The fine line between lifespan extension and shortening in response to caloric restriction

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Caloric restriction (CR) is generally linked to lifespan extension in various organisms and may limit age-associated diseases. Processes through which caloric restriction promotes lifespan include obesity-countering weight loss, increased DNA repair, control of ribosomal and telomeric DNA repeats, mitochondrial regulation, activation of antioxidants, and protective autophagy. Several of these protective cellular processes are linked to the suppression of TOR (target of rapamycin) or the activation of sirtuins. In stark contrast CR fails to extend or even shortens lifespan in certain settings. CR-dependent lifespan shortening is linked to weight loss in the non-obese, mitochondrial hyperactivity, genomic inflexibility, and several other processes. Deciphering the balance between positive and negative effects of CR is critical to understanding its ultimate impact on aging. This knowledge is especially needed in order to fulfill the promise of using CR or its mimetic drugs to counteract age-associated diseases and unhealthy aging.

Introduction

The existence of conserved longevity pathways may seem counterintuitive from an evolutionary perspective. This is because evolution selects for reproductive success rather than long life. In fact, a leading theory of the evolution of aging, antagonistic pleiotropy, even stipulates that genotypes promoting reproduction earlier in life actually accelerate the aging process later in life (Fig. 1).1 Consistent with this notion, several species experience a decline in early reproductive rate under CR conditions.1 In contrast, a recent report indicates that the same genes can confer high early-life fitness and long life.2,3 While future work is needed to improve our understanding of the evolution of aging and longevity, it is clear that various genetic and environmental conditions can alter lifespan. One of the promises of the study of conserved aging and/or longevity pathways is that it will lead to applications that help us reduce the morbidity associated with age-related diseases as well as increase overall human lifespan.

It has long been appreciated that caloric restriction (CR) is a wide-ranging and potent anti-aging intervention. As early as 1935, it was observed that reduction of caloric intake relative to ad libitum (AL, or unrestricted feeding) results in an extension in the average and maximal lifespan of laboratory mice.4 Since then, similar findings have been reported for a diverse range of organisms including yeast, nematodes, fruit flies, fish, rats, mice, and dogs, among others.5-7 The discovery of such a potent anti-aging intervention has set the stage for research into the biology of aging and its modulation by caloric restriction.8

However, contrary to the well-documented positive effects of caloric restriction, several studies reported caloric restriction to be neutral or even detrimental to lifespan. For example, studies have found that caloric restriction regimens fail to impact lifespan in rhesus monkeys,7 wild mice,9 medflies,9 an isolate of the nematode C. Remanu,10 the spider L. Hasielli,11 and some yeast strains.12-14 Even more striking, CR actually shortened lifespan in several models including houseflies,15 male butterflies,16 the rotifer Cephalodella sp.,16 ILSXISS mice strains,17 and some yeast strains (Table 1).17 The work done with ILSXISS mice is particularly poignant. A meta-analysis of all mice studies excluding the ILSXISS strains reveals an average CR-dependent increase in lifespan of 15%.18 When the ILSXISS strains are included in the meta-analysis the average increase in lifespan drops to 2.9%.18 The ILSXISS studies set CR at 60% of AL intake in agreement with common standards in the field but it remains possible that more or less substantial restrictions may promote the lifespan of both ILSXISS and other mice strains. Taken together, these studies indicate that standard caloric restriction regimens do not universally promote longevity in various organisms. In other words, several more variables may exist within the equation determining the impact of CR on lifespan than originally anticipated.

Caloric restriction has been proposed to impact lifespan by affecting genomic stability, autophagy, oxidative stress, nutrient...
Reduction of Body Weight under CR Can Positively or Negatively Affect Lifespan

A CR diet will tend to promote the loss of body mass. How this impacts lifespan depends on which tissues are catabolized and the starting weight of the individual. As they start following CR regimens, obese individuals typically increase their lifespan as they lose fat mass. Simply put, this is because obesity is correlated with a number of age-associated pathologies such as cardiovascular disease and diabetes.\(^\text{33,34}\) Consistent with this rationale, in obese human males, CR reduces body fat while also significantly decreasing obesity-related pathologies such as high blood pressure and chronic inflammation.\(^\text{35}\) In addition, in obese mice, the combination of CR and omega-3-polyunsaturated fatty acid intake simultaneously counteracts adiposity and chronic inflammation.\(^\text{36}\) Thus, within obesity settings, CR-mediated weight loss is generally beneficial.

In contrast, fat loss under CR is linked to lifespan shortening in the non-obese. For example, in non-obese mice, CR-induced fat loss is inversely correlated with lifespan.\(^\text{36}\) In addition, age-associated pathologies such as respiratory disease correlated with body weight loss in non-obese humans although CR can lower biomarkers for cardiovascular disease in this segment of the population.\(^\text{34,36}\) Although losing body weight appears to lower lifespan in non-obese humans aged 50–70 even when health status is considered, it is unclear if this applies to other age cohorts.\(^\text{34,35}\) Together, these studies and rationales suggest that the starting weight of an individual may dictate whether CR-induced fat loss positively or negatively impacts lifespan.

In addition to fat modulation, CR can also decrease muscle mass. Increased muscle mass has been shown to have an important protective effect in several age-related diseases and losing muscle mass can therefore be deleterious.\(^\text{37}\) Indeed, even in ILSXISS mice, lean/muscle body mass correlated positively with lifespan under CR conditions.\(^\text{37}\) Taken together, these studies suggest that CR-triggered losses in muscle mass can shorten lifespan.

Therefore, the impact of CR-mediated weight loss on lifespan may be bidirectional dependent on the starting weight and tissue(s) catabolized. This indicates that there may be an optimal body mass density for lifespan. In humans, recent evidence has pointed toward this as being at the high end of what is typically considered a healthy weight, 22.5–25 kg/m\(^2\).\(^\text{38}\) Alternatively, it is possible that weight loss in and of itself is a stressor whose deleterious side effects on lifespan are only mitigated if the starting weight is significantly above the ideal. Either way, these findings clearly show that CR does not universally promote lifespan.

Figure 1. The evolution of aging and dietary effects on lifespan. (A) Positive and antagonistic pleiotropy theories suggest that alterations conferring advantages in early life respectively trigger beneficial and deleterious effects at the post-reproductive age. (B) Generalized relationships between diets and lifespan.

Nutrient Balance Affects the Response to CR

Interestingly, the magnitude and quality of weight loss may also depend on the nutritional composition of the CR diet itself. In fact, the very idea that it is solely the actual decrease in caloric content that accounts for CR-dependent lifespan modulation has been questioned. Instead, it may be that the varying restriction of nutrients in different diets, which may be commonly referred to as a CR diet, can positively or negatively impact lifespan depending on the particular nutrients affected.

Studies conducted in both flies and mice lend support to this notion. The fruit fly D. melanogaster can be subjected to a yeast-based or sugar-based diet. Decreased intake of yeast or sugar increases lifespan.\(^\text{39}\) Interestingly, the positive effect on lifespan per calorie decreased was much more substantial under the yeast-based diet relative to the sugar-based counterpart.\(^\text{39}\) This suggests that it is not simply the decreased caloric content per se that solely impacts lifespan. One possibility is that restriction of yeast-based diets also limits the amount of other nutrients within this diet. Consistent with this reasoning, methionine restriction significantly increases murine lifespan independently of caloric content.\(^\text{40,41}\) It is conceivable that the restriction of several amino-acids or nutrients would have similar effects. Therefore, in addition to altering overall caloric intake, certain CR diets may also extend lifespan via restriction of various lifespan-limiting dietary components or nutrients.

Specific nutritional composition may also explain contradictory results on the impact of different CR diets on lifespan. For example, one of many possible explanations for
these apparently contradictory results on the effect of CR on rhesus monkey lifespan may be the dietary composition; the study in which the monkeys responded to CR had AL diets with higher sucrose and lower antioxidants and omega 3 polyunsaturated acids.7,42 Not surprisingly, these data imply that different nutrients may promote or limit lifespan. Thus, different CR regimens with multiple variations in overall nutrient balance are very likely to trigger a wide range of responses with respect to lifespan. For example, a CR regimen that restricts methionine while maintaining antioxidants might extend lifespan while a regimen that limits omega-3-polyunsaturated acids while maintaining sucrose may shorten lifespan.

Overall, it is not surprising that the underlying nutritional composition of a diet influences the ultimate impact of CR regimens on lifespan. Future research in organisms with complex diets should carefully control for the nutritional composition of diets in order to accurately distinguish calorie-dependent from nutrient-dependent effects on lifespan. In addition, one should revisit some of the previously published CR studies to eliminate any potential confounding factors that may be linked to background nutrient deprivation or starvation. More specifically, experiments showing a decline in longevity in response to CR have not been generally performed across a range of nutrient levels and it remains possible that a number of these reports would have revealed enhanced longevity under similar caloric but different nutrient conditions.

**Crosstalk between CR and Genome Stability**

Beyond confounding dietary designs, CR certainly influences multiple processes operating at the cellular level.43-45 For example, CR can promote genome stability by sustaining DNA repair processes and also protecting repetitive DNA loci such as telomeres and rDNA. However, pre-existing conditions in these endogenous pathways can occur in certain genetic settings, in which case the effect of CR on lifespan may rapidly turn from positive to deleterious.

Connections between CR and several DNA repair processes do exist. One of the DNA repair pathways influenced by CR is base excision repair (BER), which repairs small non-helical distorting lesions in DNA. BER is the most commonly used DNA repair pathway in mammals.46 BER declines with age but caloric restriction prevents such age-associated declines in mice.47,48 This is likely linked to the ability of CR to promote rate limiting factors in the BER pathway. For example, CR increases the enzymatic activity of apyramidine/apurinic endonuclease as well as the expression of DNA polymerase β.47,49 Caloric restriction is also capable of activating nucleotide excision repair (NER), which repairs helical-distorting DNA damage caused by large bulky adducts.50 In mice, as with BER, NER rates decline with age but the decline is prevented by CR.50 This is likely linked to the ability of CR to promote rate limiting factors in the BER pathway. For example, CR increases the enzymatic activity of apyramidine/apurinic endonuclease as well as the expression of DNA polymerase β. The autoantigen Ku is a DNA binding protein in the NHEJ pathway.52 Interestingly, Ku expression declines with age in rats but CR can counteract this phenomenon.53 Future work will show if CR impacts actual NHEJ rates and if this in turn directly affects lifespan. Taken together, CR may promote lifespan by promoting the lifelong maintenance of several DNA repair pathways.

Importantly, the effects of CR are not limited to DNA repair. In fact, CR also modulates processes that help prevent DNA damage from occurring in the first place. One area of intense investigation has been to understand the impact of CR on conserved repetitive DNA loci known to significantly affect
cellular lifespan from yeast to human. In particular, budding yeast has served as a highly valuable tool to decipher many conserved cellular aging mechanisms. Yeast lifespan can be analyzed both in terms of replicative lifespan (number of daughter cells produced by a new mother cell) and chronological lifespan (survival of non-dividing cells). Importantly, CR extends both types of yeast lifespan. Lifespan of the budding yeast *S. cerevisiae* is highly dependent on the stability of repetitive DNA loci, in particular the rDNA (rDNA) repeats as well as the telomeres. Due to their highly repetitive nature, rDNA repeats are prone to recombination. While this can be protective under extreme stress conditions, aberrant or hyperrecombinative recombination within the repeats generally leads to chromosome instability and shortens cellular lifespan. CR typically decreases recombination within the rDNA repeats via a form of rDNA silencing that represses intergenic RNA Pol II-dependent transcription and this extends lifespan. CR has been proposed to suppress recombination within rDNA repeats through multiple mechanisms including the repression of the nutrient-sensing target of rapamycin (TOR) complex as well as activation of the conserved NAD+-dependent histone deacetylase Sir2 (silent information regulator 2). Interestingly, Sir2 is required for CR-dependent extension of replicative but not chronological lifespan. More recently, CR has also been proposed to increase lifespan by suppressing the activity of rDNA origins of replication preventing them from deleteriously competing with weaker replication origins elsewhere in the genome. In human cells, the Sir2 homolog SIRT1 (Sirtuin 1) acts as a subunit of the eNOSC (energy-dependent nucleolar silencing complex) to ensure a form of rDNA silencing that represses RNA Pol I-dependent transcription in a glucose-dependent manner. SIRT1, which is also activated by CR, is linked to cell survival in human cells. It is likely that SIRT1-dependent rDNA silencing increases cellular lifespan by decreasing deleterious recombination, similarly to yeast.

On another front, telomeres are linear DNA sequences that are located at the ends of linear chromosomes and are amplified to prevent excessive chromosome shortening and subsequent genome destabilization during cell division. Telomeres also help propagate Sir2-dependent silent chromosome structures along nearby regions on chromosome arms. Importantly, it is clear that telomere length maintenance as well as downstream sirnauts-dependent silent chromatin assembly are both critical to lifespan maintenance. CR can promote subtelomeric silencing in yeast through Sir2 and this translates to a longer cellular lifespan. SIRT6 (Sirtuin 6), which is another human Sir2 homolog, also promotes telomeric silencing in human cells. As CR increases SIRT6 levels, this suggests that CR may promote mammalian lifespan in part by increasing telomeric silencing. Furthermore, in mice and rats, a CR diet helps maintain the length of telomeres over the lifetime of the animal. Telomere length maintenance is a strong predictor of lifespan and it is thus likely that CR-dependent telomere length maintenance promotes lifespan. SIRT1 may also regulate telomere length and attenuate age-associated telomere shortening, suggesting that CR acts upstream of SIRT1 to regulate telomere length and promote lifespan. However, TOR also appears to be important for telomere length maintenance and lifespan in yeast. Therefore, it is likely that CR acts through both sirtuins and TOR modulation to in order to optimize lifespan-sustaining telomeric functions. It is important to note that additional CR-dependent processes maintaining telomere/telomere stability may still exist as currently identified pathways only partly account for the beneficial effects of CR at these repetitive genomic loci. Together, current literature clearly indicates that CR activates processes operating at least at the repetitive DNA loci, rDNA, and telomeres, in order to prevent genome instability (Fig. 2). We also note that the dysregulation of other types of repetitive DNA sequences such as transposable elements have been linked to genome instability and aging. It is therefore possible that CR may somehow control these elements in order to promote lifespan. Overall, CR is a potent genome maintenance intervention that both prevents DNA damage and promotes DNA repair. Although these genome-stabilizing effects of CR can generally be viewed as beneficial, it is possible to imagine various settings in which they may ultimately shorten lifespan. For example, by decreasing DNA recombination capacity and genomic flexibility, cells often lose the ability to efficiently adapt to variable environmental conditions. In addition, cellular aging can be beneficial in multicellular organisms that need to balance new cell generation and old cell clearance within tissues and organs. Consistent with this rationale, it was recently discovered that
CR-Autophagy Connections

Calorie restriction also increases autophagy, which is the mechanism responsible for catabolizing cellular components such as organelles by targeting them for lysosome-dependent degradation.93,94 While eliminating old cellular components that may be malfunctioning and/or cytotoxic, autophagy thereby also mobilizes energy reserves in times of stress.93 Several studies suggest that CR may promote lifespan by operating in part through autophagy.

Consistent with this notion, CR mimetics fail to extend the lifespan of autophagy-deficient C. elegans.95 In addition, Arabidopsis requires autophagy genes for lifespan extension under light restriction, which is the autotrophic analog of CR.96 These data indicate that autophagy can mediate CR-dependent lifespan extension within various settings.

CR may promote autophagy through several avenues. Interestingly, these may implicate Sirtuin activation and TOR suppression.97,98 For example, absence of the essential autophagic modulator Beclin-1 abolishes the lifespan extension that has been observed in C. elegans upon overexpression of the sirtuin Sir2.1.99 We note that these effects may reflect changes to processes that are independent of Sir2.1 itself, whose overexpression may not actually underlie the extended lifespan phenotypes initially reported.97,98 CR-dependent suppression of TOR also promotes autophagy in a variety of species and does so independently of sirtuins in C. elegans.99 This may be linked to the ability of TOR to suppress adenosine monophosphate-activated protein kinase, which is a potent activator of autophagy.100,101 Thus, sirtuin activation and TOR suppression may partly underlie autophagy-dependent lifespan extension by CR.

Healthy aging is also thought to depend on the proper maintenance of adult stem cells.102 Interestingly, autophagy is essential for the lifelong maintenance of hematopoietic stem cells and for supporting an old blood system.103 This phenomenon appears to implicate a FOXO3A-dependent gene expression program and is activated by CR.

Overall, it is reasonable to conclude that autophagy underlies at least some of the beneficial effects of CR on lifespan. If specific autophagy-related processes can also be linked to the negative effects of CR on lifespan in certain settings remains unclear. One possibility may be that activating autophagy when autophagic vesicles cannot fuse with lysosomes such as in Danon disease would be deleterious as this leads to an accumulation of non-functional autophagic vesicles.93 In fact, aberrant autophagy genes are also linked to several other diseases including cancer (ovarian, breast, prostate, and colon), autoimmune diseases (lupus), asthma, Crohn disease, and others.72 This greatly increases the clinical settings in which CR-dependent activation of autophagy may simply exacerbate phenotypes.

Links between CR, Oxidative Stress, and Energy Metabolism

In addition to promoting the autophagy of organelles including mitochondria, CR can decrease oxidative stress through several distinct pathways. Oxidative stress in an organism is largely due to the accumulation of reactive oxygen species (ROS). By damaging nucleic acids, proteins, and other molecules, ROS decrease the lifespan of many organisms.106,107 CR can promote lifespan by both lowering the production of ROS as well as promoting the function of antioxidants that can repair ROS-induced damage. However, CR also promotes mitochondrial activity, which inevitably increases the chance of ROS production. Thus, a delicate balance must be maintained for CR to actually decrease ROS-induced damage and extend lifespan.

Antioxidants can scavenge ROS and generally maintain a reducing environment that promotes lifespan.108 Importantly, CR promotes the function of several antioxidants. In mice, CR activates SIRT3 (Sirtrin 3), which in turn promotes the deacetylation and consequent activation of the antioxidant enzyme Sod2 (superoxide dismutase 2).109 SIRT3 activation also promotes the glutathione antioxidant Idh2 (isocitrate dehydrogenase 2), which decreases age-associated hearing loss in mice.109 Therefore, CR may operate in part through sirtuins to promote the function of antioxidants and extend lifespan. As antioxidant activity counteracts the deleterious effects of ROS, CR-dependent upregulation of antioxidants can promote lifespan extension. Of note, several reports have suggested that the antioxidant-dependent impact of CR on lifespan may reflect tissue specific processes that can also differ between organisms.110-112

CR may also be capable of promoting lifespan by increasing mitochondrial efficiency and energy production.113 Indeed, CR increases mitochondrial respiration rates as well as the overall number of mitochondria in mouse cells.114 CR can also increase the number of mitochondria in human cells.115 While increased mitochondrial bioenergetics can be beneficial, mitochondria are in fact the major site of ROS production. So how can it be beneficial to increase mitochondrial function under CR? One explanation is that CR increases overall mitochondrial efficiency, thereby decreasing the number of electrons stalling in the electron transport chain (ETC) and preventing excessive ROS generation. Electrons stall in the ETC when their rate of entry exceeds their rate of transit.116 This then creates an environment that is prone to generate ROS.116 It was proposed that CR-dependent improvement of mitochondrial bioenergetics may prevent electron stalling by permitting mitochondria to simultaneously process a greater number of electrons.117

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Consistent with this possibility, CR-treated rats have decreased electron leaks within the ETC complex I. Thus, by increasing mitochondrial efficiency, CR may limit ROS-generating electron leaks and promote lifespan. CR can reduce electron leak by maintaining low mitochondrial membrane potential. CR maintains a low membrane potential at least in part by regulating vacuolar pH. The latter increases with age and this lowers vacuolar storage capabilities. In turn, this increases the concentration of free cytoplasmic amino acids. It is thought that mitochondrial catalysis of cytoplasmic amino acids places a burden on mitochondrial carrier proteins and this in turn may overwhelm and increase mitochondrial membrane potential. CR helps maintain a low vacuolar pH over lifespan, possibly through the lifespan-modulating usual suspect TOR. Therefore, by maintaining a high vacuolar pH, CR can lower mitochondrial membrane potential, thereby decreasing ROS production and increasing lifespan. Taken together, these studies point to CR as a suppressor of ROS production as well as an activator of antioxidant processes.

However, activation of the mitochondria is not always beneficial. In yeast Sod2 knockout cells, the switch toward respiration under CR causes a massive decrease in lifespan. Similarly, CR shortens lifespan in mice with malfunctioning Sod1. This suggests that the increase in antioxidant activity is critical to maintain lifespan under CR. Without it, the increase in mitochondrial activity will increase oxidative damage. This seems to occur even if CR can lower ROS generation during respiration in an antioxidant-independent fashion. Therefore, in these settings, the magnitude of oxidative damage caused by CR-mediated increases in respiration must be greater than the amount of oxidative damage decreased due to elevated mitochondrial efficiency and the maintenance of a low mitochondrial membrane potential.

Furthermore, CR shortens the lifespan of yeast cells lacking Vma (vacuolar membrane ATPase). Vma proteins are responsible for vacuolar H+-ATPase function and therefore maintain vacuolar acidity by proton transport. Thus, CR fails to acidify vacuoles and increase amino acid storage in Vma-deficient cells. This would then limit the ability of CR to lower mitochondrial membrane potential. The fact that CR shortens lifespan when its ability to lower mitochondrial membrane potential is impaired suggests that a low mitochondrial membrane potential is also required to compensate for the increased chance of ROS production in the presence of CR-driven mitochondrial activation (Fig. 3). Taken together, these data show that CR must maintain a low mitochondrial membrane potential and promote antioxidant functions in order to compensate for the elevation in ROS levels that typically occurs upon CR-dependent increases in mitochondrial activity.

Overall, these findings paint the picture of a very delicate balance between lifespan extension and suppression through CR’s effect on mitochondria. Cells will increase respiration and mitochondrial number, likely to compensate for decreased energy intake. This then increases the probability of ROS production. CR compensates for this via activation of antioxidant proteins and increasing mitochondrial efficiency via modulation of mitochondrial membrane potential. Overall, the combination of these changes allows CR to actually limit ROS-dependent damage. However, when CR is unable to affect antioxidants or mitochondrial membrane potential, ROS production is higher than in AL. Conditions that alter the ability of CR to positively affect mitochondrial membrane potential can thus switch the effect of CR on lifespan from positive to negative. Lifespan outcomes may also reflect the notion that the effect of CR on antioxidants can display tissue and organism specificities. Overall, this delicate balance further highlights how CR is a broad acting intervention that may just be the key to unlocking the mysteries of aging.
Calorie restriction has been proposed to impact lifespan by a great number of mechanisms, some of which are discussed here (Fig. 4). It is just emerging that various seemingly subtle changes in a genetic background can cause CR to have dramatically different consequences on lifespan. A recent study conducted in budding yeast indicates that the number of pathways the disruption of which causes the effects of CR on lifespan to change from neutral or positive to negative is substantial. These pathways include oxidative stress response, vacuolar function, and protein catabolism among others. It is important to note that different mutations impacting even the same pathway or organelle may cause CR to have different effects depending on the specific mutation. For example, although CR lowers the lifespan of superoxide dismutase mutants, it is mainly mitochondrial mutants that were found to positively respond to CR. However, several of the mutants whose lifespan responds positively to CR may reflect the ability of this broad acting dietary intervention to activate processes that correct or counteract defects triggered by the initial mutation. For example, TOR inhibition, which is also achieved by CR, can alleviate mitochondrial disease in a mouse model of Leigh syndrome. In contrast, within the setting of other mitochondrial diseases or even clinical conditions linked to dysfunctional autophagy genes, CR may actually shorten lifespan. This is likely to be only the tip of the iceberg as one can also imagine that CR will also exert unpredictable effects on cells and/or organisms carrying multiple genetic alterations or polymorphisms. Thus, the task ahead of the CR and/or aging field is really enormous.

Luckily, the fact that CR is expected to have a wide range of consequences on lifespan also implies that this dietary intervention will be beneficial within large fractions of the human population, be they healthy or suffering from various diseases. As we often strive to decrease our caloric intake in today’s health-conscious society, it will also be just as important to identify those of us who may actually be harmed rather than helped by caloric restriction.

Disclosure of Potential Conflicts of Interest
No potential conflict of interest was disclosed.

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