Published in final edited form as:
Aging Cell. 2010 April ; 9(2): 105–112. doi:10.1111/j.1474-9726.2010.00552.x.

Dietary restriction and aging, 2009

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Summary

Dietary restriction (DR) is a robust nongenetic, nonpharmacological intervention that is known to increase active and healthy lifespan in a variety of species. Despite a variety of differences in the protocols and the way DR is carried out in different species, conserved relationships are emerging among multiple species. 2009 saw the field of DR mature with important mechanistic insights from multiple species. A report of lifespan extension in rapamycin-treated mice suggested that the TOR pathway, a conserved mediator of DR in invertebrates, may also be critical to DR effects in mammals. 2009 also saw exciting discoveries related to DR in various organisms including yeast, worms, flies, mice, monkeys and humans. These studies complement each other and together aim to deliver the promise of postponing aging and age-related diseases by revealing the underlying mechanisms of the protective effects of DR. Here, we summarize a few of the reports published in 2009 that we believe provide novel directions and an improved understanding of dietary restriction.

Keywords

aging; caloric restriction; dietary restriction; lifespan; nutrients; rapamycin; TOR

Introduction

Dietary restriction (DR) is defined as a reduction of particular or total nutrient intake without causing malnutrition. Dietary restriction in this broad sense includes caloric restriction (CR), in which total food intake is reduced, as well as studies involving the restriction of major dietary components (protein, lipid or carbohydrates) or temporal variations of food intake (intermittent fasting). Understanding the molecular mechanisms of how DR slows aging and age-associated diseases has gained pace in the last few years. This is largely attributed to the utilization of the powerful genetic tools that are available in simple and short-lived model organisms like Saccharomyces cerevisiae (S. cerevisae), Caenorhabditis elegans (C. elegans) and *Drosophila melanogaster (D. melanogaster)* to determine the basic mechanisms of the protective effects of DR. These studies lay a foundation for the examination of conserved basic mechanisms in mammals. Insightful studies in mammalian systems are taking us closer to the goal of being able to employ our understanding of DR to postpone aging and age-related diseases in humans.

The contribution of individual nutrients

In practice, CR is easier to implement than DR. However, it has been observed that in certain species, restriction of individual components like protein or amino acids is sufficient for lifespan extension (Chippindale *et al.*, 1993; Orentreich *et al.*, 1993; Kapahi *et al.*, 2004; Mair *et al*., 2005; Miller *et al*., 2005; Min & Tatar, 2006). Some of the important challenges in the

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field include examining the role of individual nutrients on aging and physiology and whether these effects are conserved across species.

One of the proposed mechanisms of DR is that under poor nutrient conditions the organism reallocates resources from reproduction to somatic maintenance and thus can survive the harsher conditions of its environment (Holliday, 1989). Grandison *et al*. (2009) have examined whether DR induced loss of fecundity is invariably associated with DR induced longevity extension in *D. melanogaster*. Using a method of DR that employs restriction of yeast in a semi-synthetic diet, the authors sought to determine which components in yeast mediate its effects on lifespan and fecundity. Addition of lipids, vitamins or carbohydrates back to the DR diet had no effect on either fecundity or lifespan. However, addition of essential amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) in the DR diet was sufficient to reverse both the fecundity and lifespan changes observed. Surprisingly, adding methionine alone back to the DR diet reversed the fecundity effect but not the lifespan changes (Grandison *et al*., 2009). These observations suggest that different amino acids independently mediate the lifespan and fecundity responses to fly diet and that the lifespan extension benefits may arise by limiting only a few components, such as methionine, in a rich diet. Although the mechanisms of these findings remain unknown, they are consistent with previous findings in rodents that restriction of essential amino acids like methionine and tryptophan is sufficient to extend lifespan (De Marte & Enesco, 1986; Zimmerman *et al*., 2003; Miller *et al*., 2005).

Grandison *et al*. (2009) also examined the role of insulin signaling in mediating the effects of DR and amino acids on lifespan in *D. melanogaster*. The authors report that flies overexpressing a dominant negative form of insulin receptor have extended lifespan upon DR. However, a significantly larger percentage of lifespan extension is observed upon treatment with diets that have either double the yeast concentration of the DR diet or essential amino acids added back. These experiments suggest that the insulin signaling pathway in flies may exert its effects on lifespan both independently of DR and in a protein-dependent manner.

The role of various nutrient compositions that differ in carbohydrate to protein ratio has been examined for its effects on various physiological parameters and aging. Two recent studies examined this question using *D. melanogaster* by varying the levels of yeast (the major source of protein) and sucrose (source of carbohydrates) in the diet (Lee *et al*., 2008; Skorupa *et al*., 2008). One of the consistent findings is that calories alone are insufficient to explain the effects of nutrients on fecundity, lifespan and triglyceride accumulation in *D. melanogaster*. These studies argue that amounts and ratios of specific nutrients maximize the assayed parameters (Lee *et al*., 2008; Skorupa *et al*., 2008). Increased lifespan and triglyceride accumulation is favored by diets that have reduced yeast to carbohydrate ratio, while enhanced fecundity is favored by higher yeast to carbohydrate ratios. Interestingly, although increasing carbohydrates in the diet of flies fail to cause a significant decrease in lifespan (Chippindale *et al*., 1993; Mair *et al*., 2005; Skorupa *et al*., 2008), it was recently found that adding glucose to the diet of *C. elegans* can significantly shorten the lifespan by activating the insulin signaling pathway (Lee *et al*., 2009). These studies suggest the possibility of species-specific differences in the contribution of individual nutrients to lifespan. Further work will be needed to evaluate the effects of restriction of specific nutrients and combinations of nutrients on lifespan extension in flies, to develop testable models of the mechanisms linking nutrient levels to lifespan and to determine whether these resulting generalizations predict the outcome of analogous studies in mammals.

The role of mRNA translation regulation

Following the discoveries that TOR mediates the effects of dietary restriction in flies (Kapahi *et al*., 2004) and yeast (Kaeberlein *et al*., 2005), a number of discoveries in the last year underscore the importance of TOR as a major player in the field of aging and dietary restriction. Investigating the downstream effectors by which the TOR pathway influences lifespan is a key challenge in the aging field.

One of the known targets of TOR in multiple species is 4E-BP (eIF4E-binding protein). TOR phosphorylates 4E-BP, relieving its inhibition of eIF4E and enhancing overall mRNA translation. Recently, Zid *et al*. (2009) demonstrated that 4E-BP mediates lifespan extension by DR (restriction of yeast in the diet) in *D. melanogaster*. Dietary restriction enhances the levels of the fly ortholog of 4E-BP, and mutant flies lacking 4E-BP fail to show lifespan extension upon DR. Conversely, overexpression of an activated form of 4E-BP, a form that binds eIF4E more strongly, was able to extend lifespan of flies provided nutritionally rich diets, but did not have such an effect under DR conditions. To gain further insight into the pattern of genes whose translation is controlled by TOR and 4E-BP, Zid *et al*. (2009) conducted a genome-wide analysis of mRNA translational states in whole flies under DR. The method involved examining the differential association of specific mRNAs to ribosomes; this provides an indication of translation rate, because ribosome association is a critical rate-limiting step in protein synthesis. Although DR led to an overall decrease in protein synthesis, one subset of genes, those encoding mitochondrial proteins, showed enhanced translation under the DR condition. 5′UTRs of the mitochondrial electron transport chain (ETC) subunits and mitochondrial ribosomal proteins were found to be significantly shorter and have weaker secondary structure compared to the rest of the *D. melanogaster* genome. The mitochondrial 5′UTRs were sufficient to confer differential translational upregulation in flies with activated d4E-BP expression. Consistent with the 4E-BP results, RNAi-mediated inhibition of function of mitochondrial ETC complexes I and IV prevented the lifespan extension under DR. A similar observation with ETC complex V was also shown recently in *D. melanogaster* (Bahadorani *et al*., 2010). These data suggest that DR triggers increased translation of mitochondrial genes via 4E-BP and that this increase is required for lifespan extension in DR flies. The mechanism by which enhanced mitochondrial function extends fly lifespan remains poorly understood and is an important question in this area.

The role of mRNA translation in mediating lifespan extension by DR has also been examined in yeast. Reduction of 60S ribosomal subunit levels slows aging in *S. cerevisiae* in a manner overlapping with the effects of DR (glucose restriction) and TOR (Steffen *et al*., 2008). GCN4 was found to be translationally upregulated both after DR and upon reduced 60S subunit abundance and partially mediated the lifespan extension under these conditions (Steffen *et al*., 2008). Together, these studies suggest that a differential regulation of mRNA translation may play an important role in mediating lifespan extension upon DR. Future work in mammalian models will help address whether this is conserved across species.

Link between DR and TOR in *C. elegans*

Autophagy is a highly regulated cellular starvation response which leads to degradation of various cytoplasmic components to maintain essential nutrient levels and viability (Cecconi & Levine, 2008). Inhibition of TOR has been shown to enhance autophagy in multiple species (Diaz-Troya *et al*., 2008). The role of autophagy in mediating lifespan extension by DR and upon inhibition of TOR was examined recently in *C. elegans* (Hansen *et al*., 2008) using *eat-2* mutants, which have pharyngeal pumping defects and thus reduced food intake (Lakowski & Hekimi, 1998). Both DR and inhibition of TOR were found to enhance autophagy. In *C. elegans* increased autophagy under DR required the FOXA transcription

factor PHA-4 (Hansen *et al*., 2008). PHA-4 had previously been shown to be required for lifespan extension by DR (Panowski *et al*., 2007) and in S6K mutants (Sheaffer *et al*., 2008). The new study used RNAi to demonstrate that autophagy is also required for the maximal lifespan extension seen in *eat-2* mutants or through inhibition of TOR (Hansen *et al*., 2008). Together these experiments imply that autophagy is enhanced and required to mediate lifespan extension upon DR.

Hypoxia inducible factor (HIF-1) is a downstream target of the TOR pathway in mammalian cells (Bernardi *et al*., 2006; Hui *et al*., 2006; Wouters & Koritzinsky, 2008). In a recent study, Chen *et al*. (2009b) observed that HIF-1 also acts downstream of the TOR/S6K pathway to mediate lifespan extension by DR in *C. elegans*. A mutation in *hif-1* extends lifespan on rich nutrient conditions but does not cause further lifespan extension under DR. In this study, DR was affected by reducing the bacterial concentration of the *E. coli* lawn or by using *eat-2* mutants. Mutants in prolyl hydroxylase *egl-9*, which have elevated HIF-1 levels, failed to show maximal lifespan extension by DR or by loss of the TOR target S6 kinase. The authors proposed that HIF-1 acts in a pathway downstream of dietary restriction and TOR to repress longevity by a mechanism involving induction of endoplasmic reticulum (ER) stress response genes. Inositol requiring protein-1 (IRE-1) encodes an ER transmembrane protein that senses misfolded proteins in the ER lumen and responds by splicing the *xbp-1* mRNA, which in turn allows the translation of functional transcriptional activator Xbp-1 and expression of target genes required for increased ER stress resistance (Ron & Walter, 2007). Chen *et al*. (2009b) showed that *ire-1* was required for the lifespan extension induced either by DR or by loss of hif-1 function, suggesting a role for ER stress response pathways in both models of increased lifespan.

The role of a conserved ubiquitination pathway in DR

Recent work by Carrano *et al*. (2009) on E2/E3 ubiquitin ligase has implicated this system in lifespan extension by DR in *C. elegans*. The authors examined E3 ubiquitin ligases of the conserved Homologous to E6-AP Carboxyl Terminus (HECT) family, known to be involved in tumorigenesis in mammals (Bernassola *et al*., 2008). WWP-1 is orthologous to the WW class of HECT E3s, and a mutation in this gene caused enhanced sensitivity to heat and oxidative stress in *C. elegans*. Conversely, over-expression of *wwp-1* led to a lengthened lifespan and increased stress resistance. This lifespan extension was dependent on *pha-4*, which (see above) has previously been implicated in mediating the lifespan extension by DR (Panowski *et al*., 2007). Interestingly, the authors observed that a loss of *wwp-1* does not affect the extended lifespan of insulin/IGF and mitochondrial mutants, but the lifespan extension in *eat-2* mutants was significantly abrogated. The authors further demonstrate that WWP-1 is required for lifespan extension by DR using a method of bacterial dilution in liquid to impart lifespan extension. The authors also found that UBC-18, a protein which interacts with WWP-1, is also required for DR-induced lifespan extension. This study suggests an important and novel role for ubiquitin ligases in mediating lifespan extension upon DR and examining how the ubiquitin system interacts with other DR-related pathways will be of great interest.

Do various forms of DR extend lifespan by different mechanisms?

In *C. elegans*, DR can also be imposed by intermittent fasting. A regimen of either alternate day fasting or fasting for a day after 2 days of feeding both significantly extend lifespan by over 40% (Honjoh *et al*., 2009). Honjoh *et al*. (2009) observed that although the effects of CR induced by bacterial dilution could be mimicked by inhibition of either TOR or the TOR activating GTPase *rheb-1*, TOR inhibition, surprisingly, prevented lifespan extension induced by intermittent fasting. They demonstrated that after inhibition of *rheb-1*, intermittent fasting failed to enhance the nuclear localization of the FOXO transcription factor, DAF-16. DAF-16

has previously been shown to be required for the lifespan extension by inhibition of *daf-2*, which encodes an insulin/IGF-like receptor in *C. elegans* (Lin *et al*., 1997; Ogg *et al*., 1997). DAF-16 was required for the maximal lifespan extension by intermittent fasting (Honjoh *et al*., 2009). Micro-array analysis suggested that *rheb-1* and TOR signaling are required for the changes in gene expression induced by intermittent fasting, including the downregulation of the gene encoding insulin-like peptide INS-7. These studies suggest that different forms of DR, such as intermittent fasting and CR, may extend lifespan by different mechanisms. Furthermore, the results suggest that insulin-like signaling may play a role in mediating the lifespan extension effects of DR under certain conditions. Further support for this model comes from studies by Greer *et al*. (Greer & Brunet, 2009). These authors compared different methods of DR in *C. elegans* and observed that they may involve different mechanisms for lifespan extension. They observe that DAF-16 activity is required to obtain the maximal lifespan benefits of DR in some, but all not, DR regimens (Greer & Brunet, 2009). These studies point to the importance of studying different forms of DR to get a comprehensive understanding of the mechanisms by which these interventions extend lifespan.

TOR pathway and lifespan extension in rodents

One of the key observations from aging research in the past two decades is that some of the genetic pathways that modulate lifespan do so in multiple organisms, including yeast, worms, flies and rodents. This gives real hope to the possibility of translating the findings from invertebrate research to humans. Research in rodents allows a closer look at changes in age-related pathologies with these interventions, providing a model for dissecting the genetic pathways that modulate mammalian age-related diseases. As described earlier, a number of studies from invertebrates have demonstrated that genes in the TOR signaling pathway may play an important role in mediating the effects of DR in yeast, worms and flies. Although it remains to be seen whether the TOR pathway also mediates the effects of DR in mammalian species, some recent reports suggest that it may indeed be involved in slowing aging and age-related pathology in mammals.

Selman *et al*. (2009) showed that mice lacking S6K1, which encodes the p70 ribosomal S6 protein kinase, show a 20% extension in the median lifespan of females, although no significant extension was seen in males. S6K1 is a downstream target of both the TOR and insulin signaling pathway (Shamji *et al*., 2003). Prior studies in yeast, worms and fruit flies have demonstrated that decreased activity of mTOR and S6K1 homologs is sufficient to increase lifespan in each of these species (Fabrizio *et al*., 2001; Kapahi *et al*., 2004; Hansen *et al*., 2007; Pan *et al*., 2007), establishing S6K as a key modulator of aging in both invertebrate and now vertebrate species. Selman *et al*. (2009) also found that deleting S6K1 protects against age-related decline in motor, bone and immune dysfunction and increases insulin sensitivity at old age in mice. They showed that AMPK activity is enhanced in isolated hepatocytes from S6K1-deficient mice as well as in worms lacking S6K (Selman *et al*., 2009). The enhanced longevity resulting from loss of S6K in *C. elegans* was dependent on the nematode AMPK subunit encoded by *aak-2* (Selman *et al*., 2009). Furthermore, *aak-2* mutants also rescue the body size and fecundity defects of S6K mutants in *C. elegans* (Selman *et al*., 2009). AMPK plays a central role in integrating energy balance with metabolism and stress resistance and has been previously implicated as a longevity factor and modulator of DR-dependent lifespan extension in *C. elegans* (Apfeld *et al*., 2004; Greer *et al*., 2007; Greer & Brunet, 2009). Identifying mechanisms by which AMPK may be responsible for the effects of S6K on both growth and lifespan remains an important question and has been discussed elsewhere (Kaeberlein & Kapahi, 2009).

Translating the increase in healthspan observed in S6K1-defective mice to humans relies on identifying drugs that target the TOR pathway. Rapamycin, an inhibitor of TOR, reduces the activity of S6 kinase from yeast to human cells and is used clinically as an immunosuppressant

and as an anticancer therapeutic (Sonenberg & Hinnebusch, 2009). Rapamycin is a macrolide that binds FKBP12, leading to inhibition of mTORC1. In 2003, the National Institute on Aging (NIA) initiated the Interventions Testing Program (ITP) to evaluate drugs that putatively delay aging or prevent multiple forms of late-life disease in laboratory mice (Miller *et al*., 2007). To avoid effects specific for inbred genetic backgrounds, the ITP uses mice produced by a standardized four-way cross (Miller *et al*., 2007). The mice are exposed to selected drugs by a standardized feeding regimen, and the effects on lifespan variations are tested at three sites in parallel (Miller *et al*., 2007). The ITP found that in mice, the administration of rapamycin late in life (starting at 600 days) was sufficient to cause increased lifespan (Harrison *et al*., 2009). The effect of rapamycin on lifespan was significant in both sexes at all three sites ($P < 0.05$). Another recent study has also demonstrated that rapamycin treatment, initiated past middle age (22–24 weeks old) showed a significant increase in lifespan in mice (Chen *et al*., 2009a). This study also showed that under these conditions, rapamycin treatment can boost immune function and rejuvenate hematopoietic stem cells. Given the role of TOR in mediating the DR response in invertebrates (Rogers & Kapahi, 2006), the use of TOR inhibitors like rapamycin may help guide development of DR mimetic drugs to slow aging and age-related diseases in humans.

Caloric restriction, somatotropic axis and sirtuins

In addition to CR, mouse lifespan can also be extended by single-gene mutations involved in the regulation of the somatotropic axis. The somatotropic axis includes growth hormone (GH), upstream hypothalamic hormones, the insulin-like growth factors (IGFs) and downstream signaling molecules (Brown-Borg, 2009). Long-lived Ames mice harbor mutations in a gene called *Prop-1* that disrupts pituitary gland development; these mice display reduced levels of circulating GH and insulin-like growth factor 1 (IGF-1) (Bartke & Brown-Borg, 2004; Brown-Borg, 2009). Consistent with findings in Ames dwarf mice, mice with mutations in the growth hormone receptor (GHRKO) are also long lived (Coschigano *et al*., 2000; Brown-Borg, 2009). Caloric restriction in mice is known to downregulate somatotropic signaling in mice, as shown by a reduction in circulating IGF-1 (Al-Regaiey *et al*., 2005), suggesting that CR and mutations that block somatotropic signaling may extend lifespan in part through shared mechanisms. Consistent with the idea of shared mechanisms, GHRKO male mice receive no further extension of overall longevity when placed on a 30% CR diet (Bonkowski *et al*., 2006). Recently, Bonkowski *et al*. (2009) showed a similar effect using a protocol in which mice received food every other day (EOD; net effect 15% reduction in caloric intake). Every other day feeding led to a 16% increase in median lifespan in control male mice, but GHRKO mice showed no extension in median lifespan. This EOD feeding amplified the insulin signaling cascade in both muscle and liver in control animals, but GHRKO animals showed increased insulin signaling only in muscle. The authors propose that CR increases lifespan by enhancing insulin sensitivity in mice. In contrast to GHRKO animals, Ames dwarf mice do respond to a CR diet by lifespan extension (Bartke *et al*., 2001), but in these mice, unlike GHRKO mice, CR diets cause a significant increase in insulin sensitivity, supporting the idea that insulin sensitivity is associated with longevity in each of these models (Masternak *et al*., 2009).

In a recent study, Cohen *et al*. (2009) tested the idea that Sirt1 might mediate some of its effects on CR by regulating the somatotropic axis. Sirt1 belongs to the sirtuin family of NAD+-dependent protein deacetylases (Guarente, 2009). Genetic ablation of Sirt1 orthologs blocks the extension of lifespan by CR in yeast and flies (Lin *et al*., 2000; Rogina & Helfand, 2004). Cohen and colleagues generated a brain-specific Sirt1 knockout (BSKO) mouse (Cohen *et al*., 2009). These BSKO mice were smaller than controls and showed decreased levels of circulating GH and IGF1 levels at 4 weeks of age. The effect was specific for GH: BSKO mice had no effect on other pituitary hormones including adrenocorticotropic hormone (ACTH), prolactin (PRL) and thyroid-stimulating hormone (TSH). Male control mice responded to a

CR diet (40% CR for 7 months starting at 3 months of age) by a decrease in circulating IGF levels and an increase in insulin sensitivity, but BSKO mice did not show such effects. In addition, the CR diet increased physical activity in control mice but not in BSKO mice. Although it is still unclear how downregulation of Sirt1 in the brain regulates pituitary GH production and whether either lifespan or CR effects on lifespan are modified in BKSO mice, these animals may provide a useful tool to investigate the mechanisms by which CR extends lifespan in mammals.

Resveratrol, which activates sirtuins, has been proposed as a possible CR mimetic (Wood *et al*., 2004). To test this hypothesis, Pearson *et al*. (2008) compared the transcriptional profiles of liver, skeletal muscle, adipose tissue and heart in mice fed resveratrol and those on an EOD feeding regime. Resveratrol was found to mimic the transcriptional effects of DR and EOD in all four tissues. Pearson and colleagues also found that resveratrol delays the age-related functional decline of a number of parameters including bone health (as measured by improvement in bone density, volume and strength of femur), cataract levels, motor and vascular functions. Despite these beneficial effects, resveratrol did not lead to any increase in lifespan under these conditions (Pearson *et al*., 2008). It would be of great interest to see how combined treatments of resveratrol and other lifespan extension drugs, such as rapamycin, affect lifespan and health-span in mammals.

Caloric restriction in non-human primates

Studies from a 20-year longitudinal study recently reported that CR improves healthspan in rhesus macaques (Colman *et al*., 2009). Adult rhesus monkeys were divided into two groups and given either control or CR diet (30% reduction of total food). The CR animals looked subjectively younger and showed a statistically significant decrease in the impairment of muscle function with age, glucose homeostasis and incidence of neoplasia and cardiovascular disease. Caloric restriction also reduced the age-associated brain atrophy in regions believed to regulate motor behavioral function. Although the survival analysis is not yet complete, CR also leads to a significant increase in survival in these animals, but only when deaths considered unrelated to the aging process were eliminated from the analysis. A second major study of rhesus monkeys is being carried out at NIA. Results from the NIA group have previously demonstrated that in primates, CR exerts many of the same physiological changes seen in rodents (Verdery *et al*., 1997; Edwards *et al*., 1998; Cefalu *et al*., 1999; Lane *et al*., 2001). They also observed that long-term CR initiated during adolescence (at 3–5 years of age) can delay T-cell senescence as measured by higher numbers of circulating naïve T cells, lower numbers of inflammatory cytokine-secreting memory T cells and higher proliferative capacity of T cells (Messaoudi *et al*., 2006). Recent work from this group suggests that similar delay in T-cell senescence is not observed when CR is initiated when the monkeys are either juveniles (1–2 years of age) or late in life (> 15 years of age) (Messaoudi *et al*., 2008). The authors argue that there may be an optimal window during which CR interventions can retard aging of the immune system. Together, these studies suggest that CR in primates can slow down certain aspects of aging; further data on its lifespan extension effects are awaited.

Caloric restriction in humans

A recent study examined the long-term effects of CR on insulin action and glucose tolerance in humans. Fontana *et al*. (2009) examined a number of endpoints in three groups: (i) 28 human volunteers who had consumed a CR diet for an average of 6.9 ± 5.5 years, (ii) endurance runners who ran an average of 48 miles per week and had been training regularly for an average of 21 years and (iii) sedentary controls (regular exercise < 1 h per week) (Fontana *et al*., 2009). The authors found both CR and endurance training led to significantly reduced body weight, body mass index (BMI) and total body fat compared to controls, as well as significantly lower fasting

levels of insulin and higher insulin sensitivity. Members of the CR group also showed decreased fasting glucose levels, high serum levels of adiponectin and free fatty acids and lower serum levels of inflammatory mediators including IL-6, TNFR-I and TNFR-II. Surprisingly, around 40% of the CR individuals exhibited an exaggerated hyperglycemic response to a glucose load. This impaired glucose tolerance was found to be associated with lower circulating levels of IGF-1, total testosterone, leptin and triiodothyronine levels, previously suggested as a metabolic/hormonal adaptation to CR in rodents (Fontana & Klein, 2007).

Two recent studies have suggested that CR might reduce the risk factors for cardiovascular disease and memory decline in humans. The Comprehensive Assessment of Long-Term Effects of Reducing Intake of Energy (CALERIE) research team is looking at effects of short-term (6 months) CR on risk factors for cardiovascular disease (Lefevre *et al*., 2009). The study examined 36 individuals that were assigned randomly to one of three groups: (i) controls; (ii) CR (25% calorie restriction); and (iii) CR + EX (12.5% CR + 12.5% increase in energy expenditure via structured aerobic exercises). The group reported that CR, with or without exercise, led to significant changes in several risk factors usually associated with cardiovascular health, including the ratio of total cholesterol to HDL cholesterol and systolic blood pressure. Another study examined the effect of CR on memory function (Witte *et al*., 2009). Fifty normal to overweight elderly subjects were stratified into three groups: one consuming a 30% CR diet, one consuming an increased proportion of unsaturated fatty acids and one which served as control. The CR diet led to a 20% increase in verbal memory score, as well as decreases in fasting plasma levels of insulin and C-reactive protein (Witte *et al*., 2009).

These studies suggest CR in humans may potentially postpone age-related diseases through reduction in risk factors associated with such diseases. Furthermore, some of the endocrine changes upon CR in humans may be similar to those observed in rodents. However, the importance of these risk factors and endocrine changes in mediating lifespan extension in humans remains to be established.

Conclusion and future outlook

The last decade has seen the successful use of genetic methods to discover the molecular mechanisms of DR, largely through the exploitation of genetically malleable model organisms with short lifespans. These studies reveal the existence of multiple forms of DR which extend lifespan by distinct, but sometimes overlapping mechanisms. Future research on how various pathways integrate and interact to mediate the protective effects of DR will be invaluable to gain a more complete understanding of how genetic mutations modulate this remarkable phenomenon and to provide a better understanding of the final downstream mechanisms that slow aging upon DR. Studies from invertebrates suggest that nutrient composition, rather than total calorie restriction per se, plays an important role in lifespan extension, but more work is now needed to evaluate similar approaches in other species including humans. Collective data from various species, especially primates, demonstrating the protective effects of DR is encouraging and supports the possibility of using DR to delay at least certain aspects of aging in humans. Direct application of DR interventions on humans may be impractical because long-term adherence to extremely low-calorie diets is very poor, but studies of DR in different model systems may help to guide development of pharmaceuticals that achieve similar results through conserved pathways. The current wave of experiments testing drugs that target conserved signaling pathways is likely to provide us with pharmacological interventions that can slow the process of aging.

Acknowledgments

We thank Dr. Richard Miller for his helpful suggestions. We regret not being able to cite all of the relevant references owing to space limitations. Dr. Kapahi is supported by grants from the Hillblom foundation and the NIH/NIA.

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