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Issue: *Diet, Sulfur Amino Acids, and Health Span***Methionine restriction and life-span control**Byung Cheon Lee,^{1,*} Alaattin Kaya,^{2,*} and Vadim N. Gladyshev^{2,*}¹College of Life Sciences and Biotechnology, Korea University, Seoul, South Korea. ²Division of Genetics, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts

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Dietary restriction (DR) without malnutrition is associated with longevity in various organisms. However, it has also been shown that reduced calorie intake is often ineffective in extending life span. Selecting optimal dietary regimens for DR studies is complicated, as the same regimen may lead to different outcomes depending on genotype and environmental factors. Recent studies suggested that interventions such as moderate protein restriction with or without adequate nutrition (e.g., particular amino acids or carbohydrates) may have additional beneficial effects mediated by certain metabolic and hormonal factors implicated in the biology of aging, regardless of total calorie intake. In particular, it was shown that restriction of a single amino acid, methionine, can mimic the effects of DR and extend life span in various model organisms. We discuss the beneficial effects of a methionine-restricted diet, the molecular pathways involved, and the use of this regimen in longevity interventions.

Keywords: dietary restriction; protein restriction; methionine restriction; life-span extension

Introduction

Dietary restriction (DR) is an evolutionarily conserved strategy that was reported to extend life span in a broad range of organisms.¹ It is generally believed that reduced food intake (a term often used interchangeably with calorie restriction (CR)) is the basis for the DR effect on life span.² While the beneficial effects of DR are known for all major model organisms of aging, including yeast, fruit flies, worms, and mice, a word of caution is needed regarding the diets used in these experiments. Dietary composition necessarily differs for different experimental models and among research laboratories, which may lead to different or even opposite life-span effects.³ In addition, the duration and severity of the DR regimen that achieves maximal longevity may not be feasible outside of laboratory settings.⁴ In humans, DR may be associated with severe side effects and elevated risk

of malnutrition, especially with regard to protein and micronutrient requirements.⁵ Although analyses of those practicing CR showed that humans exhibit some of the same molecular and metabolic signatures observed in long-lived calorie-restricted rodents,⁶ currently it is impractical to directly apply CR to increase longevity and reduce the risk of age-associated diseases in humans.

In order to alleviate the detrimental consequences of DR while preserving its beneficial effects, several studies have attempted to develop modified DR regimens. In particular, a combination of optimized diet, meal frequency, and timing (prolonged fasting) was suggested to achieve the DR-associated life-span extension.⁷ Another promising DR regimen involves a decrease in the levels of protein and/or individual essential amino acids, such as methionine and tryptophan. It was suggested that these diets may support longevity without decreasing daily calorie intake;⁸ they may also reduce possible undesirable effects of DR diet in humans and avoid the risk of malnutrition. In this review, we focus on the importance of the composition of DR diets

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involving methionine restriction (MR), discuss the mechanisms involved, and provide examples that illustrate the relationship between MR and longevity through the balance of protein-based dietary regimens.

Dietary restriction: the royal road to longevity?

Since CR was first shown to be a reliable intervention for extending the life span of laboratory rats,⁹ there have been numerous studies that applied this dietary regimen to a broad range of species.^{10–15} It has been demonstrated that the incidence of many age-associated disorders could be decreased by reduced calorie intake.^{16,17} However, the benefits of this powerful regimen were also questioned by the research community because of its ineffectiveness in extending life span under many conditions, challenging researchers both to understand the basis of these contrasting effects and to develop better dietary approaches and a stronger theory.^{18–21} For instance, the effect of decreased dietary toxicity associated with DR could be easily confused with the effect of life-span extension upon reduced food intake. For example, this phenomenon is observed when highly concentrated sugar–yeast or yeast diets are used in *Drosophila*.^{18,19} To distinguish the toxic effects of diets on life span, fecundity may also be assessed in tested organisms. For example, egg production in cohorts of mated female flies may be measured, because it is decreased by toxicity associated with excessive food intake, whereas it is elevated with an increase in food intake when the diet is truly limiting and approaches malnutrition.¹⁹ However, such analyses are still insufficient and lead to further questions. What is the underlying mechanism of food toxicity? Why is the toxic effect not observed under conditions of reduced dietary intake? Is it too small to be observed by assessing egg production? In addition, DR in *Drosophila* may be achieved by simply diluting sugar–yeast levels, thereby resulting in differences in water intake. As in the case of water stress, it was reported that life-span extension by DR could be abolished by providing *ad libitum* water without altering food intake in *Drosophila* (i.e., water consumption could be a factor in assessing life-span extension by DR). Nevertheless, it is of interest that life-span extension in Canton-S flies upon reduction of dietary yeast alone was not affected by water availability.²²

Another challenge to DR is that we do not know the exact ingested amounts of food or its distribution in the organism following consumption. Compensatory feeding under DR conditions was reported in many studies and is considered as another variable for the life-span effects of DR.^{23,24} Current methods to quantify the eating behavior in *Drosophila* have limitations. Though specific dyes such as eriochlorogenic acid can be used to assess this behavior in *Drosophila*,²⁵ such analyses assess the amounts of food consumed in a short period of time, and the absorption rates of individual nutrients, their distribution in the body, and their contribution to various metabolic processes may be different and further influenced by DR.

Importance of reduced protein intake in dietary restriction

A recent evidence suggests that reduced calorie intake does not necessarily extend life span in *Drosophila*, whereas it was found that reduction of specific nutritional components, such as dietary yeast, is important in supporting life-span extension. Specifically, sugar reduction had little or no influence on life span, even though calorie intake was reduced by this regimen, whereas yeast reduction was consistently very effective in life-span extension (Fig. 1).^{18,26,27} In mammals, including humans, protein restriction (PR) diets without malnutrition also show beneficial effects: PR contributes to reduced mortality and incidence of age-associated diseases, ultimately supporting longevity.^{28–30} Interestingly, low protein intake leads to a reduction in serum IGF-1 levels in respondents aged 50–65 years and is associated with reduced cancer incidence and overall reduced mortality.²⁹ Importantly, two long-term DR studies (1 and 6 years) without malnutrition did not find reduced IGF-1 levels or the IGF:IGFBP-3 ratio in humans subjected to severe CR (CR diet group: 1800 kcal/day with 24% of calories from protein and 28% of calories from fat; Western diet group: approximately 2500 kcal/day with 16% of calories from protein and 33.6% of calories from fat), whereas IGF-1 levels could be reduced using a moderately restricted protein diet (low-protein diet: 0.76 g/kg/day, ~10% of energy intake from protein, high-protein diet: 1.73 g/kg/day, ~24% of energy intake from protein).³⁰ This finding shows that PR may be an effective regimen in humans and that it may delay aging by

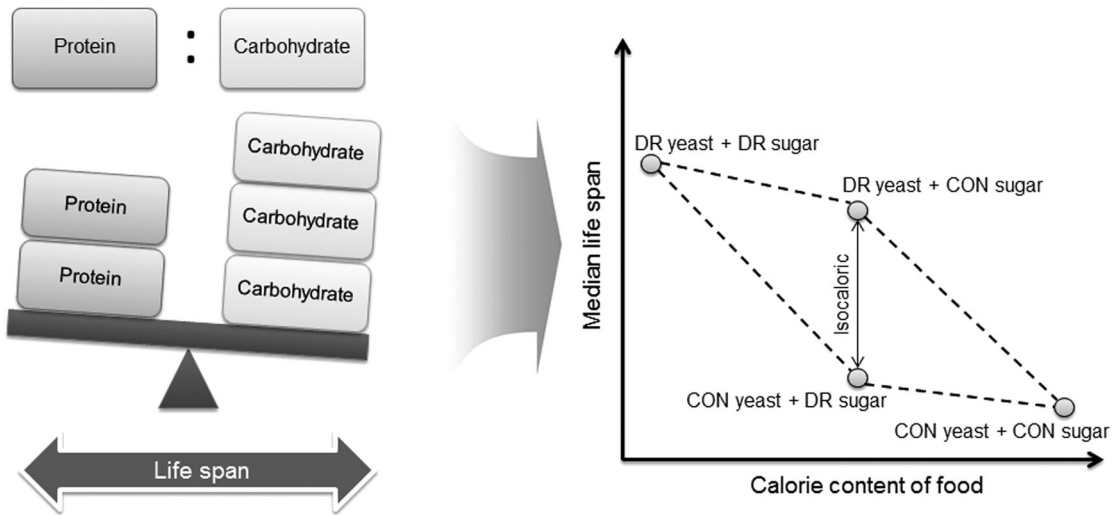


Figure 1. Protein and carbohydrate components of diet differentially influence life span in *Drosophila*. Low-protein diet (DR yeast) is more effective in extending life span than low-carbohydrate diet (DR sugar). DR, dietary restriction; CON, control diet. Modified from Ref. 27.

influencing the insulin/IGF-1 signaling pathway, an evolutionarily conserved mechanism that regulates longevity.^{30,31} For example, Ames dwarf mice, which exhibit low levels of growth hormone (GH)/IGF-1, are characterized by small body size and long life span.³² It was also reported that oxidative stress may be reduced by the PR diet. Decreased protein ingestion (by 40%) in Wistar rats leads to lower levels of reactive oxygen species (ROS), reduced oxidative damage to DNA and proteins, and decreased expression of respiratory complex I in the liver.²⁸ Overall, growing evidence regarding reduced protein intake strongly supports the idea that, among the three major nutritional components (proteins, carbohydrates, and lipids), proteins are in the unique position to regulate life span and mimic the effect of DR on aging.

With respect to the effect of a PR diet on aging, it was also reported that the protein/carbohydrate ratio needs to be considered if one wants to maximize the beneficial effects of reduced protein levels on aging. It was shown that life span in *Drosophila* is the longest on a diet with a 1:16 ratio of protein to carbohydrate and that this life span gradually decreases as the ratio increases. This finding supports the general notion that both low protein levels and the specific balance between proteins and carbohydrates are critical determinants of maximal life span of *Drosophila*.³³ Consistent with this notion,

an analysis of dietary geometry in mice showed that energy restriction achieved by food dilution in *ad libitum*-fed mice did not extend life span, probably because of the compensatory consumption of protein, but a low-protein, high-carbohydrate diet could limit such compensatory protein consumption. Here, the energy requirement was met by adding more carbohydrates and reducing protein intake, suppressing hepatic mammalian target of rapamycin (mTOR) and mitochondrial function owing to reduced circulating branched-chain amino acids.³⁴ Therefore, though PR is the main mechanism that supports the benefits of DR, compensatory food consumption due to diluted food can be a potential problem. It may lead to spurious results on life span owing to compensatory protein intake, up to a normal level. However, balanced macronutrient composition can help avoid compensatory food consumption, thereby optimizing the effect of diet on life span.

Methionine restriction and aging

With growing evidence on the importance of protein and amino acid restriction in regulating life span, several studies focused on dissecting the roles of dietary amino acids in order to identify nutrients that regulate longevity.^{35–37} In this regard, the most successful case has been the use of an MR diet. Its beneficial effect on life span was described in

rat, mouse, and fruit fly models, although it was also questioned for being inconsistent, similar to the effect of DR. For example, 2- to 4-week-old rats were examined on two diets that contained 0.86% or 0.17% methionine.²³ Compensatory food consumption occurred on the diet with the lower level of methionine that led to reduced body weight, showing the same pattern of eating behavior as in the case of DR.²³ Nevertheless, the life span of rats on the lower methionine diet was extended against increased dietary intake.³⁸ As supportive evidence, it was reported that both maximum and median life span of mice could be extended when the low-methionine diet was provided starting at 12 months of age.³⁹ It was also shown that metformin altered *Escherichia coli* methionine metabolism, leading to MR in *E. coli*, which in turn led to life-span extension of the host *Caenorhabditis elegans*.⁴⁰ In human diploid fibroblasts, MR extended replicative life span and inhibited senescence by downregulating mitochondrial protein synthesis and respiratory chain assembly.⁴¹ As such, many studies strongly support the idea that MR can be effective in extending life span in various species and that it mimics the effects of DR. On the other hand, ineffectiveness of MR has been noted in *Drosophila*, similar to the cases of DR and PR. A chemically defined diet was developed for *Drosophila* that allowed regulating the levels of individual dietary components, including methionine. Unexpectedly, initial studies did not observe a positive regulation of life span on the low-methionine diet, either when 5% or 15% glucose levels were used.⁴²

As discussed above, many of the MR, PR, and DR studies face the same challenges. For example, CR did not extend life span in rhesus macaques in a study carried out at the National Institute on Aging, whereas a study at the Wisconsin National Primate Research Center reported a positive effect of CR on life span.^{10,17} Leaving aside potential differences, including the study design, dietary regimes of control groups, housing environment, timing of food consumption, and diet composition, which may have influenced the results, a clear fact is that CR is not always successful in achieving life-span extension and may show opposing results even in very similar and well-controlled studies. These considerations must be taken into account when one considers the use of DR regimens and interprets the results. A recent approach that employs nutrient geometry

may help provide deeper insights into the DR effects on aging. Like in DR studies, the ineffectiveness of MR in extending life span could reflect the fact that both methionine itself and its balance with other nutrients are important in achieving the desired effect. A recent study tested the effect of amino acid balance on life span in *Drosophila*, examining various combinations and proportions of methionine and other amino acids. Interestingly, the life span of *Drosophila* was extended by MR under conditions of low amino acid status, while MR did not work under conditions of high amino acid status (Fig. 2).⁴³ In conclusion, as in the case of DR, methionine is not a magic bullet that regulates life span, as certain conditions must be met for its beneficial effect. For that reason, future studies may benefit from the use of diverse combinations of methionine and other nutrients.

Benefits of methionine restriction in age-related health

Aging is accompanied by organ and tissue dysfunction and elevated incidence of chronic diseases. Thus, it is not surprising that nutritional interventions involving DR and PR may also offer different benefits in preventing age-associated diseases and supporting longevity. Likewise, there have been many reports regarding the benefits of MR in health and longevity.⁴⁴ For example, a significant reduction in the development of prostatic intraepithelial neoplastic lesions in transgenic mice was observed on the 0.12% methionine diet, when compared with the 0.86% methionine diet.⁴⁵ Also, 10-week-old *ob/ob* mice fed 0.12% (MR) or 0.86% (control) methionine diets for 14 weeks were analyzed. Interestingly, mice raised on the 0.12% methionine diet were rescued from severe steatosis and significantly reduced triglyceride, serum alanine aminotransferase and aspartate aminotransferase, and plasma insulin levels.⁴⁶ It was also demonstrated that MR in mice induced secretion of cardioprotective hormones, such as adiponectin and FGF21, leading to protection against hyperhomocysteinemia.⁴⁷

Reducing oxidative stress-induced damage is also critical for pursuing healthy aging. In this regard, some studies examined how MR diets affect ROS generation. Wistar rats were maintained on an isocaloric 40% MR diet for 7 weeks and then analyzed for parameters of oxidative stress. This diet

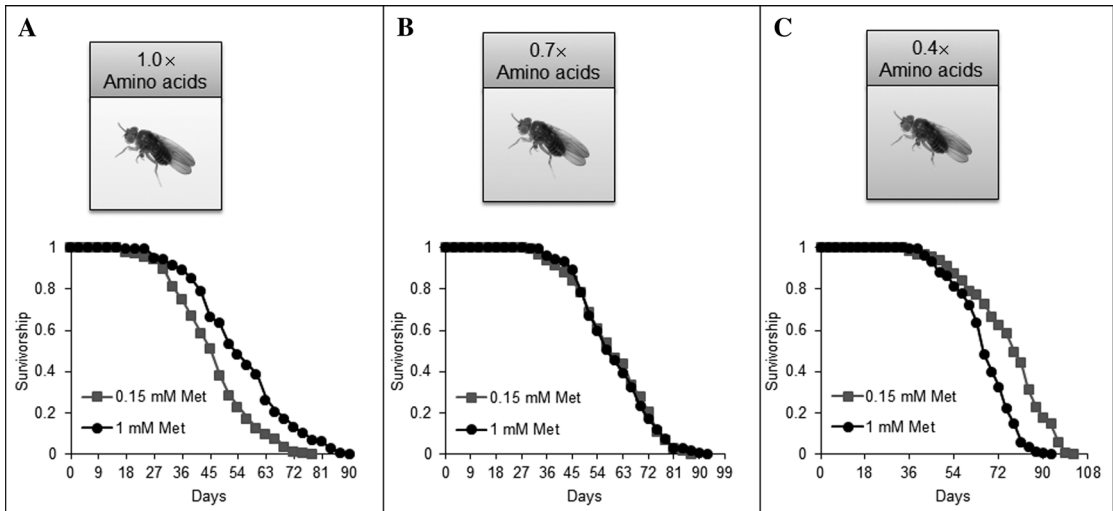


Figure 2. Methionine restriction extends *Drosophila* life span under conditions of low amino acid status. Comparison of *Drosophila* life span on 1 mM (black curves) and 0.15 mM (red curves) methionine when the levels of other amino acids are (A) 1.0 \times , (B) 0.7 \times , and (C) 0.4 \times . Modified from Ref. 43.

decreased heart mitochondrial ROS generation, particularly from complex I, as well as damage to mitochondrial DNA, proteins, and lipids.⁴⁸ It was also shown that mitochondrial ROS generation and subsequent damage to mitochondrial DNA and protein were reduced in heart, liver, brain, and kidney mitochondria in animals fed the 40% MR diet.^{49–51} In addition, an increase in glutathione levels and peroxidase activity was observed in blood and kidney of animals on the 80% MR diet.⁵² Moreover, biomarkers of oxidative stress, including plasma 8-hydroxydeoxyguanosine, 8-isoprostane, and erythrocyte protein-bound glutathione, were maintained at low levels in animals fed 80% MR diet for 6 months.⁵² Overall, lower ROS levels may contribute to maintaining healthy life span in rodent models fed MR diets, thereby leading to life-span extension.

Mechanisms of life-span extension by methionine restriction

Despite many studies, the mechanisms of life-span regulation by DR and PR remain incompletely understood. It was proposed that insulin/IGF-1 and mTOR signaling represent a conserved upstream nutrition-signaling pathway, which regulates longevity from yeast to mammals and mediates the effects of DR and PR^{53–55} (Fig. 3). Consistent with this idea, overexpression of dTsc1,

dTsc2, the dominant-negative forms of dTOR, or dS6K, all of which are involved in TOR signaling, extended *Drosophila* life span.⁴³ Moreover, life-span extension upon overexpression of dTsc2 depended on the levels of dietary yeast, supporting the idea that life-span extension by DR or PR is due to suppression of TOR signaling.¹³ This longevity strategy is also conserved in other organisms. Deletion of ribosomal S6 protein kinase 1, which acts downstream of mTOR, extended life span in mice, and the corresponding gene expression pattern mimicked that observed in response to DR.⁵⁶ It was also found that rats subjected to DR and treated with 1-methyl-1-nitrosourea to induce breast cancer showed decreased levels of phosphorylated mTOR, thereby downregulating mTOR activity and changing the phosphorylation status of p70S6K and 4E-BP1.⁵⁷ In the case of yeast, knockout of *SCH9*, which encodes a homolog of mammalian AKT and S6K and a major target of yeast TORC1, extended both chronological and replicative life spans.^{58,59} Decreased yeast TOR activity itself was also shown to extend chronological and replicative life span.⁶⁰ Finally, it was reported that CR of *SCH9* Δ or *TOR1* Δ mutants failed to further extend yeast replicative life span, suggesting that the life-span extension by CR in yeast involves suppression of TOR signaling.⁶¹

The MR diet mimics both DR and PR with regard to phenotypes associated with life-span extension

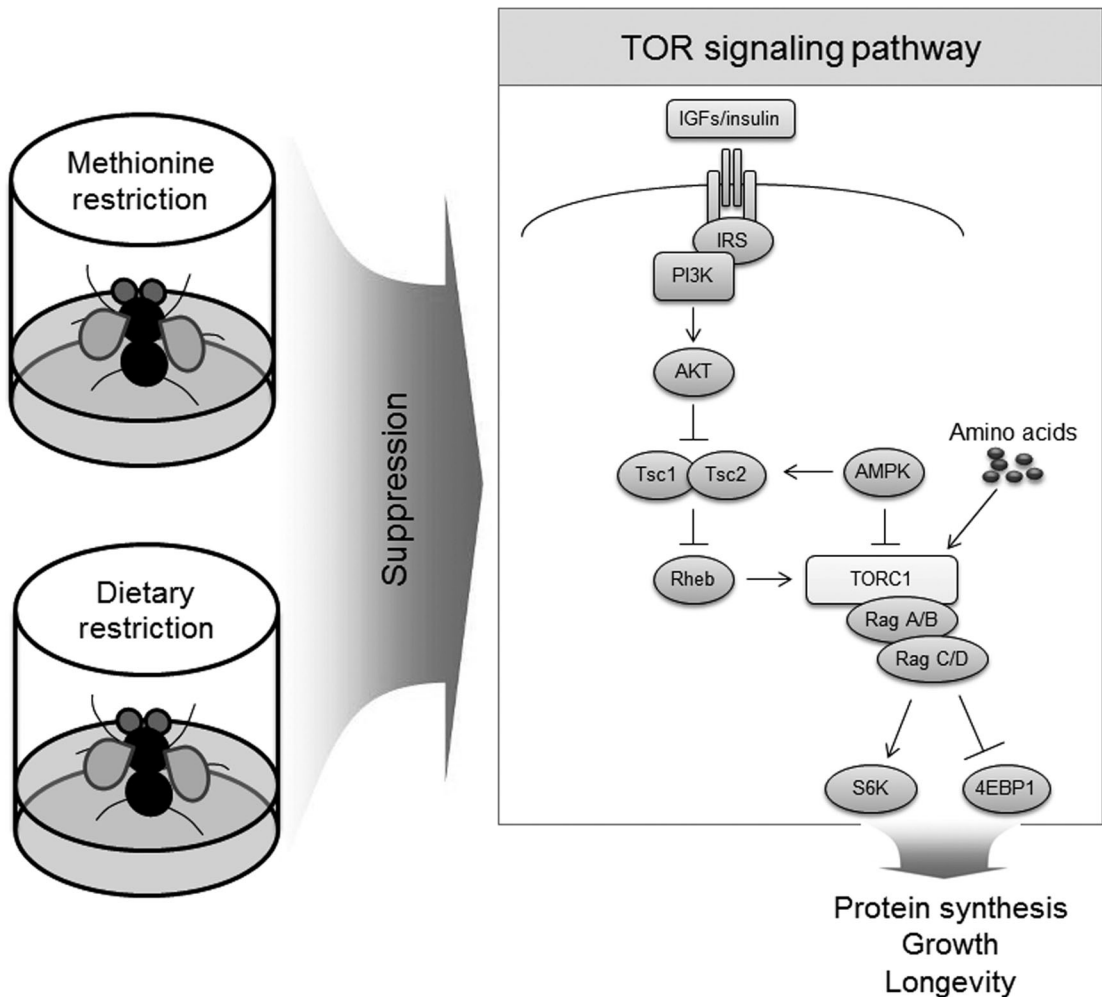


Figure 3. Dietary restriction, methionine restriction, and mTOR signaling.^{43,53–55} Life-span extension by both DR and MR depends on suppression of mTOR signaling. Insulin receptor and TSC2 were examined for their roles in life-span extension by MR under conditions of low amino acid status in *Drosophila*.

and induces similar gene expression and physiological changes.^{11,38,43,49,62,63} Thus, the underlying mechanism of life-span extension by MR may indeed involve the same pathways utilized by DR. Overexpression of dTsc2 or a dominant negative form of the insulin receptor (dInRDN) failed to further extend the life span of *Drosophila* subjected to MR under conditions of low amino acid status,⁴³ suggesting a role for mTOR signaling. However, whether this mechanism applies to MR under conditions of high amino acid levels remains unknown. On the basis of the analysis of life span in response to MR and involving graded amino acid levels, MR did

not extend *Drosophila* life span under conditions of high amino acid status.

A recent study reported that sulfur amino acid restriction increased the expression of cystathionine γ -lyase, which in turn elevated hydrogen sulfide production.⁶⁴ This effect was observed in yeast, worms, fruit flies, and mice subjected to DR media/diets, suggesting its importance for the DR-mediated longevity benefits. Consistent with this idea, MR-mediated life-span extension in fruit flies was accompanied by increased hydrogen sulfide production. Thus, the data support the notion that the life-span extension induced by MR shares

the same underlying mechanisms with the life-span extension induced by DR.

One of the conclusions from these studies is that decreased calorie intake from proteins, specifically methionine, may be a factor directly responsible for the life-span extension effect of DR.^{37,38,65} In fact, neither carbohydrate nor lipid restriction had a consistent effect on life span in rodents.^{66,67} On the basis of these findings, it is possible that these nutritional pathways also play roles in longevity regulation under MR conditions. In addition, many physiological changes in mice under MR conditions overlap with the effects of DR.⁶⁵ The involvement of mTOR together with GCN2 in amino acid-sensing mechanisms is well known.⁶⁸ Furthermore, a requirement of GCN2 for longevity extension by MR in yeast was shown. Declines in IGF-1, serum glucose, and insulin levels were also implicated in the MR effect in mice, suggesting a possible role for the GH/IGF signaling pathway in longevity control by methionine levels.⁶⁵ Moreover, additional studies support the hypothesis that Ames dwarf and GH receptor knockout mice do not respond to a 0.16% methionine diet, whereas the respective control animals and mice overexpressing GH respond with life-span extension.⁶⁹ Overall, when consumption of the essential amino acid methionine is limited, life-span extension in different organisms is observed, similar to the interventions that reduce calorie intake. However, further studies are required to better understand the molecular mechanisms involved and to apply these principles to human diet to achieve potential beneficial effects with regard to the aging process and/or age-associated chronic diseases.^{64–71}

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Conflicts of interest

The authors declare no conflicts of interest.

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