Metabolic Effects of Caloric Restriction

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Caloric restriction (CR) is a dietary intervention that robustly extends lifespan in diverse species. In mammals CR extends the period in which the animal is fit and vigorous, and attenuates age-related disease vulnerability. Benefits of CR include reduced incidence of cancer, improved cardiovascular health, increased insulin sensitivity, and resistance to neurodegenerative diseases. The fact that CR extends not only average lifespan but also maximum lifespan has led to the consensus that an optimised CR diet slows the aging process itself. Here we outline the effects of CR on physiology and metabolism and where these may fit with current theories of aging. The authors describe factors that are likely to mediate the physiological adaptations to CR, placing an emphasis on nutrient sensitive regulators of metabolism. A major incentive for research into the mechanisms of CR is the promise of novel treatments for age-related diseases and disorders that are relevant to human aging.

Introduction

The seminal experiment linking energy intake to health and longevity in rats was reported by McCay *et al*. (1935). While experiments on the effects of CR continued for the next 50 years, it was only in the 1980s that the impact of CR on the aging process began to be fully appreciated. Since that time, the effects of CR on heath and phenotypes of aging have been studied extensively in invertebrate models, laboratory rodents and nonhuman primates. The manner in which CR is implemented is species-specific, and the methodology significantly influences the outcome in terms of health and longevity. Too drastic a reduction in calories results in malnutrition and death, whereas too little CR produces negligible effects. Even within species there is considerable variation in the response to CR that may be dependent on genetic background and which may limit the degree of caloric restriction that is tolerated.

The last decade or so has seen a tremendous advance in what is known about the biology of aging and the actions of CR. Most of this work has been conducted in short-lived species where costs are low, genetic manipulation techniques are mature, and lifespan is measured in days or weeks. Recently, technical advances in genetic manipulation have made more mechanistic studies possible in mice, where lifespan is measured over 2–3 years. The translatability of CR's effects to longer lived species has also been explored in long-term studies in nonhuman primates, as well as by short-term human clinical trials. These studies confirm that multiple parameters in the physiological response to CR are evolutionarily conserved from mice to monkeys to humans. Although there are some individuals that voluntarily engage in the practice of CR, generally speaking it is not viewed as tenable lifestyle choice for the population at large. The goal of CR research is not to endorse the practice of CR in humans, but instead to use CR as a tool to explore the biology of aging and age-related disease. We propose that the mechanisms of CR have much to offer in terms of the aetiology of age-related disease and the increased vulnerability to a spectrum of diseases that occurs with advanced age in humans. **See also**: History of Research into Ageing/Senescence

How Does Caloric Restriction Work?

The mechanisms underlying the beneficial effects of a CR diet are complex, and no single pathway likely accounts for the full range of effects (Masoro, 2009). It was initially thought that CR extended lifespan passively, and that a low energy input lowers the rate of metabolism, thereby attenuating the production of damaging byproducts and diminishing the rate of aging. A prediction of this model is that CR animals would not significantly differ from control

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animals, except that age-related changes would occur later in life. While this proposal makes intuitive sense, the available evidence strongly suggests an active model, whereby an energy deficit is sensed by the organism and triggers a series of programmed responses that lead to the observed improvements in health and longevity. The active response model predicts that CR animals are not just delayed in the onset of age-related phenotypes, but that CR animals are distinct in that pathways are engaged by CR independent of the attenuation of age-related changes. The clearest evidence in favour of the active model comes from invertebrate organisms. Several genes have been identified that (1) specifically respond to CR, and (2) are absolutely required for CR's effects. Implicit in this is that deletion of the factors alone does not produce overtly negative effects under normal conditions, an animal lacking essential components may well fail to respond to CR. In mice it has been shown that the growth hormone (GH) receptor is required for increased longevity associated with CR. Several key factors associated with longevity have also been shown to be required for specific aspects of the CR response in mice, including SIRT1, SIRT3, SIRT4, endothelial nitric oxide synthase (eNOS), nuclear factor (erythroid-derived 2)-like 2 (NFE2L2), adiponectin and nicotinamide phosphoribosyltransferase (NAMPT). These data are consistent with the concept that specific pathways are engaged by CR to ultimately lead to delayed aging.

There are several hallmarks of CR, some of which are directly conserved across species and others that are similar or equivalent across species. With regards to the former, organismal size is always smaller for animals on CR; and for the latter, CR results in a reduction in fat stores and improves sensitivity to fasting/feeding signals. In mammals, including mice, monkeys and people, CR results in lower body weight, lower adiposity and enhanced insulin sensitivity.

The idea that most organisms contain a latent ability to live longer and healthier lives raises an important question: why isn't this longevity programme active all the time? A possible explanation emerges from evolutionary theories related to aging. The Disposable Soma theory of aging proposes that survival beyond the age of reproductive maturity and the creation of offspring is not selected for by evolution. Therefore, under conditions of abundant resources, evolution will favour a rapid progression to reproductive maturity at the expense of organismal longevity. These evolutionary forces may reverse when resources become limiting. During a period of famine, reproduction may become a futile exercise and a dangerous waste of resources, whereas maintaining individual health over an extended period becomes critical to eventual reproductive success. It is possible, therefore, that the beneficial effects of CR are the result of a conserved genetic programme that reallocates resources to maintenance and repair when appropriate. A second possibility is that laboratory conditions diverge from the natural habitat of animal models used in CR research. It has been argued that laboratory animals may be overfed compared to wild animals, and that a CR diet simply corrects this dietary excess. However, the primary motivation for all of these studies is to understand nutritional modulation of aging and age-related disease. Regardless of the natural food consumption by wild rodents or primates, many humans today are overfed and immersed in a veritable sea of calories.

Physiological Effects of Caloric Restriction

The physiological changes induced by CR have been extensively characterised with the hope of better understanding the basis of the beneficial effects of CR. In the next few sections, the authors highlight metabolic aspects of the CR response that they believe to be important in the mechanisms of delayed aging.

Whole body metabolism

Following food restriction, rodents go through an initial adaptation phase where metabolic rate decreases, the balance of hepatic metabolism shifts and fat stores become depleted. Over time, rodents subjected to CR shift to a lower body weight where the size of most organs is decreased roughly in proportion to body weight; however, brain weight remains relatively constant, although white adipose stores are largely depleted. Microarray experiments have demonstrated that long-term CR induces widespread changes in mitchondrial energy metabolism across tissues including adipose tissue, skeletal muscle, brain, and heart (Anderson and Prolla, 2009). A number of recent studies investigating the CR response among tissues using meta-analysis of gene expression have identified key themes including altered metabolism, activated stress response, GH signalling and suppression of immune and inflammatory pathways (Plank *et al*., 2012; Swindell, 2009). White adipose tissue in particular seems to enter a novel metabolic state that is distinct from that of control-fed animals. Whole body metabolic assessments in mice indicate that there is a preference for lipid fuel utilisation with CR (Bruss *et al*., 2010). Unexpectedly, this shift is associated with increased fatty acid synthesis, indicating increased flux in lipid metabolic pathways with CR.

In nonhuman primates overall metabolic rate adjusted for body composition is not different with long-term CR but sleeping metabolic rate, an estimate of resting energy expenditure, is lower suggesting enhanced metabolic efficiency in monkeys on CR (Yamada *et al*., 2013). In humans, a decrease in resting energy expenditure adjusted for body composition has also been reported with short-term CR. Insights into overall metabolic state can be gleaned through the study of metabolomics, by which serum metabolites can be identified using nuclear magnetic resonance analysis. In nonhuman primates the metabolic profile of CR animals is distinct from that of age and

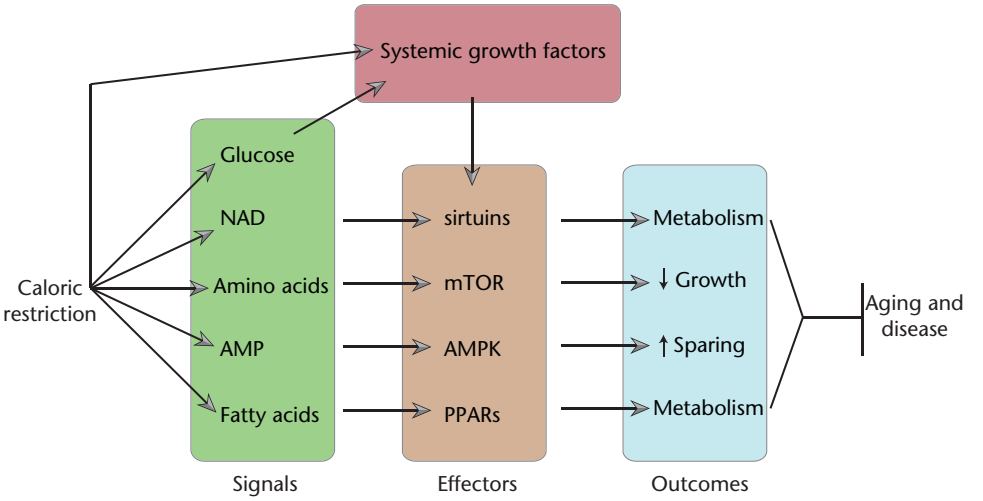

Figure 1 Simplified model of metabolic reprogramming by CR. Reduction in calorie intake extends lifespan suggesting an inverse relationship between energy and aging rate. This simple model predicts that a reduction in calorie intake induces differences in nutrient and energetic status (signals) that are detected by nutrient and energy sensitive factors (effectors) that regulate in the balance of energy use and energy sparing (outcomes). Regulation through systemic growth factors is superimposed on these cellular pathways, that together lead to changes in metabolism, growth, and energy sparing. How these changes translate to delayed aging and prevention of age-related diseases is yet to be resolved.

gender matched controls, and the trajectory of change in metabolite profile as a function of age is also distinct (Rezzi *et al*., 2009). Taken together these whole body and serum measures indicate that CR induces an altered metabolic state (Anderson and Weindruch, 2010). One working hypothesis is that nutrient sensing metabolic regulators cause a reprogramming of energy metabolism that is accompanied by reduced growth and energy sparing (**Figure 1**). These changes together influence resilience and create a nonpermissive environment for a range of age-related diseases, many of which have a basis in metabolic dysfunction. **See also**: Mitochondria as a Key Determinant of Aging

Systemic signalling

Decreased fasting blood glucose levels and increased insulin sensitivity are among the most extensively documented and robust changes induced by CR in mammals. The improvement in insulin sensitivity with CR has attracted significant attention as it has clear implications for human health. Experiments in worms, flies and mice have confirmed that genetic modulation to attenuate insulin-like signalling can extend longevity (Kenyon, 2001), a finding that at face value seems at odds with the fact that diet-induced insulin resistance (defective insulin signalling) in mammals leads to diabetes and shortens lifespan. These observations can be reconciled if diminished flux through signalling downstream of the insulin receptor is the key factor that regulates longevity. Insulin is secreted in response to nutrients, and insulin signalling is limited by negative feedback at the level of the insulin receptor. The increased sensitivity of animals subjected to CR may in part be a consequence of chronic low signalling under nonchallenging conditions and increased efficiency in the response under challenged conditions. In this way, a decrease in the overall activation of signalling downstream of the insulin receptor could be a common effect of both CR and mice with genetically attenuated insulin signalling.

While insulin sensitivity is the most scrutinised endocrine change induced by CR, it is far from the only one. There is reduced signalling through the GH/insulin-like growth factor 1 (IGF-1) axis, which shares downstream components with insulin signalling and significantly impacts longevity in mouse models. Several established genetic models of enhanced longevity share defects in the somatotropic axis (Bartke *et al*., 2013). Another significant effect of CR is on adipose tissues. Until recently white adipose tissue was seen as a biologically inert lipid storage depot; we now know that white adipose tissue plays a systemic role influencing metabolism, inflammation, and immune responses, and implemented through a suite of adipose-derived signalling factors collectively known as adipokines. The levels of many adipokines are lower in CR animals including leptin, resistin, and inflammatory mediators. Leptin is of particular interest due to its ability to influence central regulation of feeding behaviour, appetite suppression and weight maintenance. In contrast, adiponectin levels are enhanced with CR. Serum adiponectin levels correlate negatively with fat mass and are associated with increased fatty acid oxidation in adipose tissue and reduced lipid accumulation in peripheral tissues.

The impact of CR on reproductive hormones depends on gender and the extent of CR. Females on 40% CR undergo reproductive pause but retain fecundity longer than controls if moved from CR back to control diet. In males, the effect of CR on fertility is less clear, with significant gene expression changes seen in the epididymis of rats, but no apparent impact on the sperm quality of primates. Levels of testosterone are unchanged by CR in

males but increased in females on 40% CR, while levels of estradiol decline for both genders.

A modest, but consistent increase in glucocorticoid concentrations has been viewed as evidence that the body perceives CR as a mild stress. This could be an important clue as to the mechanism of CR, since the protective response induced by a number of low-intensity stresses creates a net beneficial effect on health and longevity, a process termed hormesis (Masoro, 2009). In addition, thyrotropin levels are suppressed and there are changes in the actions of many neurotransmitters that have not been fully elucidated. For example, CR has an antidepressant-like effect in mice that appears to be mediated by orexin signalling. Neuropeptide Y (NPY) expression increases in the hypothalamus, and declines in the circulation. The hypothalamus is the major target for leptin signalling and NPY, a highly abundant neurotransmitter involved in hunger sensing, is required for full implementation of the CR response (Minor *et al*., 2011). Determining which of these changes are causally related to one another, and which play important roles in the beneficial effects of CR, remains a major challenge. **See also**: Obesity Hormones in Health and Disease

Reactive oxygen species

Reactive molecules are generated during normal metabolism, primarily in the mitochondria, and damage to macromolecules increases with age. Up until recently, Denham Harman's 'Free Radical Hypothesis' was the predominant theory of the underlying cause of aging and age-related disease vulnerability. The idea is that as reactive molecules continuously damage essential cellular components, viability is eventually compromised. Supporting this concept is the observation that many animals with longer lifespans accumulate damage more slowly – with a notable exception being the naked mole rat (Lewis *et al*., 2013). The question is whether oxidative damage is a causative factor driving the aging process, or is instead simply a symptom of aging.

In the past few years increasing evidence suggest that the latter is the case. Numerous mouse models of increased oxidative stress defence show no significant effect on lifespan under conditions of normal husbandry (Perez *et al*., 2009). In a series of experiments in which mice were genetically manipulated to overexpress key proteins (including superoxide dismutases, peroxidases and thioreductase) involved in sequestering and eliminating reactive species, very few effects on lifespan were observed. Studies investigating the ability of antioxidants such as vitamin E to influence lifespan have consistently failed to demonstrate beneficial effects. As a major energy and oxygen consumer in the body, reactive oxygen species are continually being produced in skeletal muscle. Mice with disabled mechanisms for removing oxidative species exhibit accelerated muscle mass loss indicating that profound oxidative damage can drive aging-related phenotypes at the individual tissue level. Interestingly, CR can reverse this effect but data suggest that this occurs at the level of lower reactive species production, not enhanced reactive species sequestration. In support of this idea, levels of antioxidant genes are not elevated in mouse skeletal muscle with CR (Edwards *et al*., 2007) even though it has been known for some time that CR is associated with reduced levels of oxidative damage.

A new take on reactive oxygen species that has recently emerged is the theory of mitohormesis (mitochondrial hormesis). In *Caenorhabditis elegans*, CR induces increased stress resistance and promotes health and longevity via an adaptive response to the increased generation of reactive oxygen species (Ristow and Zarse, 2010), and this increase in oxidative stress is actually required for the beneficial effects of CR. It seems unlikely that this concept would translate exactly to mammalian systems in light of the evidence presented above; however, it would explain why treatment with antioxidants has sometimes proven deleterious (Selman *et al*., 2013). The connection between stress resistance and longevity has been one of the most engaging puzzles in biology of aging research to date and there is likely much more to learn. **See also**: Antioxidants; Free Radicals and Other Reactive Species in Disease

Key Signalling Pathways Implicated in Caloric Restriction

Growth signalling

The original CR study by McCay and colleagues suggested the hypothesis that decreasing body weight was the causative factor in lifespan extension. It is well-known that small dogs typically have a longer lifespan than large dogs, and a negative correlation between body weight and lifespan has been confirmed in laboratory mice (Miller *et al*., 2002). A comparison across inbred mouse strains reveals a negative correlation between lifespan and circulating IGF-1 levels (Yuan *et al*., 2009). A recently developed 'crowded litter' model is designed to stunt early growth and demonstrates lasting beneficial effects on insulin sensitivity and energy homoeostasis.

A number of strains of dwarf mice exhibit lifespan extension similar to that shown by mice on a CR diet despite having free access to food (Bartke *et al*., 2013). Two of these strains, Ames and Snell dwarf mice, have defects in the development of the anterior pituitary, making them deficient in prolactin, thyrotropin and GH. All three factors play complex roles in physiology; however, antagonising the action of GH alone is sufficient to decrease body size and extend longevity. GH deficiency has a number of downstream effects, including a reduction in IGF-1 levels, and mice heterozygous for the IGF-1 receptor have extended longevity in the absence of dwarfism (Xu *et al*., 2014). These observations suggest that the beneficial effects of downregulating the GH/IGF-1 axis in response to CR may be separable from changes in body size and

caloric intake. Notably, IGF-1 is among the genes implicated in human longevity (Pawlikowska *et al*., 2009).

It is unlikely that the mechanisms of lifespan extension in dwarf and CR mice overlap completely. The Ames dwarf mouse manifests an almost complete lack of GH and IGF-1, yet the lifespan of Ames dwarf mice is further increased by CR. However, mice lacking the GH receptor, which also have a dwarf phenotype and extended longevity, fail to respond to CR. There is extensive crosstalk between GH, IGF-1 and insulin signalling that is not fully understood and plays out in an elaborate and complex tissue-specific manner. Together, the evidence demonstrates that this axis can influence longevity and it seems highly likely that it is involved in the mechanisms of CR.

Sirtuins

Sirtuins are an evolutionarily conserved family of post-translational modification enzymes that are directly linked to metabolism through a requirement for nicotinamide–adenine dinucleotide (NAD) as a cosubstrate. NAD is a major cofactor in intermediary metabolism, where reducing equivalents produced during catabolism are accepted by NAD^+ to yield the reduced form NADH. These reducing equivalents fuel mitochondrial oxidative phosphorylation and are the principal means by which usable energy is extracted from fatty acid oxidation. Owing to their requirement for NAD as a cosubstrate, the activity of the sirtuin family of enzymes is directly linked to metabolism by the availability of NAD. NAD levels are regulated in part by the NAD salvage pathway that has been implicated in aging and in the mechanisms of CR in yeast and in mammals (Anderson *et al*., 2003; Song *et al*., 2014).

The founding member of the sirtuin family is the yeast gene SIR2 (silent information regulator 2), a histone deacetylase involved in regulating gene silencing through chromatin remodelling. SIR2 and its homologues play a role in lifespan regulation in yeast, worms, and flies, although its role in CR has been somewhat controversial (Baur *et al*., 2010; Fontana *et al*., 2010). There are seven mammalian sirtuins (SIRT1–7) that are functionally nonredundant and partitioned to distinct subcellular compartments. In contrast to the founder member of the sirtuin family, histones are not the only target for mammalian SIRT activity (**Figure 2**). Through posttranslational modification of a range of targets, the sirtuins impinge on a complex suite of metabolic and cellular processes, introducing a redox sensitive response beyond direct availability of NAD^+ as a cofactor in enzymatic reactions of intermediary metabolism.

Standard genetic approaches to test the requirement for specific factors in longevity and CR involve the 'necessary and sufficient' approach. For positive regulators, decreased levels are predicted to impair lifespan extension with CR while increased levels would enhance longevity. SIRT1-null mice are short-lived and fail to exhibit enhanced longevity when subjected to CR (Boily *et al*., 2008; Mercken *et al*., 2014). Overexpression of SIRT1

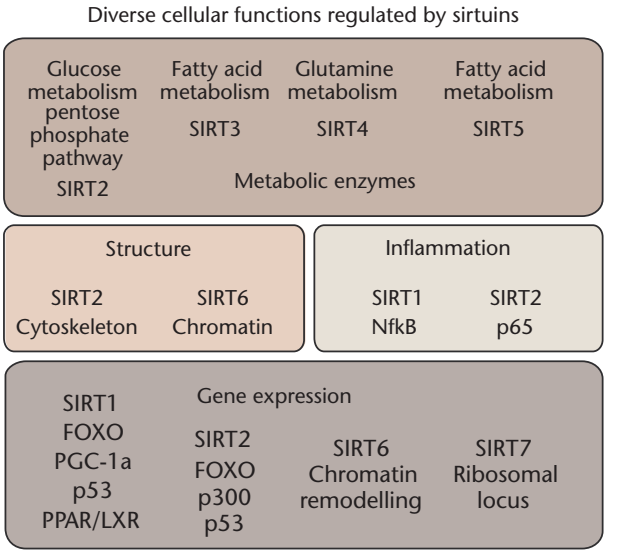

Figure 2 Diverse cellular functions regulated by sirtuins. The actions of the sirtuin family of posttranslational modification enzymes are coupled to metabolism due to their requirement for NAD as a cosubstrate. There are 7 mammalian sirtuins that populate distinct subcellular compartments, and mediate regulation of diverse metabolic processes including (1) metabolic enzymes – enzymes involved in multiple aspects of metabolism that are direct targets of sirtuin activity; (2) gene expression – regulation of transcription factors, coactivators, and regulatory factors; (3) structure – broad impact on the microtubule cytoskeleton and more specifically localised impact in chromatin; and (4) inflammation – direct regulation of NfκB inflammatory pathway.

induces phenotypes that resemble CR, and treatment with SIRT1 activators improves physiology but does not alter maximal lifespan, arguing against a direct effect on aging (Miller *et al*., 2011; Mitchell *et al*., 2014; Pearson *et al*., 2008). Critical targets of SIRT1 include factors involved in cell fate determination (p53 transcription factor, Ku DNA repair factor), adaptive metabolism (FOXO transcription factors, nuclear receptor family of transcription factors such as PPARs, PER2 circadian transcription factor and transcriptional coactivator PGC-1α), and inflammation (NfκB transcription factor), to name just a few, placing SIRT1 as a central node in the integration of cellular processes.

There are three mitochondrial sirtuins, SIRT3, SIRT4 and SIRT5 (Newman *et al*., 2012). SIRT3, like SIRT1, is a deacetylase. Targets of SIRT3 activity encompass a range of enzymes involved in intermediary metabolism, many of which are responsive to CR (Hallows *et al*., 2011; Hebert *et al*., 2013; Hirschey *et al*., 2011). SIRT3 has also been shown to influence antioxidant defence through regulation of the glutathione system and through activation of superoxide dismutase (SOD2), both of which are involved in the response to CR (Qiu *et al*., 2010; Someya *et al*., 2010). The activation of fatty acid oxidation has emerged as a key function of SIRT3 and presumably a major consequence of SIRT3 activation with CR (Hebert *et al*., 2013). SIRT4 acts as a repressor of glutamate dehydrogenase in pancreatic cells and SIRT4 expression is linked to growth signalling

through mTOR (Csibi *et al*., 2013). SIRT4 acts as a repressor of fatty acid oxidation in liver and muscle cells (Nasrin *et al*., 2010) and contributes to regulation of lipid synthesis through repression of malonyl CoA in muscle and adipose tissues (Laurent *et al*., 2013). Unlike SIRT1 and SIRT3, SIRT4 does not appear to be regulated at the protein level by CR, and a role for SIRT4 in CR has not been identified. The enzymatic activities of SIRT5 have only recently been identified. SIRT5 removes malonyl and succinyl 4-carbon groups (Du *et al*., 2011) and 5-carbon glutaryl groups (Tan *et al*., 2014) as opposed to the shorter acetyl group. The prevalence of these longer posttranslational modifications has been unappreciated, and the functional consequence of these modifications has yet to be determined. Interestingly, SIRT5 expression is regulated by PGC-1α (Buler *et al*., 2014) and is upregulated in brains of CR animals along with SIRT1 (Geng *et al*., 2011).

The predominantly nuclear SIRT6 deacetylase has also been shown to influence lifespan. SIRT6-null mice are extremely short lived and exhibit aging phenotype with severe genomic instability and metabolic defects. However, further examination clarified that the defects and short lifespan in this mouse model were primarily driven by lethal hypoglycemia. More recently, it was shown that overexpression of SIRT6 significantly increases male, but not female, lifespan (Kanfi *et al*., 2012). SIRT6 has been linked to adaptive metabolism in liver cell where its role in activation of GCN5-dependent acetylation of PGC-1α opposes the actions of SIRT1 to enhance hepatic glucose production (Dominy *et al*., 2012). The finding that SIRT6 deacetylase activity is greatly enhanced by fatty acids is a rather interesting development that suggests that intracellular free fatty acids could be endogenous ligands for SIRT6 (Feldman *et al*., 2013). SIRT7 deacetylase is also nuclear and early studies identified a role in chromatin remodelling of the ribosomal DNA locus, much like the yeast Sir2. SIRT7 deficiency results in fatty liver, a phenotype that is linked to activation of the unfolded protein reponse. SIRT2 is predominantly cytosolic and was first linked to regulation of the cytoskeleton and FOXO transcription factors (de Oliveira *et al*., 2012). More recently identified targets include metabolic enzymes involved in glycolysis and the pentose phosphate pathway (Xu *et al*., 2014; Wang *et al*., 2014). It remains to be seen what roles these less well characterised sirtuins play in aging and CR. The ability to respond to redox changes is key to metabolic adaptaion, whether fasting and feeding cycles, or adjustments to changes in energy availability or energetic demand. It seems unlikely that NAD metabolism and factors responsive to NAD flux are not involved in the mechanisms of CR, the challenge will be to untangle the crosstalk of regulatory outputs and redundancy in regulatory mechanisms to identify maleable targets for drug development.

AMPK

The standard currency of energy in the cell is adenosine triphosphate (ATP), primarily generated in the mitochondria from AMP (adenosine monophosphate) during oxidative phosphorylation. AMPK (AMP-activated protein kinase) is involved in the adaptive response to energy deficit, and is activated when levels of AMP are elevated. AMPK activation triggers increased glucose uptake, fatty acid oxidation and suppression of energy-consuming processes such as fat and protein synthesis to restore energy balance (Canto and Auwerx, 2010). In addition to being influenced by levels of cellular AMP, AMPK is also responsive to hormonal signalling, orchestrating the cellular metabolic response to diverse systemic stimuli.

AMPK induces the expression of genes involved in mitochondrial energy metabolism in skeletal muscle through activating phosphorylation of PGC-1α. AMPK also induces expression of NAMPT, which is the rate-limiting enzyme in the conversion of nicotinamide to NAD in the salvage pathway. Activation of AMPK thereby enhances SIRT1 activity that in turn activates PGC-1α by deacetylation. SIRT1 can additionally deacetylate and activate LKB1, an activating kinase upstream of AMPK, potentially creating a positive feedback loop. The AMPK/SIRT1/PGC-1α axis promotes oxidative metabolism and fatty acid fuel utilisation and is activated by adiponectin (Iwabu *et al*., 2010), one of the adipose tissue derived peptide hormones that is enhanced by CR. Moreover, the metabolic response to AMPK activation becomes blunted with age, indicating that the signalling pathway for adaptive energy metabolism becomes compromised in some way. AMPK signalling is preserved in tissues from CR animals, maintaining the integrity of energy metabolism and efficiency of fat metabolism.

In addition to activating energy metabolism through regulation of transcription factors, AMPK also promotes the utilisation of lipid fuels for energy generation through negative regulation of acetyl-CoA carboxylase, a key enzyme in lipid synthesis that inhibits fatty acid oxidation. Under conditions of energy deficit, e.g. fasting or exercise, energy stored as fat can be utilised to maintain energy balance. For this reason, activation of AMPK is an attractive therapeutic target for mobilising fat stored in tissues of overweight or obese individuals. AMPK is also linked to insulin sensitivity; metformin, the leading drug for the treatment of type 2 diabetes, improves hepatic insulin sensitivity by activating AMPK through an unknown mechanism. Treatment with metformin extends lifespan in mice, although the effect is dose dependent and possibly also dependent upon genetic background (Martin-Montalvo *et al*., 2013). AMPK is also one of the targets of resveratrol (Um *et al*., 2010), a plant polyphenol that is also effective in alleveiation of diet-induced metabolic deficiency in mice (Baur *et al*., 2006). The recent identification of longevity regulation through attenuated mTOR signalling (see below) suggests a mechanistic overlap with AMPK signalling. AMPK inhibits the mTOR signalling pathway through multiple mechanisms (**Figure 3**). First, AMPK directly phosphorylates the mTOR binding partner raptor, stimulating the inhibitory binding of raptor to 14–3–3. Second, AMPK also phosphorylates

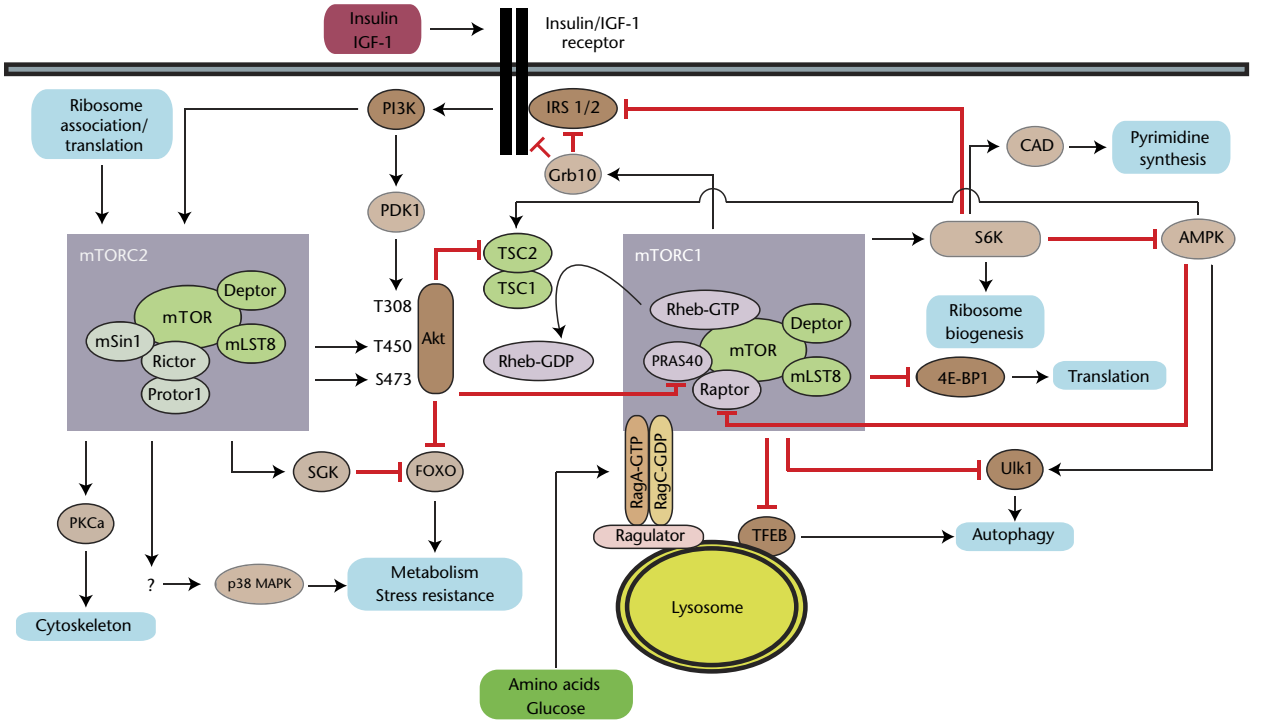

Figure 3 Integration of growth and nutrient signalling pathways through mTOR. Growth signalling and nutrient sensing are among the inputs that activate the mTOR signalling pathway. mTOR impinges on multiple key processes including protein synthesis, ribosome biogenesis, and autophagy, as part of the anabolic response. Crosstalk between the mTOR and the insulin signalling pathways is complex, with feedback inhibition of insulin signalling mediated in part by mTORC1-dependent phosphorylation of insulin receptor interacting proteins.

and stimulates the activity of TSC2, a GTPase activiating protein that inhibits mTOR signalling. Activating phosphorylation of AMPK has been reported in the hippocampus of animals subjected to long-term CR, but not the liver, heart or skeletal muscle. On balance, the data suggest a role of AMPK in the mechanisms of CR, although the manner in which AMPK signalling is regulated is likely more complex than a simple binary on/off switch. **See also**: AMP-activated Protein Kinase (AMPK)

mTOR, a master integrator of nutrient signalling pathways

A major regulator of nutrient signalling pathways downstream of the insulin/IGF-1 receptor is the mechanistic (formerly mammalian) target of rapamycin (mTOR). mTOR is a phosphatidylinositol 3-kinase (PI3K)-like serine/threonine protein kinase that is evolutionarily conserved in all eukaryotes (Lamming and Sabatini, 2013). mTOR is inhibited by rapamycin, an FDA-approved immunosuppressive and anticancer agent originally discovered in the soil of Easter Island. mTOR is found in two separate protein complexes, each with distinct cellular functions and substrates. mTOR complex 1 (mTORC1), which is acutely sensitive to rapamycin, plays a key role in the regulation of translation and cell growth via the phosphorylation of substrates that include S6 kinase and 4E-BP1. mTOR complex 2 (mTORC2), which is less sensitive to rapamycin, functions downstream of the insulin/IGF-1 receptor to promote the phosphorylation of substrates that include Akt, SGK and PKCα.

mTORC1 functions to integrate environmental cues including the availability of amino acids and glucose, growth factors, and cellular energy to regulate growth and translation. As outlined in **Figure 3**, insulin/IGF-1 signalling primarily regulates mTORC1 activity via Akt, which is itself regulated in part by mTORC2. In turn, mTORC1 activity both negatively regulates insulin receptor signalling at the level of IRS1/2, while promoting mTORC2 activity via ribosomal assembly (Hsu *et al.*, 2011). Akt, as well as many other environmental signals such as AMPK, regulate mTORC1 activity by controlling the activity of the TSC1/TSC2 complex, which in turn regulates the GTP-bound status of Rheb. mTORC1 activity is controlled by the association of mTORC1 with GTP-bound Rheb at the lysosome; this localisation is controlled in response to amino acids and glucose (Bar-Peled and Sabatini, 2014). In the face of abundant nutrients, mTORC1 promotes cell growth, protein translation and nucleotide synthesis via numerous substrates, including S6K1, S6K2, 4E-BP1 and CAD (Ben-Sahra *et al.*, 2013).

Reduction in the levels of mTOR itself, other proteins required for mTORC1 function, and mTORC1 substrates can significantly extend lifespan in yeast, worms and flies. Importantly, this work has proven to be relevant to mammalian aging; two different genetic models of reduced

mTOR signalling, as well as mice lacking the mTORC1 substrate S6K1, have extended lifespan. Treatment with the mTOR inhibitor rapamycin likewise extends lifespan in yeast, worms, and flies. Finally, rapamycin treatment begun at either 9 or 20 months of age significantly extends the lifespan of both male and female mice (Miller *et al*., 2011).

While it is clear that mTORC1 regulates lifespan, it is less clear to what extent the effects of CR are mediated by decreased mTOR signalling. In yeast, mimicking mTOR inhibition by reducing expression of many different large ribosomal subunit proteins promotes longevity in a way that is not additive with the effects of CR, and this is intriguingly dependent on increased translation of the transcriptional activator GCN4. In worms, blocking the induction of autophagy prevents lifespan extension in mTOR mutants and in response to CR. However, rapamycin treatment is additive with CR in flies, suggesting that rapamycin and CR may promote lifespan through different mechanisms (Bjedov *et al*., 2010).

While as of yet this type of lifespan experiment has not been done in a mammal, microarray studies of mice on rapamycin, CR, and rapamycin with CR demonstrate that CR and rapamycin regulate distinct transcriptional networks, and may promote longevity through different mechanisms (Fok *et al*., 2013). However, at least certain effects of CR in mice are mediated by decreased mTORC1 signalling (Yilmaz *et al*., 2012). One way in which rapamycin and CR are distinct, and which may be partially responsible for differences in gene expression, is the effect of these two interventions on insulin sensitivity. Even a relatively short, 10-week CR regime is sufficient to significantly improve glucose tolerance and insulin sensitivity in young mice. Conversely, treatment with rapamycin significantly decreases glucose tolerance due to decreased hepatic insulin sensitivity in C57BL/6 and HET3 mice strains, rats, and in humans (Lamming *et al*., 2012, 2013; Johnston *et al*., 2008). This effect is mediated by the ability of rapamycin to not only disrupt mTORC1 signalling when given acutely, but to physically disrupt mTORC2 as well during chronic rapamycin treatment (Lamming *et al*., 2012).

The protein error catastrophe theory was first advanced by Orgel in the 1960s. Orgel suggested that an error in the synthesis of a protein involved in protein translation could result in the generation of an error-prone protein. This protein could then proceed to make errors in the synthesis of many additional proteins, leading to an eventual cellular catastrophe. While the protein error catastrophe has not received much initial attention, it has been reconsidered in the light of the finding that deletion of large ribosomal subunits can lead to increased lifespan in yeast. Similarly in worms, depletion of ribosomal subunits or translation initiation factors significantly promotes longevity. However, neither chronic rapamycin treatment or deletion of S6K1, both of which extend mouse lifespan, simply dials down general translational activity. Rather, inhibition of mTOR signalling results in preferential translation of specific mRNA transcripts based on species-specific sequence identifiers. Evidence from yeast suggests that CR may work at least in part through this mechanism. Interestingly, one of the primary effects of mTOR inhibition in mammals is to inhibit the translation of mRNAs containing a 5′ terminal oligopyrimidine (TOP) motif. It will be interesting to learn if CR similarly suppresses mRNAs containing a TOP motif, and to learn which transcripts are responsible for the beneficial effects of CR. **See also**: Tuberous Sclerosis Complex and the Mammalian Target of Rapamycin Pathways

Relevance of CR to Human Aging

A major goal of aging and nutrition research is to identify the contributing factors to disease vulnerability related to diet and age. Nonhuman primates share anatomical, physiological and behavioural similarities with humans and as such promise highly translatable insights into the biology of human aging. Many of the age-related phenotypes exhibited in rhesus macaques (*Macaca mulatta*) are also observed in humans, including greying and thinning of hair, loss of vigour, the redistribution of body fat, loss of muscle tone, and loss of skin tone. Like humans, advancing age in monkeys heralds an increase in clinical manifestations of age-associated diseases and disorders, including diabetes, neoplasia, sarcopenia, bone loss, and immune function (Uno, 1997). To gain insight into the ability of CR to delay aging in primate species, three rhesus monkey Aging and CR studies were conducted at the University of Maryland, the University of Wisconsin (UW) Madison, and at the National Institute on Aging (NIA) as part of the intramural research programme.

The first evidence that CR positively influences survival in primates arose from the University of Maryland study. This study included 109 *ad libitum* fed animals and 8 animals on CR. A 2.6-fold increased risk of death in control animals compared to restricted was reported (Bodkin *et al*., 2003). The UW adult onset rhesus monkey CR study involved 76 animals randomly assigned to control or CR groups. CR delayed disease onset and mortality in rhesus monkeys with 2.9-fold decreased risk of disease and 3.0-fold decreased risk of age-related death compared to controls (Colman *et al*., 2009). In a parallel study of 120 animals conducted at the NIA, CR was initiated in young and old monkeys and the impact on survival and health was reported (Mattison *et al*., 2012). Improvements in health of CR animals were observed but did not reach significance ($p = 0.06$) and survival was not significantly different between control-fed and CR monkeys. More recently, a second survival report emerged from the UW study indicating that with less than one quarter of the animals remaining in the cohort, CR resulted in a significant reduction in risk for both age-related and all-cause deaths (Colman *et al*., 2014). A comparison of body weights at equivalent time points from the UW and NIA studies suggested that compared to UW animals the NIA controls

were modestly restricted. Furthermore, in comparison with a national primate aging database for rhesus monkeys in captivity NIA control monkeys weighed less than average for both genders and at both ages investigated. The lack of difference between NIA controls and CR suggests that for primates there may be little advantage of moderate CR over modest CR.

The health effects of CR in humans have also been investigated. A multicenter study known as CALERIE (Comprehensive Assessment of the Long-term Effects of Reducing Energy Intake) was sponsored by NIA and involved clinical trials that focused on the effects of short-term CR. The primary goal was to determine if CR could induce the same adaptive responses in humans that have been observed in rodents and nonhuman primates. The CALERIE studies were conducted in parallel at three locations: Pennington Biomedical Research Center in Baton Rouge, Washington University in St. Louis, and Human Nutrition Research Center on Aging at Tufts University in Massachusetts. In the first phase involving overweight individuals, CR was implemented for 6–12 months. Outcome measures of weight loss, enhanced glucose tolerance and insulin sensitivity, and improved serum indicators of disease risk have been reported (Larson-Meyer *et al*., 2006; Lefevre *et al*., 2008; Weiss *et al*., 2006). The second phase involved a two-year intervention and now that the study has been completed, data are expected in the near future (Rochon *et al*., 2011). Beneficial effects of long-term CR on atherosclerosis risk in humans have been reported in studies involving members of the Caloric Restriction Society who voluntarily engage in the practice of CR. Insulin sensitivity is enhanced in individuals practicing CR and levels of adiponectin are increased. These data suggest that the CR-induced metabolic changes observed in rodents are also implemented in humans and argue that insights into CR's mechanisms in short-lived animals will prove invaluable to understanding the complex process of aging and the associated increase in risk for age-related diseases and disorders. **See also**: Genetic Determinants of Human Life-Span

Conclusions and Future Directions

There is considerable interest in how diet and lifestyle interact to influence aging and the incidence of age-related disease. The recent move toward electronic records in the health care system in the United States opens the possibility for integrated databases to become a new resource for research. Aging-related variables are currently monitored in large populations over decades in discrete studies, including the Wisconsin Longitudinal Study ($>10\,000$ subjects) and the Baltimore Longitudinal Study of Aging (>1400 subjects). Longitudinal tracking of aging and health of millions of individuals across the population would advance our understanding of nutritional and social aspects of aging that are uniquely human, a key component in bringing biology of aging research to clinical practice. In laboratory studies, changes in metabolism can be tracked by monitoring selected molecules that are intermediates in cellular metabolism using high-resolution technologies such as mass spectroscopy and nuclear magnetic resonance analysis. In applying these technologies to specimens acquired over the course of human aging, the large data sets generated can then be assembled to understand metabolic changes with age, health, and with treatments for a range of diseases. Clearly there is much to learn about the relationships among metabolic pathways, the trajectories of change in humans as a function of age, and how metabolism contributes to age-related diseases and disorders. The potential for new discovery has never been greater, and the potential for scientific advance that is directly relevant to human health has never been so promising.

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