen, J. regulation of peroxisome proliferator-activated receptor-gamma activity by

mammalian target of rapamycin and amino acids in adipogenesis. *Diabetes* **2004**, *53*, 2748-2756.
61. Zhang, H.H.; Huang, J.; Duvel, K.; Boback, B.; Wu, S.; Squillace, R.M.; Wu, C.L.; Manning, B.D. Insulin stimulates adipogenesis through the Akt-TSC2-mTORC1 pathway. *PLoS One* **2009**, *4*, e6189, doi:10.1371/journal.pone.0006189.
62. Porstmann, T.; Santos, C.R.; Griffiths, B.; Cully, M.; Wu, M.; Leevers, S.; Griffiths, J.R.; Chung, Y.L.; Schulze, A. SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth. *Cell Metab* **2008**, *8*, 224-236, doi:10.1016/j.cmet.2008.07.007.
63. Wang, B.T.; Ducker, G.S.; Barczak, A.J.; Barbeau, R.; Erle, D.J.; Shokat, K.M. The mammalian target of rapamycin regulates cholesterol biosynthetic gene expression and exhibits a rapamycin-resistant transcriptional profile. *Proc Natl Acad Sci U S A* **2011**, *108*, 15201-15206, doi:10.1073/pnas.1103746108.
64. Yecies, J.L.; Zhang, H.H.; Menon, S.; Liu, S.; Yecies, D.; Lipovsky, A.I.; Gorgun, C.; Kwiatkowski, D.J.; Hotamisligil, G.S.; Lee, C.H., et al. Akt stimulates hepatic SREBP1c and lipogenesis through parallel mTORC1-dependent and independent pathways. *Cell Metab* **2011**, *14*, 21-32, doi:10.1016/j.cmet.2011.06.002.
65. Li, S.; Brown, M.S.; Goldstein, J.L. Bifurcation of insulin signaling pathway in rat liver: mTORC1 required for stimulation of lipogenesis, but not inhibition of gluconeogenesis. *Proc Natl Acad Sci U S A* **2010**, *107*, 3441-3446, doi:10.1073/pnas.0914798107.
66. Cunningham, J.T.; Rodgers, J.T.; Arlow, D.H.; Vazquez, F.; Mootha, V.K.; Puigserver, P. mTOR controls mitochondrial oxidative function through a YY1-PGC-1alpha transcriptional complex. *Nature* **2007**, *450*, 736-740, doi:10.1038/nature06322.
67. Corradetti, M.N.; Guan, K.L. Upstream of the mammalian target of rapamycin: do all roads pass through mTOR? *Oncogene* **2006**, *25*, 6347-6360, doi:10.1038/sj.onc.1209885.
68. Hay, N.; Sonenberg, N. Upstream and downstream of mTOR. *Genes Dev* **2004**, *18*, 1926-1945, doi:10.1101/gad.1212704.
69. Shahbazian, D.; Roux, P.P.; Mieulet, V.; Cohen, M.S.; Raught, B.; Taunton, J.; Hershey, J.W.; Blenis, J.; Pende, M.; Sonenberg, N. The mTOR/PI3K and MAPK pathways converge on eIF4B to control its phosphorylation and activity. *EMBO J* **2006**, *25*, 2781-2791, doi:10.1038/sj.emboj.7601166.
70. Dorrello, N.V.; Peschiaroli, A.; Guardavaccaro, D.; Colburn, N.H.; Sherman, N.E.; Pagano, M. S6K1- and betaTRCP-mediated degradation of PDCD4 promotes protein translation and cell growth. *Science* **2006**, *314*, 467-471, doi:10.1126/science.1130276.
71. Richardson, C.J.; Broenstrup, M.; Fingar, D.C.; Julich, K.; Ballif, B.A.; Gygi, S.; Blenis, J. SKAR is a specific target of S6 kinase 1 in cell growth control. *Curr Biol* **2004**, *14*, 1540-1549, doi:10.1016/j.cub.2004.08.061.
72. Blenis, J.; Kuo, C.J.; Erikson, R.L. Identification of a ribosomal protein S6 kinase regulated by transformation and growth-promoting stimuli. *J Biol Chem* **1987**, *262*, 14373-14376.
73. Kim, J.; Guan, K.L. mTOR as a central hub of nutrient signalling and cell growth. *Nat Cell Biol* **2019**, *21*, 63-71, doi:10.1038/s41556-018-0205-1.
74. Zhao, J.; Zhai, B.; Gygi, S.P.; Goldberg, A.L. mTOR inhibition activates overall protein degradation by the ubiquitin proteasome system as well as by autophagy. *Proc Natl Acad Sci U S A* **2015**, *112*, 15790-15797, doi:10.1073/pnas.1521919112.
75. Betz, C.; Stracka, D.; Prescianotto-Baschong, C.; Frieden, M.; Demaurex, N.; Hall, M.N. Feature Article: mTOR complex 2-Akt signaling at mitochondria-associated endoplasmic reticulum membranes (MAM) regulates mitochondrial physiology. *Proc Natl Acad Sci U S A* **2013**, *110*, 12526-12534, doi:10.1073/pnas.1302455110.
76. Sarbassov, D.D.; Guertin, D.A.; Ali, S.M.; Sabatini, D.M. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* **2005**, *307*, 1098-1101, doi:10.1126/science.1106148.
77. Garcia-Martinez, J.M.; Alessi, D.R. mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and activation of serum- and glucocorticoid-induced protein kinase 1 (SGK1). *Biochem J* **2008**, *416*, 375-385, doi:10.1042/BJ20081668.
78. Brunet, A.; Park, J.; Tran, H.; Hu, L.S.; Hemmings, B.A.; Greenberg, M.E. Protein kinase SGK mediates survival signals by phosphorylating the forkhead transcription factor FKHRL1 (FOXO3a). *Mol Cell Biol*

2001, *21*, 952-965, doi:10.1128/MCB.21.3.952-965.2001.
79. Kaiser, G.; Gerst, F.; Michael, D.; Berchtold, S.; Friedrich, B.; Strutz-Seebohm, N.; Lang, F.; Haring, H.U.; Ullrich, S. Regulation of forkhead box O1 (FOXO1) by protein kinase B and glucocorticoids: different mechanisms of induction of beta cell death in vitro. *Diabetologia* **2013**, *56*, 1587-1595, doi:10.1007/s00125-013-2863-7.
80. Wang, X.; Hu, S.; Liu, L. Phosphorylation and acetylation modifications of FOXO3a: Independently or synergistically? *Oncol Lett* **2017**, *13*, 2867-2872, doi:10.3892/ol.2017.5851.
81. Kaeberlein, M.; Powers, R.W., 3rd; Steffen, K.K.; Westman, E.A.; Hu, D.; Dang, N.; Kerr, E.O.; Kirkland, K.T.; Fields, S.; Kennedy, B.K. Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. *Science* **2005**, *310*, 1193-1196, doi:10.1126/science.1115535.
82. Vellai, T.; Takacs-Vellai, K.; Zhang, Y.; Kovacs, A.L.; Orosz, L.; Muller, F. Genetics: influence of TOR kinase on lifespan in C. elegans. *Nature* **2003**, *426*, 620, doi:10.1038/426620a.
83. Kapahi, P.; Zid, B.M.; Harper, T.; Koslover, D.; Sapin, V.; Benzer, S. Regulation of lifespan in Drosophila by modulation of genes in the TOR signaling pathway. *Curr Biol* **2004**, *14*, 885-890, doi:10.1016/j.cub.2004.03.059.
84. Wu, J.J.; Liu, J.; Chen, E.B.; Wang, J.J.; Cao, L.; Narayan, N.; Fergusson, M.M.; Rovira, II; Allen, M.; Springer, D.A., et al. Increased mammalian lifespan and a segmental and tissue-specific slowing of aging after genetic reduction of mTOR expression. *Cell Rep* **2013**, *4*, 913-920, doi:10.1016/j.celrep.2013.07.030.
85. Medvedik, O.; Lamming, D.W.; Kim, K.D.; Sinclair, D.A. MSN2 and MSN4 link calorie restriction and TOR to sirtuin-mediated lifespan extension in Saccharomyces cerevisiae. *PLoS Biol* **2007**, *5*, e261, doi:10.1371/journal.pbio.0050261.
86. Bjedov, I.; Toivonen, J.M.; Kerr, F.; Slack, C.; Jacobson, J.; Foley, A.; Partridge, L. Mechanisms of life span extension by rapamycin in the fruit fly Drosophila melanogaster. *Cell Metab* **2010**, *11*, 35-46, doi:10.1016/j.cmet.2009.11.010.
87. Hansen, M.; Chandra, A.; Mitic, L.L.; Onken, B.; Driscoll, M.; Kenyon, C. A role for autophagy in the extension of lifespan by dietary restriction in C. elegans. *PLoS Genet* **2008**, *4*, e24, doi:10.1371/journal.pgen.0040024.
88. Toth, M.L.; Sigmond, T.; Borsos, E.; Barna, J.; Erdelyi, P.; Takacs-Vellai, K.; Orosz, L.; Kovacs, A.L.; Csikos, G.; Sass, M., et al. Longevity pathways converge on autophagy genes to regulate life span in Caenorhabditis elegans. *Autophagy* **2008**, *4*, 330-338, doi:10.4161/auto.5618.
89. Hansen, M.; Taubert, S.; Crawford, D.; Libina, N.; Lee, S.J.; Kenyon, C. Lifespan extension by conditions that inhibit translation in Caenorhabditis elegans. *Aging Cell* **2007**, *6*, 95-110, doi:10.1111/j.1474-9726.2006.00267.x.
90. Blouet, C.; Ono, H.; Schwartz, G.J. Mediobasal hypothalamic p70 S6 kinase 1 modulates the control of energy homeostasis. *Cell Metab* **2008**, *8*, 459-467, doi:10.1016/j.cmet.2008.10.004.
91. Cota, D.; Matter, E.K.; Woods, S.C.; Seeley, R.J. The role of hypothalamic mammalian target of rapamycin complex 1 signaling in diet-induced obesity. *J Neurosci* **2008**, *28*, 7202-7208, doi:10.1523/JNEUROSCI.1389-08.2008.
92. Xiao, B.; Sanders, M.J.; Underwood, E.; Heath, R.; Mayer, F.V.; Carmena, D.; Jing, C.; Walker, P.A.; Eccleston, J.F.; Haire, L.F., et al. Structure of mammalian AMPK and its regulation by ADP. *Nature* **2011**, *472*, 230-233, doi:10.1038/nature09932.
93. Ross, F.A.; MacKintosh, C.; Hardie, D.G. AMP-activated protein kinase: a cellular energy sensor that comes in 12 flavours. *FEBS J* **2016**, *283*, 2987-3001, doi:10.1111/febs.13698.
94. Suter, M.; Riek, U.; Tuerk, R.; Schlattner, U.; Wallimann, T.; Neumann, D. Dissecting the role of 5'-AMP for allosteric stimulation, activation, and deactivation of AMP-activated protein kinase. *J Biol Chem* **2006**, *281*, 32207-32216, doi:10.1074/jbc.M606357200.
95. Davies, S.P.; Helps, N.R.; Cohen, P.T.; Hardie, D.G. 5'-AMP inhibits dephosphorylation, as well as promoting phosphorylation, of the AMP-activated protein kinase. Studies using bacterially expressed human protein phosphatase-2C alpha and native bovine protein phosphatase-2AC. *FEBS Lett* **1995**, *377*, 421-425, doi:10.1016/0014-5793(95)01368-7.
96. Fogarty, S.; Hawley, S.A.; Green, K.A.; Saner, N.; Mustard, K.J.; Hardie, D.G. Calmodulin-dependent protein kinase kinase-beta activates AMPK without forming a stable complex: synergistic effects of Ca2+

and AMP. *Biochem J* **2010**, *426*, 109-118, doi:10.1042/BJ20091372.
97. Winder, W.W.; Hardie, D.G. AMP-activated protein kinase, a metabolic master switch: possible roles in type 2 diabetes. *Am J Physiol* **1999**, *277*, E1-10, doi:10.1152/ajpendo.1999.277.1.E1.
98. Koo, S.H.; Flechner, L.; Qi, L.; Zhang, X.; Screaton, R.A.; Jeffries, S.; Hedrick, S.; Xu, W.; Boussouar, F.; Brindle, P., et al. The CREB coactivator TORC2 is a key regulator of fasting glucose metabolism. *Nature* **2005**, *437*, 1109-1111, doi:10.1038/nature03967.
99. Chen, S.; Murphy, J.; Toth, R.; Campbell, D.G.; Morrice, N.A.; Mackintosh, C. Complementary regulation of TBC1D1 and AS160 by growth factors, insulin and AMPK activators. *Biochem J* **2008**, *409*, 449-459, doi:10.1042/BJ20071114.
00. Geraghty, K.M.; Chen, S.; Harthill, J.E.; Ibrahim, A.F.; Toth, R.; Morrice, N.A.; Vandermoere, F.; Moorhead, G.B.; Hardie, D.G.; MacKintosh, C. Regulation of multisite phosphorylation and 14-3-3 binding of AS160 in response to IGF-1, EGF, PMA and AICAR. *Biochem J* **2007**, *407*, 231-241, doi:10.1042/BJ20070649.
01. Jager, S.; Handschin, C.; St-Pierre, J.; Spiegelman, B.M. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. *Proc Natl Acad Sci U S A* **2007**, *104*, 12017-12022, doi:10.1073/pnas.0705070104.
02. McGee, S.L.; van Denderen, B.J.; Howlett, K.F.; Mollica, J.; Schertzer, J.D.; Kemp, B.E.; Hargreaves, M. AMP-activated protein kinase regulates GLUT4 transcription by phosphorylating histone deacetylase 5. *Diabetes* **2008**, *57*, 860-867, doi:10.2337/db07-0843.
03. Davies, S.P.; Sim, A.T.; Hardie, D.G. Location and function of three sites phosphorylated on rat acetyl-CoA carboxylase by the AMP-activated protein kinase. *Eur J Biochem* **1990**, *187*, 183-190.
04. Davies, S.P.; Carling, D.; Munday, M.R.; Hardie, D.G. Diurnal rhythm of phosphorylation of rat liver acetyl-CoA carboxylase by the AMP-activated protein kinase, demonstrated using freeze-clamping. Effects of high fat diets. *Eur J Biochem* **1992**, *203*, 615-623.
05. Li, Y.; Xu, S.; Mihaylova, M.M.; Zheng, B.; Hou, X.; Jiang, B.; Park, O.; Luo, Z.; Lefai, E.; Shyy, J.Y., et al. AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice. *Cell Metab* **2011**, *13*, 376-388, doi:10.1016/j.cmet.2011.03.009.
06. Muoio, D.M.; Seefeld, K.; Witters, L.A.; Coleman, R.A. AMP-activated kinase reciprocally regulates triacylglycerol synthesis and fatty acid oxidation in liver and muscle: evidence that sn-glycerol-3-phosphate acyltransferase is a novel target. *Biochem J* **1999**, *338 (Pt 3)*, 783-791.
07. Clarke, P.R.; Hardie, D.G. Regulation of HMG-CoA reductase: identification of the site phosphorylated by the AMP-activated protein kinase in vitro and in intact rat liver. *EMBO J* **1990**, *9*, 2439-2446.
08. Hoppe, S.; Bierhoff, H.; Cado, I.; Weber, A.; Tiebe, M.; Grummt, I.; Voit, R. AMP-activated protein kinase adapts rRNA synthesis to cellular energy supply. *Proc Natl Acad Sci U S A* **2009**, *106*, 17781-17786, doi:10.1073/pnas.0909873106.
09. Dufer, M.; Noack, K.; Krippeit-Drews, P.; Drews, G. Activation of the AMP-activated protein kinase enhances glucose-stimulated insulin secretion in mouse beta-cells. *Islets* **2010**, *2*, 156-163, doi:10.4161/isl.2.3.11412.
10. Chavez, J.A.; Roach, W.G.; Keller, S.R.; Lane, W.S.; Lienhard, G.E. Inhibition of GLUT4 translocation by Tbc1d1, a Rab GTPase-activating protein abundant in skeletal muscle, is partially relieved by AMP-activated protein kinase activation. *J Biol Chem* **2008**, *283*, 9187-9195, doi:10.1074/jbc.M708934200.
11. Marsin, A.S.; Bouzin, C.; Bertrand, L.; Hue, L. The stimulation of glycolysis by hypoxia in activated monocytes is mediated by AMP-activated protein kinase and inducible 6-phosphofructo-2-kinase. *J Biol Chem* **2002**, *277*, 30778-30783, doi:10.1074/jbc.M205213200.
12. Jorgensen, S.B.; Nielsen, J.N.; Birk, J.B.; Olsen, G.S.; Viollet, B.; Andreelli, F.; Schjerling, P.; Vaulont, S.; Hardie, D.G.; Hansen, B.F., et al. The alpha2-5'AMP-activated protein kinase is a site 2 glycogen synthase kinase in skeletal muscle and is responsive to glucose loading. *Diabetes* **2004**, *53*, 3074-3081.
13. Hardie, D.G.; Ross, F.A.; Hawley, S.A. AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nat Rev Mol Cell Biol* **2012**, *13*, 251-262, doi:10.1038/nrm3311.
14. Leclerc, I.; Lenzner, C.; Gourdon, L.; Vaulont, S.; Kahn, A.; Viollet, B. Hepatocyte nuclear factor-4alpha involved in type 1 maturity-onset diabetes of the young is a novel target of AMP-activated protein kinase. *Diabetes* **2001**, *50*, 1515-1521, doi:10.2337/diabetes.50.7.1515.
15. Lee, J.M.; Seo, W.Y.; Song, K.H.; Chanda, D.; Kim, Y.D.; Kim, D.K.; Lee, M.W.; Ryu, D.; Kim, Y.H.; Noh, J.R.,

et al. AMPK-dependent repression of hepatic gluconeogenesis via disruption of CREB.CRTC2 complex by orphan nuclear receptor small heterodimer partner. *J Biol Chem* **2010**, *285*, 32182-32191, doi:10.1074/jbc.M110.134890.
16. Minokoshi, Y.; Alquier, T.; Furukawa, N.; Kim, Y.B.; Lee, A.; Xue, B.; Mu, J.; Foufelle, F.; Ferre, P.; Birnbaum, M.J., et al. AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. *Nature* **2004**, *428*, 569-574, doi:10.1038/nature02440.
17. Andersson, U.; Filipsson, K.; Abbott, C.R.; Woods, A.; Smith, K.; Bloom, S.R.; Carling, D.; Small, C.J. AMP-activated protein kinase plays a role in the control of food intake. *J Biol Chem* **2004**, *279*, 12005-12008, doi:10.1074/jbc.C300557200.
18. Steinberg, G.R.; Kemp, B.E. AMPK in Health and Disease. *Physiol Rev* **2009**, *89*, 1025-1078, doi:10.1152/physrev.00011.2008.
19. Wang, Z.; Wang, N.; Liu, P.; Xie, X. AMPK and Cancer. *Exp Suppl* **2016**, *107*, 203-226, doi:10.1007/978-3-319-43589-3_9.
20. Umezawa, S.; Higurashi, T.; Nakajima, A. AMPK: Therapeutic Target for Diabetes and Cancer Prevention. *Curr Pharm Des* **2017**, *23*, 3629-3644, doi:10.2174/0929867324666170713150440.
21. Greer, E.L.; Dowlatshahi, D.; Banko, M.R.; Villen, J.; Hoang, K.; Blanchard, D.; Gygi, S.P.; Brunet, A. An AMPK-FOXO pathway mediates longevity induced by a novel method of dietary restriction in C. elegans. *Curr Biol* **2007**, *17*, 1646-1656, doi:10.1016/j.cub.2007.08.047.
22. Schulz, T.J.; Zarse, K.; Voigt, A.; Urban, N.; Birringer, M.; Ristow, M. Glucose restriction extends Caenorhabditis elegans life span by inducing mitochondrial respiration and increasing oxidative stress. *Cell Metab* **2007**, *6*, 280-293, doi:10.1016/j.cmet.2007.08.011.
23. Funakoshi, M.; Tsuda, M.; Muramatsu, K.; Hatsuda, H.; Morishita, S.; Aigaki, T. A gain-of-function screen identifies wdb and lkb1 as lifespan-extending genes in Drosophila. *Biochem Biophys Res Commun* **2011**, *405*, 667-672, doi:10.1016/j.bbrc.2011.01.090.
24. Stenesen, D.; Suh, J.M.; Seo, J.; Yu, K.; Lee, K.S.; Kim, J.S.; Min, K.J.; Graff, J.M. Adenosine nucleotide biosynthesis and AMPK regulate adult life span and mediate the longevity benefit of caloric restriction in flies. *Cell Metab* **2013**, *17*, 101-112, doi:10.1016/j.cmet.2012.12.006.
25. Gonzalez, E.; McGraw, T.E. The Akt kinases: isoform specificity in metabolism and cancer. *Cell Cycle* **2009**, *8*, 2502-2508, doi:10.4161/cc.8.16.9335.
26. Oh, W.J.; Jacinto, E. mTOR complex 2 signaling and functions. *Cell Cycle* **2011**, *10*, 2305-2316, doi:10.4161/cc.10.14.16586.
27. Piscitello, D.; Varshney, D.; Lilla, S.; Vizioli, M.G.; Reid, C.; Gorbunova, V.; Seluanov, A.; Gillespie, D.A.; Adams, P.D. AKT overactivation can suppress DNA repair via p70S6 kinase-dependent downregulation of MRE11. *Oncogene* **2018**, *37*, 427-438, doi:10.1038/onc.2017.340.
28. Jia, Y.; Song, W.; Zhang, F.; Yan, J.; Yang, Q. Akt1 inhibits homologous recombination in Brca1-deficient cells by blocking the Chk1-Rad51 pathway. *Oncogene* **2013**, *32*, 1943-1949, doi:10.1038/onc.2012.211.
29. Liu, P.; Gan, W.; Guo, C.; Xie, A.; Gao, D.; Guo, J.; Zhang, J.; Willis, N.; Su, A.; Asara, J.M., et al. Akt-mediated phosphorylation of XLF impairs non-homologous end-joining DNA repair. *Mol Cell* **2015**, *57*, 648-661, doi:10.1016/j.molcel.2015.01.005.
30. Haeusler, R.A.; McGraw, T.E.; Accili, D. Biochemical and cellular properties of insulin receptor signalling. *Nat Rev Mol Cell Biol* **2018**, *19*, 31-44, doi:10.1038/nrm.2017.89.
31. Rowland, A.F.; Fazakerley, D.J.; James, D.E. Mapping insulin/GLUT4 circuitry. *Traffic* **2011**, *12*, 672-681, doi:10.1111/j.1600-0854.2011.01178.x.
32. Bevan, P. Insulin signalling. *J Cell Sci* **2001**, *114*, 1429-1430.
33. Siddle, K. Signalling by insulin and IGF receptors: supporting acts and new players. *J Mol Endocrinol* **2011**, *47*, R1-10, doi:10.1530/JME-11-0022.
34. Wong, R.H.; Sul, H.S. Insulin signaling in fatty acid and fat synthesis: a transcriptional perspective. *Curr Opin Pharmacol* **2010**, *10*, 684-691, doi:10.1016/j.coph.2010.08.004.
35. Weindruch, R. The retardation of aging by caloric restriction: studies in rodents and primates. *Toxicol Pathol* **1996**, *24*, 742-745, doi:10.1177/019262339602400618.
36. Shimokawa, I.; Komatsu, T.; Hayashi, N.; Kim, S.E.; Kawata, T.; Park, S.; Hayashi, H.; Yamaza, H.; Chiba, T.; Mori, R. The life-extending effect of dietary restriction requires Foxo3 in mice. *Aging Cell* **2015**, *14*,

707-709, doi:10.1111/acel.12340.
37. Kim, D.H.; Park, M.H.; Lee, E.K.; Choi, Y.J.; Chung, K.W.; Moon, K.M.; Kim, M.J.; An, H.J.; Park, J.W.; Kim, N.D., et al. The roles of FoxOs in modulation of aging by calorie restriction. *Biogerontology* **2015**, *16*, 1-14, doi:10.1007/s10522-014-9519-y.
38. Bluher, M.; Kahn, B.B.; Kahn, C.R. Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* **2003**, *299*, 572-574, doi:10.1126/science.1078223.
39. Dosch, J.; Meissner, U.; Rascher, W. Prolonged lifespan by defective insulin signalling? *Eur J Endocrinol* **2003**, *148*, 489-490, doi:10.1530/eje.0.1480489.
40. Tatar, M.; Bartke, A.; Antebi, A. The endocrine regulation of aging by insulin-like signals. *Science* **2003**, *299*, 1346-1351, doi:10.1126/science.1081447.
41. Kenyon, C.; Chang, J.; Gensch, E.; Rudner, A.; Tabtiang, R. A C. elegans mutant that lives twice as long as wild type. *Nature* **1993**, *366*, 461-464, doi:10.1038/366461a0.
42. Holzenberger, M.; Dupont, J.; Ducos, B.; Leneuve, P.; Geloen, A.; Even, P.C.; Cervera, P.; Le Bouc, Y. IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature* **2003**, *421*, 182-187, doi:10.1038/nature01298.
43. Taguchi, A.; Wartschow, L.M.; White, M.F. Brain IRS2 signaling coordinates life span and nutrient homeostasis. *Science* **2007**, *317*, 369-372, doi:10.1126/science.1142179.
44. Selman, C.; Lingard, S.; Choudhury, A.I.; Batterham, R.L.; Claret, M.; Clements, M.; Ramadani, F.; Okkenhaug, K.; Schuster, E.; Blanc, E., et al. Evidence for lifespan extension and delayed age-related biomarkers in insulin receptor substrate 1 null mice. *FASEB J* **2008**, *22*, 807-818, doi:10.1096/fj.07-9261com.
45. Coschigano, K.T.; Holland, A.N.; Riders, M.E.; List, E.O.; Flyvbjerg, A.; Kopchick, J.J. Deletion, but not antagonism, of the mouse growth hormone receptor results in severely decreased body weights, insulin, and insulin-like growth factor I levels and increased life span. *Endocrinology* **2003**, *144*, 3799-3810, doi:10.1210/en.2003-0374.
46. Bonkowski, M.S.; Rocha, J.S.; Masternak, M.M.; Al Regaiey, K.A.; Bartke, A. Targeted disruption of growth hormone receptor interferes with the beneficial actions of calorie restriction. *Proc Natl Acad Sci U S A* **2006**, *103*, 7901-7905, doi:10.1073/pnas.0600161103.
47. Kurosu, H.; Yamamoto, M.; Clark, J.D.; Pastor, J.V.; Nandi, A.; Gurnani, P.; McGuinness, O.P.; Chikuda, H.; Yamaguchi, M.; Kawaguchi, H., et al. Suppression of aging in mice by the hormone Klotho. *Science* **2005**, *309*, 1829-1833, doi:10.1126/science.1112766.
48. Erol, A. Insulin resistance is an evolutionarily conserved physiological mechanism at the cellular level for protection against increased oxidative stress. *Bioessays* **2007**, *29*, 811-818, doi:10.1002/bies.20618.
49. Ferrara, N.; Rinaldi, B.; Corbi, G.; Conti, V.; Stiuso, P.; Boccuti, S.; Rengo, G.; Rossi, F.; Filippelli, A. Exercise training promotes SIRT1 activity in aged rats. *Rejuvenation Res* **2008**, *11*, 139-150, doi:10.1089/rej.2007.0576.
50. Corbi, G.; Conti, V.; Scapagnini, G.; Filippelli, A.; Ferrara, N. Role of sirtuins, calorie restriction and physical activity in aging. *Front Biosci (Elite Ed)* **2012**, *4*, 768-778.
51. Rack, J.G.; Morra, R.; Barkauskaite, E.; Kraehenbuehl, R.; Ariza, A.; Qu, Y.; Ortmayer, M.; Leidecker, O.; Cameron, D.R.; Matic, I., et al. Identification of a Class of Protein ADP-Ribosylating Sirtuins in Microbial Pathogens. *Mol Cell* **2015**, *59*, 309-320, doi:10.1016/j.molcel.2015.06.013.
52. Liou, G.G.; Tanny, J.C.; Kruger, R.G.; Walz, T.; Moazed, D. Assembly of the SIR complex and its regulation by O-acetyl-ADP-ribose, a product of NAD-dependent histone deacetylation. *Cell* **2005**, *121*, 515-527, doi:10.1016/j.cell.2005.03.035.
53. Vaquero, A.; Scher, M.; Lee, D.; Erdjument-Bromage, H.; Tempst, P.; Reinberg, D. Human SirT1 interacts with histone H1 and promotes formation of facultative heterochromatin. *Mol Cell* **2004**, *16*, 93-105, doi:10.1016/j.molcel.2004.08.031.
54. Dang, W. The controversial world of sirtuins. *Drug Discov Today Technol* **2014**, *12*, e9-e17, doi:10.1016/j.ddtec.2012.08.003.
55. Houtkooper, R.H.; Pirinen, E.; Auwerx, J. Sirtuins as regulators of metabolism and healthspan. *Nat Rev Mol Cell Biol* **2012**, *13*, 225-238, doi:10.1038/nrm3293.
56. Shimazu, T.; Hirschey, M.D.; Hua, L.; Dittenhafer-Reed, K.E.; Schwer, B.; Lombard, D.B.; Li, Y.;

Bunkenborg, J.; Alt, F.W.; Denu, J.M., et al. SIRT3 deacetylates mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase 2 and regulates ketone body production. *Cell Metab* **2010**, *12*, 654-661, doi:10.1016/j.cmet.2010.11.003.
57. Hirschey, M.D.; Shimazu, T.; Goetzman, E.; Jing, E.; Schwer, B.; Lombard, D.B.; Grueter, C.A.; Harris, C.; Biddinger, S.; Ilkayeva, O.R., et al. SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation. *Nature* **2010**, *464*, 121-125, doi:10.1038/nature08778.
58. Qiu, X.; Brown, K.; Hirschey, M.D.; Verdin, E.; Chen, D. Calorie restriction reduces oxidative stress by SIRT3-mediated SOD2 activation. *Cell Metab* **2010**, *12*, 662-667, doi:10.1016/j.cmet.2010.11.015.
59. Preyat, N.; Leo, O. Sirtuin deacylases: a molecular link between metabolism and immunity. *J Leukoc Biol* **2013**, *93*, 669-680, doi:10.1189/jlb.1112557.
60. Satoh, A.; Brace, C.S.; Ben-Josef, G.; West, T.; Wozniak, D.F.; Holtzman, D.M.; Herzog, E.D.; Imai, S. SIRT1 promotes the central adaptive response to diet restriction through activation of the dorsomedial and lateral nuclei of the hypothalamus. *J Neurosci* **2010**, *30*, 10220-10232, doi:10.1523/JNEUROSCI.1385-10.2010.
61. Kaeberlein, M.; McVey, M.; Guarente, L. The SIR2/3/4 complex and SIR2 alone promote longevity in Saccharomyces cerevisiae by two different mechanisms. *Genes Dev* **1999**, *13*, 2570-2580, doi:10.1101/gad.13.19.2570.
62. Whitaker, R.; Faulkner, S.; Miyokawa, R.; Burhenn, L.; Henriksen, M.; Wood, J.G.; Helfand, S.L. Increased expression of Drosophila Sir2 extends life span in a dose-dependent manner. *Aging (Albany NY)* **2013**, *5*, 682-691, doi:10.18632/aging.100599.
63. Lin, S.J.; Defossez, P.A.; Guarente, L. Requirement of NAD and SIR2 for life-span extension by calorie restriction in Saccharomyces cerevisiae. *Science* **2000**, *289*, 2126-2128, doi:10.1126/science.289.5487.2126.
64. Rogina, B.; Helfand, S.L. Sir2 mediates longevity in the fly through a pathway related to calorie restriction. *Proc Natl Acad Sci U S A* **2004**, *101*, 15998-16003, doi:10.1073/pnas.0404184101.
65. Tissenbaum, H.A.; Guarente, L. Increased dosage of a sir-2 gene extends lifespan in Caenorhabditis elegans. *Nature* **2001**, *410*, 227-230, doi:10.1038/35065638.
66. Oberdoerffer, P.; Michan, S.; McVay, M.; Mostoslavsky, R.; Vann, J.; Park, S.K.; Hartlerode, A.; Stegmuller, J.; Hafner, A.; Loerch, P., et al. SIRT1 redistribution on chromatin promotes genomic stability but alters gene expression during aging. *Cell* **2008**, *135*, 907-918, doi:10.1016/j.cell.2008.10.025.
67. Rodgers, J.T.; Lerin, C.; Haas, W.; Gygi, S.P.; Spiegelman, B.M.; Puigserver, P. Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. *Nature* **2005**, *434*, 113-118, doi:10.1038/nature03354.
68. Nemoto, S.; Fergusson, M.M.; Finkel, T. SIRT1 functionally interacts with the metabolic regulator and transcriptional coactivator PGC-1{alpha}. *J Biol Chem* **2005**, *280*, 16456-16460, doi:10.1074/jbc.M501485200.
69. Lagouge, M.; Argmann, C.; Gerhart-Hines, Z.; Meziane, H.; Lerin, C.; Daussin, F.; Messadeq, N.; Milne, J.; Lambert, P.; Elliott, P., et al. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. *Cell* **2006**, *127*, 1109-1122, doi:10.1016/j.cell.2006.11.013.
70. Vaziri, H.; Dessain, S.K.; Ng Eaton, E.; Imai, S.I.; Frye, R.A.; Pandita, T.K.; Guarente, L.; Weinberg, R.A. hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase. *Cell* **2001**, *107*, 149-159, doi:10.1016/s0092-8674(01)00527-x.
71. Luo, J.; Nikolaev, A.Y.; Imai, S.; Chen, D.; Su, F.; Shiloh, A.; Guarente, L.; Gu, W. Negative control of p53 by Sir2alpha promotes cell survival under stress. *Cell* **2001**, *107*, 137-148, doi:10.1016/s0092-8674(01)00524-4.
72. Daitoku, H.; Hatta, M.; Matsuzaki, H.; Aratani, S.; Ohshima, T.; Miyagishi, M.; Nakajima, T.; Fukamizu, A. Silent information regulator 2 potentiates Foxo1-mediated transcription through its deacetylase activity. *Proc Natl Acad Sci U S A* **2004**, *101*, 10042-10047, doi:10.1073/pnas.0400593101.
73. van der Horst, A.; Tertoolen, L.G.; de Vries-Smits, L.M.; Frye, R.A.; Medema, R.H.; Burgering, B.M. FOXO4 is acetylated upon peroxide stress and deacetylated by the longevity protein hSir2(SIRT1). *J Biol Chem* **2004**, *279*, 28873-28879, doi:10.1074/jbc.M401138200.

74. Accili, D.; Arden, K.C. FoxOs at the crossroads of cellular metabolism, differentiation, and transformation. *Cell***2004**, *117*, 421-426, doi:10.1016/s0092-8674(04)00452-0.
75. Martins, R.; Lithgow, G.J.; Link, W. Long live FOXO: unraveling the role of FOXO proteins in aging and longevity. *Aging Cell* **2016**, *15*, 196-207, doi:10.1111/acel.12427.
76. Gross, D.N.; van den Heuvel, A.P.; Birnbaum, M.J. The role of FoxO in the regulation of metabolism. *Oncogene* **2008**, *27*, 2320-2336, doi:10.1038/onc.2008.25.
77. Frescas, D.; Valenti, L.; Accili, D. Nuclear trapping of the forkhead transcription factor FoxO1 via Sirt-dependent deacetylation promotes expression of glucogenetic genes. *J Biol Chem* **2005**, *280*, 20589-20595, doi:10.1074/jbc.M412357200.
78. Brunet, A.; Sweeney, L.B.; Sturgill, J.F.; Chua, K.F.; Greer, P.L.; Lin, Y.; Tran, H.; Ross, S.E.; Mostoslavsky, R.; Cohen, H.Y., et al. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science* **2004**, *303*, 2011-2015, doi:10.1126/science.1094637.
79. Bordone, L.; Motta, M.C.; Picard, F.; Robinson, A.; Jhala, U.S.; Apfeld, J.; McDonagh, T.; Lemieux, M.; McBurney, M.; Szilvasi, A., et al. Sirt1 regulates insulin secretion by repressing UCP2 in pancreatic beta cells. *PLoS Biol* **2006**, *4*, e31, doi:10.1371/journal.pbio.0040031.
80. Moynihan, K.A.; Grimm, A.A.; Plueger, M.M.; Bernal-Mizrachi, E.; Ford, E.; Cras-Meneur, C.; Permutt, M.A.; Imai, S. Increased dosage of mammalian Sir2 in pancreatic beta cells enhances glucose-stimulated insulin secretion in mice. *Cell Metab* **2005**, *2*, 105-117, doi:10.1016/j.cmet.2005.07.001.
81. Haigis, M.C.; Mostoslavsky, R.; Haigis, K.M.; Fahie, K.; Christodoulou, D.C.; Murphy, A.J.; Valenzuela, D.M.; Yancopoulos, G.D.; Karow, M.; Blander, G., et al. SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic beta cells. *Cell* **2006**, *126*, 941-954, doi:10.1016/j.cell.2006.06.057.
82. Bae, N.S.; Swanson, M.J.; Vassilev, A.; Howard, B.H. Human histone deacetylase SIRT2 interacts with the homeobox transcription factor HOXA10. *J Biochem* **2004**, *135*, 695-700, doi:10.1093/jb/mvh084.
83. North, B.J.; Marshall, B.L.; Borra, M.T.; Denu, J.M.; Verdin, E. The human Sir2 ortholog, SIRT2, is an NAD+-dependent tubulin deacetylase. *Mol Cell* **2003**, *11*, 437-444, doi:10.1016/s1097-2765(03)00038-8.
84. Wang, F.; Nguyen, M.; Qin, F.X.; Tong, Q. SIRT2 deacetylates FOXO3a in response to oxidative stress and caloric restriction. *Aging Cell* **2007**, *6*, 505-514, doi:10.1111/j.1474-9726.2007.00304.x.
85. Jing, E.; Gesta, S.; Kahn, C.R. SIRT2 regulates adipocyte differentiation through FoxO1 acetylation/deacetylation. *Cell Metab* **2007**, *6*, 105-114, doi:10.1016/j.cmet.2007.07.003.
86. Dryden, S.C.; Nahhas, F.A.; Nowak, J.E.; Goustin, A.S.; Tainsky, M.A. Role for human SIRT2 NAD-dependent deacetylase activity in control of mitotic exit in the cell cycle. *Mol Cell Biol* **2003**, *23*, 3173-3185, doi:10.1128/mcb.23.9.3173-3185.2003.
87. Sinclair, D.A.; Guarente, L. Extrachromosomal rDNA circles--a cause of aging in yeast. *Cell* **1997**, *91*, 1033-1042, doi:10.1016/s0092-8674(00)80493-6.
88. Starai, V.J.; Celic, I.; Cole, R.N.; Boeke, J.D.; Escalante-Semerena, J.C. Sir2-dependent activation of acetyl-CoA synthetase by deacetylation of active lysine. *Science* **2002**, *298*, 2390-2392, doi:10.1126/science.1077650.
89. Onyango, P.; Celic, I.; McCaffery, J.M.; Boeke, J.D.; Feinberg, A.P. SIRT3, a human SIR2 homologue, is an NAD-dependent deacetylase localized to mitochondria. *Proc Natl Acad Sci U S A* **2002**, *99*, 13653-13658, doi:10.1073/pnas.222538099.
90. Shi, T.; Wang, F.; Stieren, E.; Tong, Q. SIRT3, a mitochondrial sirtuin deacetylase, regulates mitochondrial function and thermogenesis in brown adipocytes. *J Biol Chem* **2005**, *280*, 13560-13567, doi:10.1074/jbc.M414670200.
91. Rose, G.; Dato, S.; Altomare, K.; Bellizzi, D.; Garasto, S.; Greco, V.; Passarino, G.; Feraco, E.; Mari, V.; Barbi, C., et al. Variability of the SIRT3 gene, human silent information regulator Sir2 homologue, and survivorship in the elderly. *Exp Gerontol* **2003**, *38*, 1065-1070, doi:10.1016/s0531-5565(03)00209-2.
92. Nasrin, N.; Wu, X.; Fortier, E.; Feng, Y.; Bare, O.C.; Chen, S.; Ren, X.; Wu, Z.; Streeper, R.S.; Bordone, L. SIRT4 regulates fatty acid oxidation and mitochondrial gene expression in liver and muscle cells. *J Biol Chem* **2010**, *285*, 31995-32002, doi:10.1074/jbc.M110.124164.
93. Michishita, E.; Park, J.Y.; Burneskis, J.M.; Barrett, J.C.; Horikawa, I. Evolutionarily conserved and nonconserved cellular localizations and functions of human SIRT proteins. *Mol Biol Cell* **2005**, *16*, 4623-

4635, doi:10.1091/mbc.e05-01-0033.
94. McCord, R.A.; Michishita, E.; Hong, T.; Berber, E.; Boxer, L.D.; Kusumoto, R.; Guan, S.; Shi, X.; Gozani, O.; Burlingame, A.L., et al. SIRT6 stabilizes DNA-dependent protein kinase at chromatin for DNA double-strand break repair. *Aging (Albany NY)* **2009**, *1*, 109-121, doi:10.18632/aging.100011.
95. Mostoslavsky, R.; Chua, K.F.; Lombard, D.B.; Pang, W.W.; Fischer, M.R.; Gellon, L.; Liu, P.; Mostoslavsky, G.; Franco, S.; Murphy, M.M., et al. Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. *Cell* **2006**, *124*, 315-329, doi:10.1016/j.cell.2005.11.044.
96. Kanfi, Y.; Naiman, S.; Amir, G.; Peshti, V.; Zinman, G.; Nahum, L.; Bar-Joseph, Z.; Cohen, H.Y. The sirtuin SIRT6 regulates lifespan in male mice. *Nature* **2012**, *483*, 218-221, doi:10.1038/nature10815.
97. Luo, L.L.; Chen, X.C.; Fu, Y.C.; Xu, J.J.; Li, L.; Lin, X.H.; Xiang, Y.F.; Zhang, X.M. The effects of caloric restriction and a high-fat diet on ovarian lifespan and the expression of SIRT1 and SIRT6 proteins in rats. *Aging Clin Exp Res* **2012**,*24*, 125-133, doi:10.3275/7660.
98. Ford, E.; Voit, R.; Liszt, G.; Magin, C.; Grummt, I.; Guarente, L. Mammalian Sir2 homolog SIRT7 is an activator of RNA polymerase I transcription. *Genes Dev* **2006**, *20*, 1075-1080, doi:10.1101/gad.1399706.
99. Vazquez, B.N.; Thackray, J.K.; Simonet, N.G.; Kane-Goldsmith, N.; Martinez-Redondo, P.; Nguyen, T.; Bunting, S.; Vaquero, A.; Tischfield, J.A.; Serrano, L. SIRT7 promotes genome integrity and modulates non-homologous end joining DNA repair. *EMBO J* **2016**, *35*, 1488-1503, doi:10.15252/embj.201593499.
00. Geng, Y.Q.; Li, T.T.; Liu, X.Y.; Li, Z.H.; Fu, Y.C. SIRT1 and SIRT5 activity expression and behavioral responses to calorie restriction. *J Cell Biochem* **2011**, *112*, 3755-3761, doi:10.1002/jcb.23315.
01. Ran, M.; Li, Z.; Yang, L.; Tong, L.; Zhang, L.; Dong, H. Calorie restriction attenuates cerebral ischemic injury via increasing SIRT1 synthesis in the rat. *Brain Res* **2015**, *1610*, 61-68, doi:10.1016/j.brainres.2015.03.043.
02. Nisoli, E.; Tonello, C.; Cardile, A.; Cozzi, V.; Bracale, R.; Tedesco, L.; Falcone, S.; Valerio, A.; Cantoni, O.; Clementi, E., et al. Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. *Science* **2005**, *310*, 314-317, doi:10.1126/science.1117728.
03. Yu, W.; Zhou, H.F.; Lin, R.B.; Fu, Y.C.; Wang, W. Shortterm calorie restriction activates SIRT14 and 7 in cardiomyocytes in vivo and in vitro. *Mol Med Rep* **2014**, *9*, 1218-1224, doi:10.3892/mmr.2014.1944.
04. Yu, W.; Qin, J.; Chen, C.; Fu, Y.; Wang, W. Moderate calorie restriction attenuates ageassociated alterations and improves cardiac function by increasing SIRT1 and SIRT3 expression. *Mol Med Rep* **2018**, *18*, 4087-4094, doi:10.3892/mmr.2018.9390.
05. Chen, L.L.; Deng, X.Q.; Li, N.X. [Effects of calorie restriction on SIRT1 expression in liver of nonalcoholic fatty liver disease: experiment with rats]. *Zhonghua Yi Xue Za Zhi* **2007**, *87*, 1434-1437.
06. Boily, G.; Seifert, E.L.; Bevilacqua, L.; He, X.H.; Sabourin, G.; Estey, C.; Moffat, C.; Crawford, S.; Saliba, S.; Jardine, K., et al. SirT1 regulates energy metabolism and response to caloric restriction in mice. *PLoS One* **2008**, *3*, e1759, doi:10.1371/journal.pone.0001759.
07. Bordone, L.; Cohen, D.; Robinson, A.; Motta, M.C.; van Veen, E.; Czopik, A.; Steele, A.D.; Crowe, H.; Marmor, S.; Luo, J., et al. SIRT1 transgenic mice show phenotypes resembling calorie restriction. *Aging Cell* **2007**, *6*, 759-767, doi:10.1111/j.1474-9726.2007.00335.x.
08. Bhullar, K.S.; Hubbard, B.P. Lifespan and healthspan extension by resveratrol. *Biochim Biophys Acta* **2015**, *1852*, 1209-1218, doi:10.1016/j.bbadis.2015.01.012.
09. Howitz, K.T.; Bitterman, K.J.; Cohen, H.Y.; Lamming, D.W.; Lavu, S.; Wood, J.G.; Zipkin, R.E.; Chung, P.; Kisielewski, A.; Zhang, L.L., et al. Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. *Nature* **2003**, *425*, 191-196, doi:10.1038/nature01960.
10. Wood, J.G.; Rogina, B.; Lavu, S.; Howitz, K.; Helfand, S.L.; Tatar, M.; Sinclair, D. Sirtuin activators mimic caloric restriction and delay ageing in metazoans. *Nature* **2004**, *430*, 686-689, doi:10.1038/nature02789.
11. Baur, J.A.; Pearson, K.J.; Price, N.L.; Jamieson, H.A.; Lerin, C.; Kalra, A.; Prabhu, V.V.; Allard, J.S.; Lopez-Lluch, G.; Lewis, K., et al. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* **2006**, *444*, 337-342, doi:10.1038/nature05354.
12. Timmers, S.; Konings, E.; Bilet, L.; Houtkooper, R.H.; van de Weijer, T.; Goossens, G.H.; Hoeks, J.; van der Krieken, S.; Ryu, D.; Kersten, S., et al. Calorie restriction-like effects of 30 days of resveratrol

supplementation on energy metabolism and metabolic profile in obese humans. *Cell Metab* **2011**, *14*, 612-622, doi:10.1016/j.cmet.2011.10.002.
13. Barger, J.L.; Kayo, T.; Vann, J.M.; Arias, E.B.; Wang, J.; Hacker, T.A.; Wang, Y.; Raederstorff, D.; Morrow, J.D.; Leeuwenburgh, C., et al. A low dose of dietary resveratrol partially mimics caloric restriction and retards aging parameters in mice. *PLoS One* **2008**, *3*, e2264, doi:10.1371/journal.pone.0002264.
14. Hagen, T.M. Oxidative stress, redox imbalance, and the aging process. *Antioxid Redox Signal* **2003**, *5*, 503-506, doi:10.1089/152308603770310149.
15. Navarro, A.; Boveris, A. The mitochondrial energy transduction system and the aging process. *Am J Physiol Cell Physiol* **2007**, *292*, C670-686, doi:10.1152/ajpcell.00213.2006.
16. Harman, D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol* **1956**, *11*, 298-300.
17. Merry, B.J. Molecular mechanisms linking calorie restriction and longevity. *Int J Biochem Cell Biol* **2002**, *34*, 1340-1354.
18. Sanz, A.; Caro, P.; Ibanez, J.; Gomez, J.; Gredilla, R.; Barja, G. Dietary restriction at old age lowers mitochondrial oxygen radical production and leak at complex I and oxidative DNA damage in rat brain. *J Bioenerg Biomembr* **2005**, *37*, 83-90, doi:10.1007/s10863-005-4131-0.
19. Yu, B.P. Aging and oxidative stress: modulation by dietary restriction. *Free Radic Biol Med* **1996**, *21*, 651-668, doi:10.1016/0891-5849(96)00162-1.
20. Barja, G. Aging in vertebrates, and the effect of caloric restriction: a mitochondrial free radical production-DNA damage mechanism? *Biological reviews of the Cambridge Philosophical Society* **2004**, *79*, 235-251.
21. Barja, G. Free radicals and aging. *Trends in neurosciences* **2004**, *27*, 595-600, doi:10.1016/j.tins.2004.07.005.
22. Forster, M.J.; Sohal, B.H.; Sohal, R.S. Reversible effects of long-term caloric restriction on protein oxidative damage. *The journals of gerontology. Series A, Biological sciences and medical sciences* **2000**, *55*, B522-529.
23. Lambert, A.J.; Merry, B.J. Lack of effect of caloric restriction on bioenergetics and reactive oxygen species production in intact rat hepatocytes. *The journals of gerontology. Series A, Biological sciences and medical sciences* **2005**, *60*, 175-180.
24. Lambert, A.J.; Portero-Otin, M.; Pamplona, R.; Merry, B.J. Effect of ageing and caloric restriction on specific markers of protein oxidative damage and membrane peroxidizability in rat liver mitochondria. *Mechanisms of ageing and development* **2004**, *125*, 529-538, doi:10.1016/j.mad.2004.06.002.
25. Chan, D.C. Mitochondria: dynamic organelles in disease, aging, and development. *Cell* **2006**, *125*, 1241-1252, doi:10.1016/j.cell.2006.06.010.
26. Kregel, K.C.; Zhang, H.J. An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. *Am J Physiol Regul Integr Comp Physiol* **2007**, *292*, R18-36, doi:10.1152/ajpregu.00327.2006.
27. Short, K.R.; Bigelow, M.L.; Kahl, J.; Singh, R.; Coenen-Schimke, J.; Raghavakaimal, S.; Nair, K.S. Decline in skeletal muscle mitochondrial function with aging in humans. *Proc Natl Acad Sci U S A* **2005**, *102*, 5618-5623, doi:10.1073/pnas.0501559102.
28. Chen, J.C.; Warshaw, J.B.; Sanadi, D.R. Regulation of mitochondrial respiration in senescence. *J Cell Physiol* **1972**, *80*, 141-148, doi:10.1002/jcp.1040800115.
29. Hansford, R.G. Lipid oxidation by heart mitochondria from young adult and senescent rats. *Biochem J* **1978**, *170*, 285-295, doi:10.1042/bj1700285.
30. Trounce, I.; Byrne, E.; Marzuki, S. Decline in skeletal muscle mitochondrial respiratory chain function: possible factor in ageing. *Lancet* **1989**, *1*, 637-639.
31. Cooper, J.M.; Mann, V.M.; Schapira, A.H. Analyses of mitochondrial respiratory chain function and mitochondrial DNA deletion in human skeletal muscle: effect of ageing. *J Neurol Sci* **1992**, *113*, 91-98.
32. Boffoli, D.; Scacco, S.C.; Vergari, R.; Solarino, G.; Santacroce, G.; Papa, S. Decline with age of the respiratory chain activity in human skeletal muscle. *Biochim Biophys Acta* **1994**, *1226*, 73-82.
33. Linnane, A.W.; Marzuki, S.; Ozawa, T.; Tanaka, M. Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases. *Lancet* **1989**, *1*, 642-645.
34. Hancock, C.R.; Han, D.H.; Higashida, K.; Kim, S.H.; Holloszy, J.O. Does calorie restriction induce

mitochondrial biogenesis? A reevaluation. *FASEB J* **2011**, *25*, 785-791, doi:10.1096/fj.10-170415.
35. Zhu, M.; de Cabo, R.; Lane, M.A.; Ingram, D.K. Caloric restriction modulates early events in insulin signaling in liver and skeletal muscle of rat. *Ann N Y Acad Sci* **2004**, *1019*, 448-452, doi:10.1196/annals.1297.082.
36. Ranhotra, H.S. Long-term caloric restriction up-regulates PPAR gamma co-activator 1 alpha (PGC-1alpha) expression in mice. *Indian J Biochem Biophys* **2010**, *47*, 272-277.
37. Corton, J.C.; Brown-Borg, H.M. Peroxisome proliferator-activated receptor gamma coactivator 1 in caloric restriction and other models of longevity. *J Gerontol A Biol Sci Med Sci* **2005**, *60*, 1494-1509, doi:10.1093/gerona/60.12.1494.
38. Puigserver, P.; Wu, Z.; Park, C.W.; Graves, R.; Wright, M.; Spiegelman, B.M. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* **1998**, *92*, 829-839, doi:10.1016/s0092-8674(00)81410-5.
39. Handschin, C.; Spiegelman, B.M. Peroxisome proliferator-activated receptor gamma coactivator 1 coactivators, energy homeostasis, and metabolism. *Endocr Rev* **2006**, *27*, 728-735, doi:10.1210/er.2006-0037.
40. Wu, Z.; Puigserver, P.; Andersson, U.; Zhang, C.; Adelmant, G.; Mootha, V.; Troy, A.; Cinti, S.; Lowell, B.; Scarpulla, R.C., et al. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* **1999**, *98*, 115-124, doi:10.1016/S0092-8674(00)80611-X.
41. St-Pierre, J.; Drori, S.; Uldry, M.; Silvaggi, J.M.; Rhee, J.; Jager, S.; Handschin, C.; Zheng, K.; Lin, J.; Yang, W., et al. Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell* **2006**, *127*, 397-408, doi:10.1016/j.cell.2006.09.024.
42. Lanza, I.R.; Zabielski, P.; Klaus, K.A.; Morse, D.M.; Heppelmann, C.J.; Bergen, H.R., 3rd; Dasari, S.; Walrand, S.; Short, K.R.; Johnson, M.L., et al. Chronic caloric restriction preserves mitochondrial function in senescence without increasing mitochondrial biogenesis. *Cell Metab* **2012**, *16*, 777-788, doi:10.1016/j.cmet.2012.11.003.
43. Hepple, R.T.; Baker, D.J.; McConkey, M.; Murynka, T.; Norris, R. Caloric restriction protects mitochondrial function with aging in skeletal and cardiac muscles. *Rejuvenation Res* **2006**, *9*, 219-222, doi:10.1089/rej.2006.9.219.
44. Miller, B.F.; Robinson, M.M.; Bruss, M.D.; Hellerstein, M.; Hamilton, K.L. A comprehensive assessment of mitochondrial protein synthesis and cellular proliferation with age and caloric restriction. *Aging Cell* **2012**, *11*, 150-161, doi:10.1111/j.1474-9726.2011.00769.x.
45. Lopez-Lluch, G.; Hunt, N.; Jones, B.; Zhu, M.; Jamieson, H.; Hilmer, S.; Cascajo, M.V.; Allard, J.; Ingram, D.K.; Navas, P., et al. Calorie restriction induces mitochondrial biogenesis and bioenergetic efficiency. *Proc Natl Acad Sci U S A* **2006**, *103*, 1768-1773, doi:10.1073/pnas.0510452103.
46. Gudiksen, A.; Pilegaard, H. PGC-1alpha and fasting-induced PDH regulation in mouse skeletal muscle. *Physiol Rep* **2017**, *5*, doi:10.14814/phy2.13222.
47. Geng, T.; Li, P.; Okutsu, M.; Yin, X.; Kwek, J.; Zhang, M.; Yan, Z. PGC-1alpha plays a functional role in exercise-induced mitochondrial biogenesis and angiogenesis but not fiber-type transformation in mouse skeletal muscle. *Am J Physiol Cell Physiol* **2010**, *298*, C572-579, doi:10.1152/ajpcell.00481.2009.
48. Leick, L.; Wojtaszewski, J.F.; Johansen, S.T.; Kiilerich, K.; Comes, G.; Hellsten, Y.; Hidalgo, J.; Pilegaard, H. PGC-1alpha is not mandatory for exercise- and training-induced adaptive gene responses in mouse skeletal muscle. *Am J Physiol Endocrinol Metab* **2008**, *294*, E463-474, doi:10.1152/ajpendo.00666.2007.
49. Vega, R.B.; Huss, J.M.; Kelly, D.P. The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor alpha in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. *Mol Cell Biol* **2000**, *20*, 1868-1876, doi:10.1128/mcb.20.5.1868-1876.2000.
50. Wang, Y.X.; Lee, C.H.; Tiep, S.; Yu, R.T.; Ham, J.; Kang, H.; Evans, R.M. Peroxisome-proliferator-activated receptor delta activates fat metabolism to prevent obesity. *Cell* **2003**, *113*, 159-170, doi:10.1016/s0092-8674(03)00269-1.
51. Perez-Schindler, J.; Summermatter, S.; Salatino, S.; Zorzato, F.; Beer, M.; Balwierz, P.J.; van Nimwegen, E.; Feige, J.N.; Auwerx, J.; Handschin, C. The corepressor NCoR1 antagonizes PGC-1alpha and estrogen-related receptor alpha in the regulation of skeletal muscle function and oxidative metabolism. *Mol Cell Biol* **2012**, *32*, 4913-4924, doi:10.1128/MCB.00877-12.

52. Hobbs, M.V.; Weigle, W.O.; Noonan, D.J.; Torbett, B.E.; McEvilly, R.J.; Koch, R.J.; Cardenas, G.J.; Ernst, D.N. Patterns of cytokine gene expression by CD4+ T cells from young and old mice. *J Immunol* **1993**, *150*, 3602-3614.
53. Riancho, J.A.; Zarrabeitia, M.T.; Amado, J.A.; Olmos, J.M.; Gonzalez-Macias, J. Age-related differences in cytokine secretion. *Gerontology* **1994**, *40*, 8-12, doi:10.1159/000213568.
54. Miller, R.A. Aging and immune function. *Int Rev Cytol* **1991**, *124*, 187-215, doi:10.1016/s0074-7696(08)61527-2.
55. Kubo, M.; Cinader, B. Polymorphism of age-related changes in interleukin (IL) production: differential changes of T helper subpopulations, synthesizing IL 2, IL 3 and IL 4. *Eur J Immunol* **1990**, *20*, 1289-1296, doi:10.1002/eji.1830200614.
56. Hayek, M.G.; Mura, C.; Wu, D.; Beharka, A.A.; Han, S.N.; Paulson, K.E.; Hwang, D.; Meydani, S.N. Enhanced expression of inducible cyclooxygenase with age in murine macrophages. *J Immunol* **1997**, *159*, 2445-2451.
57. Morgan, M.J.; Liu, Z.G. Crosstalk of reactive oxygen species and NF-kappaB signaling. *Cell Res* **2011**, *21*, 103-115, doi:10.1038/cr.2010.178.
58. Goto, M.; Katayama, K.I.; Shirakawa, F.; Tanaka, I. Involvement of NF-kappaB p50/p65 heterodimer in activation of the human pro-interleukin-1beta gene at two subregions of the upstream enhancer element. *Cytokine* **1999**, *11*, 16-28, doi:10.1006/cyto.1998.0390.
59. Kim, H.J.; Kim, K.W.; Yu, B.P.; Chung, H.Y. The effect of age on cyclooxygenase-2 gene expression: NF-kappaB activation and IkappaBalpha degradation. *Free Radic Biol Med* **2000**, *28*, 683-692, doi:10.1016/s0891-5849(99)00274-9.
60. Libermann, T.A.; Baltimore, D. Activation of interleukin-6 gene expression through the NF-kappa B transcription factor. *Mol Cell Biol* **1990**, *10*, 2327-2334, doi:10.1128/mcb.10.5.2327.
61. Yao, J.; Mackman, N.; Edgington, T.S.; Fan, S.T. Lipopolysaccharide induction of the tumor necrosis factor-alpha promoter in human monocytic cells. Regulation by Egr-1, c-Jun, and NF-kappaB transcription factors. *J Biol Chem* **1997**, *272*, 17795-17801, doi:10.1074/jbc.272.28.17795.
62. Taylor, B.S.; de Vera, M.E.; Ganster, R.W.; Wang, Q.; Shapiro, R.A.; Morris, S.M., Jr.; Billiar, T.R.; Geller, D.A. Multiple NF-kappaB enhancer elements regulate cytokine induction of the human inducible nitric oxide synthase gene. *J Biol Chem* **1998**, *273*, 15148-15156, doi:10.1074/jbc.273.24.15148.
63. Erol, A. Interleukin-6 (IL-6) is still the leading biomarker of the metabolic and aging related disorders. *Med Hypotheses* **2007**, *69*, 708, doi:10.1016/j.mehy.2007.01.021.
64. Sung, B.; Park, S.; Yu, B.P.; Chung, H.Y. Modulation of PPAR in aging, inflammation, and calorie restriction. *J Gerontol A Biol Sci Med Sci* **2004**, *59*, 997-1006, doi:10.1093/gerona/59.10.b997.
65. Ricote, M.; Huang, J.; Fajas, L.; Li, A.; Welch, J.; Najib, J.; Witztum, J.L.; Auwerx, J.; Palinski, W.; Glass, C.K. Expression of the peroxisome proliferator-activated receptor gamma (PPARgamma) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein. *Proc Natl Acad Sci U S A* **1998**, *95*, 7614-7619.
66. Jiang, C.; Ting, A.T.; Seed, B. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. *Nature* **1998**, *391*, 82-86, doi:10.1038/34184.
67. Okada, M.; Yan, S.F.; Pinsky, D.J. Peroxisome proliferator-activated receptor-gamma (PPAR-gamma) activation suppresses ischemic induction of Egr-1 and its inflammatory gene targets. *FASEB J* **2002**, *16*, 1861-1868, doi:10.1096/fj.02-0503com.
68. Barbier, O.; Torra, I.P.; Duguay, Y.; Blanquart, C.; Fruchart, J.C.; Glineur, C.; Staels, B. Pleiotropic actions of peroxisome proliferator-activated receptors in lipid metabolism and atherosclerosis. *Arterioscler Thromb Vasc Biol* **2002**, *22*, 717-726.
69. Ricote, M.; Li, A.C.; Willson, T.M.; Kelly, C.J.; Glass, C.K. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature* **1998**, *391*, 79-82, doi:10.1038/34178.
70. Narala, V.R.; Adapala, R.K.; Suresh, M.V.; Brock, T.G.; Peters-Golden, M.; Reddy, R.C. Leukotriene B4 is a physiologically relevant endogenous peroxisome proliferator-activated receptor-alpha agonist. *J Biol Chem* **2010**, *285*, 22067-22074, doi:10.1074/jbc.M109.085118.
71. Colbert, L.H.; Mai, V.; Tooze, J.A.; Perkins, S.N.; Berrigan, D.; Hursting, S.D. Negative energy balance induced by voluntary wheel running inhibits polyp development in APCMin mice. *Carcinogenesis* **2006**,

27, 2103-2107, doi:10.1093/carcin/bgl056.
72. Holloszy, J.O. Mortality rate and longevity of food-restricted exercising male rats: a reevaluation. *J Appl Physiol (1985)* **1997**, *82*, 399-403, doi:10.1152/jappl.1997.82.2.399.
73. Manini, T.M.; Everhart, J.E.; Patel, K.V.; Schoeller, D.A.; Colbert, L.H.; Visser, M.; Tylavsky, F.; Bauer, D.C.; Goodpaster, B.H.; Harris, T.B. Daily activity energy expenditure and mortality among older adults. *JAMA* **2006**, *296*, 171-179, doi:10.1001/jama.296.2.171.
74. Warburton, D.E.; Nicol, C.W.; Bredin, S.S. Health benefits of physical activity: the evidence. *CMAJ* **2006**, *174*, 801-809, doi:10.1503/cmaj.051351.
75. Laaksonen, D.E.; Lindstrom, J.; Lakka, T.A.; Eriksson, J.G.; Niskanen, L.; Wikstrom, K.; Aunola, S.; Keinanen-Kiukaanniemi, S.; Laakso, M.; Valle, T.T., et al. Physical activity in the prevention of type 2 diabetes: the Finnish diabetes prevention study. *Diabetes* **2005**, *54*, 158-165, doi:10.2337/diabetes.54.1.158.
76. Holloszy, J.O. Exercise and longevity: studies on rats. *J Gerontol* **1988**, *43*, B149-151, doi:10.1093/geronj/43.6.b149.
77. Samorajski, T.; Delaney, C.; Durham, L.; Ordy, J.M.; Johnson, J.A.; Dunlap, W.P. Effect of exercise on longevity, body weight, locomotor performance, and passive-avoidance memory of C57BL/6J mice. *Neurobiol Aging* **1985**, *6*, 17-24, doi:10.1016/0197-4580(85)90066-1.
78. Pekkanen, J.; Marti, B.; Nissinen, A.; Tuomilehto, J.; Punsar, S.; Karvonen, M.J. Reduction of premature mortality by high physical activity: a 20-year follow-up of middle-aged Finnish men. *Lancet* **1987**, *1*, 1473-1477, doi:10.1016/s0140-6736(87)92218-5.
79. Fontana, L.; Meyer, T.E.; Klein, S.; Holloszy, J.O. Long-term low-calorie low-protein vegan diet and endurance exercise are associated with low cardiometabolic risk. *Rejuvenation Res* **2007**, *10*, 225-234, doi:10.1089/rej.2006.0529.
80. Fontana, L.; Klein, S.; Holloszy, J.O. Long-term low-protein, low-calorie diet and endurance exercise modulate metabolic factors associated with cancer risk. *Am J Clin Nutr* **2006**, *84*, 1456-1462, doi:10.1093/ajcn/84.6.1456.
81. Huffman, D.M.; Moellering, D.R.; Grizzle, W.E.; Stockard, C.R.; Johnson, M.S.; Nagy, T.R. Effect of exercise and calorie restriction on biomarkers of aging in mice. *Am J Physiol Regul Integr Comp Physiol* **2008**, *294*, R1618-1627, doi:10.1152/ajpregu.00890.2007.
82. Knight, J.A. The biochemistry of aging. *Adv Clin Chem* **2000**, *35*, 1-62.
83. Park, S.Y.; Kim, Y.W.; Kim, J.E.; Kim, J.Y. Age-associated changes in fat metabolism in the rat and its relation to sympathetic activity. *Life Sci* **2006**, *79*, 2228-2233, doi:10.1016/j.lfs.2006.07.014.
84. Petersen, K.F.; Befroy, D.; Dufour, S.; Dziura, J.; Ariyan, C.; Rothman, D.L.; DiPietro, L.; Cline, G.W.; Shulman, G.I. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science* **2003**, *300*, 1140-1142, doi:10.1126/science.1082889.
85. Kenyon, C. The plasticity of aging: insights from long-lived mutants. *Cell* **2005**, *120*, 449-460, doi:10.1016/j.cell.2005.02.002.

References

1. Fernanda M. Cerqueira; Alicia J. Kowaltowski; Commonly adopted caloric restriction protocols often involve malnutrition. *Ageing Research Reviews* **2010**, *9*, 424-430, 10.1016/j.arr.2010.05.002.
2. Ken Inoki; Hongjiao Ouyang; Tianqing Zhu; Charlotta Lindvall; Yian Wang; Xiaojie Zhang; Qian Yang; Christina Bennett; Yuko Harada; Kryn Stankunas; et al.Cun-Yu WangXi HeOrmond A. MacDougaldMing YouBart O WilliamsKun-Liang Guan TSC2 Integrates Wnt and Energy Signals via a Coordinated Phosphorylation by AMPK and GSK3 to Regulate Cell Growth. *Cell* **2006**, *126*, 955-968, 10.1016/j.cell.2006.06.055.

Keywords

Caloric restriction;Nutrition;Diet