HHS Public Access

Author manuscript

Nat Rev Mol Cell Biol. Author manuscript; available in PMC 2023 January 01.

Published in final edited form as:

Nat Rev Mol Cell Biol. 2022 January ; 23(1): 56–73. doi:10.1038/s41580-021-00411-4.

Molecular mechanisms of dietary restriction promoting health and longevity

Cara L Green[1,2], **Dudley W. Lamming**[1,2], **Luigi Fontana**[3,4,5]

[1]Department of Medicine, University of Wisconsin-Madison, Madison, WI, USA

[2]William S. Middleton Memorial Veterans Hospital, Madison, WI, USA

[3]Charles Perkins Center, Faculty of Medicine and Health, University of Sydney, NSW 2006, Australia

[4]Department of Endocrinology, Royal Prince Alfred Hospital, Sydney, NSW 2006, Australia

[5]Department of Clinical and Experimental Sciences, Brescia University School of Medicine, Brescia, Italy.

Abstract

Dietary restriction with adequate nutrition is the gold-standard for delaying aging and extending healthspan and lifespan in diverse species, including rodents and non-human primates. In this Review, we discuss the effects of dietary restriction in these mammalian model organisms, and discuss accumulating data that suggests that dietary restriction results in many of the same physiological, metabolic and molecular changes responsible for the prevention of multiple age-associated diseases in humans. We further discuss how different forms of fasting, protein restriction and specific reductions in essential amino acids such as methionine and the branched-chain amino acids selectively impact AKT, FOXO, mTOR, nicotinamide adenine dinucleotide (NAD^+), AMP-activated protein kinase (AMPK) and fibroblast growth factor 21 (FGF21), which are key components of some of the most important nutrient-sensing geroprotective signaling pathways that promote healthy longevity.

Introduction

Dietary restriction (DR) without malnutrition remains the most robust non-genetic intervention to date that can maximize lifespan and healthspan in rodents. It also extends life expectancy and safeguards against obesity, cancer, neurodegeneration, frailty, and a range of cardiometabolic conditions in rhesus monkeys; and in humans, it promotes adaptations that protect against these pathologies (Figure 1). This phenomenon was first discovered in

luigi.fontana@sydney.edu.au .

Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

D.W.L has received funding from, and is a scientific advisory board member of, Aeovian Pharmaceuticals, which seeks to develop novel, selective mTOR inhibitors for the treatment of various diseases. The University of Wisconsin-Madison has applied for a patent for the use of amino acid restricted diets to promote metabolic health, for which D.W.L is an inventor.

1917 by Osborne and was followed by hundreds of DR studies that expanded on its disease preventative and pro-longevity efficacy, founding the 'biology of aging' field[1].

The mechanisms by which DR promotes health and longevity remained obscure until the 1980s, when the first single gene mutations targeting key nutrient-sensing pathways, which drastically extended the lifespans of nematode worms, were discovered[2]. Since then, several more mutations that affect longevity have been found in invertebrates and their roles in the regulation of healthspan and lifespan has been confirmed in knockout and transgenic mouse models (Table 1). Understanding the mechanisms that underpin the beneficial effects of these mutations is essential to translating these findings to the clinic, through mechanism-based therapeutic interventions.

In this review, we examine the research landscape of different forms of DR in rodents, non-human primates, and humans, focusing our attention on the metabolic and molecular adaptations that result in improved health. We also highlight new emerging scientific trends on the role of meal frequency and timing, and macronutrient composition, as potential mediators of some of the anti-aging and disease preventative effects of DR in rodents, non-human primates, and humans. As much of the mechanistic work regarding the role of specific pathways in the response to DR and macronutrients has been done in invertebrates, we will reference findings in other organisms as needed. However, the reader should remain aware that the role of these evolutionarily conserved pathways may not be precisely the same in mammals, and additional research may be needed to clarify if these pathways play the same role in DR, macronutrient restriction, or meal timing as they do in these model organisms. Understanding the molecular basis of DR and other dietary regimens may be crucial to developing dietary interventions or pharmaceuticals that can mimic some of the benefits of these regimens in the genetically heterogeneous human population.

DR and lifespan

In rodents, DR involves reducing *ad libitum* food intake by a given proportion (customarily 10–50%) without malnutrition[3]. Food restriction has a non-linear dose-dependent impact on rodent lifespan that is strain and sex specific[1,4,5]. Lifespan increases to a maximum as food intake is reduced, but then rapidly declines when the restriction becomes excessive. Elegant experiments have also shown that the degree of DR that maximizes longevity and the amplitude of this response depend on genetic and, most likely, epigenetic factors. Indeed, even among genetically identical rodents, the same degree of food restriction leads to highly variable effects on lifespan[6]. Because different mice grow (*in utero*, postnatally, and during puberty) and reproduce at different rates, it is essential to perfectly match metabolism, growth, and fecundity to the optimal intake of food and nutrients, to avoid starvation or overfeeding, while maximizing health and longevity.

Understandably, there has been significant interest into whether DR can slow aging in humans. Dozens of studies, conducted by many independent groups over the last century, have found that the benefits of DR are observed in diverse species, including yeast, worms, flies and rodents. Studies in rhesus macaques show that the benefits of DR can also be observed in non-human primates. In a study conducted at the University of Wisconsin

(UW), the hazard ratio for survival was 1.86, indicating that at any time point monkeys that followed a 30% DR regime had almost half the rate of mortality of *ad libitum* fed controls[7]. Consistently, ageing-associated methylation drift in the UW DR monkeys was markedly delayed and their estimated biological age was 7 years younger than their chronological age[8]. Another study conducted by the National Institute on Aging (NIA) reported no difference in the Kaplan-Meier estimated survival in DR monkeys (of note, the study was not statistically powered for this outcome), but the average lifespan of DR animals was extremely high: 31.8 years[9]. Whereas the median and 10% survival of rhesus monkeys in captivity is ~26 and ~35 years of age, respectively, about one third of the NIA monkeys on late-onset DR lived over 40 years, and one monkey died at 44 (the equivalent of 135 years for a human)[7,9].

Although the results and interpretations of these trials are still being debated, these findings, in conjunction with emerging new investigations in model organisms and humans, highlight two crucial points: (i) the impact of DR on reducing age-associated ailments appears conserved across species; (ii) the importance of study design, feeding regimen and diet formulation in modulating both lifespan and disease development and progression[7,10]. In Figure 2 (and more extensively in Supplementary information 1), we summarize the results of key randomized trials in rodents, monkeys, and humans on the effects of DR in preventing a wide range of diseases, highlighting similarities and differences.

DR and intrinsic aging

The reduced prevalence of chronic diseases in animals subjected to DR does not completely explain the increase in maximal lifespan, because DR animals at any time appear physiologically younger and less frail than age-matched controls. Approximately one-third of DR rodents die at a very old age without any gross histological lesion at necropsy[11], suggesting that death may be due to intrinsic aging rather than ageing-related pathologies. Similarly, 20% of Ames dwarf mice and 47% of growth hormone receptor knockout (GHR-KO) mice, which are both long-lived and share similar growth hormone signalling deficits, do not develop any obvious lethal pathological lesions (i.e. known cause of death), suggesting that organ integrity can be preserved during aging in mammals[12]. For example, normal aging is associated with a progressive decline in left ventricular diastolic function and heart rate variability, and DR counteracts these physiological changes in rodents and humans[13,14]. Lower insulin/insulin growth factor-1 (IGF-1) and transforming growth factor beta (TGFβ) signaling[13,15], increase in antioxidant mechanisms[16,17], enhanced mitochondrial function[18,19] and improved proteostasis and autophagy[20–23] may explain some of the beneficial effects of DR.

In mammals ageing is also associated with presbycusis, and DR prevents it, at least in part, by inhibiting apoptosis of the cochlear ganglion cells via sirtuin 3 (SIRT3)-induced activation of isocitrate dehydrogenase 2 (IDH2) and increased mitochondrial NADPH levels[24]. Sarcopenia is another universal aging feature, and DR delays the decline in skeletal muscle mass and strength, and the incidence of frailty by Fried's index in both rodents and primates[25,26], likely in part by upregulating proteostatic, lipid synthetic and RNA processing pathways[27]. In human skeletal muscle, long-term DR upregulates protein quality

control (as seen by an increase in the chaperones HSP-70 and Grp78) and the autophagic proteins LC3 and beclin-1, both necessary for the formation of autophagosomes and cargo engulfment[22]. Moreover, as in rodents, chronic DR induces a PCG-1α-dependent increase of genes involved in mitochondria biogenesis[18,28].

Intense investigations in multiple non-mammalian model organisms are uncovering genetic and epigenetic pathways as well as microbial factors involved in the regulation of ageing and age-associated diseases, including nutrient sensing, genomic and protein homeostasis (for example, AKT/FOXO/mTOR, FGF21/GCN2/ATF4, AMPK and sirtuin pathways) (Fig. 3)[29]. Remarkably, several of these genes and molecular determinants control mammalian longevity and nutrient-inducible pathophysiological processes, including cellular senescence, intra- and inter-cellular reprogramming, and telomere and stem-cell function. DR has also been shown to promote stem cell self-renewal and physiologic or injury-induced tissue regeneration in multiple organs (e.g. intestine, brain, skeletal muscle and bone marrow), partially through inhibition of mTOR complex 1 (mTORC1) signaling (Box 1)[30,31].

Geroprotective mechanisms of DR

Several metabolic pathways that have a role in the ageing-associated cellular and organismal decline are modulated by DR (Fig. 3).

Downregulation of growth hormone and Insulin/IGF-1 signaling

In the 1980's, a mutation in *age-1*, which encodes a subunit of phosphoinositide 3-kinase (PI3K), was found to extend the lifespan of *C. elegans*[32]; soon after, a mutation in *daf-2* was identified which more than doubled the lifespan[2]. *Daf-2* is a regulatory gene that encodes a mammalian orthologue of the insulin/IGF-1 receptor (IIR)[33] and requires *daf-16*, which encodes a FOXO transcription factor[2]. Other mutants in the insulin signaling pathway that can extend lifespan were soon identified in *Drosophila melanogaster*, including mutants in insulin receptor (InR)[34] and insulin receptor substrate (IRS)-like signaling protein, *chico*[35,36].

Overlapping these breakthroughs, the long-lived Ames Dwarf mice were discovered; these animals carry the 'longevity' gene, *Prop1*^{df} (a pituitary-specific homeodomain transcription factor) and live ~50% longer than their normal siblings[37]. These mice, as well as the long-lived Snell Dwarf mice (which have a mutation in the anterior pituitary transcriptional factor, *Pit1*, also known as *Pou1f1*), are extremely small, and have very low levels of thyroid-stimulating hormone (TSH), prolactin, growth hormone (GH) and IGF-1[37]. DR potentiates the longevity effect of dwarfism in the Ames mice, while GH treatment eradicated this beneficial effect[38]. Consistently, it was found that GH deficiency alone markedly delays aging and produces the longest-lived laboratory mouse on record[39]. As 30% DR in the GH receptor knockout mice (GHR-KO) mice failed to produce any further extension of overall or median longevity[40], suppression of the somatotropic axis may be one of the key longevity DR mechanisms.

GHR-KO mice have profoundly suppressed circulating levels of IGF-I, higher insulin sensitivity, and markedly lower cancer incidence[12]. Humans born with GHR deficiency are also protected from cancer and diabetes, but do not have an increased lifespan[41]. Improved insulin sensitivity is a widely conserved response to DR in mammals and has been proposed as a key longevity mechanism of DR[42], but recent data show that improvements in organismal insulin sensitivity are not required for DR to promote leanness, reduce frailty, and extend lifespan in mice[43].

Both long-lived Snell mice and GHR-KO mice show lower AKT activity, decreased glial fibrillary acidic protein (GFAP) phosphorylation and increased chaperone-mediated autophagy than control animals[44]. Deletion of GH-R in macrophages seems to be one of the important actors linking reduced NLRP3 inflammasome-induced inflammation and longevity in GHRKO mice; inhibition of the GHR/IGF1 axis preserves the naive T-cell pool and prevents the age-associated activation of the inflammasome in response to accumulation of cellular damage[45].

As reviewed elsewhere[1,46], these and other findings support the idea that reduced insulin/IGF-1 signaling is crucial for lifespan extension. Consistently, genetic and pharmacological interventions that reduce insulin/IGF-1 signaling also extend mice lifespan. Initial work with mice heterozygous for the IGF-1 receptor found that these mice lived approximately ~30% longer than wild-type mice[47], but the control mice in this study were comparatively short-lived; independent replication of this experiment found only a small, female-specific increase in the lifespan of *Igf1r*$^{+/-}$ mice[48]. Deletions of the insulin receptor specifically in adipose tissue, *Irs1* in the whole body, and *Irs2* heterozygosity in the whole body or selectively in the mouse brain likewise extend lifespan[48–52]. Loss of pregnancy-associated plasma protein A (PAPP-A), a metalloproteinase for IGF-1 binding proteins, extends lifespan when deleted constitutively or in adult mice[53,54]. Finally, late-life inhibition of IGF-1 signaling using an antibody targeted to the IGF-1 receptor was recently shown to improve longevity[55]. Overall, it is likely that reduced insulin/IGF-1 signaling contributes to the beneficial effects of DR, and strategies to reduce signaling through this pathway may have translatable potential to promote healthy aging.

Reduced mTORC1 signaling is a conserved mechanism for lifespan extension

One of the most important DR-induced molecular mechanisms downstream of insulin/IGF-1 signaling is the mTOR serine/threonine protein kinase (Fig. 2). mTOR is the catalytic core of two distinct protein complexes, mTORC1 and mTORC2, each of which are composed of distinct protein subunits that phosphorylate different substrates[56]. Briefly, mTORC1 is responsive to a wide range of environmental stimuli, including the availability of amino acids, glucose, oxygen, cholesterol, and insulin/IGF-1, whereas mTORC2 is primarily an effector of PI3K signaling. mTORC1 is acutely sensitive to the drug rapamycin, whereas mTORC2, owing to its structure[57,58], is inhibited only by high levels of rapamycin over extended periods of time[59–61]. mTORC1 integrates numerous environmental signals that indicate when conditions are favorable for the anabolic processes it controls, which include ribosomal biogenesis, protein translation, autophagy, lipogenesis and nucleotide biogenesis. The regulation of mTORC1 has been reviewed in detail[56] and is briefly outlined in Box 1.

mTORC1 activity is reduced in Ames and Snell Dwarf mice[62], and several different genetic models of reduced mTORC1 signaling, including mice heterozygous for *mTOR* and *mLST8* (which encodes a subunit of mTORC1), mice expressing a hypomorphic allele of mTOR, and those lacking the mTORC1 substrate S6K1, have extended lifespan[59,63,64]. The initial studies on mTOR were conducted in invertebrates (reviewed in[65]), but in 2009 it was found that rapamycin extends lifespan in aged mice[66]. Since then several independent laboratories have confirmed the ability of rapamycin to prolong lifespan in multiple strains of mice, even when treatment is intermittent or conducted for only a short period of time[67,68] (and reviewed in[69]). As summarized in Supplementary information 2, rapamycin and DR have similar positive effects on many age-related pathologies in mice; and in a recent small study, rapamycin even improved diastolic and systolic cardiac function in middle-aged dogs[70].

There has been significant interest in rapamycin as a potential DR mimetic, but rapamycin has several side effects that have precluded its wide-scale use; these include the dysregulation of blood glucose and lipid homeostasis as well as immunosuppression[59,71–73]. These side effects are probably mediated by "off-target" inhibition of mTORC2, which is disrupted by chronic treatment with rapamycin *in vivo* in mice[59,60]. Although whole body or tissue-specific (brain, liver, or adipose tissue) deficiency of mTORC2 signaling impairs metabolic health and reduces lifespan in wild-type and long-lived mice[43,74–76], mTORC2 activity has been positively associated with longevity in flies and in some (but not all) studies using worms[77–80]. Two drugs that extend mice lifespan, acarbose and 17-α estradiol, boost hepatic mTORC2 signaling[81], and mTORC2 activity is elevated in long-lived Snell dwarf mice and *GHR-KO* mice[62,81]. There has been significant interest in identifying rapamycin dosing regimens or drugs with greater specificity for mTORC1 that could deliver the beneficial DR-mimetic effects of rapamycin while minimizing negative side effects[67,72,73,82,83].

Given the similarity of the beneficial effects of DR and mTORC1 inhibition, and the clear link between DR and reduced mTORC1 activity, it is widely accepted that mTORC1 has a role in the response to DR. In yeast, deletion of *TOR1* is epistatic with DR; that is, DR does not further extend the lifespan of yeast lacking *TOR1*[84]. However, genetic analysis of the interaction between DR and TOR in worms and flies has not clearly demonstrated an epistatic relationship. In *C. elegans*, several studies have linked various DR regimens to TOR signaling, but also identified TOR-independent longevity mechanisms activated by DR[85–88], whereas in flies, rapamycin extends lifespan at every level of calorie intake[89]. Extensive mammalian metabolomic and transcriptomic studies in blood, liver, and white adipose tissue suggest that rapamycin and DR have distinct, largely non-overlapping effects[90–94]. Additional research is warranted to fully evaluate the impact of different forms of DR on mTORC1 signaling.

Activation of GCN2 and reduced protein synthesis

GCN2 is another evolutionarily conserved serine/threonine kinase that functions as an amino-acid sensing metabolic switch to control various nutritionally responsive mechanisms, including immune system homeostasis[95] and tumor cell growth[96], and to coordinate integrated stress responses and the inflammasome[97]. When GCN2 is activated

through binding to uncharged tRNAs or by ribosome stalling[98–100], the protein kinase phosphorylates the α-subunit of eukaryotic initiation factor 2 (eIF2)[101], blocking the translation of most mRNAs[101], while stimulating the translation of a select set of proteins, including activating transcription factor 4 (ATF4)[102,103] (Fig. 3). ATF4 is a transcription factor that upregulates genes necessary to adapt to amino acid or protein restriction (a dietary regimen distinct from DR that involves the specific reduction of protein intake while overall calories are not restricted; see below), including the energy balance hormone fibroblast growth factor 21 (FGF21)[104].

Gcn2-knockout mice (*Gcn2* is also known as *Eif2ak4*) that are subjected to protein restriction have a delayed metabolic response to protein restriction, including a two week delay in the induction of FGF21 and FGF21 mediated metabolic phenotypes (see below)[105]. While the response to acute protein restriction may be dependent on activation of FGF21 through the GCN2-ATF4 axis, chronic protein restriction works upstream of GCN2 to directly activate ATF4, thereby stimulating FGF21 through alternate pathways[105]. Although activation of GCN2 is not sufficient to directly inhibit mTORC1, it is required for mTORC1 inhibition upon leucine and arginine deprivation[106]. Interestingly, this mTORC1 inhibition seems to occur, at least initially, independently of ATF4 activation, as it is not required for early repression of mTORC1 during leucine deprivation; however, phosphorylation of eif2α by GCN2 is essential, though not sufficient alone, to inhibit mTORC1[106].

While GCN2 has not been heavily investigated as a mediator of DR in mammals, GCN2 in *C. elegans* is required for both DR and TOR inhibition to extend lifespan, linking these two key amino acid sensing and longevity-regulating pathways[107]. Understanding how GCN2 regulates the response to DR in mammals will clearly be an important area for future research.

Multiple effects of FGF21 signalling

Liver-derived FGF21 is implicated in many key metabolic pathways that are altered under nutritional stress[108]. FGF21 is a potent regulator of the effects of protein as well as specific amino acids restriction on metabolism, notably by increasing insulin sensitivity and energy expenditure[109]. FGF21 stimulates insulin-independent glucose uptake in the cells of both mice and humans[109,110], and facilitates adaptive behavioral changes in feeding through signaling to the brain[111].

The importance of FGF21 in the response to PR has been seen across multiple rodent models as well as in humans. In male Sprague Dawley rats and C57BL/6J mice, chronic PR induces an increase in both hepatic expression and circulating levels of FGF21[109,112]. In humans, 4–6 weeks of protein restriction is sufficient to increase circulating FGF21[109,112]. Changes in hepatic *Fgf21* expression can be induced within 24 hours by switching rats to a low protein diet, followed by a 10-fold increase in circulating FGF21 after only 4 days[109]. In mice lacking *Fgf21*, protein restriction is unable to provoke shifts in food intake, energy expenditure and weight gain[109,111].

As FGF21 is also powerfully induced by fasting, there has been significant interest in understanding if FGF21 mediates the effects of DR. Unfortunately, a straightforward

assessment of how FGF21 levels are regulated by DR is confounded by time of feeding effects[113]. However, treatment of mice with recombinant FGF21 or transgenic expression of FGF21 promotes glucose tolerance and insulin sensitivity, and FGF21 overexpressing mice are resistant to diet-induced obesity[110]. Moreover, FGF21 transgenic overexpression extends mouse lifespan without reducing calorie intake or modulating mTORC1 signaling[114]. While future research will be needed to further clarify the role of FGF21 in the response to protein restriction and DR, FGF21 analogues are being pursed as a possible therapy for age-related diseases, including diabetes[115], non-alcoholic fatty liver disease[116], and Alzheimer's disease[117].

Activation of sirtuins, conserved regulators of lifespan

The lifespan extending effects of sirtuins, a family of nicotinamide adenine dinucleotide (NAD^+)–dependent deacetylases, was originally discovered when a mutation in the silencing regulating gene *Sir4* was found to extend the replicative lifespan of yeast. It was later demonstrated that this effect on yeast lifespan required silent information regulator 2 (Sir2), the homologue of mammalian SIRT1, and that overexpression of *Sir2* increased yeast replicative lifespan[118]. Importantly, Sir2 and several yeast Sir2 homologues are required for DR to extend yeast lifespan[119,120]. Sir2 homologues also regulate the lifespan of worms and flies and play a part in their response to DR[121,122], although these effects have not been observed consistently in different studies[123].

There are seven mammalian sirtuin family members (SIRT1–7), with different subcellular localizations. SIRT1 (with highest sequence homology to yeast *Sir2*), SIRT6 and SIRT7 are nuclear; SIRT3, SIRT4 and SIRT5 are mitochondrial; and SIRT2 is largely cytoplasmic, but shuttles in and out of the nucleus[124,125]. While all of the mammalian sirtuins are (NAD^+)–dependent deacetylases, other enzymatic activities have been reported for some of these enzymes, including ADP-ribosylation (SIRT 4 and 6)[126,127] and demalonylation and desuccinylation (SIRT5)[128,129]. SIRT1 and SIRT2 have also been reported to act as lysine decrotonylases, and SIRT1–4 can remove lipoic acid from lysine[130].

Sirtuins are linked to diet and metabolism via their need for NAD^+. The ability of DR to extend the lifespan of yeast is dependent not only on Sir2, but also on the NAD^+ synthesis pathway enzyme Npt1[119]. *Pnc1*, a key enzyme in the NAD^+ salvage pathway, is induced by DR in yeast via a TOR-mediated pathway and is required for DR-mediated lifespan extension in both yeast and worms[131–133]. Adipose-specific overexpression of NAMPT, which is the rate limiting enzyme in a major NAD^+ synthesis pathway, was recently shown to elevate NAD^+ levels in multiple tissues, improve multiple measures of metabolic health, cognition, and physical performance, and extend the lifespan of female mice[134]. Nutritional supplementation with NAD^+ or NAD^+ precursors is being actively investigated as a way to promote healthy aging and intervene in diseases by activating sirtuins (reviewed in[135]).

A number of studies have found that DR induces SIRT1 expression in multiple tissues in rats and in human cells[136], and humans on DR have increased levels of SIRT1 in skeletal muscle[137]. SIRT3 and SIRT5 are also induced by DR in mice[138,139]. Conversely, overnutrition by high fat diet feeding lowers SIRT1 expression in mouse adipose tissue[140],

and SIRT1 levels are lower in the adipose tissue of obese humans[141], as well in Alzheimer's disease[142].

The role of mammalian sirtuins has been primarily investigated in mice in which sirtuins are deleted or overexpressed, either in specific tissues or universally. Whole body deletion of *Sirt1* blocks the ability of DR to extend lifespan, although these results are complicated by the short lifespan of *Sirt1*$^{-/-}$ mice[143]. Mice genetically modified to overexpress SIRT1 are lean, metabolically active with reduced circulating cholesterol, insulin and improved glucose tolerance[144]. While whole body overexpression of SIRT1 does not increase murine lifespan[145], hypothalamic-specific overexpression caused a significant extension of lifespan in both males and females[146]. SIRT2 is a tumor suppressor[147], and its overexpression rescues the lifespan of progeroid mice lacking *BubR1*[148]. Deletion of Sirt3 shortens lifespan[149], while increased Sirt3 levels improve the regenerative capacity of hematopoietic stem cells[150]. Deletion of SIRT6 and SIRT7 increases frailty and shortens lifespan[151,152], while healthspan and lifespan is increased in transgenic male mice overexpressing SIRT6 (MOSES)[153,154]; overexpression of SIRT7 extends the lifespan of a mouse model of Hutchinson-Gilford Progeria Syndrome[155].

The molecular mechanisms by which sirtuins regulate metabolism have been actively investigated. SIRT1 deacetylates and activates the transcriptional coactivator PGC1α by promoting its nuclear localization[156,157]. SIRT1 in the liver up-regulates the transcription of the mTORC2 component *Rictor*, enhancing glucose homeostasis, while liver-specific deletion of *Sirt1* increases oxidative stress[158]. SIRT1 overexpression suppresses senescence, whereas its inhibition accelerates it in human endothelial cells[159,160]. Sirt3 is essential for the DR-mediated reduction in oxidative damage, via enhancement of the mitochondrial glutathione antioxidant defense system, not only in the cochlear cell, but also in the neocortex and liver[24]. This may be due to an inability of SIRT3-KO mice to lower their acetyl CoA levels in response to DR[161]. Interestingly, a polymorphism in the *SIRT3* gene has been associated with male centenarians in a European population[162]. SIRT6 promotes DNA stability and suppresses senescence by enhancing DNA double-strand break repair, perhaps via activation of PARP[127,163,164]. SIRT6-dependent repression of LINE1 elements, which induce inflammation and DNA damage, may be another important mechanism[165].

Taken together, the data collected thus far suggests that the sirtuin family of enzymes likely plays an important role in the response to DR. Research is continuing to define the targets and enzymatic functions of the sirtuins, and the role of each sirtuin in regulating metabolism, healthspan, and longevity.

Oxidative Stress and AMPK signalling

The oxidative stress theory of aging postulates that the accumulation of oxidative damage shortens lifespan. As reviewed elsewhere[166], several rodent studies have shown that aged DR animals and long-lived mouse mutants have reduced markers of oxidative damage. Similar reduced oxidation has been found in humans subjected to DR[17]. In support of this theory, mice overexpressing human catalase, which protects from oxidative stress by converting hydrogen peroxide to water and oxygen, localized to the mitochondria are long-lived[167].

This theory is not supported by experiments manipulating many other oxidative stress response genes. The lifespan of *Sod1*$^{-/-}$ mice lacking CuZn superoxide dismutase is reduced[168,169], but mice one copy of *Sod2* deleted, leading to increased DNA damage, or lacking *Sod3,* which have increased lipid peroxidation and higher sensitivity to oxidative stress, have a normal lifespan[169,170]. Deletion of *Gpx1* (encoding Glutathione peroxidase 1) or loss of *MsrA* (encoding Methionine sulfoxide reductase A) also result in increased lipid or protein peroxidation and increased susceptibility to oxidative stress without affecting lifespan[171,172]. Studies crossing these mice to induce deletions of multiple oxidative stress genes have confirmed that increased oxidative stress is generally not highly deleterious to lifespan[169,171,173].

New surprising findings in *C. elegans* suggest that DR may increase reactive oxygen species and induce an oxidative stress resistance response that is essential for its pro-longevity effect[16,174]. Intriguingly, this effect, named mitochondrial hormesis or mitohormesis, is dependent on AMPK (AMP-dependent kinase), a sensor of nutrient status and mitochondrial stress that regulates many cellular processes, including mTORC1 via phosphorylation of Raptor and TSC2[175,176]. While the mitohormesis hypothesis has not been formally tested in mice, chemical inhibition of glycolysis by D-glucosamine extends the lifespan of *C. elegans* and C57BL/6 mice, possibly via increasing reactive oxygen species[177]. Moreover, supplementation with antioxidants block the beneficial effects of exercise in humans[178,179], and might even increase cancer risk[180]. In summary, the data suggests that the benefits of DR are probably not mediated by reduced oxidative stress.

Effects of specific dietary manipulations

Until recently, reduced intake of calories, rather than of specific nutrients, was considered key for the life-extension effects of DR. It is now clear that the old adage '*a calorie is just a calorie*' is incorrect, as new data support a model whereby diet composition as well as timing of food intake have crucial roles in regulating key aging pathways[181,182].

Protein Restriction

Early studies in rodents on protein restriction had mixed results, probably owing to differences in dietary protein quality and the degree of restriction[182]. More recently, interest in PR has been rekindled by the finding that, in flies, total protein restriction or specific essential amino acid restrictions can extend lifespan independently of calorie intake[183–185]. Studies in rodents have confirmed that protein restriction independent of caloric intake promotes longevity[186,187], and as described in Supplementary information 3, PR can impact a range of ageing-related conditions in both rodents and humans. These observations, and work on fruit flies done primarily by the Partridge laboratory, has resulted in a strengthening of the theory that it is the reduction in protein, and not calories, that drives the lifespan extension of DR[184]. However, the amount of protein restriction during DR studies is smaller than during carefully controlled protein restriction studies, and recent analyses suggest that these interventions act through independent mechanisms[188]. Indeed in recent studies on rodents short-term protein restriction did not replicate the physiological and metabolic

effects of DR[189,190], and long-term protein restriction only extends the lifespan of male mice, while DR extends the lifespan of both sexes[43,187].

Estimated daily protein intake for adults in many Western Societies is approximately 90–100g (of which ~70–85% is animal protein), roughly twice the amount recommended by the US Institute of Medicine[191]. Accumulating data indicate that excessive protein intake may cause insulin resistance and type 2 diabetes and induce other long-term negative health consequences by overstimulating the the AKT−mTOR pathway and inhibiting FGF21 signaling[192]. In a weight loss trial, consuming a relatively high protein diet (1.3 g/kg/day) completely prevented the improved insulin sensitivity observed in women consuming a normal protein diet (0.8 g/kg/day) who lost the same amount of body weight[193]. High protein intake, therefore, counteracts the beneficial effects of weight loss on insulin resistance, and potentiates the pro-aging and pro-cancer effects induced by compensatory hyperinsulinemia in the face of significant reductions in abdominal and liver adiposity.

In contrast, long-term adherence to physiologically adequate protein diets (0.8–1 g/kg per day) has been found to be beneficial in retrospective and prospective studies[192], and a recent randomized clinical trial found that short-term PR significantly reduced fat mass and improved blood glucose levels in middle-aged overweight and mildly obese males without caloric restriction[112]. The benefits of lower protein consumption may start in early life: newborns fed a lower protein-content formula (similar to that found in human milk) had a reduced risk of childhood obesity than infants consuming an isocaloric high protein-content formula[194]. Although the effect of protein restriction on human lifespan is unknown, the longest living population in the world, the Okinawans, have traditionally eaten a diet containing 9% calories from protein[195].

As dietary amino acids are reduced by protein restriction, the activity of mTORC1, which is responsive to amino acid levels, is also reduced[186,196]. As discussed above, protein restriction increases FGF21 levels in rodents and humans via activation of GCN2 and ATF4[105,109,111,112,197]. GCN2 is essential for the acute response to protein restriction. *Gcn2* knockout mice have increased FGF21 levels after only 2 weeks on protein restriction[105], indicating that GCN2 is only required for the initial induction of FGF21.

Restriction of specific amino acids: methionine

It has been proposed that a selective reduction of specific, essential amino acids might be sufficient to extend healthspan and lifespan, independent of total protein and calorie intake. Methionine, threonine, tryptophan and branched chain amino acids (BCAAs) have all been identified as potential candidates.

In 1993, Orentreich and colleagues observed that lifelong ~80% methionine restriction (MR) resulted in a 30% increase in lifespan in male rats[198]; these findings were subsequently confirmed in mice[199]. As illustrated in Supplementary information 4, methionine reduction has strong beneficial effects on the metabolic health of rodents, and reduced consumption of methionine potentially contributes to the health of humans consuming vegan diets. Methionine plays a unique role in translation, as methionine is specified by the AUG start codon and is thus required for translation initiation of most proteins. Methionine

restriction thus has a dramatic effect on protein translation, strongly downregulating protein synthesis[200]. In addition to these effects, which may be beneficial for healthy aging, methionine also has important and unique metabolic roles and effects that have been implicated in the benefits of methionine restriction.

Many of the metabolic adaptations to methionine reduction have been attributed to the actions of the hormone FGF21, levels of which are upregulated by methionine restriction or depletion in both young and aged mice[201–203]. As with protein restriction, a reduced dietary intake of methionine increases energy expenditure by promoting a FGF21-dependent browning of white adipose tissue[204]. Browning refers to a phenotypic switch where white adipose tissue becomes enriched in mitochondria and upregulates expression of uncoupling protein 1 (UCP1). This promotes thermogenesis and energy expenditure, resulting in reduced adiposity. This effect is independent of GCN2, and induced by the activation of the eIF2a kinase PERK[205] as a consequence of methionine restriction-induced oxidative stress resulting from a depletion of glutathione. Dietary supplementation with cysteine blocks the effect of methionine restriction on FGF21 levels, adiposity, and energy expenditure[205,206].

Alterations in dietary methionine levels strikingly and rapidly lead to changes in the levels of its metabolites — the universal methyl donor SAM and cysteine, a key precursor of antioxidant glutathione and the gaseous messenger hydrogen sulfide (H_2S)[207]. SAM is crucial for histone and DNA methylation; and methionine restriction causes substantial reductions of SAM levels and alterations to both DNA and histone methylation[208–210]. Changes in the levels of metabolites such as SAM and S-adenosylhomocysteine (SAH) are thought to drive the protection against hepatic DNA hypomethylation with age in adult mice[208], and might account for the stronger effects on metabolic health seen with methionine restriction rather than leucine restriction on hepatic lipogenic gene expression and circulating FGF21[211]. H_2S, a powerful vasodilator, is endogenously produced via the trans-sulfuration pathway, and may help protect from multiple age-related diseases[207,212]. Production of H_2S is required for DR to extend *C.elegans* lifespan[213]. Finally, methionine restriction activates AMPK in mice[214], which is required for the lifespan extension induced by increased synthesis of the SAM in yeast[215].

SAM levels are also sensed by the mTORC1 protein kinase via SAMTOR, and a reduction in SAM leads to decreased mTORC1 signaling[216]. However, even complete methionine depletion does not significantly alter hepatic mTORC1 activity, and mice lacking liver *TSC1*, which have constitutively active hepatic mTORC1, respond normally to methionine-deprived diet[203]. Thus, at least in the liver, reduced mTORC1 signaling does not mediate many of the metabolic effects of methionine restriction. Further research will be required to fully define the molecular mechanisms underlying the geroprotective effects of methionine restriction.

Restriction of BCAAs

As summarized in Supplementary information 4, increased blood levels of BCAAs (leucine, isoleucine, and valine) are associated with obesity and diabetes in humans. In mice, dietary BCAA restriction recapitulates many of the beneficial effects of protein restriction, including reduced adiposity, improved glucose tolerance, and elevated energy expenditure,

but not increased FGF21[112,217]. These effects can be largely recapitulated by restricting isoleucine, and the beneficial metabolic effects of protein restriction are dependent on isoleucine restriction[218]. Conversely, high BCAA diets are associated with enlarged fat mass, increased body weight, hyperphagia, and insulin resistance[217,219,220]. BCAAs are potent mTORC1 agonists, and dietary and circulating BCAAs levels are correlated with mTORC1 activation in mouse liver[186]. Reintroduction of BCAAs to a protein restricted diet blocks the effects on mTORC1 signaling[221], highlighting the critical role of dietary BCAAs in mTORC1 activity. Although the Ames dwarf mouse has low levels of circulating BCAAs[222], the effects of BCAAs on the longevity and healthspan in this long-lived genetic model are only now being explored.

A low BCAA diet was recently shown to increase survival in *Drosophila*[223], and a 67% restriction of dietary BCAAs increases the survival of two different progeroid mouse models[187]. Similarly, initiation of a BCAA-restricted diet very early in life (3 weeks of age) not only improved metabolic heath, but in wild-type male mice reduced frailty and extended lifespan by over 30%[187]. This lifespan extension was associated with a male-specific reduction in mTORC1 activity. However, beginning a 50% or 80% BCAAs restricted diet at 12 weeks of age, or a 67% BCAA restricted diet at 16 months of age, improved body composition and glucose homeostasis, but did not increase mouse lifespan[187,219]. Finally, further supporting a negative effect of dietary BCAAs on mammalian healthspan and lifespan, dietary supplementation with additional BCAAs results in a hyperphagia-induced reduction in mouse lifespan[219].

Tryptophan or Threonine Restriction

Studies in the 1970's and 1980's reported that tryptophan restriction increases overall and maximum lifespan of mice and rats, and delays aging-associated pathologies including cancers[224–226]. However, it was only recently found that tryptophan restriction may be an evolutionarily conserved geroprotective intervention as administration of ibuprofen, an inhibitor of tryptophan uptake, extends the lifespan of yeast, worms and flies[227]. Tryptophan is catabolized via the kynurenine pathway, and its metabolites include NAD^+, which is a key regulator of metabolism and an essential co-factor for the activity of the sirtuin family of enzymes[228].

While the effects of tryptophan restriction on aging in mammals has not been the direct subject of investigation again until recently, one recent study found that the serum level of tryptophan was associated with onset of diabetes[229]. There is also a negative correlation between tryptophan levels and cognitive function in humans[230]. Finally, restriction of dietary tryptophan induces expression of FGF21 and recapitulates metabolic effects of a protein restriction diet in mice[231]. Much work remains to be done to examine if tryptophan restriction can promote healthy aging in mice or in humans.

A recent study surveyed the nine dietary essential amino acids to try and identify the amino acids responsible for the metabolic effects of a protein restricted diet, as modeled by a diet in which 5% of the calories were derived from casein. Intriguingly, in this dietary setting, restriction of threonine mimicked the effects of protein restriction, increasing energy expenditure and increasing insulin sensitivity[231]. Restriction of threonine induces

the hormone FGF21, and the effects of threonine restriction were shown to be FGF21-dependent[231]. Levels of an enzyme in the threonine catabolic pathway, GCAT (glycine-C-acetyltransferase), decline with age in *C. elegans* and mice, and downregulation of this gene with RNAi extends the healthspan of *C. elegans*[232]. Overall, while this work is suggestive that threonine restriction may promote health and longevity, this remains to be formally tested in mice and in humans.

Impact of meal frequency and timing

Unlike most humans practicing DR who typically cut calories at every single meal, hungry DR rodents devour their once-a-day restricted food allotment in 1 to 4 hours (depending on the mouse strain) followed by a daily prolonged period of fasting[233,234]. Recently, and as discussed below, interventions such as meal feeding that involve the imposition of a daily fasting period have been shown to have metabolic benefits and extend the lifespan of mice[235–237]. We recently utilized a series of dietary regimens to dissect the contributions of reduced calorie intake and an imposed fasting period to the metabolic, molecular, and geroprotective effects of DR[238]. We found that imposed daily fasting is required to observe DR-induced changes in insulin sensitivity and fuel selection, as well as for the geroprotective effects of DR on frailty, cognition, and lifespan[238]. Moreover, we determined that a prolonged daily fast without restricting calories is sufficient to recapitulate the metabolic phenotypes and transcriptional signature of a DR[238]. Thus, fasting between meals is a critical component of DR in rodents.

These observations coupled with the beneficial effect on murine healthspan and lifespan of alternate-day fasting, even independently of weight loss, has opened a new exciting field of translational research that is gaining momentum. Intermittent fasting in rodent models usually refers to a 24-hr complete fast every other day, while in humans it refers to a variety of regimes such as complete fasting or severe DR (e.g. 500–600 calories per day) on alternate days or 2 non-consecutive days per week (5:2 diet). Another form of intermittent fasting is time-restricted feeding, which involves consuming all daily food in a 4- to 12-h time window and fasting for the remainder of the day. Prolonged, periodic fasting, lasting for two to 7 consecutive days, and repeated cyclically, is an extreme form of DR that might have benefits for specific clinical indications.

An often overlooked problem in translating rodent findings to humans is that humans can fast for much, much longer than mice (Fig. 4). A 24-hr fast-feed cycle in mice most likely equates to recurrent ~5 days fast-feed cycles in humans. Another consideration is that laboratory rodents during the feast days consume a nutritionally balanced chow; in contrast, most people practicing different forms of intermittent fasting, prolonged, periodic fasting or time-restricted feeding eat the unhealthy Western-like obesogenic food that has been shown to cause negative consequences on metabolic and gut microbiome health even during DR, and potentiate vitamin and mineral deficiencies[193,239–241]. Finally, unlike laboratory rodents many obese adults take a range of medications (e.g., antidiabetic and antihypertensive agents) that could have serious negative and potentially fatal consequences when coupled with fasting, including hypotension and severe hypoglycemia[242].

Impact of intermittent fasting on lifespan and healthspan

A 24-hour fast every other day or twice per week extends lifespan in mice and rats. The ability of IF to extend lifespan, as well as the magnitude of life extension, depends on background strain and age of initiation; intermittent fasting started at 10 months of age caused a 15% reduction in mean and maximal lifespan of A/J mice[243]. Intermittent fasting in rodents also reduces the incidence of a wide range of chronic diseases, including stroke, cardiomyopathy, hypertension, diabetes, and several neurodegenerative diseases due to its stimulatory effect on synaptic plasticity[244]. Cancer protection, however, is not universal, and several studies (especially in laboratory rats)[245,246] report a cancer promoting effect of intermittent fasting that may be mediated at least in part by TGF-β1[247].

Several common pathways are induced by chronic DR and intermittent fasting. These include activation of multiple transcriptional factors induced by metabolic/hormonal modifications that lead to reduced oxidative stress and inflammation, and enhanced autophagy, mitophagy, and tissue repair capacity[248,249]. In mice, a 24-hr fast increases intestinal stem cell function through induction of fatty acid oxidation; molecular deletion of carnitine acyltransferase I (the rate-limiting enzyme in fatty acid oxidation) reduces the numbers and function of gut stem cells[250]. Alterations of gut microbiota composition seem to play a role in mediating some of the effects of intermittent fasting on energy expenditure by selectively upregulating monocarboxylate transporter 1 and UCP1 expression in brown adipose tissue in rodents[251], but not in humans[252].

A peculiar characteristic of intermittent fasting that does not apply to chronic DR (when the restricted allotment of food is equally distributed during the day) is the metabolic switch with a transient elevation of plasma non-esterified fatty acids and ketone bodies that occur during fasting. β-OH butyrate binds to two G protein-coupled receptors, GPR109a and GPR41,[253,254] and by acting as an endogenous histone deacetylase inhibitor causes a wide range of modifications of gene expression and downstream signaling pathways that protects against oxidative stress[255]. However, mice and humans regulate ketones differently in response to acute fasting; in adult C57BL/6J mice, plasma ketones begin to increase after just 4–7 hours of fasting, and peak at about 24 hours[256]. In contrast, in adult humans the production of ketone bodies after an overnight fast is negligible; plasma β-OH-B levels start to creep up after 18–24 hours, and progressively increase (more rapidly in women than in men), peaking at 4–7 mM after 2 weeks fasting[257258]. Intermittent fasting in rodents exerts neuroprotective effects by increasing Brain-derived neurotrophic factor (BDNF) concentrations[259]. However, several clinical trials of intermittent fasting have reported a significant reduction of circulating BDNF levels[260,261], confirming that the metabolic adaptations to alternate day fasting in mammals with a very high-metabolic rate such as young rodents cannot be compared with those of middle-aged human adults.

While there have been no studies using intermittent fasting in non-human primates, several short-term randomized clinical trials (summarized in Supplementary information 5) have shown potentially favorable effects of fasting in humans. However, not all studies have demonstrated beneficial effects, and unlike in rodents where some of the metabolic adaptations of intermittent fasting are independent of food intake and weight loss, well-conducted human studies suggest that energy restriction is required to improve health[262].

Thus, the translatable lessons that can be learned from intermittent fasting studies in rodents may be limited.

Prolonged, periodic fasting

In contrast to intermittent fasting, which consists of frequent short periods without or with limited amounts of food, prolonged, periodic fasting lasts more than 24 hours and is repeated once or twice a month. Because water-only fasting for more than 48–60 hours is deadly in mice, an alternative is to feed them reduced portions of a diet low in protein and carbohydrate, and high in unsaturated fat, providing between 10 and 50% of normal *ad libitum* intake. 16-mo old C57BL/6 mice fed a fasting-mimicking diet for four days twice per month had reduced weight and visceral fat accumulation, while preserving lean and bone mass. This fasting-mimicking diet regimen reduced cancer burden, rejuvenated the immune system, improved motor and memory performance, and increased median, though not maximum lifespan by 11%[263].

Weekly 3-days of fasting-mimicking diet suppresses autoimmunity, and induces oligodendrocyte precursor cell regeneration and axonal remyelination in a murine experimental autoimmune encephalomyelitis model[264]. This suggests that a fasting-mimicking diet might work by activating stem cell-based or other regenerative processes through transient inhibition of the AKT, PKA and mTOR pathways[265]. Similarly, weekly 4-days of fasting-mimicking diet can cure both type 2 and type 1 diabetes, restoring pancreatic insulin production in a streptozotocin mouse model of type 1 diabetes by causing a stepwise expression of SOX17 and PDX1, followed by NGN3-driven generation of insulin-producing β cells[266]. Whether or not a fasting-mimicking diet can regenerate β-cells in patients with type 1 diabetes can be easily tested in a clinical study, because unlike multiple sclerosis, this autoimmune disease is not relapsing-remitting, and C-peptide is an excellent marker of therapeutic efficacy.

Preclinical data have shown that both prolonged fasting and fasting-mimicking diets can induce a differential stress resistance response in tumor-bearing mice that has the potential to maximize chemotherapy toxicity to cancer cells while protecting normal cells[267,268]. Increased resistance to stress during fasting is associated with improved activity of chemotherapeutic agents by reducing circulating IGF-1, insulin and leptin and by inhibiting AKT–mTOR signalling via upregulation of EGR1 and PTEN[269]. By inhibiting the stress-responsive enzyme heme oxygenase-1 (HO-1), fasting increases T cell-dependent targeted killing of cancer cells in murine models of breast and melanoma cancer[270].

In a recent randomized clinical trial of women with HER2-negative stage II/III breast cancer, 4 days of fasting-mimicking diet a week was able to reinforce the radiological and pathological tumor response to neoadjuvant chemotherapy and reduced DNA damage to T-cells[271]. In this trial (in contrast to previous results reported in a non-randomized parallel study[263]), a fasting-mimicking diet significantly reduced circulating insulin levels but did not change serum IGF-1 or IGFBP-3 concentrations, while increasing inflammation as assessed by C-reactive protein[271]. Very small preliminary clinical studies suggest that fasting before and during chemotherapy may reduce adverse events of chemotherapy and improve quality of life[272,273].

Time restricted feeding

Preclinical and epidemiological data show that consuming food out of sync with the day/night cycle and/or over an extended time frame impairs metabolic health[274]. In mice, time restricted feeding (8-hour daily access to food) is thought to regenerate disrupted circadian clock rhythms, and protect against obesity, fatty liver disease, insulin resistance, hyperinsulinemia and inflammation independently of caloric and fat intake[235]. These positive metabolic effects are attributed to a fine tuning of circadian clock genes in response to metabolic modulation of mTOR, CREB and AMPK pathway activity[235,236], even when weekend *ad libitum* "cheat days" are permitted. Disrupting circadian oscillations by genetic manipulations of clock genes that encode circadian rhythmicity (e.g. *Clock*, *Per1*, and Cry genes) induces obesity, alters glucose metabolism and reduces lifespan in mouse models[275–277], and irregular feeding uncouples peripheral clocks from the central pacemaker inducing insulin resistance and glucose intolerance[278].

Data on early time-restricted feeding (from 6am to 2pm) in non-human primates are limited to a study of geriatric vervet monkeys showing a significant increase in HDL-cholesterol and reverse cholesterol efflux but not change in adiposity[275]. This is consistent with findings from a randomized clinical trial involving women affected by polycystic ovary syndrome showing that early meal timing (980 kcal breakfast, 640 kcal lunch, and 190 kcal dinner) is associated with more weight loss, higher insulin sensitivity, lower circulating testosterone, and increased ovulation rate than controls eating isocaloric diets with a later meal pattern (190 kcal breakfast, 640 kcal lunch, and 980 kcal dinner)[279]. A five-week, randomized, crossover, isocaloric and eucaloric controlled feeding trial in males with prediabetes found that independently of weight loss early time-restricted feeding (6-hr feeding period, with dinner before 3 pm) did not improve glucose, IL-6 and C-reactive protein levels, but ameliorated insulin sensitivity, β cell responsiveness and blood pressure; however, early- time-restricted feeding markedly increased plasma triglycerides and total cholesterol[280]. A number of small short-term (2–4 months) pilot studies in overweight individuals suggest a beneficial effect of time-restricted feeding (~8–12 hours per day) on body weight and composition[281], but the large TREAT randomized clinical trial challenges these findings. It shows that overweight or obese men and women randomized to time-restricted feeding diets(access to food for 8 hours per day) did not experience weight loss or any cardiometabolic or glucose homeostatic improvements over 3 months[282]. While the underlying molecular changes that may dictate the beneficial effect of time-restricted feeding in humans is largely unknown, 30 consecutive days of dawn till dusk fasting (~14 hour fast) in healthy humans showed an anti-cancer and anti-diabetes serum proteomic signature[283]. Long-term randomized clinical trials are warranted to establish the efficacy of time-restricted feeding to improve metabolic health in primary and secondary prevention of other cardiometabolic conditions.

Conclusions

Many questions about the effects of DR and its clinical translatability remain unanswered. Research over the next decade will focus on understanding how precise dietary components – amino acids, specific sugars, fats and microbial metabolites – regulate health and

longevity, and how these components interact with a DR diet, exercise and cognitive training and other lifestyle factors in a mechanistic way. In addition, the continued development of tools for conditional gene inactivation and mutation will permit a greater range of epistasis experiments to identify pathways required for DR to extend healthspan and lifespan. While we believe that promoting healthy eating and habits is the most cost-effective way to prevent multiple chronic diseases and promote human and environmental health[284], identification of these pathways may also help to further the development of geroprotective agents that might potentiate the effects of healthy lifestyles.

Finally, a crucial point to consider is that humans are genetically heterogeneous, and experiments examining the interaction of diet and strain in mice have demonstrated that genetic and epigenetic background determines the response to dietary interventions, including DR. Our hope is that in the near future, these findings will be clinically translated using a personalized food-as-medicine approach, to identify how each person can best improve their health and potentially extend their lifespan by optimizing what, when and how much they eat.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors specially thank Aimee Yang Xin Dai for helping with the creation of figures. The Lamming laboratory is supported by the US National Institute on Aging (AG056771, AG061635, and AG062328 to D.W.L.), the US National Institute of Diabetes and Digestive and Kidney Diseases (DK125859 to D.W.L.), and by funding from the University of Wisconsin-Madison School of Medicine and Public Health and Department of Medicine to D.W.L. C.L.G. is a Glenn Foundation for Medical Research Postdoctoral Fellow. The Lamming laboratory and C.L.G. were supported in part by a generous gift from Dalio Philanthropies. The Lamming laboratory is also supported by the U.S. Department of Veterans Affairs (I01-BX004031), and this work was supported using facilities and resources from the William S. Middleton Memorial Veterans Hospital. The Fontana laboratory is supported by grants from the Australian NHMRC Investigator Grant (APP1177797), Australian Youth and Health Foundation, and Bakewell Foundation. The content is solely the responsibility of the authors and does not necessarily represent the official views of NIH or the Department of Veterans Affairs or the United States Government. The authors apologize for the omission of relevant work owing to space constraints.

References

1. Fontana L, Partridge L & Longo VD Extending Healthy Life Span—From Yeast to Humans. Science 328, 321–326 (2010). [PubMed: 20395504]
2. Kenyon CJ The genetics of ageing. Nature 464, 504–512 (2010). [PubMed: 20336132]
3. Speakman JR & Mitchell SE Caloric restriction. Mol Aspects Med 32, 159–221 (2011). [PubMed: 21840335]
4. Liao CY, Rikke BA, Johnson TE, Diaz V & Nelson JF Genetic variation in the murine lifespan response to dietary restriction: from life extension to life shortening. Aging Cell 9, 92–95 (2010). [PubMed: 19878144]
5. Mitchell SJ et al. Effects of Sex, Strain, and Energy Intake on Hallmarks of Aging in Mice. Cell Metab 23, 1093–1112 (2016). [PubMed: 27304509]
6. Weindruch R & Sohal RS Seminars in medicine of the Beth Israel Deaconess Medical Center. Caloric intake and aging. The New England journal of medicine 337, 986–994 (1997). [PubMed: 9309105]
7. Mattison JA et al. Caloric restriction improves health and survival of rhesus monkeys. Nature communications 8, 14063 (2017).

8. Maegawa S et al. Caloric restriction delays age-related methylation drift. Nature communications 8, 539 (2017).
9. Stonebarger GA et al. Amyloidosis increase is not attenuated by long-term calorie restriction or related to neuron density in the prefrontal cortex of extremely aged rhesus macaques. Geroscience 42, 1733–1749 (2020). [PubMed: 32876855]
10. Austad SN Mixed results for dieting monkeys. Nature 489, 210–211 (2012). [PubMed: 22932269]
11. Shimokawa I et al. Diet and the Suitability of the Male Fischer 344 Rat as a Model for Aging Research. Journal of Gerontology 48, B27–B32 (1993). [PubMed: 8418135]
12. Ikeno Y et al. Reduced Incidence and Delayed Occurrence of Fatal Neoplastic Diseases in Growth Hormone Receptor/Binding Protein Knockout Mice. The Journals of Gerontology: Series A 64A, 522–529 (2009).
13. Meyer TE et al. Long-Term Caloric Restriction Ameliorates the Decline in Diastolic Function in Humans. Journal of the American College of Cardiology 47, 398–402 (2006). [PubMed: 16412867]
14. Stein PK et al. Caloric restriction may reverse age-related autonomic decline in humans. Aging Cell 11, 644–650 (2012). [PubMed: 22510429]
15. Wang M et al. Calorie Restriction Curbs Proinflammation That Accompanies Arterial Aging, Preserving a Youthful Phenotype. J Am Heart Assoc 7, e009112 (2018). [PubMed: 30371211]
16. Ristow M & Zarse K How increased oxidative stress promotes longevity and metabolic health: The concept of mitochondrial hormesis (mitohormesis). Exp Gerontol 45, 410–418 (2010). [PubMed: 20350594]
17. Il'yasova D et al. Effects of 2 years of caloric restriction on oxidative status assessed by urinary F2-isoprostanes: The CALERIE 2 randomized clinical trial. Aging Cell 17 (2018).
18. Nisoli E et al. Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. Science 310, 314–317 (2005). [PubMed: 16224023]
19. Lopez-Lluch G et al. Calorie restriction induces mitochondrial biogenesis and bioenergetic efficiency. Proc Natl Acad Sci U S A 103, 1768–1773 (2006). [PubMed: 16446459]
20. Hansen M, Rubinsztein DC & Walker DW Autophagy as a promoter of longevity: insights from model organisms. Nat Rev Mol Cell Biol 19, 579–593 (2018). [PubMed: 30006559]
21. Cuervo AM Calorie restriction and aging: the ultimate "cleansing diet". J Gerontol A Biol Sci Med Sci 63, 547–549 (2008). [PubMed: 18559626]
22. Yang L et al. Long-Term Calorie Restriction Enhances Cellular Quality-Control Processes in Human Skeletal Muscle. Cell reports 14, 422–428 (2016). [PubMed: 26774472]
23. Hetz C, Zhang K & Kaufman RJ Mechanisms, regulation and functions of the unfolded protein response. Nat Rev Mol Cell Biol 21, 421–438 (2020). [PubMed: 32457508]
24. Someya S et al. Sirt3 mediates reduction of oxidative damage and prevention of age-related hearing loss under caloric restriction. Cell 143, 802–812 (2010). [PubMed: 21094524]
25. Marzetti E, Lees HA, Wohlgemuth SE & Leeuwenburgh C Sarcopenia of aging: underlying cellular mechanisms and protection by calorie restriction. BioFactors (Oxford, England) 35, 28–35 (2009).
26. Yamada Y et al. Caloric Restriction and Healthy Life Span: Frail Phenotype of Nonhuman Primates in the Wisconsin National Primate Research Center Caloric Restriction Study. J Gerontol A Biol Sci Med Sci 73, 273–278 (2018). [PubMed: 28398464]
27. Rhoads TW et al. Molecular and Functional Networks Linked to Sarcopenia Prevention by Caloric Restriction in Rhesus Monkeys. Cell Syst 10, 156–168 e155 (2020). [PubMed: 31982367]
28. Mercken EM et al. Calorie restriction in humans inhibits the PI3K/AKT pathway and induces a younger transcription profile. Aging Cell 12, 645–651 (2013). [PubMed: 23601134]
29. Lopez-Otin C, Blasco MA, Partridge L, Serrano M & Kroemer G The hallmarks of aging. Cell 153, 1194–1217 (2013). [PubMed: 23746838]
30. Mana MD, Kuo EY & Yilmaz Ö H Dietary Regulation of Adult Stem Cells. Current stem cell reports 3, 1–8 (2017). [PubMed: 28966904]
31. Yilmaz OH et al. mTORC1 in the Paneth cell niche couples intestinal stem-cell function to calorie intake. Nature 486, 490–495 (2012). [PubMed: 22722868]

32. Friedman DB & Johnson TE A mutation in the age-1 gene in Caenorhabditis elegans lengthens life and reduces hermaphrodite fertility. Genetics 118, 75–86 (1988). [PubMed: 8608934]
33. Kimura KD, Tissenbaum HA, Liu Y & Ruvkun G daf-2, an insulin receptor-like gene that regulates longevity and diapause in Caenorhabditis elegans. Science 277, 942–946 (1997). [PubMed: 9252323]
34. Tatar M et al. A mutant Drosophila insulin receptor homolog that extends life-span and impairs neuroendocrine function. Science 292, 107–110 (2001). [PubMed: 11292875]
35. Clancy DJ et al. Extension of Life-Span by Loss of CHICO, a *Drosophila* Insulin Receptor Substrate Protein. Science 292, 104–106 (2001). [PubMed: 11292874]
36. Tu M-P, Epstein D & Tatar M The demography of slow aging in male and female Drosophila mutant for the insulin-receptor substrate homologue chico. Aging Cell 1, 75–80 (2002). [PubMed: 12882356]
37. Brown-Borg HM, Borg KE, Meliska CJ & Bartke A Dwarf mice and the ageing process. Nature 384, 33 (1996).
38. Gesing A, Al-Regaiey KA, Bartke A & Masternak MM Growth hormone abolishes beneficial effects of calorie restriction in long-lived Ames dwarf mice. Exp Gerontol 58, 219–229 (2014). [PubMed: 25152388]
39. Coschigano KT, Clemmons D, Bellush LL & Kopchick JJ Assessment of growth parameters and life span of GHR/BP gene-disrupted mice. Endocrinology 141, 2608–2613 (2000). [PubMed: 10875265]
40. Bonkowski MS et al. Disruption of Growth Hormone Receptor Prevents Calorie Restriction from Improving Insulin Action and Longevity. PLoS ONE 4, e4567 (2009). [PubMed: 19234595]
41. Bartke A, Sun LY & Longo V Somatotropic signaling: trade-offs between growth, reproductive development, and longevity. Physiol Rev 93, 571–598 (2013). [PubMed: 23589828]
42. Lamming DW & Anderson RM in eLS (John Wiley & Sons, Ltd, 2014).
43. Yu D et al. Calorie-Restriction-Induced Insulin Sensitivity Is Mediated by Adipose mTORC2 and Not Required for Lifespan Extension. Cell reports 29, 236–248 e233 (2019). [PubMed: 31577953]
44. Endicott SJ, Boynton DN Jr., Beckmann LJ & Miller RA Long-lived mice with reduced growth hormone signaling have a constitutive upregulation of hepatic chaperone-mediated autophagy. Autophagy, 1–14 (2020).
45. Spadaro O et al. Growth Hormone Receptor Deficiency Protects against Age-Related NLRP3 Inflammasome Activation and Immune Senescence. Cell reports 14, 1571–1580 (2016). [PubMed: 26876170]
46. Lamming DW Diminished mTOR signaling: a common mode of action for endocrine longevity factors. SpringerPlus 3, 735 (2014). [PubMed: 25674466]
47. Holzenberger M et al. IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. Nature 421, 182–187 (2003). [PubMed: 12483226]
48. Bokov AF et al. Does reduced IGF-1R signaling in Igf1r+/− mice alter aging? PLoS One 6, e26891 (2011). [PubMed: 22132081]
49. Bluher M, Kahn BB & Kahn CR Extended longevity in mice lacking the insulin receptor in adipose tissue. Science 299, 572–574 (2003). [PubMed: 12543978]
50. Taguchi A, Wartschow LM & White MF Brain IRS2 signaling coordinates life span and nutrient homeostasis. Science 317, 369–372 (2007). [PubMed: 17641201]
51. Selman C et al. Evidence for lifespan extension and delayed age-related biomarkers in insulin receptor substrate 1 null mice. FASEB J 22, 807–818 (2008). [PubMed: 17928362]
52. Selman C, Partridge L & Withers DJ Replication of extended lifespan phenotype in mice with deletion of insulin receptor substrate 1. PLoS One 6, e16144 (2011). [PubMed: 21283571]
53. Conover CA & Bale LK Loss of pregnancy-associated plasma protein A extends lifespan in mice. Aging Cell 6, 727–729 (2007). [PubMed: 17681037]
54. Bale LK, West SA & Conover CA Inducible knockdown of pregnancy-associated plasma protein-A gene expression in adult female mice extends life span. Aging Cell 16, 895–897 (2017). [PubMed: 28600811]

55. Mao K et al. Late-life targeting of the IGF-1 receptor improves healthspan and lifespan in female mice. Nature communications 9, 2394 (2018).
56. Liu GY & Sabatini DM mTOR at the nexus of nutrition, growth, ageing and disease. Nat Rev Mol Cell Biol 21, 183–203 (2020). [PubMed: 31937935]
57. Chen X et al. Cryo-EM structure of human mTOR complex 2. Cell Res 28, 518–528 (2018). [PubMed: 29567957]
58. Scaiola A et al. The 3.2-A resolution structure of human mTORC2. Sci Adv 6 (2020).
59. Lamming DW et al. Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. Science 335, 1638–1643 (2012). [PubMed: 22461615]
60. Sarbassov DD et al. Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. Mol Cell 22, 159–168 (2006). [PubMed: 16603397]
61. Schreiber KH et al. Rapamycin-mediated mTORC2 inhibition is determined by the relative expression of FK506-binding proteins. Aging Cell 14, 265–273 (2015). [PubMed: 25652038]
62. Dominick G et al. Regulation of mTOR activity in Snell dwarf and GH receptor gene-disrupted mice. Endocrinology 156, 565–575 (2015). [PubMed: 25456069]
63. Selman C et al. Ribosomal protein S6 kinase 1 signaling regulates mammalian life span. Science 326, 140–144 (2009). [PubMed: 19797661]
64. Wu JJ et al. Increased mammalian lifespan and a segmental and tissue-specific slowing of aging after genetic reduction of mTOR expression. Cell reports 4, 913–920 (2013). [PubMed: 23994476]
65. Johnson SC, Rabinovitch PS & Kaeberlein M mTOR is a key modulator of ageing and age-related disease. Nature 493, 338–345 (2013). [PubMed: 23325216]
66. Harrison DE et al. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature 460, 392–395 (2009). [PubMed: 19587680]
67. Arriola Apelo SI, Pumper CP, Baar EL, Cummings NE & Lamming DW Intermittent Administration of Rapamycin Extends the Life Span of Female C57BL/6J Mice. J Gerontol A Biol Sci Med Sci 71, 876–881 (2016). [PubMed: 27091134]
68. Bitto A et al. Transient rapamycin treatment can increase lifespan and healthspan in middle-aged mice. eLife 5 (2016).
69. Arriola Apelo SI & Lamming DW Rapamycin: An InhibiTOR of Aging Emerges From the Soil of Easter Island. J Gerontol A Biol Sci Med Sci 71, 841–849 (2016). [PubMed: 27208895]
70. Urfer SR et al. A randomized controlled trial to establish effects of short-term rapamycin treatment in 24 middle-aged companion dogs. Geroscience 39, 117–127 (2017). [PubMed: 28374166]
71. Powell JD, Pollizzi KN, Heikamp EB & Horton MR Regulation of immune responses by mTOR. Annual review of immunology 30, 39–68 (2012).
72. Schreiber KH et al. A novel rapamycin analog is highly selective for mTORC1 in vivo. Nature communications 10, 3194 (2019).
73. Arriola Apelo SI et al. Alternative rapamycin treatment regimens mitigate the impact of rapamycin on glucose homeostasis and the immune system. Aging Cell 15, 28–38 (2016). [PubMed: 26463117]
74. Arriola Apelo SI et al. Ovariectomy uncouples lifespan from metabolic health and reveals a sex-hormone-dependent role of hepatic mTORC2 in aging. eLife 9 (2020).
75. Chellappa K et al. Hypothalamic mTORC2 is essential for metabolic health and longevity. Aging Cell 18, e13014 (2019). [PubMed: 31373126]
76. Lamming DW et al. Depletion of Rictor, an essential protein component of mTORC2, decreases male lifespan. Aging Cell 13, 911–917 (2014). [PubMed: 25059582]
77. Soukas AA, Kane EA, Carr CE, Melo JA & Ruvkun G Rictor/TORC2 regulates fat metabolism, feeding, growth, and life span in Caenorhabditis elegans. Genes Dev 23, 496–511 (2009). [PubMed: 19240135]
78. Chang K et al. TGFB-INHB/activin signaling regulates age-dependent autophagy and cardiac health through inhibition of MTORC2. Autophagy 16, 1807–1822 (2020). [PubMed: 31884871]
79. Robida-Stubbs S et al. TOR signaling and rapamycin influence longevity by regulating SKN-1/Nrf and DAF-16/FoxO. Cell Metab 15, 713–724 (2012). [PubMed: 22560223]

80. Mizunuma M, Neumann-Haefelin E, Moroz N, Li Y & Blackwell TK mTORC2-SGK-1 acts in two environmentally responsive pathways with opposing effects on longevity. Aging Cell 13, 869–878 (2014). [PubMed: 25040785]
81. Garratt M, Bower B, Garcia GG & Miller RA Sex differences in lifespan extension with acarbose and 17-alpha estradiol: gonadal hormones underlie male-specific improvements in glucose tolerance and mTORC2 signaling. Aging Cell 16, 1256–1266 (2017). [PubMed: 28834262]
82. Strong R et al. Rapamycin-mediated mouse lifespan extension: Late-life dosage regimes with sex-specific effects. Aging Cell, e13269 (2020). [PubMed: 33145977]
83. Mahoney SJ et al. A small molecule inhibitor of Rheb selectively targets mTORC1 signaling. Nature communications 9, 548 (2018).
84. Kaeberlein M et al. Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. Science 310, 1193–1196 (2005). [PubMed: 16293764]
85. Hansen M et al. Lifespan extension by conditions that inhibit translation in Caenorhabditis elegans. Aging Cell 6, 95–110 (2007). [PubMed: 17266679]
86. Heintz C et al. Splicing factor 1 modulates dietary restriction and TORC1 pathway longevity in C. elegans. Nature 541, 102–106 (2017). [PubMed: 27919065]
87. Greer EL & Brunet A Different dietary restriction regimens extend lifespan by both independent and overlapping genetic pathways in C. elegans. Aging Cell 8, 113–127 (2009). [PubMed: 19239417]
88. Wu Z et al. Dietary Restriction Extends Lifespan through Metabolic Regulation of Innate Immunity. Cell Metab 29, 1192–1205 e1198 (2019). [PubMed: 30905669]
89. Bjedov I et al. Mechanisms of life span extension by rapamycin in the fruit fly Drosophila melanogaster. Cell Metab 11, 35–46 (2010). [PubMed: 20074526]
90. Fok WC et al. Combined treatment of rapamycin and dietary restriction has a larger effect on the transcriptome and metabolome of liver. Aging Cell 13, 311–319 (2014). [PubMed: 24304444]
91. Fok WC et al. Mice fed rapamycin have an increase in lifespan associated with major changes in the liver transcriptome. PLoS One 9, e83988 (2014). [PubMed: 24409289]
92. Fok WC et al. Short-term rapamycin treatment in mice has few effects on the transcriptome of white adipose tissue compared to dietary restriction. Mech Ageing Dev 140, 23–29 (2014). [PubMed: 25075714]
93. Fok WC et al. Short-term treatment with rapamycin and dietary restriction have overlapping and distinctive effects in young mice. J Gerontol A Biol Sci Med Sci 68, 108–116 (2013). [PubMed: 22570137]
94. Yu Z et al. Rapamycin and dietary restriction induce metabolically distinctive changes in mouse liver. J Gerontol A Biol Sci Med Sci 70, 410–420 (2015). [PubMed: 24755936]
95. Bunpo P et al. The eIF2 kinase GCN2 is essential for the murine immune system to adapt to amino acid deprivation by asparaginase. The Journal of nutrition 140, 2020–2027 (2010). [PubMed: 20861212]
96. Ye J et al. The GCN2-ATF4 pathway is critical for tumour cell survival and proliferation in response to nutrient deprivation. The EMBO Journal 29, 2082–2096 (2010). [PubMed: 20473272]
97. Ravindran R et al. The amino acid sensor GCN2 controls gut inflammation by inhibiting inflammasome activation. Nature 531, 523–527 (2016). [PubMed: 26982722]
98. Wek SA, Zhu S & Wek RC The histidyl-tRNA synthetase-related sequence in the eIF-2 alpha protein kinase GCN2 interacts with tRNA and is required for activation in response to starvation for different amino acids. Molecular and cellular biology 15, 4497–4506 (1995). [PubMed: 7623840]
99. Dong J, Qiu H, Garcia-Barrio M, Anderson J & Hinnebusch AG Uncharged tRNA activates GCN2 by displacing the protein kinase moiety from a bipartite tRNA-binding domain. Mol Cell 6, 269–279 (2000). [PubMed: 10983975]
100. Harding HP et al. The ribosomal P-stalk couples amino acid starvation to GCN2 activation in mammalian cells. eLife 8 (2019).
101. Dever TE et al. Phosphorylation of initiation factor 2α by protein kinase GCN2 mediates gene-specific translational control of *GCN4* in yeast. Cell 68, 585–596 (1992). [PubMed: 1739968]

102. Harding HP et al. Regulated Translation Initiation Controls Stress-Induced Gene Expression in Mammalian Cells. Molecular Cell 6, 1099–1108 (2000). [PubMed: 11106749]
103. Vattem KM & Wek RC Reinitiation involving upstream ORFs regulates *ATF4* mRNA translation in mammalian cells. Proceedings of the National Academy of Sciences of the United States of America 101, 11269 (2004). [PubMed: 15277680]
104. De Sousa-Coelho AL, Marrero PF & Haro D Activating transcription factor 4-dependent induction of FGF21 during amino acid deprivation. Biochem J 443, 165–171 (2012). [PubMed: 22233381]
105. Laeger T et al. Metabolic Responses to Dietary Protein Restriction Require an Increase in FGF21 that Is Delayed by the Absence of GCN2. Cell reports 16, 707–716 (2016). [PubMed: 27396336]
106. Averous J et al. GCN2 contributes to mTORC1 inhibition by leucine deprivation through an ATF4 independent mechanism. Scientific reports 6, 27698 (2016). [PubMed: 27297692]
107. Rousakis A et al. The general control nonderepressible-2 kinase mediates stress response and longevity induced by target of rapamycin inactivation in Caenorhabditis elegans. Aging Cell 12, 742–751 (2013). [PubMed: 23692540]
108. Nishimura T, Nakatake Y, Konishi M & Itoh N Identification of a novel FGF, FGF-21, preferentially expressed in the liver. Biochim Biophys Acta 1492, 203–206 (2000). [PubMed: 10858549]
109. Laeger T et al. FGF21 is an endocrine signal of protein restriction. J Clin Invest 124, 3913–3922 (2014). [PubMed: 25133427]
110. Kharitonenkov A et al. FGF-21 as a novel metabolic regulator. J Clin Invest 115, 1627–1635 (2005). [PubMed: 15902306]
111. Hill CM et al. FGF21 Signals Protein Status to the Brain and Adaptively Regulates Food Choice and Metabolism. Cell reports 27, 2934–2947 e2933 (2019). [PubMed: 31167139]
112. Fontana L et al. Decreased Consumption of Branched-Chain Amino Acids Improves Metabolic Health. Cell reports 16, 520–530 (2016). [PubMed: 27346343]
113. Thompson AC et al. Fibroblast growth factor 21 is not required for the reductions in circulating insulin-like growth factor-1 or global cell proliferation rates in response to moderate calorie restriction in adult mice. PLoS One 9, e111418 (2014). [PubMed: 25369265]
114. Zhang Y et al. The starvation hormone, fibroblast growth factor-21, extends lifespan in mice. eLife 1, e00065 (2012). [PubMed: 23066506]
115. Gaich G et al. The effects of LY2405319, an FGF21 analog, in obese human subjects with type 2 diabetes. Cell Metab 18, 333–340 (2013). [PubMed: 24011069]
116. Lee JH et al. An engineered FGF21 variant, LY2405319, can prevent non-alcoholic steatohepatitis by enhancing hepatic mitochondrial function. Am J Transl Res 8, 4750–4763 (2016). [PubMed: 27904677]
117. Ruhlmann C et al. Neuroprotective Effects of the FGF21 Analogue LY2405319. Journal of Alzheimer's disease : JAD 80, 357–369 (2021). [PubMed: 33554901]
118. Imai S & Guarente L NAD(+) and sirtuins in aging and disease. Trends Cell Biol. 24, 464–471 (2014). [PubMed: 24786309]
119. Lin SJ, Defossez PA & Guarente L Requirement of NAD and SIR2 for life-span extension by calorie restriction in Saccharomyces cerevisiae. Science 289, 2126–2128 (2000). [PubMed: 11000115]
120. Lamming DW et al. HST2 mediates SIR2-independent life-span extension by calorie restriction. Science 309, 1861–1864 (2005). [PubMed: 16051752]
121. Houtkooper RH, Pirinen E & Auwerx J Sirtuins as regulators of metabolism and healthspan. Nature Reviews Molecular Cell Biology 13, 225–238 (2012). [PubMed: 22395773]
122. Wood JG et al. Sirt4 is a mitochondrial regulator of metabolism and lifespan in Drosophila melanogaster. Proc Natl Acad Sci U S A 115, 1564–1569 (2018). [PubMed: 29378963]
123. Burnett C et al. Absence of effects of Sir2 overexpression on lifespan in C. elegans and Drosophila. Nature 477, 482–485 (2011). [PubMed: 21938067]
124. Haigis MC & Sinclair DA Mammalian sirtuins: biological insights and disease relevance. Annu Rev Pathol 5, 253–295 (2010). [PubMed: 20078221]

125. Eldridge MJG, Pereira JM, Impens F & Hamon MA Active nuclear import of the deacetylase Sirtuin-2 is controlled by its C-terminus and importins. Scientific reports 10, 2034 (2020). [PubMed: 32042025]
126. Haigis MC et al. SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic beta cells. Cell 126, 941–954 (2006). [PubMed: 16959573]
127. Mao Z et al. SIRT6 promotes DNA repair under stress by activating PARP1. Science 332, 1443–1446 (2011). [PubMed: 21680843]
128. Du J et al. Sirt5 Is a NAD-Dependent Protein Lysine Demalonylase and Desuccinylase. Science 334, 806 (2011). [PubMed: 22076378]
129. Peng C et al. The first identification of lysine malonylation substrates and its regulatory enzyme. Mol Cell Proteomics 10, M111 012658 (2011).
130. Feldman JL, Baeza J & Denu JM Activation of the protein deacetylase SIRT6 by long-chain fatty acids and widespread deacylation by mammalian sirtuins. J Biol Chem 288, 31350–31356 (2013). [PubMed: 24052263]
131. Anderson RM, Bitterman KJ, Wood JG, Medvedik O & Sinclair DA Nicotinamide and PNC1 govern lifespan extension by calorie restriction in Saccharomyces cerevisiae. Nature 423, 181–185 (2003). [PubMed: 12736687]
132. Medvedik O, Lamming DW, Kim KD & Sinclair DA MSN2 and MSN4 link calorie restriction and TOR to sirtuin-mediated lifespan extension in Saccharomyces cerevisiae. PLoS Biol 5, e261 (2007). [PubMed: 17914901]
133. Moroz N et al. Dietary restriction involves NAD(+) -dependent mechanisms and a shift toward oxidative metabolism. Aging Cell 13, 1075–1085 (2014). [PubMed: 25257342]
134. Yoshida M et al. Extracellular Vesicle-Contained eNAMPT Delays Aging and Extends Lifespan in Mice. Cell Metab 30, 329–342 e325 (2019). [PubMed: 31204283]
135. Covarrubias AJ, Perrone R, Grozio A & Verdin E NAD(+) metabolism and its roles in cellular processes during ageing. Nat Rev Mol Cell Biol 22, 119–141 (2021). [PubMed: 33353981]
136. Cohen HY et al. Calorie Restriction Promotes Mammalian Cell Survival by Inducing the SIRT1 Deacetylase. Science 305, 390 (2004). [PubMed: 15205477]
137. Civitarese AE et al. Calorie Restriction Increases Muscle Mitochondrial Biogenesis in Healthy Humans. PLOS Medicine 4, e76 (2007). [PubMed: 17341128]
138. Shi T, Wang F, Stieren E & Tong Q SIRT3, a Mitochondrial Sirtuin Deacetylase, Regulates Mitochondrial Function and Thermogenesis in Brown Adipocytes. Journal of Biological Chemistry 280, 13560–13567 (2005).
139. Nakagawa T, Lomb DJ, Haigis MC & Guarente L SIRT5 Deacetylates carbamoyl phosphate synthetase 1 and regulates the urea cycle. Cell 137, 560–570 (2009). [PubMed: 19410549]
140. Chalkiadaki A & Guarente L High-fat diet triggers inflammation-induced cleavage of SIRT1 in adipose tissue to promote metabolic dysfunction. Cell Metab 16, 180–188 (2012). [PubMed: 22883230]
141. Pedersen SB, Ølholm J, Paulsen SK, Bennetzen MF & Richelsen B Low Sirt1 expression, which is upregulated by fasting, in human adipose tissue from obese women. Int J Obes (Lond) 32, 1250–1255 (2008). [PubMed: 18560370]
142. Lutz MI, Milenkovic I, Regelsberger G & Kovacs GG Distinct patterns of sirtuin expression during progression of Alzheimer's disease. Neuromolecular Med 16, 405–414 (2014). [PubMed: 24464653]
143. Boily G et al. SirT1 Regulates Energy Metabolism and Response to Caloric Restriction in Mice. PLOS ONE 3, e1759 (2008). [PubMed: 18335035]
144. Bordone L et al. SIRT1 transgenic mice show phenotypes resembling calorie restriction. Aging Cell 6, 759–767 (2007). [PubMed: 17877786]
145. Herranz D et al. Sirt1 improves healthy ageing and protects from metabolic syndrome-associated cancer. Nature communications 1, 3 (2010).
146. Satoh A et al. Sirt1 extends life span and delays aging in mice through the regulation of Nk2 homeobox 1 in the DMH and LH. Cell metabolism 18, 416–430 (2013). [PubMed: 24011076]

147. Park SH et al. SIRT2 is a tumor suppressor that connects aging, acetylome, cell cycle signaling, and carcinogenesis. Transl Cancer Res 1, 15–21 (2012). [PubMed: 22943040]

148. North BJ et al. SIRT2 induces the checkpoint kinase BubR1 to increase lifespan. EMBO J 33, 1438–1453 (2014). [PubMed: 24825348]

149. Benigni A et al. Sirt3 Deficiency Shortens Life Span and Impairs Cardiac Mitochondrial Function Rescued by Opa1 Gene Transfer. Antioxid Redox Signal 31, 1255–1271 (2019). [PubMed: 31269804]

150. Brown K et al. SIRT3 reverses aging-associated degeneration. Cell reports 3, 319–327 (2013). [PubMed: 23375372]

151. Kawahara TL et al. SIRT6 links histone H3 lysine 9 deacetylation to NF-kappaB-dependent gene expression and organismal life span. Cell 136, 62–74 (2009). [PubMed: 19135889]

152. Vakhrusheva O et al. Sirt7 Increases Stress Resistance of Cardiomyocytes and Prevents Apoptosis and Inflammatory Cardiomyopathy in Mice. Circulation Research 102, 703–710 (2008). [PubMed: 18239138]

153. Kanfi Y et al. The sirtuin SIRT6 regulates lifespan in male mice. Nature 483, 218–221 (2012). [PubMed: 22367546]

154. Roichman A et al. SIRT6 Overexpression Improves Various Aspects of Mouse Healthspan. J Gerontol A Biol Sci Med Sci 72, 603–615 (2017). [PubMed: 27519885]

155. Sun S et al. Vascular endothelium-targeted Sirt7 gene therapy rejuvenates blood vessels and extends life span in a Hutchinson-Gilford progeria model. Sci Adv 6, eaay5556 (2020).

156. Anderson RM et al. Dynamic regulation of PGC-1alpha localization and turnover implicates mitochondrial adaptation in calorie restriction and the stress response. Aging Cell 7, 101–111 (2008). [PubMed: 18031569]

157. Rodgers JT et al. Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. Nature 434, 113–118 (2005). [PubMed: 15744310]

158. Wang RH et al. Hepatic Sirt1 deficiency in mice impairs mTorc2/Akt signaling and results in hyperglycemia, oxidative damage, and insulin resistance. J Clin Invest 121, 4477–4490 (2011). [PubMed: 21965330]

159. Menghini R et al. MicroRNA 217 Modulates Endothelial Cell Senescence via Silent Information Regulator 1. Circulation 120, 1524–1532 (2009). [PubMed: 19786632]

160. Ota H et al. Sirt1 modulates premature senescence-like phenotype in human endothelial cells. J Mol Cell Cardiol 43, 571–579 (2007). [PubMed: 17916362]

161. Hebert AS et al. Calorie restriction and SIRT3 trigger global reprogramming of the mitochondrial protein acetylome. Mol Cell 49, 186–199 (2013). [PubMed: 23201123]

162. Rose G et al. Variability of the SIRT3 gene, human silent information regulator Sir2 homologue, and survivorship in the elderly. Exp Gerontol 38, 1065–1070 (2003). [PubMed: 14580859]

163. Van Meter M et al. JNK Phosphorylates SIRT6 to Stimulate DNA Double-Strand Break Repair in Response to Oxidative Stress by Recruiting PARP1 to DNA Breaks. Cell reports 16, 2641–2650 (2016). [PubMed: 27568560]

164. Chen J et al. Sirt6 overexpression suppresses senescence and apoptosis of nucleus pulposus cells by inducing autophagy in a model of intervertebral disc degeneration. Cell Death & Disease 9, 56 (2018). [PubMed: 29352194]

165. Simon M et al. LINE1 Derepression in Aged Wild-Type and SIRT6-Deficient Mice Drives Inflammation. Cell Metab 29, 871–885 e875 (2019). [PubMed: 30853213]

166. Salmon AB, Richardson A & Perez VI Update on the oxidative stress theory of aging: does oxidative stress play a role in aging or healthy aging? Free Radic Biol Med 48, 642–655 (2010). [PubMed: 20036736]

167. Schriner SE et al. Extension of murine life span by overexpression of catalase targeted to mitochondria. Science 308, 1909–1911 (2005). [PubMed: 15879174]

168. Elchuri S et al. CuZnSOD deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later in life. Oncogene 24, 367–380 (2005). [PubMed: 15531919]

169. Sentman ML et al. Phenotypes of mice lacking extracellular superoxide dismutase and copper- and zinc-containing superoxide dismutase. J Biol Chem 281, 6904–6909 (2006). [PubMed: 16377630]

170. Van Remmen H et al. Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. Physiological genomics 16, 29–37 (2003). [PubMed: 14679299]

171. Zhang Y et al. Mice deficient in both Mn superoxide dismutase and glutathione peroxidase-1 have increased oxidative damage and a greater incidence of pathology but no reduction in longevity. J Gerontol A Biol Sci Med Sci 64, 1212–1220 (2009). [PubMed: 19776219]

172. Salmon AB et al. Lack of methionine sulfoxide reductase A in mice increases sensitivity to oxidative stress but does not diminish life span. FASEB J 23, 3601–3608 (2009). [PubMed: 19487311]

173. Van Remmen H et al. Multiple deficiencies in antioxidant enzymes in mice result in a compound increase in sensitivity to oxidative stress. Free Radic Biol Med 36, 1625–1634 (2004). [PubMed: 15182862]

174. Schulz TJ et al. Glucose restriction extends Caenorhabditis elegans life span by inducing mitochondrial respiration and increasing oxidative stress. Cell Metab 6, 280–293 (2007). [PubMed: 17908557]

175. Gwinn DM et al. AMPK phosphorylation of raptor mediates a metabolic checkpoint. Mol Cell 30, 214–226 (2008). [PubMed: 18439900]

176. Inoki K, Zhu T & Guan KL TSC2 mediates cellular energy response to control cell growth and survival. Cell 115, 577–590 (2003). [PubMed: 14651849]

177. Weimer S et al. D-Glucosamine supplementation extends life span of nematodes and of ageing mice. Nature communications 5, 3563 (2014).

178. Ristow M et al. Antioxidants prevent health-promoting effects of physical exercise in humans. Proc Natl Acad Sci U S A 106, 8665–8670 (2009). [PubMed: 19433800]

179. Bjornsen T et al. Vitamin C and E supplementation blunts increases in total lean body mass in elderly men after strength training. Scand J Med Sci Sports 26, 755–763 (2016). [PubMed: 26129928]

180. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG & Gluud C Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. The Cochrane database of systematic reviews, Cd007176 (2012).

181. Fontana L & Partridge L Promoting health and longevity through diet: from model organisms to humans. Cell 161, 106–118 (2015). [PubMed: 25815989]

182. Green CL & Lamming DW Regulation of metabolic health by essential dietary amino acids. Mech Ageing Dev 177, 186–200 (2019). [PubMed: 30044947]

183. Soultoukis GA & Partridge L Dietary Protein, Metabolism, and Aging. Annual review of biochemistry 85, 5–34 (2016).

184. Mair W, Piper MD & Partridge L Calories do not explain extension of life span by dietary restriction in Drosophila. PLoS Biol 3, e223 (2005). [PubMed: 16000018]

185. Grandison RC, Piper MD & Partridge L Amino-acid imbalance explains extension of lifespan by dietary restriction in Drosophila. Nature 462, 1061–1064 (2009). [PubMed: 19956092]

186. Solon-Biet SM et al. The ratio of macronutrients, not caloric intake, dictates cardiometabolic health, aging, and longevity in ad libitum-fed mice. Cell Metab 19, 418–430 (2014). [PubMed: 24606899]

187. Richardson NE et al. Lifelong restriction of dietary branched-chain amino acids has sex-specific benefits for frailty and life span in mice. Nature Aging 1, 73–86 (2021). [PubMed: 33796866]

188. Speakman JR, Mitchell SE & Mazidi M Calories or protein? The effect of dietary restriction on lifespan in rodents is explained by calories alone. Exp Gerontol 86, 28–38 (2016). [PubMed: 27006163]

189. Mitchell SE et al. The effects of graded levels of calorie restriction: I. impact of short term calorie and protein restriction on body composition in the C57BL/6 mouse. Oncotarget 6, 15902–15930 (2015). [PubMed: 26079539]

190. Mitchell SE et al. The effects of graded levels of calorie restriction: II. Impact of short term calorie and protein restriction on circulating hormone levels, glucose homeostasis and oxidative stress in male C57BL/6 mice. Oncotarget 6, 23213–23237 (2015). [PubMed: 26061745]
191. Gardner CD, Hartle JC, Garrett RD, Offringa LC & Wasserman AS Maximizing the intersection of human health and the health of the environment with regard to the amount and type of protein produced and consumed in the United States. Nutr Rev 77, 197–215 (2019). [PubMed: 30726996]
192. Mittendorfer B, Klein S & Fontana L A word of caution against excessive protein intake. Nature reviews. Endocrinology 16, 59–66 (2020).
193. Smith GI et al. High-Protein Intake during Weight Loss Therapy Eliminates the Weight-Loss-Induced Improvement in Insulin Action in Obese Postmenopausal Women. Cell reports 17, 849–861 (2016). [PubMed: 27732859]
194. Weber M et al. Lower protein content in infant formula reduces BMI and obesity risk at school age: follow-up of a randomized trial. Am J Clin Nutr 99, 1041–1051 (2014). [PubMed: 24622805]
195. Willcox BJ et al. in Healthy Aging and Longevity Vol. 1114 Annals of the New York Academy of Sciences (eds Weller NJ & Rattan SIS) 434–455 (2007).
196. Lamming DW et al. Restriction of dietary protein decreases mTORC1 in tumors and somatic tissues of a tumor-bearing mouse xenograft model. Oncotarget 6, 31233–31240 (2015). [PubMed: 26378060]
197. Hill CM et al. Low protein-induced increases in FGF21 drive UCP1-dependent metabolic but not thermoregulatory endpoints. Scientific reports 7, 8209 (2017). [PubMed: 28811495]
198. Orentreich N, Matias JR, DeFelice A & Zimmerman JA Low methionine ingestion by rats extends life span. The Journal of nutrition 123, 269–274 (1993). [PubMed: 8429371]
199. Miller RA et al. Methionine-deficient diet extends mouse lifespan, slows immune and lens aging, alters glucose, T4, IGF-I and insulin levels, and increases hepatocyte MIF levels and stress resistance. Aging Cell 4, 119–125 (2005). [PubMed: 15924568]
200. Mazor KM et al. Effects of single amino acid deficiency on mRNA translation are markedly different for methionine versus leucine. Scientific reports 8, 8076 (2018). [PubMed: 29795412]
201. Lees EK et al. Methionine restriction restores a younger metabolic phenotype in adult mice with alterations in fibroblast growth factor 21. Aging Cell 13, 817–827 (2014). [PubMed: 24935677]
202. Perrone CE et al. Genomic and metabolic responses to methionine-restricted and methionine-restricted, cysteine-supplemented diets in Fischer 344 rat inguinal adipose tissue, liver and quadriceps muscle. J Nutrigenet Nutrigenomics 5, 132–157 (2012). [PubMed: 23052097]
203. Yu D et al. Short-term methionine deprivation improves metabolic health via sexually dimorphic, mTORC1-independent mechanisms. FASEB J 32, 3471–3482 (2018). [PubMed: 29401631]
204. Douris N et al. Central Fibroblast Growth Factor 21 Browns White Fat via Sympathetic Action in Male Mice. Endocrinology 156, 2470–2481 (2015). [PubMed: 25924103]
205. Wanders D et al. Role of GCN2-Independent Signaling Through a Noncanonical PERK/NRF2 Pathway in the Physiological Responses to Dietary Methionine Restriction. Diabetes 65, 1499–1510 (2016). [PubMed: 26936965]
206. Elshorbagy AK et al. Cysteine supplementation reverses methionine restriction effects on rat adiposity: significance of stearoyl-coenzyme A desaturase. J Lipid Res 52, 104–112 (2011). [PubMed: 20871132]
207. Hine C & Mitchell JR Calorie restriction and methionine restriction in control of endogenous hydrogen sulfide production by the transsulfuration pathway. Exp Gerontol 68, 26–32 (2015). [PubMed: 25523462]
208. Mattocks DA et al. Short term methionine restriction increases hepatic global DNA methylation in adult but not young male C57BL/6J mice. Exp Gerontol 88, 1–8 (2017). [PubMed: 27940170]
209. Haws SA, Leech CM & Denu JM Metabolism and the Epigenome: A Dynamic Relationship. Trends Biochem Sci (2020).
210. Haws SA et al. Methyl-Metabolite Depletion Elicits Adaptive Responses to Support Heterochromatin Stability and Epigenetic Persistence. Mol Cell 78, 210–223 e218 (2020). [PubMed: 32208170]

211. Lees EK et al. Direct comparison of methionine restriction with leucine restriction on the metabolic health of C57BL/6J mice. Scientific reports 7, 9977 (2017). [PubMed: 28855637]

212. Das A et al. Impairment of an Endothelial NAD(+)-H2S Signaling Network Is a Reversible Cause of Vascular Aging. Cell 176, 944–945 (2019). [PubMed: 30735637]

213. Hine C et al. Endogenous hydrogen sulfide production is essential for dietary restriction benefits. Cell 160, 132–144 (2015). [PubMed: 25542313]

214. Wang SY et al. Methionine restriction delays senescence and suppresses the senescence-associated secretory phenotype in the kidney through endogenous hydrogen sulfide. Cell Cycle 18, 1573–1587 (2019). [PubMed: 31164038]

215. Ogawa T et al. Stimulating S-adenosyl-l-methionine synthesis extends lifespan via activation of AMPK. Proc Natl Acad Sci U S A 113, 11913–11918 (2016). [PubMed: 27698120]

216. Gu X et al. SAMTOR is an S-adenosylmethionine sensor for the mTORC1 pathway. Science 358, 813–818 (2017). [PubMed: 29123071]

217. Cummings NE et al. Restoration of metabolic health by decreased consumption of branched-chain amino acids. The Journal of physiology 596, 623–645 (2018). [PubMed: 29266268]

218. Yu D et al. The adverse metabolic effects of branched-chain amino acids are mediated by isoleucine and valine. Cell Metab 33, 905–922 e906 (2021). [PubMed: 33887198]

219. Solon-Biet SM et al. Branched chain amino acids impact health and lifespan indirectly via amino acid balance and appetite control. Nat Metab 1, 532–545 (2019). [PubMed: 31656947]

220. Newgard CB et al. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metab 9, 311–326 (2009). [PubMed: 19356713]

221. Maida A et al. Repletion of branched chain amino acids reverses mTORC1 signaling but not improved metabolism during dietary protein dilution. Molecular metabolism 6, 873–881 (2017). [PubMed: 28752051]

222. Wijeyesekera A et al. Metabotyping of Long-Lived Mice using 1H NMR Spectroscopy. Journal of Proteome Research 11, 2224–2235 (2012). [PubMed: 22225495]

223. Juricic P, Gronke S & Partridge L Branched-Chain Amino Acids Have Equivalent Effects to Other Essential Amino Acids on Lifespan and Aging-Related Traits in Drosophila. J Gerontol A Biol Sci Med Sci 75, 24–31 (2020). [PubMed: 30891588]

224. Ooka H, Segall PE & Timiras PS Histology and survival in age-delayed low-tryptophan-fed rats. Mech Ageing Dev 43, 79–98 (1988). [PubMed: 3374178]

225. Segall PE & Timiras PS Patho-physiologic findings after chronic tryptophan deficiency in rats: a model for delayed growth and aging. Mech Ageing Dev 5, 109–124 (1976). [PubMed: 933560]

226. De Marte ML & Enesco HE Influence of low tryptophan diet on survival and organ growth in mice. Mech Ageing Dev 36, 161–171 (1986). [PubMed: 3784629]

227. He C et al. Enhanced longevity by ibuprofen, conserved in multiple species, occurs in yeast through inhibition of tryptophan import. PLoS Genet 10, e1004860 (2014). [PubMed: 25521617]

228. Imai S, Armstrong CM, Kaeberlein M & Guarente L Transcriptional silencing and longevity protein Sir2 is an NAD- dependent histone deacetylase. Nature 403, 795–800 (2000). [PubMed: 10693811]

229. Chen T et al. Tryptophan Predicts the Risk for Future Type 2 Diabetes. PLoS One 11, e0162192 (2016). [PubMed: 27598004]

230. Ramos-Chavez LA et al. Low Serum Tryptophan Levels as an Indicator of Global Cognitive Performance in Nondemented Women over 50 Years of Age. Oxid Med Cell Longev 2018, 8604718 (2018). [PubMed: 30584466]

231. Yap YW et al. Restriction of essential amino acids dictates the systemic metabolic response to dietary protein dilution. Nature communications 11, 2894 (2020).

232. Ravichandran M et al. Impairing L-Threonine Catabolism Promotes Healthspan through Methylglyoxal-Mediated Proteohormesis. Cell Metab 27, 914–925 e915 (2018). [PubMed: 29551589]

233. Acosta-Rodriguez VA, de Groot MHM, Rijo-Ferreira F, Green CB & Takahashi JS Mice under Caloric Restriction Self-Impose a Temporal Restriction of Food Intake as Revealed by an Automated Feeder System. Cell Metab 26, 267–277 e262 (2017). [PubMed: 28683292]

234. Bruss MD, Khambatta CF, Ruby MA, Aggarwal I & Hellerstein MK Calorie restriction increases fatty acid synthesis and whole body fat oxidation rates. Am J Physiol Endocrinol Metab 298, E108–116 (2010). [PubMed: 19887594]

235. Hatori M et al. Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. Cell Metab 15, 848–860 (2012). [PubMed: 22608008]

236. Chaix A, Zarrinpar A, Miu P & Panda S Time-restricted feeding is a preventative and therapeutic intervention against diverse nutritional challenges. Cell Metab 20, 991–1005 (2014). [PubMed: 25470547]

237. Mitchell SJ et al. Daily Fasting Improves Health and Survival in Male Mice Independent of Diet Composition and Calories. Cell Metab 29, 221–228 e223 (2019). [PubMed: 30197301]

238. Pak HH et al. Distinct roles of fasting and calories in the metabolic, molecular, and geroprotective effects of a calorie restricted diet. Nature Metabolism (2021, in press).

239. Trepanowski JF et al. Effect of Alternate-Day Fasting on Weight Loss, Weight Maintenance, and Cardioprotection Among Metabolically Healthy Obese Adults: A Randomized Clinical Trial. JAMA Intern Med 177, 930–938 (2017). [PubMed: 28459931]

240. Griffin NW et al. Prior Dietary Practices and Connections to a Human Gut Microbial Metacommunity Alter Responses to Diet Interventions. Cell Host Microbe 21, 84–96 (2017). [PubMed: 28041931]

241. Dey N et al. Regulators of gut motility revealed by a gnotobiotic model of diet-microbiome interactions related to travel. Cell 163, 95–107 (2015). [PubMed: 26406373]

242. Carter S, Clifton PM & Keogh JB The effects of intermittent compared to continuous energy restriction on glycaemic control in type 2 diabetes; a pragmatic pilot trial. Diabetes Res Clin Pract 122, 106–112 (2016). [PubMed: 27833048]

243. Goodrick CL, Ingram DK, Reynolds MA, Freeman JR & Cider N Effects of intermittent feeding upon body weight and lifespan in inbred mice: interaction of genotype and age. Mech Ageing Dev 55, 69–87 (1990). [PubMed: 2402168]

244. Mattson MP, Longo VD & Harvie M Impact of intermittent fasting on health and disease processes. Ageing research reviews 39, 46–58 (2017). [PubMed: 27810402]

245. Caderni G, Perrelli MG, Cecchini F & Tessitore L Enhanced growth of colorectal aberrant crypt foci in fasted/refed rats involves changes in TGFbeta1 and p21CIP expressions. Carcinogenesis 23, 323–327 (2002). [PubMed: 11872640]

246. Tomasi C et al. Effect of fasting/refeeding on the incidence of chemically induced hepatocellular carcinoma in the rat. Carcinogenesis 20, 1979–1983 (1999). [PubMed: 10506114]

247. Tessitore L & Bollito E Early induction of TGF-beta1 through a fasting-re-feeding regimen promotes liver carcinogenesis by a sub-initiating dose of diethylnitrosamine. Cell Prolif 39, 105–116 (2006). [PubMed: 16542346]

248. Yang W et al. Alternate-day fasting protects the livers of mice against high-fat diet-induced inflammation associated with the suppression of Toll-like receptor 4/nuclear factor kappaB signaling. Nutr Res 36, 586–593 (2016). [PubMed: 27188904]

249. Bagherniya M, Butler AE, Barreto GE & Sahebkar A The effect of fasting or calorie restriction on autophagy induction: A review of the literature. Ageing Res Rev 47, 183–197 (2018). [PubMed: 30172870]

250. Mihaylova MM et al. Fasting Activates Fatty Acid Oxidation to Enhance Intestinal Stem Cell Function during Homeostasis and Aging. Cell Stem Cell 22, 769–778 e764 (2018). [PubMed: 29727683]

251. Li G et al. Intermittent Fasting Promotes White Adipose Browning and Decreases Obesity by Shaping the Gut Microbiota. Cell metabolism 26, 672–685.e674 (2017). [PubMed: 28918936]

252. Liu B, Page AJ, Hutchison AT, Wittert GA & Heilbronn LK Intermittent fasting increases energy expenditure and promotes adipose tissue browning in mice. Nutrition (Burbank, Los Angeles County, Calif.) 66, 38–43 (2019).

253. Kimura I et al. Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). Proc Natl Acad Sci U S A 108, 8030–8035 (2011). [PubMed: 21518883]

254. Taggart AK et al. (D)-beta-Hydroxybutyrate inhibits adipocyte lipolysis via the nicotinic acid receptor PUMA-G. The Journal of biological chemistry 280, 26649–26652 (2005). [PubMed: 15929991]

255. Shimazu T et al. Suppression of oxidative stress by β-hydroxybutyrate, an endogenous histone deacetylase inhibitor. Science (New York, N.Y.) 339, 211–214 (2013).

256. Schreiber RA & Yeh YY Temporal changes in plasma levels and metabolism of ketone bodies by liver and brain after ethanol and/or starvation in C57BL/6J mice. Drug Alcohol Depend 13, 151–160 (1984). [PubMed: 6723514]

257. Haymond MW, Karl IE, Clarke WL, Pagliara AS & Santiago JV Differences in circulating gluconeogenic substrates during short-term fasting in men, women, and children. Metabolism 31, 33–42 (1982). [PubMed: 7043160]

258. Cahill GF Jr. et al. Hormone-fuel interrelationships during fasting. The Journal of clinical investigation 45, 1751–1769 (1966). [PubMed: 5926444]

259. Mattson MP & Arumugam TV Hallmarks of Brain Aging: Adaptive and Pathological Modification by Metabolic States. Cell metabolism 27, 1176–1199 (2018). [PubMed: 29874566]

260. Schübel R et al. Effects of intermittent and continuous calorie restriction on body weight and metabolism over 50 wk: a randomized controlled trial. The American journal of clinical nutrition 108, 933–945 (2018). [PubMed: 30475957]

261. Catenacci VA et al. A randomized pilot study comparing zero-calorie alternate-day fasting to daily caloric restriction in adults with obesity. Obesity (Silver Spring, Md.) 24, 1874–1883 (2016).

262. Hutchison AT et al. Effects of Intermittent Versus Continuous Energy Intakes on Insulin Sensitivity and Metabolic Risk in Women with Overweight. Obesity (Silver Spring) 27, 50–58 (2019). [PubMed: 30569640]

263. Brandhorst S et al. A Periodic Diet that Mimics Fasting Promotes Multi-System Regeneration, Enhanced Cognitive Performance, and Healthspan. Cell Metab 22, 86–99 (2015). [PubMed: 26094889]

264. Choi In Y. et al. A Diet Mimicking Fasting Promotes Regeneration and Reduces Autoimmunity and Multiple Sclerosis Symptoms. Cell reports 15, 2136–2146 (2016). [PubMed: 27239035]

265. Cheng CW et al. Prolonged fasting reduces IGF-1/PKA to promote hematopoietic-stem-cell-based regeneration and reverse immunosuppression. Cell Stem Cell 14, 810–823 (2014). [PubMed: 24905167]

266. Cheng C-W et al. Fasting-Mimicking Diet Promotes Ngn3-Driven β-Cell Regeneration to Reverse Diabetes. Cell 168, 775–788.e712 (2017). [PubMed: 28235195]

267. Raffaghello L et al. Fasting and differential chemotherapy protection in patients. Cell Cycle 9, 4474–4476 (2010). [PubMed: 21088487]

268. Lee C et al. Fasting cycles retard growth of tumors and sensitize a range of cancer cell types to chemotherapy. Sci Transl Med 4, 124ra127 (2012).

269. Caffa I et al. Fasting-mimicking diet and hormone therapy induce breast cancer regression. Nature 583, 620–624 (2020). [PubMed: 32669709]

270. Di Biase S et al. Fasting-Mimicking Diet Reduces HO-1 to Promote T Cell-Mediated Tumor Cytotoxicity. Cancer cell 30, 136–146 (2016). [PubMed: 27411588]

271. de Groot S et al. Fasting mimicking diet as an adjunct to neoadjuvant chemotherapy for breast cancer in the multicentre randomized phase 2 DIRECT trial. Nature communications 11, 3083 (2020).

272. Bauersfeld SP et al. The effects of short-term fasting on quality of life and tolerance to chemotherapy in patients with breast and ovarian cancer: a randomized cross-over pilot study. BMC cancer 18, 476 (2018). [PubMed: 29699509]

273. Safdie FM et al. Fasting and cancer treatment in humans: A case series report. Aging 1, 988 (2009). [PubMed: 20157582]

274. Mattson MP et al. Meal frequency and timing in health and disease. Proceedings of the National Academy of Sciences 111, 16647–16653 (2014).

275. Kavanagh K, Bashore AC, Davis M, Sherrill C & Parks J EARLY TIME RESTRICTED FEEDING IMPROVES HIGH DENSITY LIPOPROTEIN FUNCTION IN GERIATRIC MONKEYS. Innovation in Aging 3, S104 – S104 (2019).

276. Turek FW et al. Obesity and metabolic syndrome in circadian Clock mutant mice. Science (New York, N.Y.) 308, 1043–1045 (2005).

277. Rudic RD et al. BMAL1 and CLOCK, two essential components of the circadian clock, are involved in glucose homeostasis. PLoS Biol 2, e377 (2004). [PubMed: 15523558]

278. Arble DM, Bass J, Laposky AD, Vitaterna MH & Turek FW Circadian timing of food intake contributes to weight gain. Obesity (Silver Spring) 17, 2100–2102 (2009). [PubMed: 19730426]

279. Jakubowicz D, Barnea M, Wainstein J & Froy O Effects of caloric intake timing on insulin resistance and hyperandrogenism in lean women with polycystic ovary syndrome. Clin Sci (Lond) 125, 423–432 (2013). [PubMed: 23688334]

280. Sutton EF et al. Early Time-Restricted Feeding Improves Insulin Sensitivity, Blood Pressure, and Oxidative Stress Even without Weight Loss in Men with Prediabetes. Cell metabolism 27, 1212–1221.e1213 (2018). [PubMed: 29754952]

281. St-Onge MP et al. Meal Timing and Frequency: Implications for Cardiovascular Disease Prevention: A Scientific Statement From the American Heart Association. Circulation 135, e96–e121 (2017). [PubMed: 28137935]

282. Lowe DA et al. Effects of Time-Restricted Eating on Weight Loss and Other Metabolic Parameters in Women and Men With Overweight and Obesity: The TREAT Randomized Clinical Trial. JAMA Internal Medicine 180, 1491–1499 (2020). [PubMed: 32986097]

283. Mindikoglu AL et al. Intermittent fasting from dawn to sunset for 30 consecutive days is associated with anticancer proteomic signature and upregulates key regulatory proteins of glucose and lipid metabolism, circadian clock, DNA repair, cytoskeleton remodeling, immune system and cognitive function in healthy subjects. Journal of Proteomics 217, 103645 (2020). [PubMed: 31927066]

284. Fontana L, Fasano A, Chong YS, Vineis P & Willett WC Transdisciplinary research and clinical priorities for better health. PLoS Med 18, e1003699 (2021). [PubMed: 34314418]

285. Flurkey K, Papaconstantinou J, Miller RA & Harrison DE Lifespan extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production. Proceedings of the National Academy of Sciences 98, 6736 (2001).

286. Flurkey K, Papaconstantinou J & Harrison DE The Snell dwarf mutation Pit1(dw) can increase life span in mice. Mech Ageing Dev 123, 121–130 (2002). [PubMed: 11718806]

287. Bartke A et al. Extending the lifespan of long-lived mice. Nature 414, 412 (2001). [PubMed: 11719795]

288. Bartke A et al. Effects of Soy-derived diets on plasma and liver lipids, glucose tolerance, and longevity in normal, long-lived and short-lived mice. Horm Metab Res 36, 550–558 (2004). [PubMed: 15326565]

289. Ikeno Y, Bronson RT, Hubbard GB, Lee S & Bartke A Delayed occurrence of fatal neoplastic diseases in ames dwarf mice: correlation to extended longevity. J Gerontol A Biol Sci Med Sci 58, 291–296 (2003). [PubMed: 12663691]

290. Bartke A, Chandrashekar V, Bailey B, Zaczek D & Turyn D Consequences of growth hormone (GH) overexpression and GH resistance. Neuropeptides 36, 201–208 (2002). [PubMed: 12359510]

291. Coschigano KT et al. 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 144, 3799–3810 (2003). [PubMed: 12933651]

292. Yan L et al. Type 5 Adenylyl Cyclase Disruption Increases Longevity and Protects Against Stress. Cell 130, 247–258 (2007). [PubMed: 17662940]

293. Zhang HM, Diaz V, Walsh ME & Zhang Y Moderate lifelong overexpression of tuberous sclerosis complex 1 (TSC1) improves health and survival in mice. Scientific reports 7, 834 (2017). [PubMed: 28400571]

294. Miskin R & Masos T Transgenic mice overexpressing urokinase-type plasminogen activator in the brain exhibit reduced food consumption, body weight and size, and increased longevity. J Gerontol A Biol Sci Med Sci 52, B118–124 (1997). [PubMed: 9060969]

295. Nojima A et al. Haploinsufficiency of akt1 prolongs the lifespan of mice. PLoS One 8, e69178 (2013). [PubMed: 23935948]

296. Uneda K et al. Angiotensin II Type 1 Receptor-Associated Protein Regulates Kidney Aging and Lifespan Independent of Angiotensin. Journal of the American Heart Association 6, e006120. [PubMed: 28751545]

297. Meng J & Ferguson SM GATOR1-dependent recruitment of FLCN-FNIP to lysosomes coordinates Rag GTPase heterodimer nucleotide status in response to amino acids. J Cell Biol 217, 2765–2776 (2018). [PubMed: 29848618]

298. Petit CS, Roczniak-Ferguson A & Ferguson SM Recruitment of folliculin to lysosomes supports the amino acid-dependent activation of Rag GTPases. J Cell Biol 202, 1107–1122 (2013). [PubMed: 24081491]

299. Wu X et al. FLCN Maintains the Leucine Level in Lysosome to Stimulate mTORC1. PLoS One 11, e0157100 (2016). [PubMed: 27280402]

300. Martinez-Carreres L et al. CDK4 Regulates Lysosomal Function and mTORC1 Activation to Promote Cancer Cell Survival. Cancer Res 79, 5245–5259 (2019). [PubMed: 31395606]

301. Hesketh GG et al. The GATOR-Rag GTPase pathway inhibits mTORC1 activation by lysosome-derived amino acids. Science 370, 351–356 (2020). [PubMed: 33060361]

302. Efeyan A et al. Regulation of mTORC1 by the Rag GTPases is necessary for neonatal autophagy and survival. Nature 493, 679–683 (2013). [PubMed: 23263183]

303. Wolfson RL et al. KICSTOR recruits GATOR1 to the lysosome and is necessary for nutrients to regulate mTORC1. Nature 543, 438–442 (2017). [PubMed: 28199306]

304. Orozco JM et al. Dihydroxyacetone phosphate signals glucose availability to mTORC1. Nat Metab (2020).

305. Yang H et al. Mechanisms of mTORC1 activation by RHEB and inhibition by PRAS40. Nature 552, 368–373 (2017). [PubMed: 29236692]

306. Dibble CC et al. TBC1D7 is a third subunit of the TSC1-TSC2 complex upstream of mTORC1. Mol Cell 47, 535–546 (2012). [PubMed: 22795129]

307. Yang S et al. The Rag GTPase Regulates the Dynamic Behavior of TSC Downstream of Both Amino Acid and Growth Factor Restriction. Dev Cell (2020).

308. Budanov AV & Karin M p53 target genes sestrin1 and sestrin2 connect genotoxic stress and mTOR signaling. Cell 134, 451–460 (2008). [PubMed: 18692468]

Box 1:

Regulation of mTORC1 Activity by Amino Acids and Growth Factors

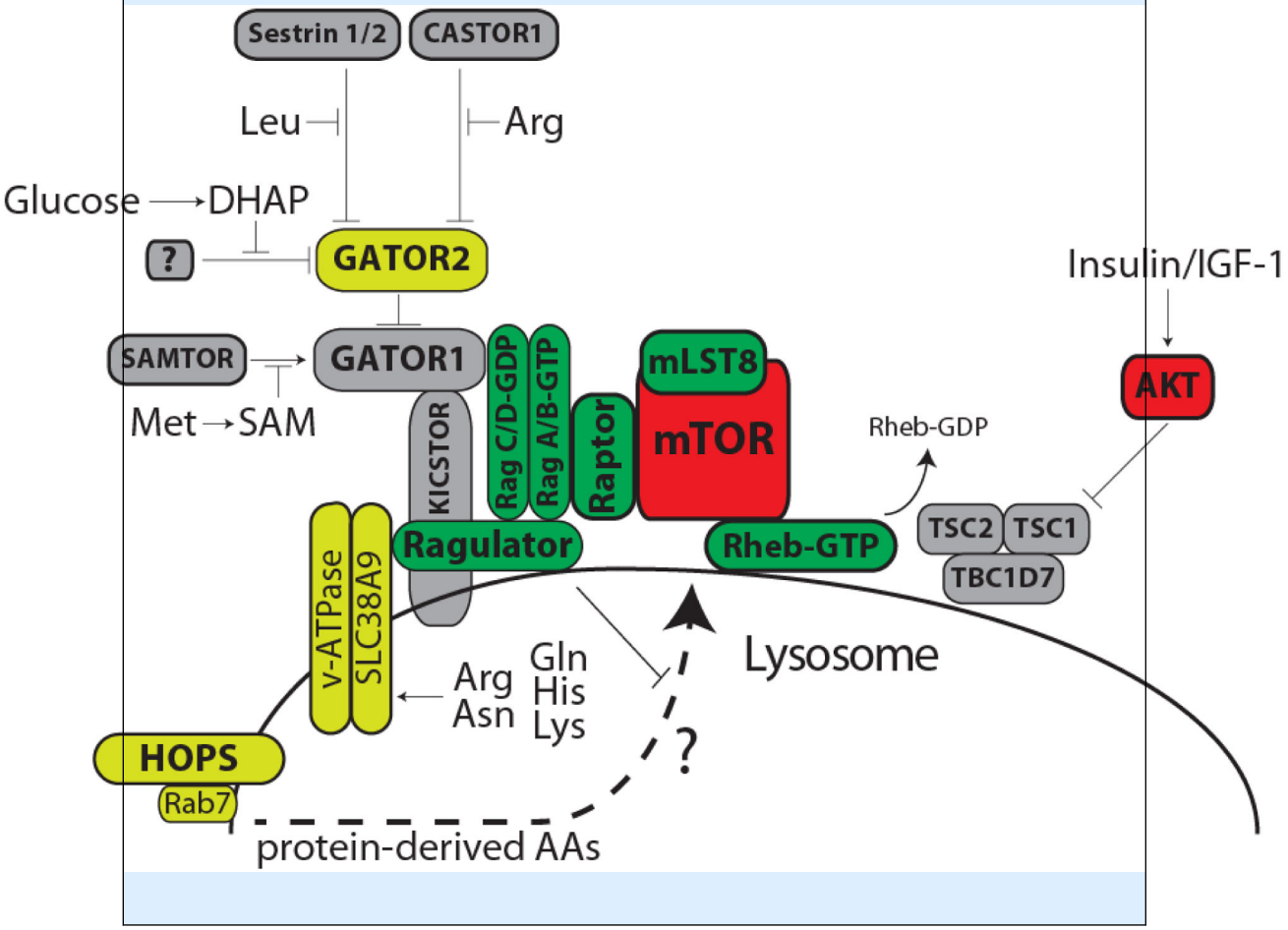
mTORC1 is recruited to the lysosomal surface by heterodimers of the Rag family of small GTPases, which interact with the mTORC1 subunit Raptor when RagA/RagB is bound to GTP and RagC/RagD is bound to GDP (see the figure). The nucleotide binding state of the Rags is controlled by amino acids via several different protein complexes with guanine nucleotide exchange factor (GEF) or GTPase activating protein (GAP) activity, each of which is sensitive to different amino acids. These complexes include the Ragulator, a GEF activity for RagA and RagB; GATOR1, a GAP for RagA and RagB, and the FLCN complex, a GAP for Rag C and Rag D. Despite the massive amount of work already done to define the molecular mechanisms by which nutrients and environmental cues act to regulate mTORC1, more continues to be discovered, and key recent discoveries are highlighted below.

The GEF activity of Ragulator is modulated by multiple amino acids via lysosmal v-ATPase and SLC38A9. Specific sensors for the amino acids leucine (SESTRIN1, SESTRIN2, and SESTRIN3) and arginine (CASTOR1 and CASTOR2) have been identified; when levels of the sensed amino acids are low, these proteins inhibit GATOR2 activity to indirectly modulate the GAP activity of GATOR1 towards RagA and RagB. SAMTOR is a recently discovered indirect methionine sensor for mTORC1; SAMTOR regulates GATOR1 activity in response to levels of the methionine metabolite S-adenosylmethionine (SAM)[216], which is extremely responsive to methionine levels both in cell culture and *in vivo*[210]. Another recent finding was the discovery that GATOR1 action upon RagA/B allows the recruitment of the FLCN complex to the lysosome[297,298]. At the lysosome, FLCN acts to preserve lysosomal levels of leucine and mTORC1 activity by blocking accumulation of PAT1, a lysosomal amino acid transporter[299]; phosphorylation of FLCN by CDK4 is required for FLCN to depart the lysosome and allow the Rags to recruit mTORC1[300]. Finally, a new study suggests that while the RagGTPases are critical for the sensing of exogenous amino acids, lysosomal-derived amino acids activate mTORC1 via a RagGTPase-independent mechanism that requires the homotypic fusion and vacuole protein sorting (HOPS) tethering complex[301]. Surprisingly, in the context of lysosomal derived amino acids the Rag-GATOR pathway acts as a negative regulator of mTORC1. A detailed mechanism for the regulation of mTORC1 by lysosomal-derived amino acids remains to be determined.

The Rags also play a role in glucose sensing by mTORC1[302,303]. Glucose itself is not sensed by mTORC1; instead, the glycolytic intermediate dihydroxyacetone phosphate (DHAP) is detected via a GATOR2 and GATOR1 dependent mechanism[304]. The precise molecular sensor of DHAP that modulates GATOR activity and the nucleotide-loading status of the Rags remains unknown.

At the lysosome, mTORC1 is activated by the Rheb-GTPase, which binds to the mTOR protein kinase and allosterically realigns the kinase-site residues, activating its ability to phosphorylates substrates[305]; disruption of this interaction inhibits mTORC1 signaling[83]. The nucleotide loading status of Rheb is controlled by the Tuberous Sclerosis Complex

(TSC, which comprises TSC1, TSC2 and TBC1D7[306]), which acts as a GAP for Rheb; the activity of TSC is controlled by many different kinases, which phosphorylate different residues and proteins within the TSC complex[56]. Intriguingly, the RagGTPases help to recruit TSC to lysosomes in response to amino acid or growth factor restriction[307]. Many details around this process are unknown, but GATOR2 seems to act as a regulator of TSC2 phosphorylation via this process, and Sestrin2 has likewise been implicated in the phosphorylation of TSC2[308].



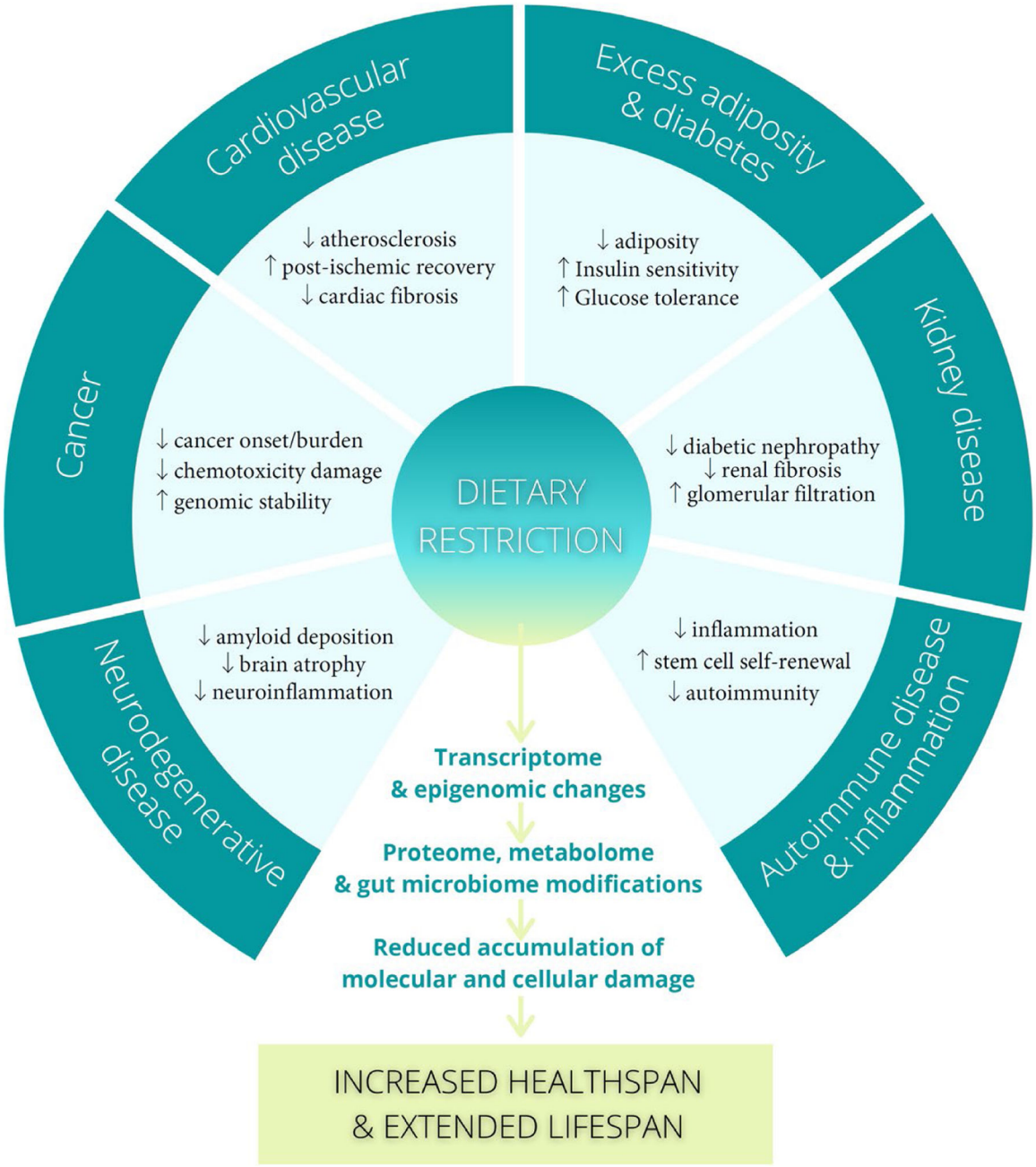


Figure 1 |. The Hallmarks of dietary restriction.

This schematic enumerates the proven biological adaptations induced by dietary restriction (DR) that have a protective effect against ageing-related pathologies and diseases across rodents, non-human primates, and humans. These protective effects include the prevention of obesity and diabetes, cardiovascular disease, cancer, kidney disease, autoimmune and inflammatory conditions and cancer, leading to increased healthspan and lifespan. It is not yet clear what combination of transcriptional, epigenetic, proteomic, metabolomic, and microbiota changes drive such benefits of DR on healthspan and lifespan. Relevant references can be found in Supplementary information 1.

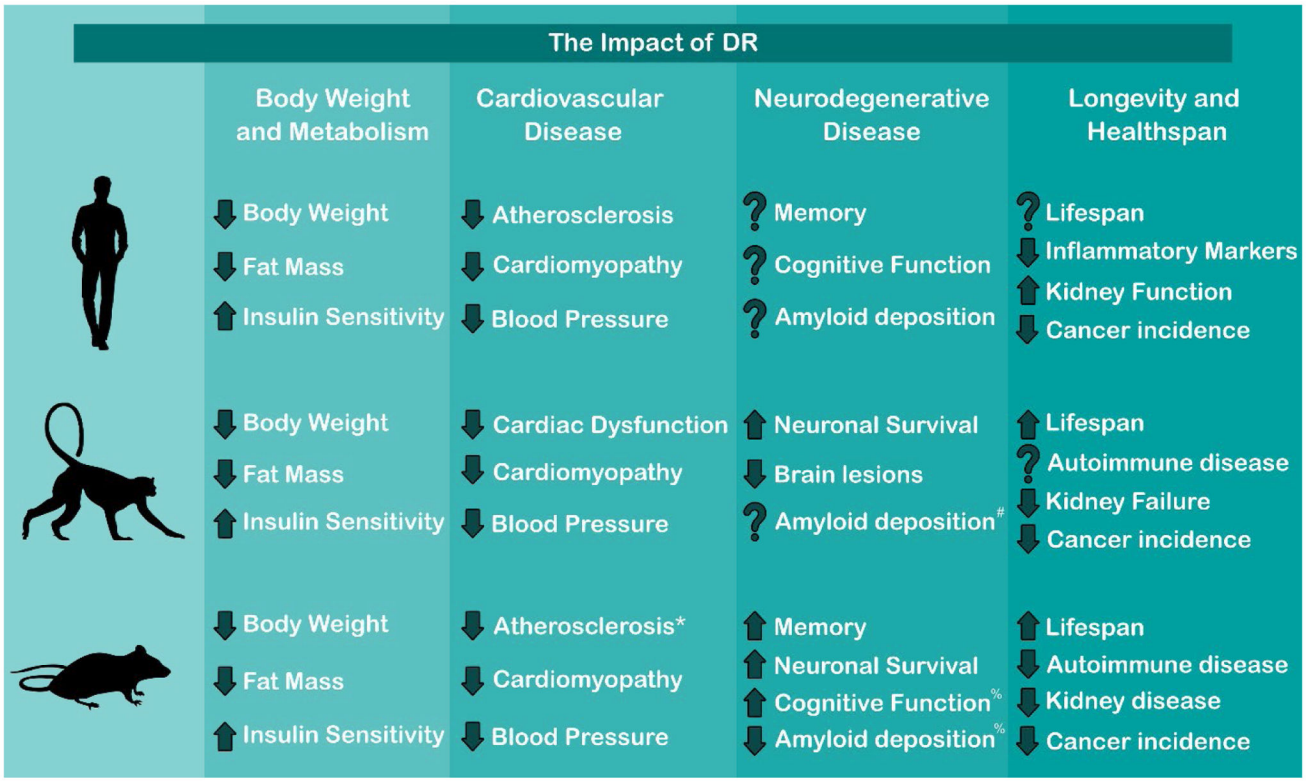


Figure 2 |. Multiple molecular pathways engaged by dietary restriction.

Dietary restriction (DR) results in reduced consumption of most macronutrients, including carbohydrates and specific amino acids, the building blocks of proteins. Reduced levels of glucose and its catabolite dihydroxyacetone phosphate (DHAP) are sensed by AMP-activated protein kinase (AMPK) and mTOR complex 1 (mTORC1), resulting in increased AMPK activity and decreased mTORC1 signaling, mediated through activation of TSC as well as modulation of the Rag-GATOR pathway that controls lysosomal localization of mTORC1. Downstream of mTORC1, ribosomal biogenesis and protein synthesis are downregulated and autophagy is increased. Decreased levels of methionine, branched-chain amino acids (BCAAs), or of protein similarly reduce mTORC1 signaling via the Rag-GATOR pathway. Decreased levels of protein and amino acids are also sensed by the integrated stress response pathway via GCN2, eukaryotic translation initiation factor eIF2α and cAMP-dependent transcription factor ATF4, leading to the induction of the pro-longevity hormone fibroblast growth factor 21 (FGF21). Reduced levels of carbohydrates and calories lead to decreased insulin/insulin-like growth factor 1 (IGF-1) signaling, which leads to decreased activity of the PI3K/mTOR complex 2 (mTORC2)/AKT signaling cascade that normally inhibits forkhead box protein O (FOXO)-dependent gene transcription, as well as decreased mTORC1 activity. Decreased levels of methionine lead to decreased levels of the metabolite S-adenosyl methionine (SAM), altering DNA and histone methylation. Collectively, DR induces repair and recycling pathways, including autophagy, mitophagy, DNA repair, and oxidant defense, and enhances stem cell function. As a result, cell senescence is downregulated and proteostasis is improved. Together these positive effects on cell and tissue function (shown in blue) contribute to extension of lifespan and

healthspan. Proteins or protein complexes with kinase activity are depicted in red. SIRT1, sirtuin 1.

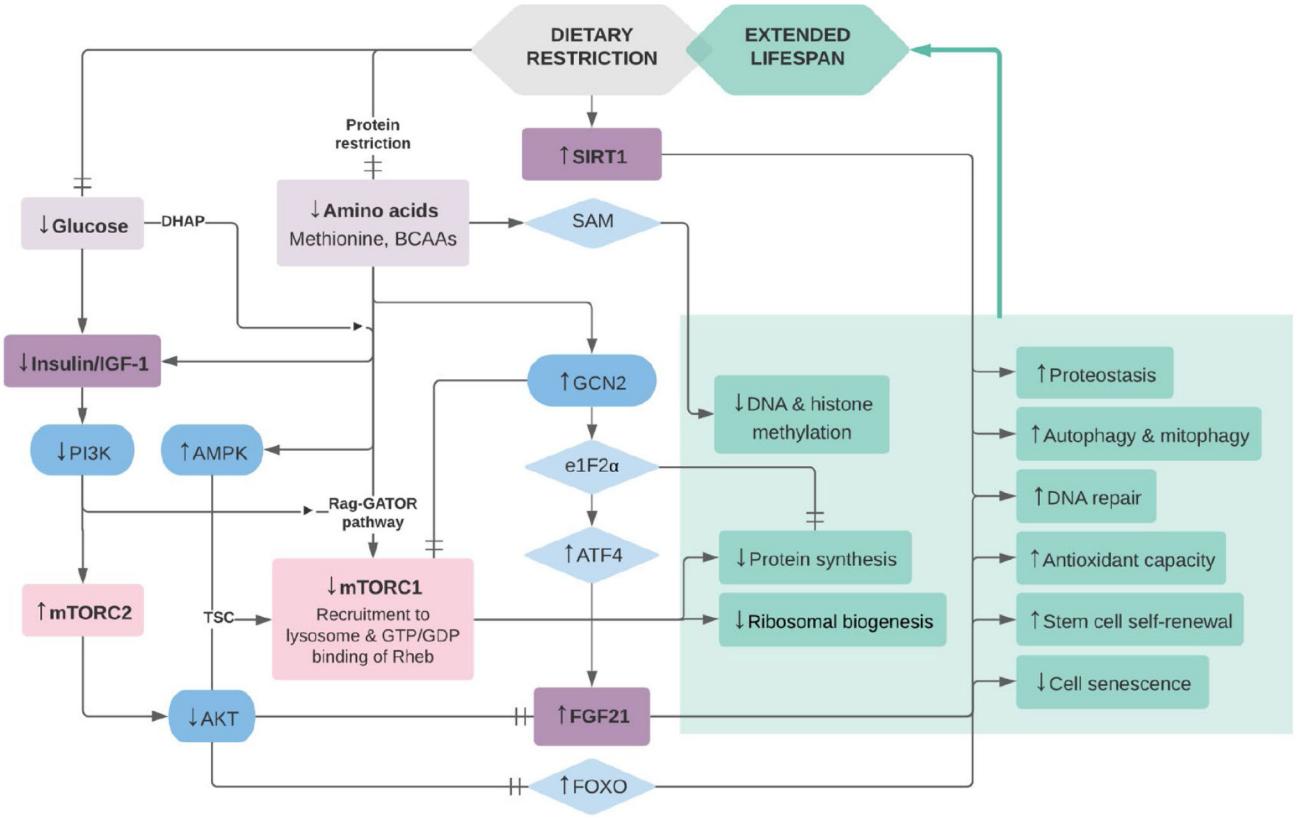


Figure 3 |. Species-specific effects of fasting on ketone bodies production and survival.

Remarkable differences in biological adaptations to fasting exist between mice and humans that should be considered when determining how studies from rodent models can inform human trials. Because of their high-energy metabolism, most strains of mice starve to death after a 48–60 hour fast. In contrast, even lean men and women can undergo a 57–73 days of water-only fasting before death occurs, and some severely obese individuals can fast for more than a year. Similarly, serum ketone levels increase after approximately 4–7 hours of fasting and peak after 24 hours in rodents, whereas in humans ketone bodies usually start to increase after 18–24 hours of fasting and do not peak until 2 weeks.

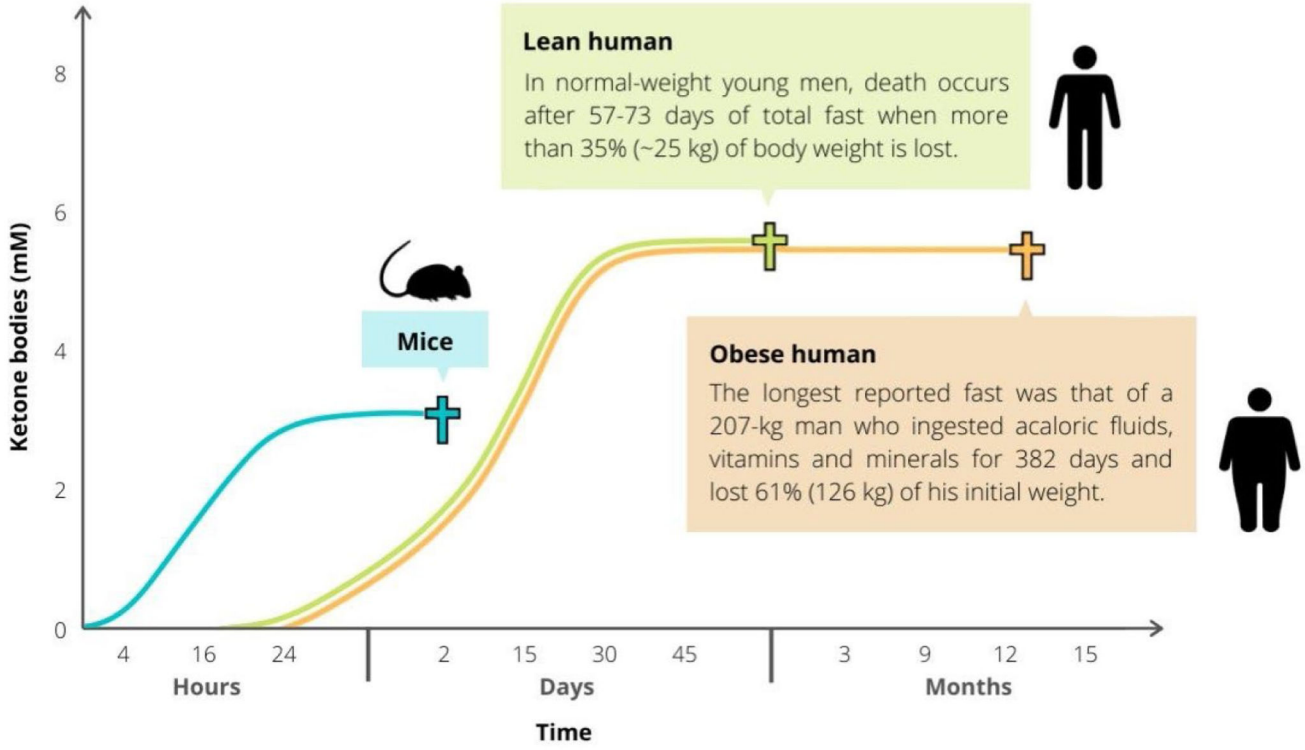


Table 1:

Genetic mouse models of extended longevity

Genetic Intervention	Genetic background	Sex	Lifespan extension	Phenotype	Ref
Inhibition of growth hormone signaling					
Snell dwarf mice (dw/dw)	Snell dwarf mice (C3H/HeJ × DW/J)F_1 background	Male and female	Average lifespan increased by 42%	Reduced body size, impaired growth hormone defects. Homozygous loss of function mutation in Pit-1	285,286
Ames dwarf mice (df/df)	Heterogeneous genetic background.	Male and female	Average lifespan increased by 64% (F) and 49% (M)	Reduced body size, impaired growth hormone defects Homozygous loss of function mutation in Prop-1	37,287–289
Little mice (lit/lit)	C57BL/6J	Male and female	Average lifespan increased by 23% (M) and 25% (F)	Slow growth excessive fat. Mutation in GH-releasing hormone (GHRH) receptor gene. Very low GH. Low fat diet for obesity prevention.	
GH Receptor Knockout (Laron Dwarf) Mice GHR/BP −/−	129Ola and BalbC	Male and female	Average lifespan increased by 55% (M) and 38% (F)	Slow growth and reduced body weight after birth. Disruption of the GH receptor/GH-binding protein (GHR/GHBP). Redced body size. Significantly lower IGF-1 levels	39,290,291
AC5 KO	129/SvJ × C57BL/6	Male and female	Median lifespan increased by ~30%	Resistant to cardiac stress. Lower BW and GH.	292
Transgenic overexpression of *FGF21*	C57BL/6J	Male and female	Median lifespan increased by 30% (M) and 39% (F)	Blunts GH/IGF-1 signaling pathway in liver.	114
Inhibition of mTOR signaling					
$mTOR^{(\Delta/\Delta)}$ mTOR expressed at 25% of WT	129S1 and C57BL/6Ncr	Male and female	Median lifespan increased by 22% (M) and 19% (F)	Reduced mTORC1 and mTORC2 activity. Smaller than WT.	64
$mtor^{+/-}$ $mlst8^{+/-}$	C57BL6/129S5	Male and female	Median lifespan increased by n.s. (M) and 14.4% (F)	Decreased mTORC1 activity. Normal glucose tolerance and insulin sensitivity	59
$S6K1^{-/-}$	C57BL/6	Male and female	Median lifespan increased by n.s. (M) and 19% (F)	Reduced fat mass and increased food intake (F)	63
Transgenic overexpression of human *TSC1*		Male and female	Median lifespan increased by n.s. (M) and 12.3% (F)		293
αMUPA mice	NIH FVB/N inbred mouse line	Female	Median lifespan increased by 16%	Consume 20% less food and exhibit 20% reduced body weight. Overproduce urokinase-type plasminogen activator (uPA) in the brain.	294
Inhibition of iInsulin/IGF-1 signaling					
Igf1r+/− mice	129/Sv genetic background	Female	Average lifespan increased by 16% (M, n.s.) and 33% (F)	Heterozygous IGF-1 receptor knockouts. Greater resistance to oxidative stress	47
FIRKO mice	C57BL/6J × FVB/NJ	Male and female	Average lifespan increased by 18%	Fat-specific insulin receptor knockout. Reduced fat mass.	49
$Irs1^{-/-}$ mice	C57BL/6J × FVB/NJ	Male and female	Median lifespan increased by 16% (M) and 32% (F)	Delayed age-sensitive markers in female $Irs1^{-/-}$ mice	51,52

Genetic Intervention	Genetic background	Sex	Lifespan extension	Phenotype	Ref
Irs2$^{+/-}$ mice	C57BL/6J	Male and female	Median lifespan increased by 17%	Insulin resistant	50
Brain-specific *Irs2*$^{+/-}$ mice	C57BL/6J	Male and female	Median lifespan increased by18%	Insulin resistant, increased metabolic flexibility	50
Brain-specific *Irs2*$^{-/-}$ mice	C57BL/6J	Male and female	Median lifespan increased by 14%	Insulin resistant, increased metabolic flexibility	50
Irs2$^{-/-}$ mice	C57BL/6J × FVB/NJ	Male and female	Median lifespan decreased by 86% (M) and 23% (F)	Significantly shortens lifespan	51
Akt1$^{+/-}$ mice	C57BL/6	Male and female	Average lifespan increased by 8% (M) and 15% (F)	Decreased TOR signaling and suppressed mitochondrial activity	295
Altered sirtuin function or expression					
Whole body Sirt1 overexpression	C57BL6/CBA	Male and female	No significant change in lifespan	No change in longevity, protected from development of age-associated diseases	145
Brain specific *Sirt1*-overexpressing (BRASTO) transgenic mice	C57BL/6J	Male and female	Median lifespan increased by9% (M) and 16% (F)	Enhanced neural activity	146
Sirt3−/−	C57BL6/J × 129Sv	Male	Median lifespan decreased by 19%	Shortened lifespan and severe cardiac damage	149
Sirt6-transgenic mice	C57BL/6J and BALB/cOlaHsd	Male and female	Average lifespan increased by 15.7% (M) and n.s. (F)	Reduced serum IGF-1 (M)	153
ATRAP-KO	C57BL/6	?	Median lifespan decreased by 18%	Age-related pathological changes in the kidney correlated with decreased expression of the prosurvival gene, *Sirtuin1*.	296
Adipose-specific overexpression of NAMPT	C57BL/6J	Male and female	Median lifespan increased by n.s. (M) and 13.4% (F)	Increased wheel running, and better sleep quality, glucose tolerance, pancreatic beta cell function, and cognitive function with aging	134

n.s.: Not significant; ?: Not stated in the original research.

Table 2 |

Impact of dietary restriction on common ageing-associated pathologies and physiological decline on different mammalian species.

Organism	Human	Non-human primates	Rodents
Effects on body weight and metabolism			
Body weight	↓	↓	↓
Fat mass	↓	↓	↓
Insulin sensitivity	↑	↑	↑
Inflammation	↓	↓	↓
Effects on cardiovascular disease			
Atherosclerosis	↓	?	↓ [a]
Diastolic dysfunction	↓	?	↓
Cardiomyopathy	?	↓	↓
Blood pressure	↓	↓	↓
Cholesterol	↓	↓	↓
Heart rate variability	↑	?	↑
Effects on neurodegenerative disease			
Memory	?	?	↑
Cognitive function	?	?	↑ [c]
Neuronal survival	?	↑	↑
Amyloid deposition	?	? [b]	↓ [c]
Effects on longevity and other chronic diseases			
Lifespan	?	↑	↑
Obesity	↓	↓	↓
Type 2 diabetes	↓	↓	↓
Fatty liver disease	↓	?	↓
Kidney disease	?	?	↓
Cancer incidence	↓	↓	↓
Autoimmune disease	?	?	↓
Sarcopenia and frailty	?	↓	↓

Footnote: Up arrows: increase or improvement; down arrows: decrease or decline; question marks: unknowns.

[a] in apolipoprotein E deficient mice.

[b] Amyloid peptides were decreased in DR squirrel monkeys but were unchanged in DR rhesus macaques.

[c] In Alzheimer's Disease mouse model. Relevant references can be found in Supplementary information 1.