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The Conserved Role for Protein Restriction During Aging and Disease

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Abstract

Purpose of review—Dietary interventions are effective and cost efficient strategies for preventing disease and promoting healthspan. Many of the effects of dietary restriction are linked to amino acid and protein availability sensed by nutrient-signaling pathways. Thus, protein restriction is a promising therapeutic strategy for treating aging-related diseases and extending lifespan.

Recent findings—Studies in yeast and flies have shown that amino acid restriction promotes longevity and cytoprotection. In rodents, protein restriction extends lifespan and alleviates detrimental aging phenotypes. Finally, clinical trials in middle-aged adults have demonstrated the utility of a protein-restricted diet in promoting healthspan, such as reducing cancer, heart disease and diabetes. Interestingly, the elderly population over the age of 65 do not benefit from protein restriction potentially due to increased physiological decline that leads to muscle wasting.

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 healthspan and longevity.

Keywords

healthspan; longevity; CR; PR

Introduction

Early studies evaluating diet and longevity in a number of organisms concluded that restriction of calories (CR) was the major contributing factor for the extension of lifespan. However, more recent results indicate that CR may have both beneficial and detrimental effects, which can even shorten lifespan. Furthermore, a growing number of studies indicate that specific nutrients, independent of caloric intake, are capable of regulating aging (1)

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Conflicts of interest

V.D.L. has equity interest in L-Nutra, a company that develops medical food.

These studies range from work in yeast and invertebrates, to mammals and clinical trials. Several recent studies have indicated that modulation of amino acid and protein intake results in decreased aging-related pathologies and increased healthspan and lifespan in mice (1–4). However, epidemiological data suggests 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 restriction in model organisms and discuss current trends in human clinical trials 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 mechanisms of dietary restriction and their link to healthspan and lifespan (Longo, Shadel et al. 2012). Multiple pathways have been found to play a role in nutrient-sensing and the subsequent activation of pro-growth and pro-aging signaling (6). The Tor1-Sch9 and Ras2-cAMP-PKA pathways are activated by amino acids and glucose, respectively (6). In the presence of nutrients, activation of these pathways results in the inhibition of Rim15, a positive regulator of Msn2/4 and Gis1 stress resistance transcription factors. Alternatively, deletion of Tor1-Sch9 or Ras2 in yeast has been shown to extend chronological lifespan, reduce age-related genome instability, and promote multi-stress resistance.

The use of biochemically-defined media has also demonstrated the role for glucose and amino acids on Tor-Sch9 and Ras2 signaling, including their subsequent impact on healthspan and lifespan (7–9). Restriction of both glucose and amino acids results in an increase in lifespan (8, 9). Specifically, amino acid scarcity stalls translation, which promotes the accumulation of uncharged tRNAs. 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 (Figure 1) (9).

Interestingly, the limitation of a single amino acid is enough to impact the signaling of pro-growth and pro-aging pathways. Restriction of methionine or an increase in glutamic acid has been shown to increase lifespan in yeast (10). Stress resistance, a hallmark of healthy aging, is also impacted by nutrient signaling. Recent work in our laboratory indicated that glucose sensitized yeast to oxidative stress in a Ras-dependent manner, while threonine, valine, and serine promoted cellular sensitization and reduced longevity by activating upstream regulators of Sch9 (8). Conversely, amino acid limitation was sufficient to increase lifespan and also resulted in the decline of aging-dependent DNA damage (5, 8).

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 (11). Alternatively, nucleocytoplasmic Acs2-dependent generation of Acetyl-CoA results in histone acetylation changes and the down-regulation of cytoprotective genes (Figure 1) (11, 12).

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 works have shown that nutrient limitation is a potent method for promoting stress resistance and extending lifespan in yeast.

Amino Acid Restriction Promotes Lifespan 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 (DR) extends lifespan, which may be mediated in part by reducing the activity of the TOR pathway (6, 13, 14). Inactivation of TOR can occur through the limitation of amino acids, as well as nitrogen and carbon sources (6, 15–17). Recent work in *C. elegans* has indicated that the type of bacterial food source has a significant impact on lifespan (18)*. However, this phenomenon may be attributed to pathogen stress, rather than diet. Worms are well-known for their utility in genetic engineering studies in order to elucidate longevity pathways. For instance, *eat-2 (ad465)* mutants have a pharyngeal pumping defect which results in a mechanically restricted diet that extends lifespan via inhibition of TOR signaling (16, 19). In *C. elegans*, insulin like signaling is regulated by *daf-2/Inr*. Both *daf-2/Inr* and *tor* mutations cause lifespan extension by modulating nutrient signaling and increasing the activity of stress resistance transcription factors PHA-4/FOXA (16, 20). Similar to yeast, the GCN2 kinase is conserved in *C. elegans* and has been shown to mediate longevity during DR and during inhibition of TOR signaling by activating cytoprotective genes through PHA-4/FOXA (19, 21).

While research suggests that amino acid sensing pathways are conserved in *C. elegans*, due to limited ability to alter the diet, researchers have yet to distinguish between the impacts of reduced calorie intake versus amino acid restriction in worms (2). However, in flies, DR can be implemented without impacting calorie content by modulating dietary sugar, yeast, and other macronutrients. Lifespan extension is achieved for flies exposed to DR conditions. However an important observation was that, the supplementation of essential amino acids in DR flies resulted in a loss of lifespan extension, while the supplementation of non-essential acids had a minimal impact on lifespan (22).

In addition to modulation of specific amino acids, DR-dependent lifespan extension can be attained by changing the ratio of protein to other macronutrients (23–25). In a study where diets were chemically composed to control specific free amino acids, those that were rich in protein and lower in carbohydrates had a negative impact on the lifespan of the Queensland fruit fly (23). Careful studies in *D. melanogaster* that modulated specific dietary proteins and sugars found similar results (Bruce, Hoxha et al. 2013). Therefore, the protein: carbohydrate ratio significantly impacts lifespan in flies.

Protein Restriction in Rodents and Non-human Primates

The evolutionarily conserved role for nutrient-sensing pathways in longevity and stress resistance also extends to rodent models and non-human primates. Mice with a GH-insulin/IGF-1 signaling deficiency exhibit increased insulin sensitivity and a delayed manifestation of fatal neoplasms (6). 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 AD-related pathologies (6, 26). Inhibition of mTOR/S6K signaling also resulted in increased lifespan and reduction of detrimental aging phenotypes (Figure 1) (6, 13).

In rodents, restriction of tryptophan results in delayed tumor onset, increased healthspan, and protection against ischemia/reperfusion kidney and liver injuries (1, 2, 27). 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. Restriction of leucine improves insulin sensitivity, but has not been shown to impact lifespan (2, 28).

Amino acid-response pathways control other cytoprotective processes such as autophagy, immune function, and energy metabolism, which have an additive impact that contributes to the overall benefits of protein restriction (2). These pathways likely evolved independently, but play critical roles in managing metabolic demands and optimizing growth potential in response to translational control by GCN2, IGF-1, and TOR through amino acid availability (2, 13, 27).

Nutrient starvation leads to reduced acetyl-CoA availability, which results in reduction of histone acetylation, and induction of autophagy-dependent cellular homeostasis that promotes lifespan extension (11, 12). In contrast, supplementation of media with Leu has been shown to maintain acetyl-CoA and histone acetylation that suppresses the activation of autophagy in nutrient-starved cells and subduing lifespan benefits (Eisenberg, Schroeder et al. 2014). Similarly, in yeast studies excess Leu in media directly correlates with increase in the extracellular acetate concentration, which has been shown to impact lifespan, indicating a conserved role for some branched-chain amino acids in regulating longevity (Figure 1) (29, 30).

In rodents, both total protein and specific amino acid restriction results in food aversion and reduced food intake. While studies control for this artifact using pair-wise feeding of control animals, it increases the possibility that caloric restriction is a confounding variable (1). A recent paper by Robertson *et al.* addressed this concern by removing essential AAs or non-essential AAs known to influence IGF-1, TOR, and GCN2 signaling and replacing them with isocaloric levels of other amino acids in order to maintain an equal level of calorie and nitrogen intake (31)*. 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 measures for metabolic health and longevity (32, 33). Results of these studies have indicated that protein and carbohydrate intake, rather than fats, are the predominating factors for driving food consumption to meet biological requirements (33). Specifically, of the different combinations of macronutrients tested, a low protein/high carbohydrate diet resulted in the longest lifespan and was accompanied by an increase in hepatic mTOR activation (33). These results are in agreement with the finding that changes in some amino acids and possibly glucose can regulate the activation of mTOR (34).

While a number of studies have been conducted to understand the biological impact of dietary restriction in invertebrates and rodents, few studies have evaluated diet in non-human primates. Only two major non-human primate studies have been conducted to address the impact of caloric restriction on lifespan. Both studies, from the University of Wisconsin and the National Institute on Aging, used rhesus monkeys to investigate the impact of a 30% reduction in calories on lifespan and healthspan (35, 36). While the studies had conflicting results in regards to lifespan extension (the WNPRC study showed increased lifespan while the NIA study showed no difference), both the WNPRC and NIA investigations demonstrated healthspan benefits (35)*. In the WNPRC study, aging-related diseases manifested at the rate of three times higher in the control vs the CR group and age-related disease and all cause mortality were reduced in the CR group while the NIA study reported reduced incidences of cancer in the CR group (35, 36).

One difference in experimental conditions was that the protein source for the NIA study was derived from wheat, corn, soybean, fish, and alfalfa meal, while 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 suggest that a more plant-based protein source diet (as used in the NIA study) may have lower risk for aging-related mortality factors compared to animal-based protein sources (as used in the WNPRC study). However, the effects of lower sucrose levels in the NIA study (3.95% in comparison to 28.5%) cannot be discounted.

Protein Restriction in Clinical Trials and Epidemiological Studies

Several human clinical trials and epidemiological studies have investigated the benefits of protein intake on healthspan 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 (37). 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 (37). 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 (37).

While 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 (38, 39). 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 (40), colorectal cancer (41), and diabetes (42).

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

While the majority of studies suggest a negative correlation between high protein diets and aging-related disease, the fact that some studies do not may be due to trade offs 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 also found that individuals over 65 that consumed a low protein diet demonstrated increased mortality compared to individuals with a higher protein intake (5). This mechanism was confirmed in mice, demonstrating 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 is indicative that low protein intake in the elderly may benefit some and not others.

In Ecuador, the inhabitants of a small town exhibit a rare case of symmetrical dwarfism, which is due to a deficiency in GHR and IGF-1. Longitudinal studies of this population have linked the absence of aging-related pathologies, such as cancer and diabetes, to the protective properties of a disrupted GHR/IGF-1 axis, as demonstrated in other model organisms (44). The collective knowledge acquired from both human and non-human studies support the hypothesis that lower protein intake results in lower activity of the GHR/IGF-1 and Tor-S6K pathways, thus enabling cellular and organismal protection against aging-related pathologies.

Conclusions

In this review, we have highlighted some of the most recent work investigating protein intake and longevity in a variety of model organisms, including yeast, worms, flies, and rodents, as well as human and non-human primates. The current body of knowledge strongly suggests that the pathways regulating metabolism, growth, and aging are connected and complex. It is clear that lower protein consumption, specifically those derived from red meat and other types of animal sources, have many health benefits and that the recommended daily allowance for protein intake is different for younger adults compared to 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 human patients to further understand

the effects of protein intake 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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Key Points

- Pro-growth and nutrient-sensing pathways, such as TOR, S6K, and IGF-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 CR can be achieved by protein restriction, making it easier to implement in a modern lifestyle.
- Dietary restriction is not “one size fit all” and has shown to have very different effects in the young and elderly populations, thus highlighting different macronutrient requirements in different age groups.

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, GH/IGF-1 axis and downstream effectors are affected by reduction of protein/amino leading to FOXO activation. Similarly, specific amino acids are shown to regulate acetyl-CoA production, Tor and GCN2. Availability 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 due to scarcity of amino acids activates GCN2 resulting in inhibition of TOR signaling by activating cytoprotective genes through PHA-4/FOXO.