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Cutting back on the essentials; can manipulating intake of specific amino acids modulate health and lifespan?

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Abstract

With few exceptions, nutritional and dietary interventions generally impact upon both old-age quality of life and longevity. The life prolonging effects, commonly observed with dietary restriction reportedly are linked to alterations in protein intake and specifically limiting the dietary intake of certain essential amino acids. There is however a paucity of data methodically evaluating the various essential amino acids on health- and lifespan and the mechanisms involved. Rodent diets containing either lower methionine content, or tryptophan, than that found in commercially available chow, appear to elicit beneficial effects. It is unclear whether all of these favorable effects associated with restricted intake of methionine and tryptophan are due to their specific unique properties or if restriction of other essential amino acids, or proteins in general, may produce similar results. Considerably more work remains to be done to elucidate the mechanisms by which limiting these vital molecules may delay the onset of age-associated diseases and improve quality of life at older ages.

Keywords

methionine restriction; tryptophan; dwarf mice; naked mole-rat; delayed aging; healthspan

1.1 Introduction

For more than eighty years, a key focus in aging research has centered on the impact of dietary restriction on modulating lifespan. Across evolutionary distant phyla containing yeast, flies, worms, mice, monkeys and potentially humans, nutritional and dietary interventions generally impact upon longevity and quality of life at older ages. With few exceptions, life extending benefits of caloric restriction have been described numerous times in both vertebrates and invertebrates and with benefits evident at various levels of dietary restriction and/or intermittent feeding regimes and even when these interventions start in middle age (Liao et al., 2010; see Masoro, 2005; Le Couteur et al., 2016; Lee and Longo,

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2016 for reviews). Although these improvements in longevity are attributed to simply a reduction in calorie intake, several studies gaining traction have challenged this dogma and shown that, life prolonging effects are not due to carbohydrate and lipid dietary restriction but rather appear to be specifically due to restricting protein intake (Grandison et al., 2009; Levine et al., 2014; Mair et al., 2005; Min and Tatar, 2006; reviewed in Sanchez-Roman and Barja, 2013). Indeed, in 90% of the studies involving protein restriction in laboratory rats and mice, a ~20% lifespan extension was observed (Pamplona and Barja, 2006). Very few studies have dissected this relationship between protein restriction and lifespan so as to assess the role of the individual essential amino acids (EAA; i.e., those that cannot be synthesized *de novo* in humans and require dietary sources). Indeed, in mammals there are relatively few reports in which a specific amino acid has been selectively reduced in the diet followed by examination of physiological parameters related to aging and life extension. Despite this large gap in our understanding of the role of the various EAAs, the delay of age-associated diseases and life prolonging effects of protein restriction are primarily attributed to restricting two of the EAAs, namely tryptophan (Segall, 1977; Sidransky, 1986) and methionine (Zimmerman et al., 2003; Orentreich et al., 1993). Understanding the mechanisms that are altered by restriction of these amino acids and how they impact upon aging may provide critical information towards finding mechanisms to modulate both lifespan and healthspan.

1.2 Tryptophan restriction

Tryptophan is commonly found in protein rich foods such as turkey, chicken, fish eggs, and red meat. However, it is also abundant in dairy products, chocolate, oats, dried dates, bananas, seeds and nuts (e.g., almonds, sunflower seeds, pumpkin seeds). Tryptophan is of critical importance in growth, development and reproduction and is also the precursor for serotonin, a signaling molecule involved in numerous blood, bone, bowel, and brain functions (Fernstrom and Wurtman, 1997). Tryptophan may thus influence memory, mood and learning through this serotonin pathway as well as affect stress responses and aging (Markus, 2008; Ruddick et al., 2006). In excess, tryptophan can be both toxic and carcinogenic, traits attributed to the effects of excess serotonin and its modulation of cell growth, fibrosis and inflammation and its direct effects on pituitary function, in particular the adrenocorticotrophin and thyroid axes (Hiraku et al., 1995; King et al., 1997; Welford et al., 2016).

Work performed 40 years ago by Timiras and coworkers (Zimmerman et al., 2003; Segall and Timiras, 1976; Segall et al., 1983; Ooka et al., 1988) described studies in rats where tryptophan-deficiency was created by limiting this amino acid to 30–40% in the diet from weaning to 24–30 months of age. Aging features appeared to be delayed in some organs like liver, heart and ovary but not in others (kidney, lung, aorta) while survival appeared to be increased. However, longevity was assessed following a return to the control diet partway through the experiments, thus it is unknown how long the animals may have survived if left on a tryptophan deficient diet. Early deaths occurred in the tryptophan deficient group but rats surviving past the first year outlived control groups. Also, low levels of tryptophan caused reductions in overall diet intake thus confounding the results.

Other studies on tryptophan-poor diets revealed that rats increased their period of fertility and fecundity and showed higher levels of testosterone. Metabolic rate is also increased with dietary tryptophan depletion as is the pelage condition and hair growth (Segall et al., 1983; Ooka et al., 1988; Ashley and Curzon, 1981). These indicators of extended healthspan are likely linked to the reported increased lifespan observed in lab rodents (Segall et al., 1983).

Marked decreases in brain serotonin as the underlying mechanism for the delayed aging was mostly ruled out as pharmacologic antagonists did not affect growth to the same extent as tryptophan restriction while side effects impacted the length of these studies. Of note is that gut microbes contribute to the tryptophan levels in mice, as microbiota depletion experiments have shown that the tryptophan metabolic pathway is altered in adult brains (Desbonnet et al., 2015). In addition, tryptophanase activity is increased in germ-free mice (microbiome-depleted) thus increasing serum levels of tryptophan (Wikoff et al., 2009). Many studies have been conducted using acute tryptophan deficiency but outcomes were not aging related. Overall, it will be important to validate the longevity data in rodents on tryptophan deficient diets to solidify the potential contribution of this amino acid to our understanding of its role in aging-related processes.

1.3 Methionine restriction

An abundance of work has been reported examining aging-related outcomes and longevity in rodents consuming methionine deficient diets. Foods with the highest amounts of methionine per total protein content include beef, cereals, dairy, eggs, and brazil nuts. In contrast, foods with the lowest levels by comparison would be those that predominantly make up a low protein, vegan diet. Symbiotic microorganisms in the gastrointestinal tract can synthesize methionine, but it not known whether this serves as a significant source of methionine for humans (Lee and Hase, 2014). The microbiome serves as an important source of methionine and other proteins in *Caenorhabditis elegans* and other organisms (Cabreiro et al., 2013). This microbial food source is most notably important for herbivores maintained on a low quality (low protein/high fiber) diet (Bennett and Faulkes, 2000; Torrallardona et al., 1996).

Methionine is notably the first amino acid present in nuclear-encoded proteins, as the methionine codon signals the start of protein translation. Methionine is essential for the production of cysteine which in turn is the precursor of glutathione (GSH) a key component of detoxification pathways, and S-adenosylmethionine (SAM), a critical methyl-donor for methylation of various molecules including DNA and proteins (Figure 1). Activation of the transsulfuration pathway, downstream of methionine recycling, leads to the production of hydrogen sulfide (H_2S) gas, that is itself implicated in the beneficial effects of methionine restriction (Hine et al., 2015).

1.4 Methionine catabolism: The transsulfuration pathway

The liver is the main organ involved in methionine metabolism. During the methionine cycle, methionine is converted to SAM, S-adenosylhomocysteine (SAH), and homocysteine, and thereafter it is either recycled via remethylation to methionine or further modified via

the irreversible transsulfuration pathway to form cysteine. Cysteine, rather than methionine, may be the critical player inducing direct affects upon body size, adiposity and associated endocrine signaling. In support of this premise is the observation that dietary supplementation with cysteine attenuates the metabolic effects and energy balance perturbations observed during methionine restriction (Elshorbagy et al., 2011; Gomez et al., 2015). These effects could be directly linked to changes in the transsulfuration pathway. Both cystathionine beta-synthase (CBS), and cystathionine gamma-lyase (CSE) enzymes are upregulated during methionine restriction giving rise to elevated levels of H_2S and other downstream effectors such as glutathione and related compounds (Kabil and Banerjee, 2010; McIsaac et al., 2012; Petti et al., 2012). H_2S is considered a gasotransmitter, modulating many molecular pathways involved in mitochondrial function, cytoprotection, inflammation and apoptosis (Wallace and Wang, 2015). It also regulates second messenger systems and activates ATP-dependent potassium ion channels, thereby affecting many physiological and pathophysiological processes. H_2S levels are elevated in cardiovascular disease, rheumatoid arthritis and tumor growth (Peter et al., 2013; Muniraj et al., 2014; Hellmich et al., 2015). Despite this increase in pathological states, numerous reports suggest that high levels of H_2S may extend longevity. For example, *C. elegans* based studies in which exogenous levels of H_2S are elevated lead to an increase in the lifespan (Miller and Roth, 2007). Similarly, augmented levels of H_2S have been associated with increased lifespan in Drosophila and also with caloric restriction (Hine et al., 2015; Kabil et al., 2011). In contrast the naked mole-rat has very low circulating levels of H_2S and low CBS activity under non-stressed conditions but also shows increased sensitivity of CBS to SAM activation (Dziegelewska et al., 2016).

Enhanced activity of the transsulfuration pathway and resulting H_2S production may have hormetic effects activating various cytoprotective and/or nutrient signaling pathways (e.g., target of rapamycin [TOR], eukaryotic initiation factor 2 [eIF2α]). For example, H_2S induces protein S-sulfhydration whereby cysteine residues within proteins are modified such that the -SH from the sufhydryl donor forms a persulfide (-SSH) that may impact upon its function. S-sulfhydration of Kelch-like ECH-associated protein 1 (KEAP1), the negative regulator of the master cytoprotective transcription factor, nuclear factor erythroid 2-related factor 2 (NRF2), results in the activation of this important cytoprotective factor. This leads to increased detoxification, molecular chaperone levels and augmented ubiquitin proteasome system (UPS) proteolysis and in doing so helps protect against senescence (Yang et al., 2013). Another possible mechanism involves mitochondrial cytochrome enzymes, as H_2S binds with iron and inhibits both respiration and ROS formation conferring protection against oxidative damage (Pietra et al., 2011). Similar protective effects of elevated H_2S are also observed in invertebrates, fungi, and torpid animals (Robertson et al., 2015; Miller and Roth, 2007; Blackstone et al., 2005). Moreover, the preternaturally long-lived naked mole-rats have high levels of expression of the critical enzymes involved in cysteine and H_2S formation, namely CSE and CBS, suggestive of elevated levels of H_2S, although surprisingly observed serum levels are very low (Lewis et al., 2013). Resultant enhanced sulfhydration and antioxidant properties (Salmon et al., 2005) may also confer resistance to endogenous ROS and other oxidative stressors (Harper et al., 2011) and also contribute to extended longevity and stress resistance commonly observed in longer lived mutants of model

organisms, including yeasts and worms (Hine et al., 2015; Miller and Roth, 2007; Salmon et al., 2005; Harper et al., 2011; Calvert et al., 2009). In humans, high H_2S levels reportedly improve clinical outcome in response to ischemic reperfusion injury. However, high and moderately elevated levels of H_2S have also been shown to inhibit cytochrome c oxidase, react with other heme- and sulfur-containing proteins and contribute to disease thus, the amount of this gasotransmitter is likely tightly controlled and needs further study (Ueki et al., 2011; Roman et al., 2013; Dorman et al., 2002; Goubern et al., 2007). Moreover, enhanced resistance to oxidative stress is only one piece of the puzzle in terms of lifespan extension (Perez et al., 2009; Dai et al., 2014; Cunningham et al., 2015; Speakman et al., 2015). Methionine restriction may itself directly activate NRF2. Nrf2 is a key regulator of several hundred cytoprotective molecules, including molecular chaperones, antioxidants, detoxicants and proteasome subunits (Lewis et al., 2010). Efficient removal of damaged proteins via the UPS may contribute substantially to a well maintained proteome and prolonged healthspan that may similarly contribute to lifespan extension (Calvert et al., 2009; Hourihan et al., 2013).

Both *CYS3* and *CYS4* are highly conserved in naked mole-rats (Altschul et al., 1990) and it has been shown that the gene product, CBS, overexpression is sufficient to extend lifespan in worms as can exogenous H_2S (Hine et al., 2015). In animals, especially animals consuming a large proportion of plant material, it is important to note that the bacteria and protozoa symbionts of the gut also produce H_2S, and while tempting to speculate that this is an integral component of the health benefits of a vegetarian diet, it has not as yet been determined if H_2S produced by the microbiome causally contributes to longevity and if it has similar effect to that produced endogenously. While tantalizing, the beneficial effects of H_2S associated with methionine restriction remain poorly understood. Nevertheless, the genes associated with the transsulfuration pathway may be potential therapeutic targets in the quest for ways to retard the aging process and extend the period of good health.

1.5 Rodent studies of methionine restriction

The earliest aging studies restricting methionine in rodents were conducted by Orentreich and colleagues (Zimmerman et al., 1993; Orentreich et al., 1993; Richie et al., 1994) and showed that lifespan was significantly extended in animals subjected to an 80% restriction. Since that time, many reports have focused on physiological parameters contributing to delayed aging as well as potential disease resistance following short and long-term intake of diets deficient in methionine. Lifespan extension is observed both in mice started on these diets shortly after weaning and in mice started on the diets at 12 months of age, suggesting that the underlying mechanisms are not necessarily growth or developmentally regulated (Sun et al., 2009; Brown-Borg et al., 2014a; Miller et al., 2005). Although absolute differences exist in the changes reported in mice, they trend in the same direction and may be partly due to the particular background strains utilized.

Rats and mice fed diets with low methionine weigh less and have altered body composition when compared to rodents fed methionine-replete food. Introduction of the methionine-restricted diet either at one month or 12 months of age resulted in lower body weights compared to animals on normal chow (Orentreich et al., 1993; Sun et al., 2009; Miller et al.,

2005). Moreover, several studies suggest these rodents are more physically active than those fed methionine-sufficient diets but also show improved healthspan (Plaisance et al., 2010; Lees et al., 2014). Also, cataract development appears to be retarded slightly but significantly in mice on methionine-restricted diets (Miller et al., 2005). There is an age-dependent increase in T-cell subsets in mice and with methionine restriction, this age-related change seems to be slowed in comparison to mice ingesting higher amounts of methionine (0.43%; Miller et al., 2005). Although there is an abundance of pathology and necropsy data in pro-longevity mutant mice, few studies have systematically examined animals on methionine-restricted diets. One study observed that mice fed methionine-restricted diets exhibited similar types of illnesses as control mice but at slightly later ages (45 days) while others showed that methionine restriction delays prostate cancer development in a prostate cancer model (Miller et al., 2005; Sinha et al., 2014). In rats, chemically induced colon cancer is inhibited with methionine-restricted diets (Komninou et al., 2006). In short-living, growth hormone transgenic mice, methionine restriction significantly decreased the incidence of liver and kidney tumors in addition to lengthening lifespan by more than 50% (Brown-Borg et al., 2014b). Significant anti-inflammatory gene expression profiles in both liver and white adipose tissue were observed in mice consuming methionine-restricted diets (Wanders et al., 2014). Clearly, overall rodent health appears to be improved with this dietary intervention.

1.5.1 Alterations in hormone profiles with methionine restriction

Not only did methionine restriction in mice lead to a 10–20% increase in maximum lifespan (Orentreich et al., 1993; Miller et al., 2005) and improve healthspan, but sustained insulin sensitivity and metabolic rates were also observed during aging. Strikingly, although methionine restricted mice ate more than their experimental controls, within three weeks of methionine restriction, they showed improved responses to glucose tolerance testing rapidly removing the glucose bolus from the blood and presumably funneling this into cells (Lees et al., 2014; Malloy et al., 2006).

Rodents consuming low methionine diets exhibit reductions in plasma IGF1, insulin, leptin, thyroxine, and increases in adiponectin and fibroblast growth factor 21. Insulin like growth factor 1 levels are decreased by about one-third in mice and two-thirds in rats when compared to animals fed normal chow (Plaisance et al., 2010; Miller et al., 2005; Malloy et al., 2006). Insulin concentrations in the plasma are markedly decreased in both rats and mice fed methionine-restricted diets (by 85% and 75%, respectively) while thyroxine or T4 levels are reduced by 25% in mice and no difference was found in methionine-restricted rats (Miller et al., 2005; Malloy et al., 2006). Insulin sensitivity is greater in animals subjected to methionine restriction as glucose levels are lower and adiponectin and FGF21 are increased (Miller et al., 2005; Lees et al., 2014; Malloy et al., 2006). Directional changes in expression of both this growth factor and IGF1 may contribute to the observed changes in body composition and size of methionine-restricted mice as well as concomitant resistance to various cancers (Ables et al., 2012).

The levels of endocrine hormones in methionine-restricted animals are very similar to the levels observed in several long-living strains of mice supporting their underlying role in

delaying or slowing processes that contribute to aging. Dwarf mice share many phenotypes in common with methionine-restricted mice. Both mouse models show similar increases in longevity and resistance to cancer as well as enhanced stress resistance (Brown-Borg et al., 1996). It is possible that this shared phenotype reflects similar perturbations in methionine metabolism, in keeping with the hypothesis that upregulation of the methionine pathway and its downstream components (e.g., the transsulfuration pathway and formation of cysteine) are key players in extending both lifespan and the period of good health (Uthus and Brown-Borg, 2006). One significant finding is that growth hormone signaling is necessary for methionine restriction to contribute to lifespan extension in rodents as long living GH deficient (Ames dwarf) and GH resistant (growth hormone receptor knockout) mice do not benefit with further life extension when fed methionine-restricted diets (Brown-Borg et al., 2014b).

1.5.2 Changes in lipid metabolism with methionine restriction

Reductions in dietary methionine intake also impact lipid metabolism and nutrient signaling. Components of lipid metabolism are altered via reductions in hormones due to the decrease in fat mass while serum cholesterol and triglyceride levels are observed to be lower in comparison to normal chow-fed animals (Elshorbagy et al., 2011; Malloy et al., 2006). The direction of change in fatty acid composition of CNS and liver membranes in methionine-restricted mice are indicative of an increased resistance to oxidative damage (Jove et al., 2013). In this same study, glycerophospholipid and sphingolipids were altered thereby influencing lipid raft assembly and cellular signaling in methionine-restricted mice. These changes in sphingolipids and its associated ceramide signaling pathways may also contribute to improved tolerance to oxidative stress. Similarly, methionine restriction modulates membrane phospholipid composition and this too may influence susceptibility to lipid peroxidation (Jove et al., 2013). The bi-lipid layer of cell membranes from methionine-restricted mice has a lower double bond index and less oxidation-prone docasohexanoic acid [DHA] resulting in membrane phospholipids that are more resistant to oxidative damage (Jove et al., 2013; Hulbert et al., 2006). Similarly, long-lived species such as naked mole-rats (Mitchell et al., 2007; Hulbert et al., 2006) and parrots (Pamplona et al., 1996; Pamplona et al., 2005) as well as Ames dwarf mice show lower proportions of DHA in their cell membranes and a lower peroxidation index is observed in their tissues (Pamplona et al., 2005; Hulbert et al., 2007; Montgomery et al., 2012; Valencak and Ruf, 2013).

1.5.3 Altered energy balance with methionine restriction

Food consumption is increased in animals on methionine-restricted diets whether expressed in absolute terms or per gram of body weight (Orentreich et al., 1993; Miller et al., 2005; Malloy et al., 2006; Hasek et al., 2010). Fat mass is significantly reduced however lean mass does not appear to be affected by these diets (Malloy et al., 2006; Hasek et al., 2010; Elshorbagy, 2014). In terms of bone mass, rodents consuming a methionine-restricted diet had lower bone mineral content and a lower cancellous bone volume but also exhibited lower bone resorption markers, and bone mineralization rates (Huang et al., 2014). Thus, in balance, methionine restriction impacts bone mineralization but not bone turnover per se and decreases fat mass resulting in lighter weight animals (Huang et al., 2016).

Mice consuming low methionine diets exhibit higher levels of average daily energy expenditure compared to control fed animals. Enhanced metabolic flexibility (i.e., effectiveness of substrate switching between fasting and fed states; fat to carbohydrate utilization) may be linked to improvement in glucose metabolism for fatty acid oxidation appears to be downregulated in methionine restriction (Plaisance et al., 2010; Hasek et al., 2010; Anthony and Gietzen, 2013; Orgeron et al., 2014; Wanders et al., 2015). Increased metabolic rate of methionine-restricted mice is however not observed in mice lacking uncoupling protein (UCP1; Wanders et al., 2015), suggesting that methionine-restricted mice exhibit higher levels of thermogenesis and possibly may have higher body temperatures. Increased thermogenesis rather than ATP formation may be causally linked to the observed decline in oxidative damage in methionine-restricted rats (Maddineni et al., 2013; Sanchez-Roman et al., 2012). Redox signaling through ubiquinone 9 and NRF2-dependent phase II antioxidants, including NAD(P)H dehydrogenase quinone 1 (NQO1), glutathione-S-transferase (GST), and heme oxygenase 1 (HO1) is also upregulated in methionine-restricted mice, traits shared with other long-lived vertebrates (Lewis et al., 2010; Jove et al., 2013; Mitchell et al., 2007; Brown-Borg et al., 2015).

Fat deposition was shown to be limited by methionine restriction even when fed to obesity-prone animals due to the increase in total energy expenditure (Valencak and Ruf, 2013). Many of these changes reflect the altered costs of maintenance and growth when dietary methionine is low. Growth factor signaling and nutrient signaling are therefore clearly integrated and detect nutrient levels influencing the organism's responses to its environment. Low amino acid levels are detected by mammalian target of rapamycin (mTOR), an intracellular sensor and regulator of protein synthesis, cell growth and proliferation. Downstream effectors of mTOR, TORC1 and TORC2 coordinate anabolic and catabolic processes in response to energy status, nutrients and growth (Laplante and Sabatini, 2012). In contrast to other longevity mutants however, phosphorylated mTOR and AMPK (energy status sensor) are similar between methionine restriction and mice fed methionine-replete diets (Sun et al., 2009; Brown-Borg, 2015; Dominick et al., 2015; Gesing et al., 2011; Sharp and Bartke, 2005; Wang and Miller, 2012; Kim and Guan, 2011). The lack of a difference in AMPK may explain the decrease in fatty acid oxidation described in these animals as opposed to the upregulation found in long living dwarf mice (Perrone et al., 2012; Stauber et al., 2005; Bartke and Westbrook, 2012). Adiponectin and FGF21 are higher in methionine-restricted mice and typically activate AMPK in peripheral tissues stimulating fatty acid oxidation and utilization (Chau et al., 2010). Other mechanisms likely contributing to the methionine-restricted-mediated increase in energy expenditure include beta adrenergic receptor activation and UCP1 (Plaisance et al., 2010). It appears that either some of the sensors of nutrients and energy status do not detect the lack of methionine nor the change in energy status or that mRNA and protein levels of these sensors are not indicative of their action suggesting that the effects of methionine restriction are complex. Recently, Leib and Knight (2015) showed that the proposed amino acid sensor, GCN2, plays no role in dietary essential amino acid sensing, contrary to earlier reports.

1.5.4 Changes in mitochondrial function with methionine restriction

Mitochondria are intimate players in aging and lifespan and exhibit altered function in several mouse models of extended longevity (aging mutants). Mitochondrial encoded proteins of the respiratory chain complexes are enriched for methionine in mice, with reports that methionine itself serves as an antioxidant when ROS production is high (Bender et al., 2008). Altered mitochondrial function and concomitant reduced production of ROS and toxic oxidized macromolecules has been proposed to be causally linked to the increased lifespan of methionine-restricted mice (Carol et al., 2008; Lopez-Torres and Barja, 2008).

Uncoupled respiration is increased in tissues from rodents consuming methionine-restricted diets while enzyme components of oxidative phosphorylation are reduced or unchanged (Plaisance et al., 2010; Hasek et al., 2010; Caro et al., 2008; Sanz et al., 2006). Mitochondrial reactive oxygen species (ROS) generation and oxidative damage to proteins and DNA are also lower in methionine-restricted animals (Sanchez-Roman and Barja, 2013; Maddineni et al., 2013; Bender et al., 2008; Caro et al., 2008; Lopez-Torres and Barja, 2008; Sanz et al., 2006; Caro et al., 2009; Yang et al., 2015). Glutathione is decreased but no differences in antioxidative enzymes have been observed (Richie et al., 1994; Maddineni et al., 2013). Aspects of mitochondrial function reported for methionine-restricted animals are similar to some of the other interventions that impact aging and lifespan such as mild uncoupling of respiration and oxidative phosphorylation, reduced ROS production as well as reduced oxidative damage yet other mechanisms are present as antioxidant protection is less apparent.

1.5.5 Enhanced cytoprotection in methionine restricted rodents

Cytoprotection is an important physiological component that plays a pivotal role in attenuating the functional declines that occur during aging and is causally linked to species longevity (Lewis et al., 2010; Brown-Borg, 2006). Similarly enhanced stress resistance has been linked to prolonged lifespans in numerous studies and is common in longevity mutants and interventions that promote long-life (Kapahi et al., 1999; Murakami et al., 2003; Harper et al., 2006; 2007; 2011; Lewis et al., 2012). Studies using skin fibroblasts from methionine-restricted mice were not found to be resistant to cytotoxic agents in contrast to similar studies in long-living mice (Salmon et al., 2005; Harper et al., 2006; Murakami et al., 2003). However, hepatocytes from methionine-restricted mice showed contrary results and resisted the toxic effects of acetaminophen when compared to normal chow fed and dwarf mouse cells (Harper et al., 2006). Both *in vivo* and *in vitro* studies reveal that the activity of the master regulator of cytoprotective factors, NRF2, is elevated in methionine-restricted mice, a feature shared with long-lived species (Zhang et al., 2010; Lin et al., 2012; Lewis et al., 2014). Increased Nrf2 activity and concomitant enhanced expression of genes with an antioxidant response element would lead to enhanced antioxidant and detoxification capacities, although the exact mechanisms behind this relationship are unknown. Increased Nrf2 activity thus likely explains the higher levels of glutathione S-transferase, heme oxygenase 1, and NAD(P)H dehydrogenase quinone 1 in methionine-restricted mice (Jove et al., 2013). Clearly, methionine-restricted rodents share these stress resistance mechanisms with other long-living experimental mouse models, implicating common mechanisms in their lifespan extension, lower incidence of cancer and later onset of age-associated diseases

(Komninou et al., 2006; Sinha et al., 2014). What is not known is if these common mechanisms of extending mouse lifespan are equally applicable to long-lived humans and other long-lived species, or if these are mechanisms employed by short-lived animal models that naturally have poor defenses against endogenous and environmental stressors and thus, aging.

1.6 Other long-lived model organisms

To date methionine restriction has not been undertaken in any long–lived mammal and indeed almost nothing is known about methionine biology outside that of laboratory rodents. Very little is known about methionine and the effects of methionine restriction on metabolism in non-traditional model organisms. Circulating levels of methionine in the long-lived naked mole-rat are one-third of that observed in C57Bl/6 mice and liver tissue methionine levels are also markedly lower than those measured by mass spectrometry in mouse livers (McIsaac et al., 2016; Ma et al., 2015). The physiological relevance of these low levels is not known. This may reflect divergent regulation of methionine metabolism although it may also simply indicate the low levels of methionine present in their fruit and vegetable diet. In addition, tissue and plasma levels of methionine are only part of the picture. The flux of methionine within tissues in various organisms lends important information about utilization and regulation of this pathway. The flux of methionine is markedly higher in long-living Ames mice compared to wild type mice indicating that both transmethylation and transsulfuration are enhanced (Uthus and Brown-Borg, 2006). Further work in long-living mammals will address the importance of these findings.

1.7 Methionine restriction in humans

Published reports on the effects of methionine restriction on human health are few and far between. More common are case reports identifying the effects of mutations in components of the methionine pathway. Fortunately, many of these are rare. Hypermethioninemia is caused when mutations in methionine adenosyltransferase, glycine N-methyltransferase, adenosylhomocysteine hydrolase or cystathionine beta synthase genes occur (Baric et al., 2004; Furujo et al., 2012; Chien et al., 2015; Motzek et al., 2016). Low methionine diets are prescribed for patients with hypermethioninemia that are considered high risk for a cardiovascular event or neurologic deficit keeping in mind the potential to impact S-adenosylmethionine and potential neurological outcomes.

In vitro studies using human cell lines strongly suggest a similar effect of methionine restriction on replicative lifespan and delayed senescence, to that observed in yeasts where both chronological and replicative lifespan are increased in response to methionine restriction (Johnson and Johnson, 2014; Koziel et al., 2013). A thirty-fold reduction in methionine content of the cell culture media resulted in a 1.75 increase in the Hayflick limit, number of cumulative population doublings before a cell becomes senescent (Koziel et al., 2014). This increase in replicative lifespan of human cells was accompanied by a lower growth rate as well as reduced p16 levels and decreased oxygen consumption (Koziel et al., 2014). The one *in vivo* study involving humans reported that methionine restriction can increase fat oxidation in obese adults with metabolic syndrome (Plaisance et al., 2011).

Clinically, more relevant work is needed to understand the potential therapeutic use of methionine restriction to delay age-related physiological decline.

1.8 Branched chain amino-acid diets

There have been studies showing that different macronutrient ratios within the diet impact longevity. Solon-Biet and coworkers (2014, 2015) showed that longevity could be increased and metabolic outcomes improved in ad libitum fed animals by changing the relative proportions of macronutrients to induce mTOR inhibition. As the scientific community looks to further understand the role of dietary restriction and protein restriction, in particular, related studies describing the role of branched chain amino acid (BCAA) diets on metabolism and aging have been conducted (Valerio et al., 2011; Fontana et al., 2016). Rodent diets that specifically reduce the BCAA improved glucose tolerance, pyruvate tolerance, and body composition but did not induce an increase in serum FGF21 (Fontana et al., 2016). These investigators also conducted a clinical trial feeding low protein to 19 males for six weeks and found that this diet decreased body weight, fat mass, lowered fasting blood glucose levels and increased FGF21 in the serum. Additional studies indicate that FGF21 may be the key regulator of the metabolic effects induced by low protein diets (Laeger et al., 2016). A prospective cohort study in humans also suggests that in addition to the amount of the protein in the diet, the source of that protein is also important in terms of metabolic outcomes and mortality (Song et al., 2016). This report concluded that there was a positive association with high animal protein intake and mortality while high plant-based protein intake was inversely associated with mortality when at least one life style risk factor was present.

1.9 Conclusion

Substantial evidence has accumulated in laboratory rodents that there are numerous benefits to a diet lower in methionine and possibly also tryptophan, than found in commercially available chow. It is not known whether the beneficial effects associated with restricted intake of these two amino acids are due to their unique properties or if restriction of other essential amino acids may produce similar phenotypes. Recent, albeit limited, evidence suggests that reducing other essential amino acids or protein in general, may induce similar effects at least under stressful conditions such as renal ischemia (Fontana et al., 2016; Song et al., 2016; Ables et al., 2014). Substantially more work remains to be done to elucidate if all individual essential amino acids behave similarly and to understand the mechanisms by which limiting these vital molecules improve health span and delay the onset of age-associated maladies.

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Highlights

- In rodent diets, methionine and tryptophan restriction benefit health and lifespan.
- Low dietary methionine affects multiple systems including glucose, lipid and energy metabolism.
- Possible mechanisms include alterations in methionine metabolism, mTOR and Nrf2 signaling.

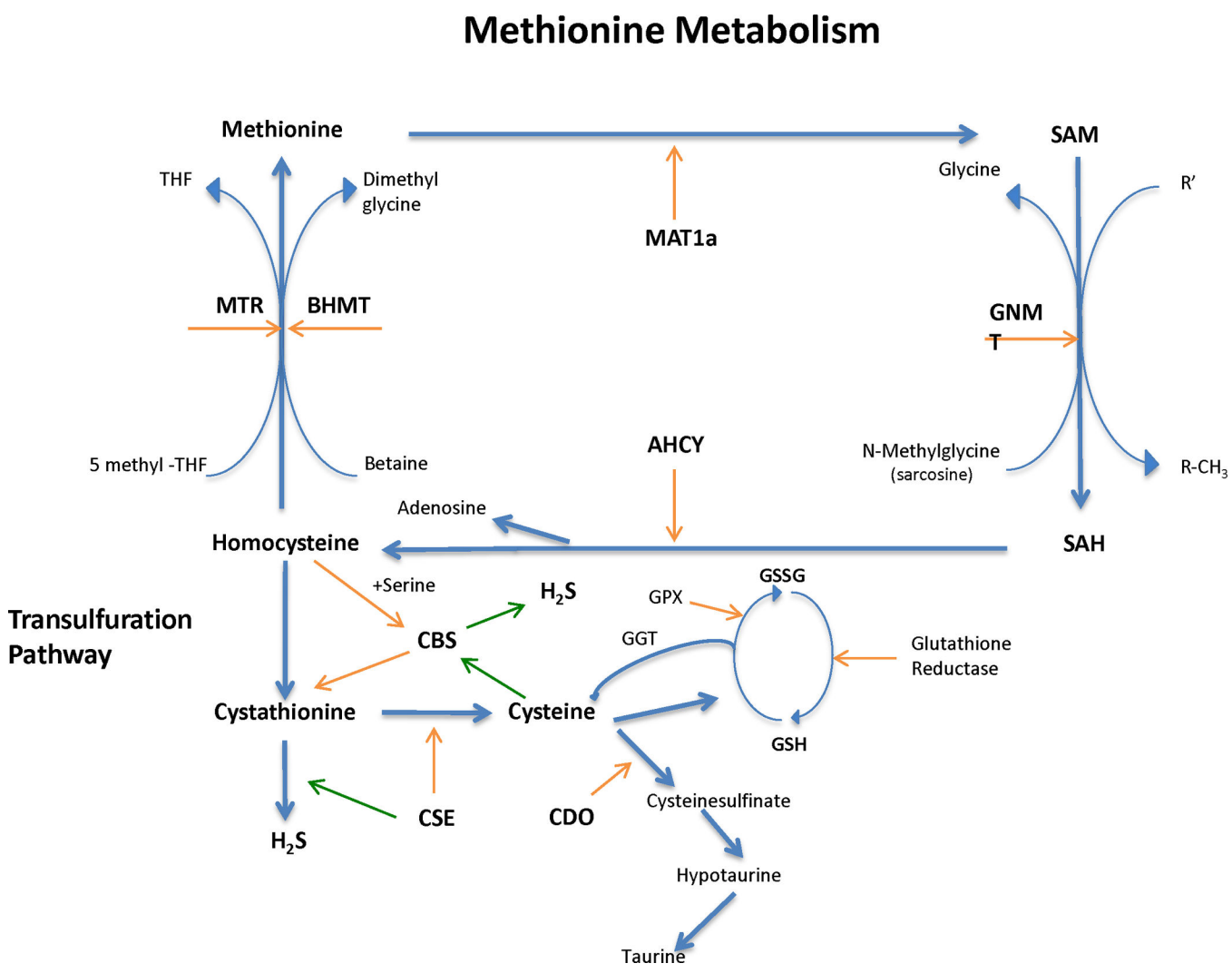

Figure 1.

The methionine metabolic pathway. We begin with methionine and include remethylation of homocysteine to methionine and transsulfuration of homocysteine to cysteine. In addition, the fate of cysteine incorporation to cysteine sulfinate through to taurine and hydrogen sulfide (green arrows) is shown as well as the contribution of cysteine from glutathione degradation pathways. MAT1a - methionine adenosyltransferase 1a; SAM – S-adenosylmethionine; GNMT – glycine N-methyltransferase; SAH – S-adenosyltransferase; AHCY – adenosylhomocysteine hydrolase; CBS – cystathionine beta-synthase; CSE – cystathionine gamma-lyase; CDO – cysteine dioxygenase; GGT – gamma-glutamyl transpeptidase; GPX – glutathione peroxidase; GSH – glutathione; GSSG – glutathione disulfide; H_2S – hydrogen sulfide; MTR – methionine synthase; BHMT – betaine homocysteine S-methyltransferase; THF - tetrahydrofolate.