# The conserved role of protein restriction in aging and disease

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**Purpose of review**
Dietary interventions are effective strategies for preventing disease and promoting health span. Many of the effects of dietary restriction are linked to amino acid and protein levels and their regulation of nutrient-signaling pathways. Thus, protein restriction is a promising therapeutic strategy for preventing aging-related diseases and extending life span.

**Recent findings**
Studies in yeast and flies have shown that amino acid restriction promotes longevity and protection. In rodents, protein restriction extends life span and alleviates detrimental aging phenotypes. Finally, clinical trials in middle-aged adults have demonstrated the role of a protein-restricted diet in promoting health span. Interestingly, the population over the age of 65 may not benefit from severe protein restriction potentially because of the increased physiological decline that leads to decreased amino acid absorption and altered protein synthesis.

**Summary**
Protein restriction can have profound effects on health and longevity, but excessive restriction is detrimental, particularly in the very old. The investigation of the mechanisms that modulate nutrient-sensing pathways is important to understand how regulation of protein intake can optimize health span and longevity.

**Keywords**
health span, restriction of calories, longevity, protein restriction

## INTRODUCTION

Early studies evaluating diet and longevity in a number of organisms concluded that restriction of calories is the major contributing factor for the extension of life span. However, more recent results indicate that restriction of calories may have both beneficial and detrimental effects, which can even shorten life span. Furthermore, a growing number of studies suggest that specific nutrients, independently of caloric intake, are capable of regulating aging [1]. These studies range from work in yeast and invertebrates, to studies in mice and humans. Several recent studies have proposed that modulation of amino acid and protein intake results in decreased aging-related pathologies and increased health span and life span in mice [1–4]. However, epidemiological data suggest that the benefits of protein restriction are age-dependent or at least are affected by age [5▪]. In this review, we will summarize recent research on protein and amino acid restriction in model organisms and discuss current results for both middle-aged and older adults.

### Nutrient-signaling pathways and amino acid availability in yeast

The model organism *Saccharomyces cerevisiae* has been used extensively to study the mechanisms of dietary restriction and their link to health span and life span [6]. Multiple pathways have been found to play a role in nutrient sensing and the subsequent activation of pro-growth and pro-aging signaling [7]. The Tor1-Sch9 (functional ortholog of the mammalian S6 kinase) and Ras2 (homologous to RAS protooncogene)-cAMP (cyclic adenosine monophosphate)-protein kinase A (PKA) pathways are activated by amino acids and glucose, respectively

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## KEY POINTS

- Pro-growth and nutrient-sensing proteins such as target of rapamycin, S6 kinase, and insulin like growth factor 1, play a major role in longevity and stress resistance.
- Dietary restriction is a powerful method for promoting stress resistance and alleviating detrimental aging phenotypes in yeast, invertebrates, mice, and primates, including humans.
- Many of the beneficial effects of restriction of calories can be achieved by protein restriction, making it easier to implement.
- Dietary restriction can have very different effects in the young and elderly populations, thus highlighting different macronutrient requirements in different age groups.

[7]. In the presence of nutrients, activation of these pathways results in the inhibition of regulator of IME2 (Rim15), a positive regulator of Msn2/4 and Gis1 (glycogenin-like gene 1-2 suppressor) stress resistance transcription factors. Alternatively, inhibition of Tor1-Sch9 or Ras2-PKA signaling in yeast has been shown to extend chronological life span, reduce age-related genome instability, and promote multistress resistance.

The use of biochemically defined media has also helped to dissect the role for glucose and amino acids on Tor-Sch9 and Ras2 signaling, and determine their subsequent impact on health span and life span [8–10]. Restriction of both glucose and amino acids results in an increase in yeast life span [9,10]. Specifically, amino acid scarcity stalls translation, which promotes the accumulation of uncharged tRNAs. General control nonderepressible 2 (GCN2) is a protein kinase that senses nutrient deprivation by binding to these uncharged tRNAs, resulting in slowed growth and the restoration of metabolic homeostasis (Fig. 1) [10].

Interestingly, the limitation of a single amino acid is enough to impact the signaling of progrowth and proaging pathways. Restriction of methionine or an increase in glutamic acid has been shown to increase life span in yeast [11]. Stress resistance, a hallmark of healthy aging, is also impacted by nutrient signaling. Recent work indicated that glucose sensitized yeast to oxidative stress in a Ras-dependent manner, whereas threonine, valine, and serine-promoted cellular sensitization, and reduced longevity by activating upstream regulators of Sch9 [9]. Conversely, amino acid limitation was sufficient to increase life span and also resulted in the decline of aging-dependent DNA damage [5▪,9].

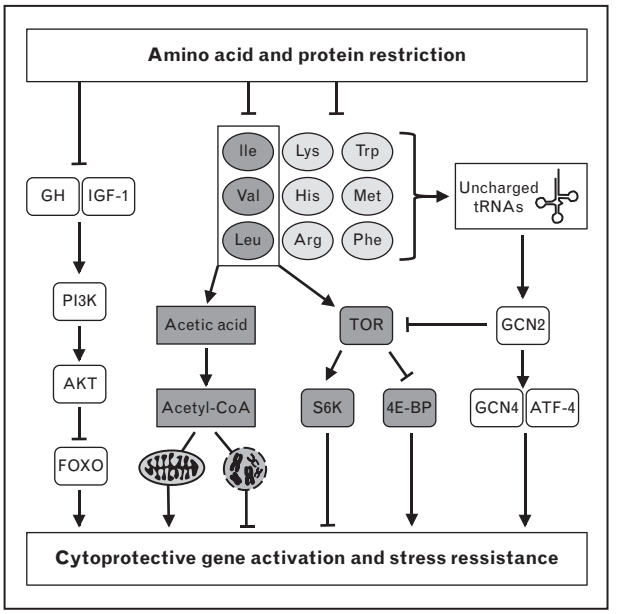


**FIGURE 1.** Nutrient signaling pathways are impacted by protein and amino acid restriction, resulting in the modulation of cytoprotective genes and changes in stress resistance. On the left, growth hormone/insulin like growth factor 1 (IGF-1) axis and downstream effectors are affected by reduction of protein/amino leading to Forkhead box protein O (FOXO) activation. Similarly, specific amino acids are shown to regulate acetyl-CoA production, Tor, and general control nonderepressible 2 (GCN2). Availability of acetyl-CoA affects many aspects of the cell including providing the acetyl group for histone acetylation, which in turn affects autophagy-dependent cellular homeostasis. Moreover, accumulation of uncharged tRNAs as a result of scarcity of amino acids activates GCN2 resulting in inhibition of target of rapamycin (TOR) signaling by activating cytoprotective genes through defective pharyngeal development protein 4 (PHA-4)/Forkhead box protein A (FOXA).

A single amino acid can also play a multifaceted role in longevity signaling. Catabolism of leucine results in the production of acetic acid, which can be utilized by the mitochondria or the nucleus to produce acetyl-CoA. Mitochondrial Ach1-dependent generation of acetyl-CoA promotes the storage of stress resistance carbon sources [12▪]. Alternatively, nucleo-cytosolic Acs2-dependent generation of acetyl-CoA results in histone acetylation changes and the down-regulation of cytoprotective genes (Fig. 1) [12▪,13].

These biochemical and genetic studies in yeast have indicated that glucose and amino acids are critical factors in the activation of a network of pro-aging and pro-growth nutrient-sensing pathways. Moreover, these investigations have shown

that nutrient limitation is a potent method for promoting stress resistance and extending life span.

## Amino acid restriction promotes life span and cytoprotection in invertebrates

Nutrient-sensing pathways are well conserved in invertebrate models, such as *Caenorhabditis elegans* and *Drosophila melanogaster*. In both models, dietary restriction extends life span, which may be mediated in part by reducing the activity of the target of rapamycin (TOR) pathway [7,14,15]. Inactivation of TOR can occur through the limitation of amino acids, as well as nitrogen and carbon sources [7,16–18]. Recent work in *C. elegans* has indicated that the type of bacterial food source has a significant impact on life span [19▪]. However, this phenomenon may be attributed to pathogen stress, in addition to diet.

Worms are well known for their value in genetic studies to elucidate longevity pathways. For instance, *eat-2 (ad465)* mutants have a pharyngeal pumping defect which results in a mechanically restricted diet that extends life span via inhibition of TOR signaling [17,20]. In *C. elegans*, insulin-like signaling is regulated by *daf-2/Inr*. Both *daf-2/Inr* and *tor* mutations cause life span extension by modulating nutrient signaling and increasing the activity of stress resistance transcription factors defective pharyngeal development protein 4 (PHA-4)/Forkhead box protein A (FOXA) [17,21]. Similar to yeast, the GCN2 kinase is conserved in *C. elegans* and has been shown to mediate longevity during dietary restriction and during inhibition of TOR signaling by activating cytoprotective genes through PHA-4/FOXA [20,22].

Although research suggests that amino acid sensing pathways are conserved in *C. elegans*, because of their limited ability to alter the diet, researchers have yet to distinguish between the impacts of reduced calorie intake versus amino acid restriction [2]. In contrast in flies, dietary restriction can be implemented without impacting calorie content by modulating dietary sugar, yeast, and other macronutrients. Life span extension is achieved for flies exposed to dietary restriction conditions. However, an important observation was that the supplementation of essential amino acids in dietary restricted flies results in a loss of life span extension, whereas the supplementation of nonessential amino acids has a minimal impact on life span [23].

In addition to the modulation of specific amino acids in flies, life span extension can be attained by changing the ratio of protein to other macronutrients [24–26]. In a study where diets were chemically composed to control the levels of specific macronutrients, those that were rich in protein and lower in carbohydrates, had a negative impact on the life span of the Queensland fruit fly [24]. Careful studies in *D. melanogaster* that modulated specific dietary proteins and sugars reached similar conclusions [26]. Therefore, the protein:carbohydrate ratio significantly impacts life span in flies, with optimal lifespan in the flies consuming a high carbohydrate low protein diet.

## Protein restriction in rodents and nonhuman primates

The evolutionarily conserved role for nutrient-sensing pathways in longevity and stress resistance also extends to rodent models and nonhuman primates. Mice, with a growth hormone-insulin/insulin like growth factor 1 (IGF-1) signaling deficiency, exhibit increased insulin sensitivity and a delayed manifestation of fatal neoplasms [7]. In an Alzheimer's mouse model, a protein-restricted diet was shown to reduce IGF-1 and phosphorylated Tau, resulting in a decline in cognitive impairment and Alzheimer's disease-related pathologies [7,27]. Inhibition of mTOR/S6 kinase (S6K) signaling also results in increased life span and reduction of detrimental aging phenotypes (Fig. 1) [7,14].

In rodents, restriction of tryptophan results in delayed tumor onset, increased health span, and protection against ischemia/reperfusion dependent kidney, and liver injuries [1,2,28]. Restriction of methionine results in lowered serum glucose, insulin, and IGF-1 levels, as well as decreased mitochondrial-dependent oxidative stress and reduced adiposity [1,2] in mice. Furthermore restriction of leucine improves insulin sensitivity, but has not been shown to impact life span [2,29].

Amino acid response pathways control other cytoprotective processes such as autophagy, immune function, and energy metabolism, which have an additive impact as part of overall benefits of protein restriction [2]. These pathways, which have likely evolved independently, play critical roles in managing metabolic demands and optimizing growth potential in response to translation regulation by GCN2, IGF-1, and TOR through amino acid availability [2,14,28].

Nutrient starvation leads to reduced acetyl-CoA availability, which results in reduction of histone acetylation, and induction of autophagy-dependent cellular homeostasis and life span extension [12▪,13]. In contrast, supplementation of media with Leu has been shown to maintain the acetyl-CoA levels and histone acetylation that suppresses the activation of autophagy and life span benefits in nutrient-starved cells [30]. Similarly, in yeast

studies, excess Leu in media directly correlates with an increase in the extracellular acetate concentration, which has been shown to impact life span, indicating a conserved role for some branched-chain amino acids in regulating longevity (Fig. 1) [30,31].

In rodents, both total protein and specific amino acid restriction results in food aversion and reduced food intake. Although studies control for this artifact using pairwise feeding of control animals, caloric restriction may be a confounding variable [1]. A recent study by Robertson *et al.* [32▪] addressed this concern by removing essential amino acids or nonessential amino acids known to influence IGF-1, TOR, and GCN2 signaling and replacing them with isocaloric levels of other amino acids to maintain an equal level of calorie and nitrogen intake. In a different study, a method called Geometric Framework has been used in ad libitum-fed mice to evaluate the impact of different combinations of dietary macronutrients on food and energy intake, as well as on measures for metabolic health and longevity [33,34]. Results of these studies indicate that protein and carbohydrate intake, rather than fats, are the predominating factors driving food consumption to meet biological requirements [34]. Specifically, of the different combinations of macronutrients tested, a low-protein/high-carbohydrate diet was accompanied by an increase in hepatic mTOR activation and resulted in the longest life span [34]. These results are in agreement with the finding that changes in some amino acids, and possibly in glucose levels can regulate the activation of mTOR [35].

Although a number of studies have been conducted to understand the biological impact of dietary restriction in invertebrates and rodents, few studies have evaluated CR in nonhuman primates. Only two major nonhuman primate studies have been conducted to address the impact of caloric restriction on life span. Both the study at the University of Wisconsin and at the National Institute on Aging (NIA), monitored rhesus monkeys to investigate the impact of chronic calorie restriction on life span and health span [36▪,37]. Although the studies had conflicting results in regards to the effect of CR on life span extension [the Wisconsin National Primate Research Center (WNPRC) study showed increased life span whereas the NIA study showed no difference], and both the WNPRC and NIA investigations demonstrated health span benefits [36▪]. In the WNPRC study, aging-related diseases manifested at a rate that was three times higher in the control versus that in the restriction of calories group; and age-related disease and all-cause mortality were reduced in the restriction of calorie restricted group in the WNPRC study whereas the NIA study reported reduced incidences of cancer in the restricted group [36▪,37].

One difference in experimental conditions was that the protein source for the NIA study was derived from wheat, corn, soybean, fish, and alfalfa meal, whereas the protein source for the WNPRC study was from lactalbumin obtained from milk whey. Comparing only the control groups of NIA and WNPRC, these studies raise the possibility that a more plant-based protein source diet (as used in the NIA study) may have a protective effect against aging-related mortality factors compared with the animal-based protein source diet (as used in the WNPRC study). However, the potential role of lower sucrose levels in the NIA study (3.95% in comparison to 28.5%) cannot be discounted.

## Clinical trials and epidemiological studies

Several human clinical trials and epidemiological studies have investigated the benefits of protein intake on health span measures. A 26-year follow-up of the 'Nurses' Health Study' (NHS) and a 20-year follow-up of the 'Health Professionals' Follow-up Study' (HPFS) suggested a positive correlation between a low-carbohydrate diet and decline of aging-related disease [38]. For the two studies including 85 168 women (NHS) and 44 548 men (HPFS), there were a total of 21 233 deaths, 41% of which were from cancer and 25% from cardiovascular disease [38]. When macronutrient intake was evaluated, diets high in animal-based protein and fats and low in carbohydrates were associated with the higher cases of mortality for both men and women. In contrast, vegetable-based low-carbohydrate diets resulted in the lowest mortality and cardiovascular disease mortality rates for both men and women [38]. Although this study did not analyze nor did it reach any conclusion about the role of proteins in age-related diseases, it is supportive of a role of proteins in increasing the incidence of several diseases.

Although an independent analysis of the HPFS cohort revealed no significant correlation between protein intake and ischemic heart disease (IHD) or stroke events, comparison of the top and bottom quintile protein source groups revealed an inverse correlation between plant-based protein intake and IHD/stroke incidence, and a negative correlation between animal-based protein intake [39,40]. Furthermore, independent multivariable analyses of the NHS cohort and others have found a positive correlation between red meat and high-fat dairy consumption and risks for aging-related diseases, such as IHD [41], colorectal cancer [42], and diabetes [43].

A Swedish study investigating a cohort of 43 396 women found that a 10% decrease in carbohydrate intake or a 10% increase in protein intake was correlated with a significant increase in cardiovascular disease incidences [44]. Similar to yeast, invertebrate, and rodent studies, the relationship between proteins and carbohydrates has a critical role in health span measures. For the Swedish cohort, a 10% reduction in carbohydrate or increase in protein intake corresponded to a 5 g increase in protein consumption or a 20 g decrease in carbohydrate consumption, ultimately changing the protein : carbohydrate ratio [44]. This appeared to be as a result of the fact that individuals substituted carbohydrates with animal protein, thereby changing the overall protein intake.

Although the majority of studies suggest a negative correlation between high-protein diets and aging-related disease, the fact that some studies do not, may indicate a trade-off caused by low-protein intake at older ages. Recent work has indicated that for individuals aged 50 or older, there is no correlation between protein intake and increased mortality [5▪]. A higher protein diet was associated with an increase in mortality for individuals younger than 65, only when the cohort was divided into groups ranging from 50 to 65 or 65 and older [5▪]. Interestingly, the individuals that consumed a high-protein diet also had higher levels of IGF-1. Because IGF-1 decreases with age, it is possible that the oldest members of the cohort actually benefited from the increase in protein intake. In support of this conclusion, this study found that individuals over 65 that consumed a low-protein diet demonstrated increased mortality compared with individuals with a higher protein intake [5▪]. This mechanism was confirmed in mice, by experiments that showed that young mice could maintain a healthy weight after being switched to a low-protein diet, whereas older mice could not. However, the increased mortality in over 65 individuals reporting low-protein intake does not imply that a low protein intake is detrimental in the elderly but possibly that a very low protein intake is not sufficient for old individuals. It is also possible that the cohort of older subjects reporting very low protein intake included more individuals who are sick, frail and malnourished than those who maintain a low protein but high nourishment and generally healthy diet.

In Ecuador, approximately 100 individuals are known to be homozygous carriers of mutations in the growth hormone receptor (GHR) which cause deficiency in IGF-1. Longitudinal studies of this population indicate a very low incidence of aging-related pathologies, including cancer and diabetes, as demonstrated in model organisms [45]. The collective knowledge acquired from both human and nonhuman studies support the hypothesis that lower protein intake results in lower activity of the GHR/IGF-1 and Tor-S6K pathways, and consequently in cellular and organismal protection against aging-related pathologies.

## CONCLUSION

In this review, we have highlighted some of the most recent work investigating amino acid and/or protein intake and longevity in a variety of model organisms, including yeast, worms, flies, and rodents, as well as human and nonhuman primates. The current body of knowledge strongly suggests that the pathways regulating metabolism, growth, and aging are connected and complex. It is clear that a high consumption of proteins specifically those derived from red meat and other types of animal sources has a pro-aging and disease effect and that the recommended daily allowance for protein intake should be lower for younger adults than that for adults over the age of 65. Therefore, it is crucial to continue research on both the molecular mechanisms of protein restriction and its impact on animal models and humans to further understand the effects of amino acids on aging and diseases. In fact, dietary interventions are among the most promising and cost-effective means to prevent and, in some cases, treat a wide variety of aging-related diseases, especially those exacerbated by a Western lifestyle.

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### Conflicts of interest

*V.D.L. has equity interest in L-Nutra, a company that develops medical food. H.M. and R.R. have no conflicts of interest.*

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