Dietary Protein, Metabolism, and Aging

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Abstract

Dietary restriction (DR), a moderate reduction in food intake, improves health during aging and extends life span across multiple species. Specific nutrients, rather than overall calories, mediate the effects of DR, with protein and specific amino acids (AAs) playing a key role. Modulations of single dietary AAs affect traits including growth, reproduction, physiology, health, and longevity in animals. Epidemiological data in humans also link the quality and quantity of dietary proteins to long-term health. Intricate nutrient-sensing pathways fine tune the metabolic responses to dietary AAs in a highly conserved manner. In turn, these metabolic responses can affect the onset of insulin resistance, obesity, neurodegenerative disease, and other age-related diseases. In this review we discuss how AA requirements are shaped and how ingested AAs regulate a spectrum of homeostatic processes. Finally, we highlight the resulting opportunity to develop nutritional strategies to improve human health during aging.

Contents

INTRODUCTION

Diet and Health

Obesity and its associated metabolic diseases are a global health problem, linked to reduced life expectancy. Both quantity and quality of food intake are important in the development of obesity, with excess fat (1) and carbohydrate intake detrimental to health and life span in flies (2), mice (3), and humans (4). Clearly, diets that promote obesity should be avoided, as should those that induce nutritional deficiencies. But what dietary compositions best promote health, and why? Does the optimal balance of macronutrients vary with age, gender, genotype, or disease state? Defining the macronutrient composition of a healthy diet and identifying the molecular and physiological mechanisms by which it promotes health are important challenges. In this review, we focus particularly on the roles of dietary protein.

Dietary Restriction

DR: dietary restriction

Energy homeostasis: a set of balancing adjustments aimed at metabolic equilibrium

A nutritional intervention with clear health benefits is dietary restriction (DR), a moderate reduction in food intake that protects against multiple aging-related diseases and impairments and extends life span in most captive animals tested. The severity of DR can range from ~10% to 50% of ad libitum intake levels, and the life span increase can be as modest as a few percentage points or as high as three-fold (5). In rodents and primates, DR protects against aging-related loss of function and disease, including cardiovascular disease, obesity, multiple cancers, neurodegeneration, nephropathy, diabetes, and loss of sensory, motor, and immune function (5, 6). Short-term DR in humans also benefits glucose and energy homeostasis, increasing insulin sensitivity and reducing body fat (5). However, DR is not a practical intervention for most humans because it is difficult to implement and sustain. Moreover, DR can decrease wound-healing capacity and

increase susceptibility to viral infections (5). Thus, an important aim is to identify the nutrients that mediate the health benefits of DR. Understanding the physiological and molecular mechanisms by which these key nutrients exert their effects may pave the way to DR-mimicking diets as well as pharmacological interventions to improve health during aging with minimal side effects.

AA: amino acid

Nutrient-sensing: a collection of systemic, neural, and cellular autonomous signals elicited in response to nutrient and/or energy status

IIS: insulin and insulin-like growth factor signaling

Dietary Protein and AAs

Recent findings have increasingly pointed to a causal role of the protein component of the diet in promoting the health and life span benefits of DR. In the fruit fly *Drosophila*, restriction of dietary yeast, the fly's usual protein source, but much less the restriction of carbohydrate or total calories, extends life span (7), an effect attributable to the amino acids (AAs) (8) and the protein-to-carbohydrate ratio of the diet (2). In mice (3) and rats (9), reducing dietary protein, thereby decreasing the diet's protein-to-carbohydrate ratio, also increases health in later life and life span. The beneficial effects of protein restriction outweigh those of carbohydrate or fat restriction (3, 10, 11). Indeed, dietary carbohydrates and fats are largely interchangeable without detrimental effects in many species (1, 12). Moreover, specific AAs or their ratio can determine health and aging, because reduced methionine in *Drosophila* (8) or of methionine or tryptophan in rodents (13, 14) results in improved health during aging and increased life span. Additionally, increases in circulating branched-chain AAs (BCAAs) stimulate the target of rapamycin (TOR) and insulin/insulin-like growth factor (IGF) signaling (IIS) pathways in rodents (3, 15), which may be detrimental for health, because suppression of TOR and IIS signaling is often beneficial for a healthy life span (5). The mechanisms by which individual dietary AAs affect metabolism and health are starting to be understood and are revealing potential targets for improvement of organismal health during aging.

DIETARY RESTRICTION, HEALTH, AND AGING

Protective Effects of Dietary Restriction

The physiological, metabolic, and molecular changes through which DR increases health later in life and life span are becoming clearer, although a complete account is lacking for any organism. Reduction in nutrient intake triggers modulations in the activity of nutrient-sensing pathways, which stimulate protective mechanisms over most aspects of health during aging, with an extensive array of protective metabolic changes that include an increase in stress resistance, detoxification capacity, and genome stability and the promotion of proteostasis and energy homeostasis (5, 16). The IIS and TOR pathways are also conserved in humans, and so are their responses to DR (5). Long-term and short-term DR trials in humans result in marked reductions in IIS and TOR signaling, decreasing multiple risk factors such as obesity, insulin resistance, and cardiovascular disease (17). These evolutionarily conserved responses to DR present an opportunity for significant health-promoting applications in human nutrition (11). Apart from its effects on energy homeostasis, DR also reduces cancer propensity. A reduction in tumor incidence (lymphomas, pituitary, and thyroid neoplasms) accompanies DR treatment in mice (6). Furthermore, primate studies also implicate dietary protein in health during aging. Two recent rhesus monkey life span trials found conflicting results, with one study [by the Wisconsin National Primate Research Centre (WNPRC)], but not the other [by the National Institute on Aging (NIA)], reporting a life span extension as a response to DR (11), although both studies found multiple improvements in health in the DR animals. Among several experimental differences between the two trials, contrasts in the amount and type of dietary protein were prominent (5, 11).

In humans, DR also reduces levels of IGF-1 and decreases the risk of cancer (17). However, DR in humans lowers circulating IGF-1 levels only if protein intake is also restricted (5). Human trials also highlight a role for protein quality, because diets containing low, plant-based AAs promote multiple aspects of health (18). Such findings emphasize a difference between protein sources, for example plant versus animal protein (discussed in the section on Health Biomarkers of Specific AA Imbalances). Moreover, increased activity of the transsulfuration pathway is required for the extension of life span by DR in *Drosophila*, and hydrogen sulfide production by the transsulfuration pathway is associated with extension of life span by DR in yeast, flies, and mice (19). Thus, dietary protein, and specific AAs, play an emerging role in modulating the health benefits of DR in humans, suggesting that interventions in protein intake may improve human health.

Dietary amino acid (AA) imbalance: an imbalance between the intake of AAs and the AA requirements of an organism for a specific trait

Macronutrient ratio: the relative proportions of protein, carbohydrates, and fats in a diet

Protein Content and AA Imbalance

In agreement with what is observed in DR animals, restriction of dietary protein or AAs reduces wound-healing capacity and increases susceptibility to viral infections (20). Also in accord with what is seen in DR animals, protein-restricted mice have protected cognitive function and live longer (3, 21). In contrast to DR animals, however, protein-restricted mice can show an increase in body fat and insulin resistance (3). Decreasing dietary protein also increases body fat in humans (22), but a high intake of dietary protein and AAs promotes insulin resistance and adversely perturbs glucose homeostasis (23). Consequently, only 10–15% of energy intake as protein is recommended (24), although for weight loss management the absolute amount of protein consumption is of greater importance than the percentage of energy (22).

Effective protein uptake depends on the efficiency of a protein's usage, which is determined by the combined effects of (*a*) how much protein is ingested and metabolized, (*b*) its essential-to-nonessential AA ratio (EAA:NEAA ratio), and (*c*) its precise AA composition (25). Adequate protein intake can be achieved with lower amounts of high-quality protein than of low-quality protein, with quality determined by its efficiency for anabolic traits. The effects of a dietary AA imbalance are more severe when overall AA intake is low (26), as AA imbalances further decrease AA usage. A low EAA:NEAA ratio can also be inefficient for anabolic traits at low AA intake levels. Therefore, the protein content and effective macronutrient ratio of protein to carbohydrates or to fats depends on the AA proportions of the ingested protein. Consequently, the AA proportions of a diet are critical for health, through both the effects on total protein usage and the mechanisms mediated by specific AAs. In the following sections, we highlight the metabolic fate of AAs, from ingestion to effects upon metabolism and health.

Multivariate Complexity of Defining a Dietary AA Imbalance

Identifying a diet with a healthy mixture of nutrients is complicated by the multivariate nature of diets, which poses a challenge for experimentation, and by the synergistic effects of multiple essential macro- and micronutrients (2). Accordingly, complex interactions between individual AAs render phenotypic responses to different AA ratios hard to interpret. Moreover, an interaction of AAs with other nutrients, such as vitamins, can also modulate metabolism (27), which further increases the complexity of nutritional space and confounds biological interpretations. Consequently, defining a balanced AA intake is challenging.

To simplify the nutritional landscape, recent methods dissect nutritional interactions and their physiological effects in multidimensional space (2). Such a representation of nutritional space, termed the geometric framework, can better describe the responses of metabolic, life span, and other traits and can reconcile apparently contradicting results (2). Multidimensional approaches

are desirable but sometimes impractical, and progress can also be impeded owing to the lack of standardized dietary nutrients and methods of measurement. In flies, the recent development of holidic diets has enabled accurate analysis of the effects of single dietary AAs (28), and such tools would benefit work in other model organisms such as mice and rats and aid the systematic analysis of multivariate AA interactions and their effects on health and aging.

EAA: essential amino acid

It is difficult to discern the physiometabolic effects of subtle AA imbalances that occur under conditions of normal nutrition, which is usually characterized by the intake of varied dietary protein sources. Even in laboratory model organisms, with carefully controlled conditions using chemically defined diets, complications can occur. Autoclaving and irradiation, both common sterilization steps in laboratory rodent food preparation, can degrade certain AAs including lysine, methionine, and cysteine, as well as vitamins including A and B_1 (27). In everyday human nutrition, food processing such as cooking can alter the AA contents of a protein source. Food texture can also affect metabolism. For instance soft foods increase nutrient efficiency and adiposity. In humans, aging also leads to anorexia and weight loss, largely owing to the progressive functional decline of the digestive system (29), which also probably results in deceased AA absorption. All these factors render practical diet design challenging.

Anabolic Traits and Their Experimental Assessment

Although health and life span responses to AA mixtures are sometimes evaluated, experimentally the balance of an AA source is usually defined by its ability to maximize production traits (27). To define the growth effect of single EAA limitations in vertebrates, the principle of the minimum is typically applied. This principle states that, if all essential nutrients required for growth are abundant in a diet except for one, the limiting essential nutrient, then incremental additions of only this limiting essential nutrient will increase growth (30). For EAAs, this principle has been repeatedly demonstrated experimentally in rodents (25), and it is coupled to the law of diminishing returns, according to which each succeeding increment of the limiting essential nutrient will produce a smaller increment of growth than the preceding increment (30, 31). However, the nature of the link between anabolic traits and long-term health is complex and is discussed below with respect to protein and AA intake levels.

Link Between Anabolic Traits and Long-Term Health?

Several lines of observation suggest a connection between anabolic traits and long-term health. However, the precise nature and mechanics of any such connection are still under investigation. Some important findings are described below.

Growth. Both DR and protein restriction can suppress growth and extend life span in rodents (3, 5, 25). The developmental theory of aging holds that a prolonged life span is caused by retarded development, and this notion was quickly adopted as an explanation of the life span response to DR (32). In flies and mice, respectively, the reduced growth observed upon DR or protein restriction seems to be effected by a reduced cell number, implicating the suppression of IIS (33, 34). Life-extending tryptophan or methionine restrictions also reduce growth in mice and rats via reduction in circulating IGF-1 (13, 14). Reduced IGF-1 signaling modulates the negative correlation between body size and life span in mice (35) and dogs (36). Several rodent studies show a negative correlation between body size and longevity in both genders (35, 37), as do several genetic models of extended longevity under reduced IIS (38). All these observations strongly suggest an inverse correlation between growth in body size and life span.

Amino acid bioavailability: the fraction of an absorbed amino acid that reaches systemic circulation

However, growth depression is not a prerequisite for life span extension. Body growth has been uncoupled from longevity both in mice and in flies (38). Thus, manipulations of growth signaling can extend longevity with no effect on body growth. Moreover, although anabolic traits are often used in the nutritional evaluation of a dietary protein, such traits are not reliable predictors of health during aging. In rodents, some AA imbalances that do not cause growth depression can be detrimental for health and cause fatty infiltration of the liver (25). Therefore, reduction in growth signals can cause growth suppression and life span extension, but the former is not a prerequisite for the latter. From this perspective it remains possible that specific dietary AA intake levels could optimize anabolic traits and life span, avoiding trade-offs between them.

Reproduction. Fecundity depends on nutrient utilization, and animal models are used to evaluate the link between fecundity and life span. In flies, DR reduces fecundity and increases life span (39). Fecund females allocate much of their ingested nutrients to reproductive processes, a proportion diminished in DR flies (40). In rodents, some long-lived models show a marked reproductive capacity reduction (39). Apart from extending rodent life span, protein restriction also suppresses fecundity (27). Reproductive output is also negatively associated with life span in dogs (41) and humans (42); however, in several fly models life span extension is not characterized by a lower reproductive output (39). Supplementing methionine in a methionine-restricted fly diet can rescue fecundity with no life span shortening (8), indicating that dietary modulation of AAs can promote longevity without impairing fecundity. Therefore, suppression of reproduction may not be indispensible for life span extension, and the design of diets that optimize both traits is possible.

AAS: FROM INGESTION TO REGULATION OF METABOLISM AND HEALTH

Absorption and Systemic Availability of AAs

The intake of AAs is achieved through the consumption of either whole protein or free AAs in the diet. Here we discuss the various factors that determine AA absorption and availability.

Dietary protein and AA absorption. Following their ingestion, the identity and amount of the AAs that become available to cells and tissues depends on AA absorption by epithelial enterocytes. The digestibility of whole dietary proteins is confounded by numerous factors (43), making it difficult to establish the identity and amount of bioavailable AAs after the ingestion of whole protein foods (44). Yet, whether derived from digested peptides or free AA diets, AAs show substrate antagonism and other physicochemical properties that can complicate estimations of their availability (43–45). In contrast to whole protein diets, free AA diets avoid many of the confounding factors influencing whole protein digestion and are more readily absorbable (28, 46). Moreover, in contrast to oligopeptide transporters, characterization of the free AA transporters in the human intestinal epithelium is comprehensive (45). Therefore, free AA diets are more suitable for the quantitative assessment of the postabsorptive effects of dietary AAs upon metabolism, health, and aging.

Dietary AAs and the microbiota. The uptake of AAs is influenced by gut microbes, and this can greatly affect the organism's response to a dietary protein or AA source. Free AAs in the gut's lumen may first encounter gut microbes, instead of epithelial enterocytes. Gut microorganisms are both consumers and producers of AAs, but the net exchanges between host and microbiota of specific

AAs and the factors that influence this exchange are not yet fully understood (47, 48). Nevertheless, many AA exchanges between the host and the microbiota have been characterized. Gut bacteria synthesize all EAAs and contribute up to 10% of mammalian plasma metabolites, including the EAAs tryptophan, phenylalanine, and lysine (48, 49). Although such contributions for other EAAs are not yet described, they are likely to occur. In addition, during the host-colon nitrogen cycle, microbes further contribute toward AA reabsorption by the host (48). However, when rodents with a gut microbiota are fed single EAA-deficient diets, their health quickly deteriorates, suggesting that bacterial EAA contributions are modest (25). The effects of the deficiency also depend on the identity of the deprived AA (25) and on how much of each EAA gut bacteria can provide to the host. Ruminants with a rumen microbial load able to synthesize all EAAs, however, still require an ample dietary AA supply to achieve high levels of growth or milk production (50). Thus, the contribution of EAAs from gut bacteria to the host appears limited. In contrast, the microbiota consumes substantial amounts of AAs, with up to 50% of fecal nitrogen being of bacterial origin (1, 4). More work is needed to establish how much of each ingested AA can be used by gut bacteria (47). Taken together, these observations demonstrate that the gut microbiota can shape AA availability to the host. This influence can be especially important in low-protein diets, because small changes in AA availability can have a proportionally greater effect on the available AA profile. In turn, this availability can also influence the useable dietary protein and the macronutrient ratio, thereby shaping health and aging.

SB: splanchnic bed

Changes in the microbiota are also associated with the risk for obesity, diabetes, and heart disease, and in mice DR enriches microbiota phylotypes associated with increased longevity (51). Gut bacteria adapt to ingested nutrients and can shift focus from dietary carbohydrate to dietary AA metabolism (52), although they also benefit from ample dietary fiber, which increases fecal nitrogen and decreases net AA uptake by the host (47). By adapting to macronutrients and metabolizing fiber, gut bacteria can stimulate the secretion of intestinal growth factors or satiety hormones (53, 54). Apart from the health risks of a chronically overstimulated growth axis, such interactions also complicate estimations of efficiency of dietary AA sources that rely on the evaluation of anabolic traits (growth) or behavioral traits (food intake). Also, the metabolism of AAs by gut bacteria may be influenced by circadian rhythms or the host's age and immune status. Owing to such complications, quantifying the net AA exchanges between host and microbiota is easier in animals with a small number of gut bacteria species and chemically defined diets, such as fruit flies (28, 53). Our understanding of the factors affecting AA usage by gut bacteria, the mechanisms by which gut microbes influence AA availability to the host, and the health consequences will be aided by approaches in which the amount or composition of the microbiome is experimentally manipulated.

The splanchnic bed and systemic AA availability. The amount of each AA that becomes systemically available is critical for metabolism, affecting health through AA-sensing mechanisms discussed in the following sections (see section on Detection of AA Limitations or Excesses). However, this amount greatly depends on how many AAs are metabolized immediately after absorption in the gut. Once absorbed by enterocytes, free AAs enter into the splanchnic bed (SB), which comprises the gut, liver, spleen, and pancreas and is where free AAs can be metabolized. In general, up to a third of all dietary AAs are metabolized by the SB (47), greatly shaping the AA profile that reaches circulation to become systemically available. First pass metabolism of AAs in the SB depends on AA identity. Despite arterial supply of AAs, enterocytes greatly rely on dietary AAs (55), and a low AA intake may contribute to enteral atrophy. Enteral usage of threonine is particularly high, presumably for the synthesis of threonine-rich mucins (47, 55). In the liver, methionine enters many transsulfation, transmethylation, and folate metabolism reactions (55). Glutamate, valine, isoleucine, leucine, and phenylalanine are also largely used by the SB. For all

BCAA: branched-chain amino acid

these AAs, an estimated 35–100% of dietary intake is used by the SB and never reaches systemic circulation (47, 55). In contrast, arginine, alanine, tyrosine, and proline undergo minimal usage by the SB (55). An AA's conformation can also determine its SB usage. In flies, mice, and rats, D- and L-methionine are highly bioavailable, whereas in humans D-methionine is only ~30% bioactive (25, 56, 57). In contrast, all other AAs are fully usable only in the L- form across the four species (25, 56). Thus, SB metabolism of free AAs substantially affects their systemic availability depending on the individual AA's identity.

Following their passage through the SB, free AAs pass into circulation and become part of the free AA pool. Free AAs represent a very small fraction of a body's total AA contents but are metabolically significant as they form the systemically available AA profile. Indeed, free AAs are associated with life span across species. In flies, low levels of glutamine, lysine, and alanine are linked to extended longevity (58). In mice, circulating metabolites including glutamine, methionine, and proline decrease with age (59). This decrease is countered by acute DR, which increases circulating methionine, glutamine, alanine, and valine, indicating a shift toward gluconeogenesis and energy conservation (60). However, an opposite metabolic shift has been suggested in dogs, in which lower levels of isoleucine, leucine, phenylalanine, and valine are associated with the health benefits induced by DR (61). The same association has also been made in humans, as a plasma decrease in isoleucine, leucine, valine, lysine, phenylalanine, and histidine has been linked to a reduced carbohydrate metabolism and an increased AA catabolism (62). Moreover, depleted levels of circulating methionine and BCAAs have been observed in long-lived IIS mutant mice (63). Finally, in mice, elevated circulating BCAAs stimulate their catabolism in the liver (15). Thus, it is currently difficult to interpret plasma AA changes with age or with DR and the consequences of these changes for health. Some possible mechanisms linking such free AA modulations to life span are discussed in more detail in following sections (see section on Detection of AA Limitations or Excesses).

From circulation, free AAs can enter interstitial fluids and cells to become part of tissue intracellular pools. Although the circulated free AA pool is available to all tissues reached by circulation, cell-specific AA availability depends on AA transporters whose abundance can vary between cell types (64, 65). Abundance of transporters is well characterized for enterocytes, hepatocytes, pancreocytes, nephrocytes, and in the brain, as is substrate antagonism between AAs for transporters (45, 65, 66). Thus, despite equilibrium between circulatory and intracellular pools for most free AAs, substantial differences in concentrations between the two pools are seen in some cases. In humans, glycine, glutamate, and glutamine are 10–50 times more concentrated in intracellular pools (4). It is also noteworthy that the free AAs in a tissue do not match the AA composition of the tissue's proteome. In rat muscle, compared with protein-bound AAs, levels of phenylalanine, methionine, and BCAAs are depleted (4). Therefore, circulating, intracellular, and protein-bound AA profiles differ significantly, but circulating and intracellular AAs fluctuate more dynamically and play a prominent metabolic role.

The dynamics between transporter abundance and tissue-specific AA availability need more clarification, as do the effects of bidirectional transport between specific AAs, such as glutamine and leucine (67), upon tissue-specific AA availability. Nonetheless, some physiological effects of AA antagonisms are clear. Antagonisms between the BCAAs can result in growth depression upon supplementation of one of the three BCAAs (leucine, isoleucine, and valine) in the diet (25). Similarly, antagonisms between lysine and arginine can suppress growth upon addition of lysine or arginine only in the diet (25). Excess leucine or methionine depresses rat growth independent of food intake, and excess leucine increases the growth requirement for tryptophan (25). However, long-term studies are lacking, and the effects of an AA imbalance–induced decrease in growth signaling upon health and life span await further study.

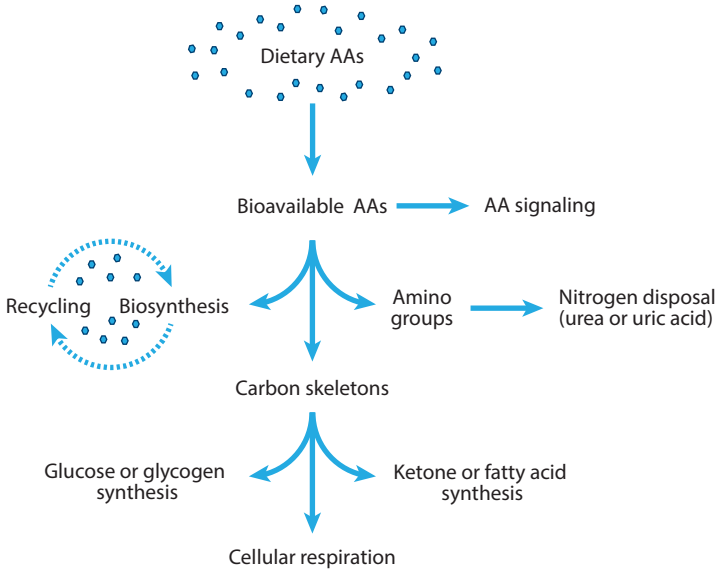

Figure 1

The metabolic fate of ingested amino acids (AAs). Figure modified from Reference 150.

Metabolic fate of ingested AAs. The metabolic fate of intracellular AAs is important in determining the effects of AA intake upon health and aging. An outline of AA metabolism is given in **Figure 1**. Once in the SB, free AAs can be used for protein biosynthesis (e.g., in liver or intestinal muscle cells) or can be broken down to their carbon skeleton and amine groups. Amine groups are typically excreted, but carbon skeletons can have a diverse fate. They can be used in the biosynthesis of acetyl-coenzyme A (acetyl-CoA) or acetoacetyl-CoA, the main precursors of fatty acids, which are in turn stored as triacylglycerides (TAGs) in adipose tissue. Alternatively, carbon skeletons can be used to synthesize pyruvate and oxaloacetate, the precursors of glucose (stored as glycogen), thereby fueling the tricarboxylic acid (TCA) cycle. Finally, catabolism of the carbon skeleton can also be used for cellular respiration and energy production in the form of adenosine triphosphate (ATP). Those AAs not metabolized in the SB can enter circulation, from which they can be absorbed by cells and tissues. Consequently, the proportion of AAs in the diet can affect many of these metabolic pathways. This aspect is discussed separately for dietary AA limitations and excesses in the following sections.

Detection of AA Limitations or Excesses

The sensing of both circulating and intracellular free AAs occurs through various mechanisms, both cellular and systemic. These two modes of AA sensing determine many of the metabolic and physiological responses to fluctuations in AA availability.

Dietary amino acid (AA) limitation: AA intake below the organism's requirement for a given trait

Dietary amino acid (AA) excess: AA intake exceeding the organism's requirement for a given trait

GCN2-dependent detection of AA limitation. A metabolic response to limited AAs can occur only after their limitation is detected. In flies, nutrient perception involves chemosensory sensillae and enteroendocrine and gustatory signals (68). The consequences of the fly's nutrient sensing can be uncoupled from its actual food intake, because stimulating odorant receptors can reverse the benefits of DR upon life span independent of food or protein intake (69). The fly's selection of an AA source is partly mediated by the serine/threonine-protein kinase general control

nonderepressible 2 (GCN2) acting in dopaminergic neurons in the brain (70). In mammals, the brain's anterior prepiryform cortex (APC) contains a GCN2-dependent chemosensor that perceives decreased levels of circulating EAAs (71). Low levels of EAAs stimulate GCN2, which suppresses anabolism and promotes catabolism through the AA response (AAR) pathway (71, 72, 92) (**Figure 2**). This GCN2 activation is independent of the AA's identity, because GCN2 senses

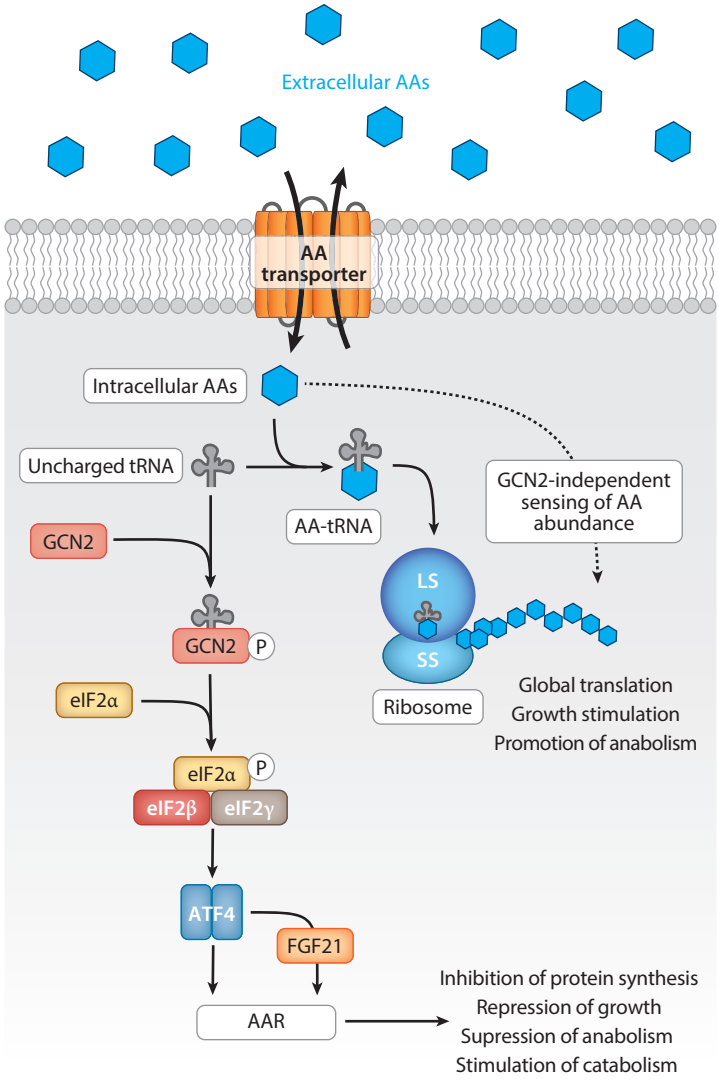

Figure 2

GCN2-dependent sensing of AA limitations. Intracellular AAs can activate their cognate tRNA, which is then available for ribosomal protein synthesis (LS, large subunit; SS, small subunit). In contrast, uncharged tRNAs bind to and activate by phosphorylation GCN2, which in turn phosphorylates eIF2α. This activates the eIF2 complex to stimulate ATF4, inducing FGF21 to trigger the AAR, which inhibits anabolic processes and promotes catabolism. Abbreviations: AA, amino acid; AAR, amino acid response; ATF4, activating transcription factor 4; eIF2, eukaryotic initiator factor 2; FGF21, fibroblast growth factor 21; GCN2, general control nonderepressible 2.

the AA deficiency by binding nonspecifically to any uncharged transfer RNA (71, 73). However, BCAAs, and in particular leucine, the most abundantly used AA in mammalian proteomes, appear to play a predominant role (74). Upon binding an uncharged tRNA, GCN2 changes its conformation to promote inhibitory phosphorylation of its primary downstream translation activator, the eukaryotic initiator factor 2α (eIF2α) (71). This change leads to global downregulation of transcription and translation through changes in mRNA levels or mRNA stabilization, growth arrest and reductions in lipid and carbohydrate anabolism, activation of AA transporters [e.g., asparagine synthetase (ASNS)], and changes in neuronal glutamatergic activity, intracellular calcium, and GABAergic (γ-aminobutyric acid-ergic) signaling (64, 71, 72, 75). A common downstream effector of GCN2 activation upon methionine or leucine restriction is fibroblast growth factor 21 (FGF21), which represses liver fatty acid synthesis and increases fatty acid mobilization (13, 76).

GCN2: general control nonderepressible 2

AAR: amino acid response

ASNS: asparagine synthetase

Although a key modulator of the systemic response to decreased levels of circulating AAs, GCN2 is also expressed across tissues and may also act in a cell-autonomous manner (71). In rodents, three isoforms of GCN2 are found: α, β, and γ. The α and γ isoforms have no functional GI (GCN2/impact) domain to bind GCN2 to its activator GCN1 and are expressed tissue specifically, whereas the β isoform has a functional GI domain and is expressed similarly across tissues (73). However, mice lacking GCN2 specifically in the brain fail to show the normal aversive behavior toward AA imbalanced diets. AA sensing in the APC thus overrides peripheral GCN2 activity with regard to feeding behavior (72). Therefore, low-circulating AAs result in stimulation of the AAR pathway through the activation of GCN2, which orchestrates cell-autonomous and non-cell-autonomous effects to promote catabolism, suppress anabolism, and thereby induce a metabolic maintenance mode.

GCN2-independent detection of AA limitation. Although much evidence supports a role for GCN2 in the sensing of ingested AAs, some studies cast doubt over its significance in the physiological and behavioral responses to AA-deficient foods. Recently, GCN2 has been shown to have no effect on the detection of AA-deficient diets in mice (77). In addition, sensing of AAs such as alanine or glycine in hypothalamic neurons also occurs via excitatory signals that are modulated within seconds from the moment an AA is supplied to these cells (78). Such response mechanisms are GCN2-independent, as transcriptional changes modulated by GCN2 would require a longer time period. Indeed, some preliminary findings in GCN2 knockout mice indicate that the response to methionine restriction is not dependent on GCN2 (13). Moreover, a central role in leucine sensing, but not valine sensing, has also been located in the mediobasal hypothalamus and the nucleus of the tractus solitaries (NTS) (79). Therefore, given the central role of the hypothalamus in modulating feeding behavior, obesity, and metabolism it will be interesting to see how different ingested AAs give rise to an organism's hypothalamic and consequent metabolic response to ingested AAs independent of the APC's GCN2 response.

Dietary AA restriction also regulates gene expression via multiple GCN2-independent pathways, including transcriptional (e.g., Cxcl10) or posttranscriptional (e.g., Dusp16) responses (72, 80). Although the molecular mechanisms behind the activation of such responses by low AA levels are not well known, such changes are able to increase catabolic processes and metabolic efficiency independent of the GCN2-mediated AAR response. For example, efficiency of AA uptake is increased by upregulation of ASNS or of plasma membrane AA transporters such as the neutral AA transporters SNAT2 and LAT-1 and the cationic AA transporter CAT-1 (64, 72, 81). Other GCN2-independent responses can include transcription factor adjustments [activating transcription factors 2–5 (ATF2–5), CAAT/enhancer-binding protein (C/EBP), and other ATF/cAMP response element–binding protein (CREB) transcription factors] and changes in ribosomal proteins that affect translation (64, 72, 81). Although GCN2 is the only kinase exclusively

AMPK: adenosine monophosphate–activated protein kinase

responsive to AA deprivation, phosphorylation of eIF2α can also be affected by other kinases, including heme-regulated inhibitor kinase (HRI), double-stranded RNA-activated protein kinase (PKR), and PKR-like endoplasmic reticulum-resident kinase (PERK), and considerable overlap has emerged in the activation of downstream effectors between PERK and GCN2 upon methionine restriction in mouse liver (75). Moreover, internal ribosomal entry sites (IRESs), such as those in the CAT-1 mRNA, allow preferential translation by phosphorylated eIF2α, and the role of such IRESs in PERK or GCN2 activation also requires further characterization (75). Another GCN2-independent mechanism may involve the AMP-activated protein kinase (AMPK), which senses low-energy states by detecting high AMP levels (82). AMPK functions in both cell-autonomous and non-cell-autonomous ways and is also activated upon low AA status (82, 83). Importantly, increased AMPK activation extends worm and fly life spans (82).

The dynamics of GCN2-independent responses can vary. As mentioned, sensing of supplied AAs by hypothalamic neurons can occur within seconds, thereby comprising a rapid response (78). In contrast, other modulators can occur across a wide range of times. For ASNS, a translational surge upon AA limitation is followed by a more sustained transcriptional activation (64). Moreover, in response to a dietary AA limitation, the limited AA initially drops in the plasma but after several days its levels may be restored (84). In response to an AA-imbalanced diet, growth and food intake depression also subside after several days (85), perhaps through reconstitution of hormonal homeostasis (see section on TOR-Independent Detection of AA Abundance). In contrast, short-term responses relying on GCN2 may not require hormonal adjustments (71). Thus, although several GCN2-independent responses to limited AAs have emerged, further clarification of the molecular mechanisms and characterization of the short-term versus long-term responses are needed.

Effects of AA limitations on aging. All limitations or excesses of dietary AAs are sensed in a way that modulates anabolic and catabolic processes and, ultimately, homeostasis. Suppression of anabolism and growth signaling can extend life span and is induced by AA limitations. The identity of EAAs is conserved between rodents and humans, and human cell culture work on AA limitations shows consistent results to murine cell systems, implying conserved molecular mechanisms (86). In rodents, methionine and tryptophan limitations suppress anabolism and translation and promote catabolic processes (13). Glucose, insulin, thyroid hormones, and IGF-1 levels are also reduced in methionine-restricted mice. Yet, generally, low levels of circulating EAAs reduce IGF-1 function largely independently of EAA identity (87). Lack of the AA building blocks for anabolic traits also results in induction of apoptosis by IGF-1, which activates the apoptosis-inducer CHOP (CCAAT/-enhancer-binding protein homologous protein) (64). Stimulation of apoptosis aids the recycling of molecular building blocks, including AAs. Therefore, AA limitations deplete growth signaling and can thus induce a maintenance mode that benefits long-term health.

By increasing catabolism, methionine or tryptophan restrictions also reduce fat storage in rodents (13, 14). Although across multiple organisms and humans DR results in leanness or rescue from obesity and confers multiple metabolic advantages favoring longevity, the role of fat loss per se in promoting health is not clear. For instance, diets low in protein increase adiposity in mice because of increased food intake, but these mice are as healthy as DR mice (3). Additionally, the ability of animals to maintain their adiposity despite DR appears to mediate the beneficial effects of DR (88). The decline of mammalian target of rapamycin (mTOR) expression with age in rat white adipose tissue is also prevented by DR (89). Therefore, given the unclear role of fat deposition in DR and the different effects of different types of fat on health, the role of fat deposition in mediating the health benefits of protein or single AA restriction requires further investigation.

TOR-dependent detection of AA abundance. In mammals, the cell-autonomous AA response upon dietary AA excess relies primarily on mTOR, which occurs in two complexes, mTORC1 (rapamycin/nutrient sensitive) and mTORC2 (rapamycin/nutrient insensitive). Absence of AAs results in the TORC1-inhibitory recruitment of the tuberous sclerosis protein TSC2 onto the lysosomal membrane (90). Mechanisms of activation of mTORC1 in the presence of AAs are shown in **Figure 3**. Sensing involves a complex interplay among numerous molecules recruited in

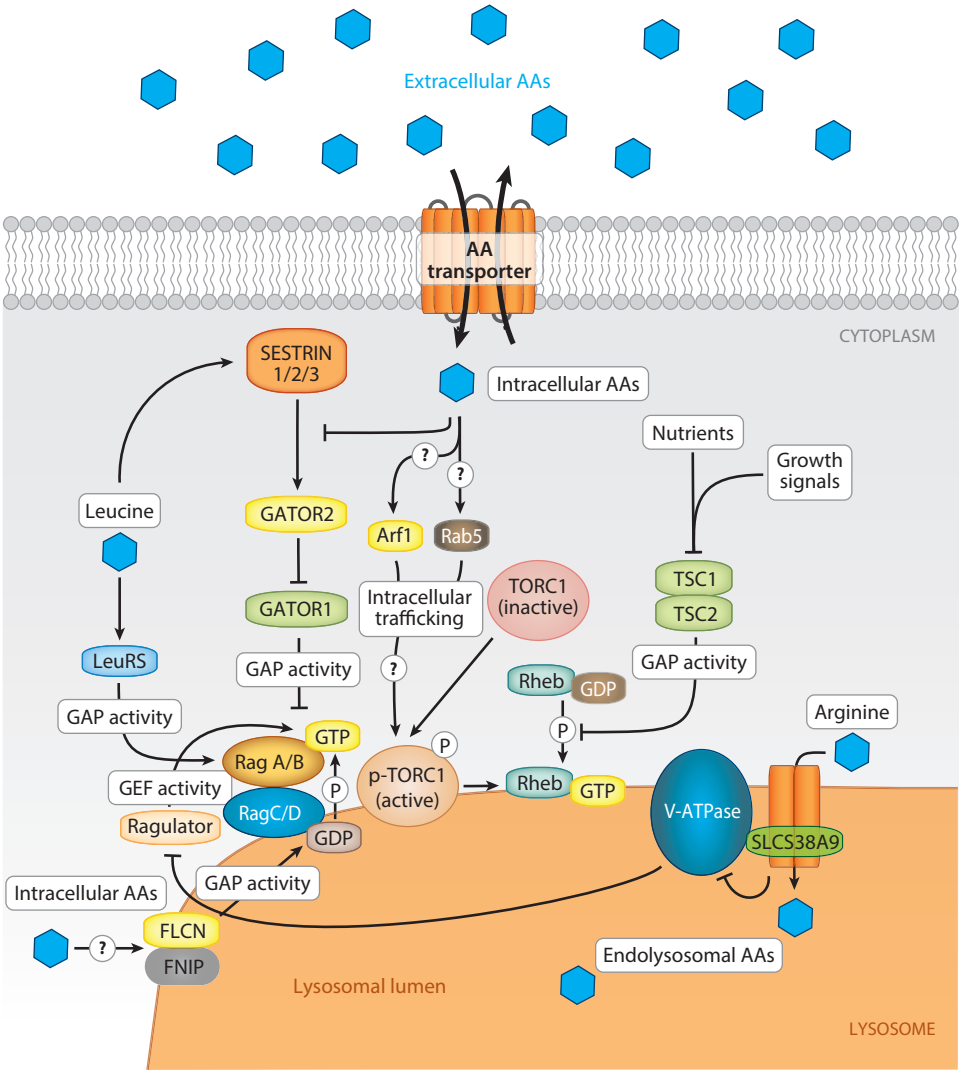

Figure 3

TOR-dependent sensing of AAs. Intracellular AAs activate TORC1 through multiple TOR-associated factors. Abbreviations: AA, amino acid; Arf1, ADP-ribosylation factor 1; FLCN–FNIP, folliculin–folliculin-interacting protein; GAP, GTPase-activating protein; GATOR 1/2, GTPase-activating proteins toward Rags 1/2; GDP, guanosine diphosphate; GEF, guanine nucleotide exchange factor; GTP, guanosine triphosphate; LeuRS, leucyl-tRNA synthetase; Rab5, Ras-related protein 5; SLC38A9, sodium-coupled neutral amino acid transporter 9; TOR, target of rapamycin; V-ATPase, vacuolar-type H+-ATPase.

mTOR: mammalian target of rapamycin

GPCR: G protein–coupled receptor

Transceptors: transmembrane transporters of nutrients, including amino acids, that can also act as receptors, therefore having signaling capacity

or around the lysosome, which is a key site of AA recycling and of intracellular/intravacuolar AA sensing (74, 91). Intracellular AAs prevent the inhibitory association of sestrins with the GATOR2 (GTPase-activating proteins toward Rags 2) complex (92, 93). This lowers GATOR1's GAP activity upon RagA–RagB, which is bound to RagC–RagD and is important for the activation and translocation of TORC1 onto the lysosomal membrane (92). Intracellular AAs are taken into the lysosome by transporters such as SLC38A9 (sodium-coupled neutral amino acid transporter 9), which has a particularly high affinity for arginine (94). This transport induces conformational changes in the endolysosomal V-ATPase (vacuolar-type H^+-ATPase), which dissociates from the Ragulator–Rag complex (94). The Ragulator then enables the activation of RagA–RagB through its guanine nucleotide exchange factor activity (94). Intracellular AAs can also promote the GTPase activity of the folliculin complex FLCN–FNIP (folliculin–folliculin-interacting protein) (95), which results in the RagC–RagD complex being loaded with GDP (guanosine diphosphate), and stimulate Arf1 (ADP-ribosylation factor 1) and Rab5 (Ras-related protein 5), which are involved in intracellular trafficking that induces TORC1 activation (96).

The identity of the AA also determines how it is sensed by TORC1. Leucine activates RagA–RagB through Sestrin 2 (93); glutamine sensing involves Arf1 and the V-ATPase, but not RagA–RagB; and arginine sensing involves SLC38A9 (97). Other factors involved in TORC1 activation by AAs may involve the kinases Vps34 and SH3BP4 (91). Importantly, inhibition of mTORC1 activation extends life span and, although TOR is critical in regulating adiposity by inducing lipid synthesis (91), insulin resistance is effected primarily by mTORC2 (98). The interplay between TOR and AMPK in sensing AAs also requires further characterization. Inhibition of rat muscle mTORC1 with rapamycin has no effect on AMPK, but activation of AMPK suppresses mTOR signaling and insulin resistance (99). Accordingly, reduced AMPK activity precedes mTOR activation by glucose or leucine, leading to insulin resistance (99).

As discussed for GCN2, not all AAs stimulate mTORC1 equally. In rodents, leucine is a particularly strong activator (74). Although intracellular leucine may be sensed by leucyl-tRNA synthetase (LeuRS) to activate mTORC1 (**Figure 3**) (100), it is now known that Sestrin2 possesses a leucine pocket to bind to, and sense, cytoplasmic leucine levels (101). Some obese animal models deplete their circulating glucogenic AAs, leaving higher levels of circulating sulfur (102) and BCAAs including leucine (15), which can result in chronic TOR activation. In contrast, a low-protein diet decreases circulating BCAAs and mTOR activation (3) and sensitizes animals to AA imbalances (25). Importantly, TOR activation can stimulate the secretion of hunger or satiety hormones in the gastrointestinal tract and brain (hormones such as ghrelin and leptin, respectively) (23), and recent evidence suggests TOR is a mediator of the enteroendocrine hormonal responses to dietary proteins and AAs (103). Therefore, a regularly high dietary intake of AAs induces chronic mTOR activation, which is detrimental to health and life span, whereas AA imbalances or limitations can inhibit TOR. However, TOR activation with respect to single AA modulations requires further elucidation both in invertebrates and vertebrates.

TOR-independent detection of AA abundance. Multicellular organisms have both intracellular and extracellular AA sensors, as well as neural sensors that respond to the intake of nutrients. Although TORC1 senses endocellular AAs and can stimulate satiety signals, extracellular AA sensors in the gastrointestinal tract also play a prominent role. At least some mammalian G protein–coupled receptors (GPCRs) are transceptors. Transceptors are transmembrane transporters of nutrients, including AAs, that also act as receptors involved in inducing intracellular signaling. In mammals, GPCR receptors of the T1R family are activated mostly by L-AAs in the digestive tract (104) and even modulate TOR signaling independent of intracellular AA levels (65). Transmembrane GPCRs in enteroendocrine cells can stimulate the release of appetite-regulating

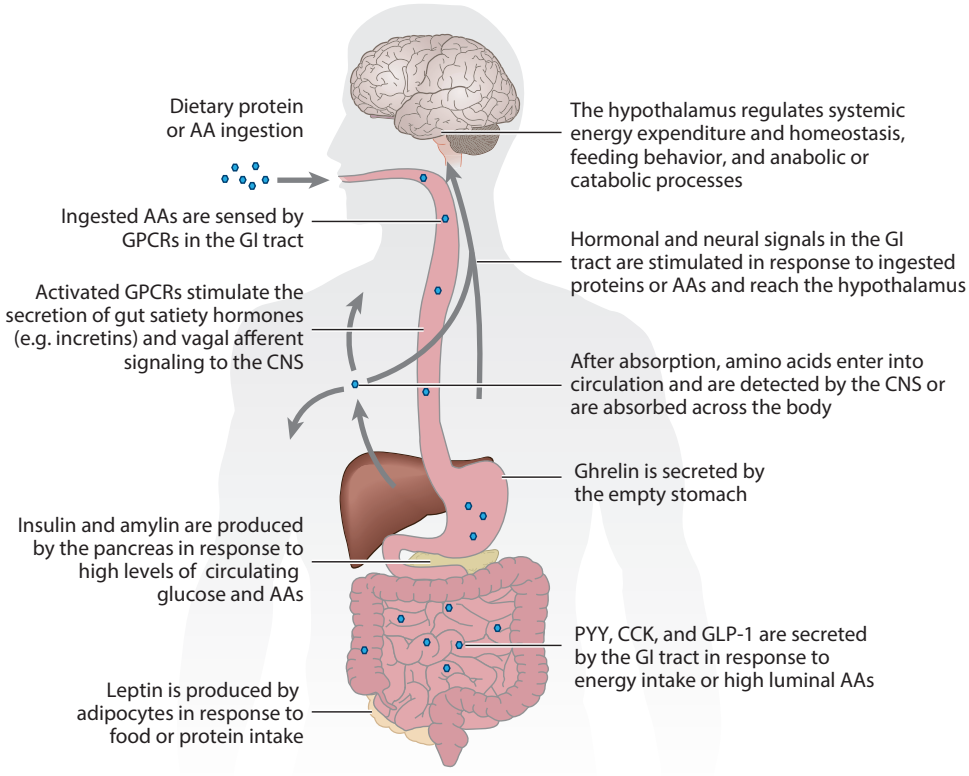

Figure 4

Sensing of dietary proteins and AAs along the GI tract. Ingested AAs activate GPCRs (e.g., transceptors) that relay hormonal and neural signals of satiety to the brain. Protein or AA intake can also stimulate the secretion of a range of appetite suppressors, including PYY, CCK, and GLP-1. Such hormonal signals are targeted to the hypothalamus, which regulates a range of systemic and metabolic processes that are associated with homeostasis and health during aging. Absorbed AAs can also be sensed by the CNS once in circulation. Abbreviations: AA, amino acid; CCK, cholecystokinin; CNS, central nervous system; GI tract, gastrointestinal tract; GLP-1, glucagon-like peptide 1; GPCR, G protein–coupled receptor; PYY, peptide YY.

incretins or decretins (105, 106). Specifically, high luminal AA concentrations increase secretion of satiety incretin hormone GLP-1 (glucagon-like peptide 1) by enterocytes (107). Additionally, in response to food or AA intake, density receptors, stretch receptors, and chemoreceptors in the gastrointestinal tract release neural signals of satiety to the central nervous system (CNS) (54, 108). Cholecystokinin (CCK) is secreted in response to luminal AAs, and CCK receptors stimulate vagal afferent signals to the NTS of the brainstem, which relays signals to the hypothalamus (108). Other peptides secreted by the gastrointestinal tract or along the SB include leptin, insulin, and peptide YY (PYY), which suppress appetite in response to bulk food or protein intake, whereas ghrelin increases it (108) (**Figure 4**). Furthermore, recent evidence strongly supports unique ingested AA-specific signaling to the CNS, involving vagal afferents and the area postrema (109). All the above responses have some degree of conservation between invertebrates and vertebrates, as similar mechanisms are involved in the fly's intestinal nutrient sensing (68). Thus, neural and hormonal modulations in the gut can function independently of intracellular AA sensing by TOR.

In this way, ingested AAs trigger the release of hormones to regulate homeostatic processes in the whole organism (**Figure 4**).

Responses to AA surpluses that affect physiology and aging. High dietary protein intake increases satiety and decreases food intake in many organisms, including flies (2), mice (2), and humans (22), an effect referred to as protein leverage (2). In this effect, the main driver of appetite is a target protein intake. Therefore, the reduction in both obesity and insulin resistance of animals and humans fed a high protein diet ad libitum can be explained by decreased food intake (23). However, dietary AA-induced chronic stimulation of the IIS/TOR pathways is detrimental for health (3, 23, 83, 91, 110). In yeast and worms, AA restrictions can inhibit TOR and extend life span (5, 111, 112). Inhibition of TOR by rapamycin or of S6 kinase (S6K), a downstream effector of TOR, also extends fly and rodent life span (110). Another main effector of mTOR is the translation repressor 4E-BP (4E binding protein 1), which is activated upon TOR inhibition by DR in flies (110) or methionine restriction in rodents (13). In humans, high levels of protein or AA intake also result in TOR activation (23) and insulin secretion (22), whereas excess acidifying AAs or sulfur AAs also raise blood pressure (22). With aging, mTOR activity in mouse hypothalamic neurons increases, silencing anorexic neurons and contributing to age-related obesity (16). Moreover, TOR function can affect diverse systemic processes, including cell and tissue growth signaling, immune function, proteostasis, neurodegeneration and cognitive function, tissue and stem cell physiology, and others (91, 110). Thus, activation of TOR by high AA intake levels can in the long term be detrimental for health, promoting age-related disease such as neurodegeneration (113).

Although AAs promote growth signaling and TOR, excess AAs can also suppress growth, and therefore growth signaling, through antagonistic interactions. Mechanistically, such interactions may occur if an AA is ingested in amounts that saturate specific AA transporters due to the AA's higher abundance, substrate affinity, or kinetics. Thus, in some tissues intracellular levels of outcompeted AAs may become limited, thereby triggering the AAR to inhibit anabolic processes. Specific examples of moderate additions of AAs inhibiting growth in rodents were discussed above. Importantly, such inhibitions of growth signaling may also affect health and longevity. In worms, addition of some AAs extends life span significantly through TOR inhibition (111). However, the identity of the AA is important, as addition of some AAs to the worms' diet had no effect or even decreased life span drastically (111). In rats, an excess of threonine can be well tolerated, but a similar excess of tyrosine can cause pathological lesions (25). Therefore, the identity of the AA ingested in excess determines its effects upon health. More lifelong studies will further clarify these interactions upon long-term health and aging.

Convergence of AA-sensing pathways. There are many interactions among the multiple nutrient-sensing and AA-sensing pathways discussed above (80). Phosphorylation of some translation initiation factors by TOR changes their conformation to allow accessibility by other kinases or phosphatases (including GCN2 downstream effectors) (114). Protein synthesis inhibition by GCN2/eIF2α stimulation occurs in conjunction with mTOR inhibition, and some cancer drugs deplete circulating AAs and trigger GCN2 to decrease mTORC1 signaling (115). In yeast, the AAR pathway is most responsive when mTORC1 is inhibited by rapamycin (73). In worms, GCN2 and TOR converge upon AA limitation toward inhibition of global translation and downregulation of FOXO (forkhead box O) transcription factors (116). Activation of FOXO transcription factors mediates the life-extending effect of IIS downregulation across species (5, 16). In mammalian cells, GCN2 induces Sestrin2 to suppress mTORC1 during AA deprivation (117). Therefore, the orchestration of these two nutrient-sensing pathways (TOR and GCN2) modulates AA sensing,

although a detailed characterization of this interaction, especially with respect to individual AAs, remains to be established.

TCA cycle: tricarboxylic acid cycle

Food aversion, protein leverage, and growth signaling. Because imbalanced protein sources prevent the usage of excess and therefore total AAs, adequate protein intake levels can be achieved with lesser amounts of high-quality protein than of low-quality protein. In order to achieve the target AA intake as driven by protein leverage, a less usable protein will therefore be consumed in greater amounts than a highly usable protein. For instance, whey promotes growth more than casein and also induces a higher satiating effect in humans (118, 119). However, imbalanced AA sources can result in deficiencies for specific essential AAs, and so animals must also have protective aversive responses to direct them to alternative, balanced AA sources (26). Thus, the motive for increasing the intake of an imbalanced AA diet to achieve a target protein consumption may conflict with the motive for avoiding a detrimentally imbalanced AA intake. The thresholds distinguishing between such conflicting motives are unclear, as is the impact of the imbalanced AA's identity on such effects.

Rodents are more sensitive to limited than they are to excess AAs. Very small AA limitations are detectable by rats, representing a 0.009% w/w (weight of AA per weight of food) change in the diet (71). Such limitations are not reflected in the plasma but are seen in the APC region within 15 minutes of feeding on the imbalanced diet, resulting in loss of appetite (71). In contrast, growth suppression is detectable upon changes that represent >0.1% w/w of the limiting AA in the diet (25). In addition, rodents fed ad libitum on severely AA-limited diets decrease their food intake and growth, but if the animals are made to eat equal amounts, growth returns to normal (25). Therefore, appetite appears more malleable in response to ingested AAs than is growth signaling. Moreover, responses to ingested AAs also depend on the identity of the imbalanced AA. Restriction of specific AAs (lysine, threonine, or isoleucine) alters food preference but not food intake in rats (71). In mice, excess consumption of some AAs (e.g., methionine, tryptophan) suppresses food intake and growth more than excess intake of others does (e.g., threonine) (25). Therefore, the response to imbalanced AA diet depends on the identity of the imbalanced AA(s) and on the physiological (e.g., growth) or behavioral (e.g., appetite, food choice) trait assessed. Further understanding of these aspects and interactions will be important in elucidating how AA modulations regulate metabolism and aging and in designing nutritional applications for humans.

Distinct bioenergetic and metabolic roles of AAs. Because of their different molecular structures, free AAs are broken down through distinct biochemical reactions. According to their catabolism, AAs can be glucogenic (all AAs except lysine and leucine), leading to the generation of glucose, or ketogenic (lysine, leucine), resulting in ketone bodies, although some AAs can be both (isoleucine, threonine, phenylalanine, tyrosine, and tryptophan). Glucose and ketones are the body's main energy sources, and cellular energy production from AA catabolism can represent 10–15% of total energy production (22). Importantly, the energy density of glucose is typically lower than that of ketones. Moreover, the energy expenditure for the metabolism of different AAs varies. Glutamate is the most energetically efficient AA (120), which may explain the central role of glutamate in providing TCA cycle precursors (66). Each AA also has a different metabolic efficiency for anaplerotic reactions (i.e., reactions that produce TCA cycle intermediates from precursors including AAs). Therefore, the metabolism of specific AA can uniquely affect energy homeostasis, which may in turn affect health during aging. In worms, dietary supplementation with the ketogenic β-hydroxybutyrate (111), the ketone derivative α-ketoglutate (121), or several TCA cycle metabolites, extends life span (111). This longevity gain in worms is thought to be mediated by anaplerotic reactions (111, 121). The energy sensor AMPK senses and modulates the metabolic

and energy homeostasis changes of these long-lived worms, and the FOXO transcription factor DAF-16 is also activated by higher levels of TCA cycle intermediates (111).

FOXO: transcription factor forkhead box O

The ketogenic or glucogenic potential of ingested AAs may also affect long-term health and aging in rodents and humans. In mice, highly ketogenic diets reduce the catabolism of ketogenic AAs to prevent further ketogenesis but do not alter life span (13). However, a modest increase in the intake of ketogenic compounds may be beneficial for mouse life span. Increasing the intake of the ketogenic AA leucine contributes to the mouse life span extension by BCAA supplementation (122), whereas in a mouse cancer model two different ketogenic compounds, butanediol and ketone ester, significantly increased survival independently of DR (123). In humans, ketogenic or leucine-supplemented diets may decrease food intake, adiposity, insulin resistance, sarcopenia, and cognitive deterioration with age (124–127). However, the TCA cycle is amphibolic (i.e., it is both anaplerotic and cataplerotic), which makes it difficult to quantify its bioenergetic modulations upon intake of different AAs. Therefore ketogenesis, TCA metabolite levels, and energy flux are coordinated by ingestion of different AAs to induce health and longevity across species. However, more investigations are needed to further elucidate how AA catabolism affects health and aging through such modulations.

Health biomarkers of specific AA imbalances. The AA profile of a dietary protein is generally the primary determinant of the protein's nutritional value. Several studies have identified effects of different dietary proteins with distinct AA profiles upon health and aging. Soy and whey proteins improve a range of health markers and longevity, including increased insulin sensitivity and reduced adiposity (**Table 1**). In contrast, milk or casein proteins increase circulating IGF-1, insulin, and satiety hormones compared with other protein sources, and such chronic IIS overstimulation can be detrimental for aging (**Table 1**). The molecular mechanisms mediating the effects of such different protein sources implicate their AA contents. Soy and whey proteins are low in methionine and tryptophan content (1, 128), whereas casein has a higher methionine content than soy protein. Tryptophan (129) and methionine (13) promote growth hormone secretion, so diets with lower levels of these AAs decrease IGF-1, thereby promoting long-term health (128). Whey protein is also high in the BCAAs leucine and isoleucine, which may explain its growth-promoting and appetite-suppressing effects in animals (119) and its prevention of muscle loss in older humans (126). Egg protein is a high-quality protein for growth (119) but is not necessarily optimal for long-term health as it causes high postprandial levels of circulating glucose accompanied by low appetite suppression (118). Therefore, specific protein sources with distinct AA profiles can downregulate IIS and increase health during aging and longevity.

Other endocrine modulations involve thyroid hormones, with soy protein lowering parathyroid hormone secretion (**Table 1**), which in humans is linked to body mass index and mortality, at least under some pathological conditions. Along with growth hormones, secretion of thyroid hormones is also reduced by DR (6) and tryptophan restriction (14). In mice, increased plasma levels of BCAAs are associated with decreased life span (3), but high dietary levels of BCAAs have also extended life span presumably through different protective mechanisms (122) that require more detailed investigation. In humans, increased plasma levels of BCAAs are linked to insulin resistance and type 2 diabetes (130).

Fish protein is also linked to human health benefits, including increased insulin sensitivity and reduced levels of circulating low-density lipoproteins (**Table 1**) (22, 23). A comparison of the AA content in several fish species shows that the two most limiting AAs in fish are tryptophan and methionine, with cysteine as the most limiting NEAA. Some long-lived human populations, like Okinawans, Sardinians, or Ikarians (131), are located in areas were fish is a predominant

Table 1 List of findings relating dietary protein sources to health span and life span, including effects upon the IIS and TOR pathways and on circulating metabolites linked to health-related parameters

Dietary protein source(s) tested/compared	Species	Observed physiological effects	Measured effect on health and/or life span (protein source that the effect refers to)	Reference(s)
Milk, dairy	*Homo sapiens*	Increased circulating IGF levels, mTORC1 activation, circulating AAs, and growth rate; reduced insulin sensitivity; and changed calcium signaling	Negative (milk, dairy)	29, 129, 146
Soy	*Homo sapiens*	Reduced plasma lipid levels and adiposity, reduced incidence of prostate and breast cancers, and improved insulin and glucose homeostasis	Positive (soy)	23
Soy, animal meat	*Homo sapiens*	Soy protein decreased protein synthesis, protein oxidation, energy expenditure, and thermogenesis compared with animal meat	n.d.	22
Soy, whey, casein	*Homo sapiens*	Whey protein increased circulating GLP-1 levels, thereby increasing satiety	n.d.	22
Soy, casein	*Rattus norvegicus*	Soy protein reduced serum parathyroid hormone levels and incidence of nephropathy	Positive (soy)	143
Fish	*Homo sapiens*	Increased insulin sensitivity, reduced plasma low-density lipoprotein levels, and increased high-density lipoprotein levels	Positive (fish)	23
Whey, casein, soy	*Rattus norvegicus*	Whey protein reduced plasma and liver cholesterol levels	Positive (whey)	142
Whey, casein	*Mus musculus*	Increased mean life span, liver and heart glutathione levels, and insulin sensitivity	Positive (whey)	144, 145
Whey	*Homo sapiens*	Reduced adiposity; increased muscle protein and protection from high blood pressure	Positive (whey)	127, 147
Casein, wheat, corn	*Rattus norvegicus*	Casein protein increased circulating IGF levels	Negative (casein)	148
Plant-based protein	*Homo sapiens*	Decreased circulating IGF-1 levels compared with animal proteins; increased levels of the IGF-1 inhibitor IGFBP-3	Positive (plant and soy proteins)	128, 149
Egg, turkey, fish, whey	*Homo sapiens*	Whey protein increased satiety, decreased postprandial levels of circulating glucose, and decreased subsequent food intake compared with egg and turkey proteins	Positive (whey)	118

Abbreviations: AA, amino acid; GLP-1, glucagon-like peptide 1; IGF, insulin-like growth factor; IIS, insulin/IGF; n.d., no data; TOR, target of rapamycin.

protein source (132, 133). Thus, it is tempting to draw a link between the reduced sulfur AAs and tryptophan and the insulin sensitivity and life span extension observed in these populations.

In humans, several cohort studies show that high intake levels of mammal protein, which is typically methionine rich, are positively associated with chronic and age-related disease, and this association is abolished when the dietary protein source is plant based (11). However, the age of an individual also determines the health response to the ingested protein (11), as discussed below (see section on Variation in Requirement for Amino Acids).

The bioenergetics of different AA sources may also contribute to health effects. The metabolic efficiency of different dietary proteins integrates their AA composition and the energy used in the catabolism of each AA to produce one ATP molecule (120). A comparison of the metabolic efficiency of different dietary proteins shows that proteins linked to beneficial effects for health in later life and life span in animals and humans (**Table 1**) tend to have an essential AA profile that has a higher metabolic efficiency (**Figure 5*a***). The calculated percentage energy efficiency is lower for lactalbumin, egg, and casein and higher for soy and fish proteins. Therefore it is possible, although not yet established, that a link between metabolic efficiency of AA catabolic reactions and health exists.

Regarding the two recent studies of DR in primates, the WNPRC diet had higher contents of tryptophan and BCAAs (lactalbumin) than the NIA diet (fish, soybean, wheat, corn, alfalfa) (**Figure 5*b***). Milk and dairy proteins such as lactalbumin can induce TOR activation and insulin resistance in humans (23, 129). Thus, AA intake differences between the two studies could have contributed to differences in mortality and cancer incidence, as a higher intake of BCAAs and tryptophan could lead to a chronically higher TOR/IIS stimulation (5).

In conclusion, a number of observations suggest an important role of dietary AA intake upon health and aging in humans. The pattern of ingested AAs influences nutrient-sensing pathways and dictates several physiometabolic processes. Nonetheless, more controlled studies are needed to confirm or disprove some possible connections between ingested AAs and health parameters.

OPTIMAL AA INTAKE

Variation in Requirement for AAs

The AA needs of individuals, populations, and species are influenced dynamically by internal state and environmental factors. Such factors can lead to variations in the requirement for AAs, resulting in individualized optimal AA patterns. The main known sources leading to variation in AA requirements are discussed here.

Although not much studied beyond inborn errors of metabolism, genetic variation between and within species can greatly affect the response to AA consumption. Interstrain variability for the requirement of some AAs (e.g., glycine) has been shown to be significant in *Drosophila* (79). In rodents and humans, genotype profoundly affects body size, AA requirements, and food intake (4, 27). Wild-derived strains of worms and flies live longer upon DR (134), but the responses of mice of different strains to a single DR regime can be highly variable (135) and genetic differences may have contributed to differences in the DR response observed in recent primate studies (5). Single DR regimes do not show the full response of a mouse strain across different restriction levels (2), but they do indicate that genetic constitution affects the response to reduced intake of nutrients, including AAs. Recent studies have assessed single nucleotide polymorphisms (SNPs) to identify specific genes and to explain how genetic variation in inbred mouse populations determines traits of interest (136). Similar approaches could be employed to evaluate the role of genetic determinants in the DR response, both in rodents and in humans. Such information will inform our understanding

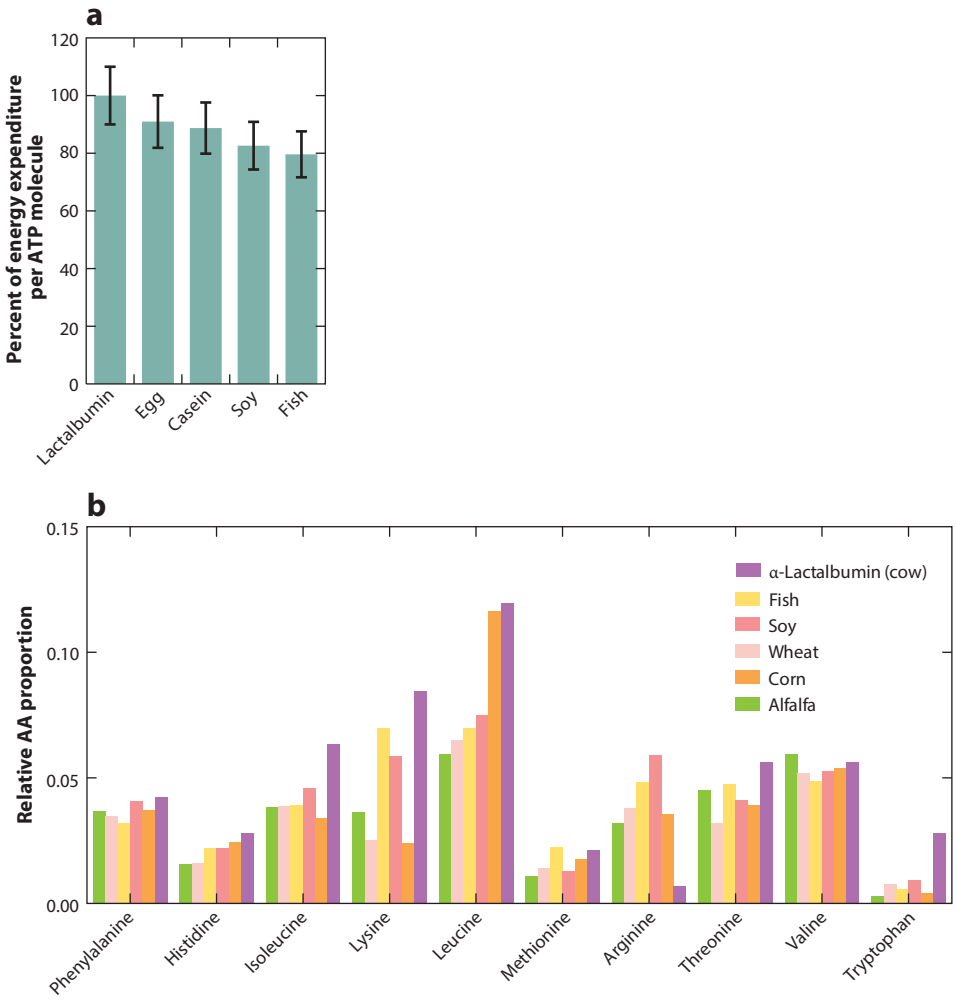

Figure 5

Comparison of the calculated percentage energy expenditure and of the molar AA proportions for a range of dietary protein sources. (*a*) The calculated percentage energy expenditure for the production of one ATP molecule based on nonintegral P/O ratios (120), shown for the essential AA composition of five common types of dietary protein: lactalbumin, egg, casein, soy, and fish. Differences are owing to the range of carbon chain and cofactors that result from essential AA catabolism. Some protein sources associated with health benefits (**Table 1**) appear to have a higher proportional metabolic efficiency than proteins associated with detrimental effects. (*b*) Comparison of the approximate EAA content of the six protein sources used in the two primate studies. Lactalbumin has particularly high levels of tryptophan and isoleucine, as well as leucine. Abbreviations: AA, amino acid; ATP, adenosine triphosphate; EAA, essential amino acid.

of how natural genetic variation predisposes the DR response both in model organisms and in humans and even aid the design of individualized nutritional interventions.

All growing or reproducing mammals, including humans, have higher AA requirements than adults (24, 27). Consequently, young children are more susceptible to protein malnutrition and related diseases such as kwashiorkor. In laboratory animals, the distinction between diets optimized for breeding or growth stages as opposed to long-term maintenance is clear (27). Adjusting

the dietary protein supply to match AA requirements with age promotes health and longevity. Providing high amounts of dietary protein to young animals and lower amounts to mature ones extends life span in rats (9, 137) and mice (6). In rodents, protein absorption declines with age and older rats show a decreased ability to digest proteins and AAs (138). Moreover, mature rodents fail to show some of the adverse effects of ingesting AA-imbalanced diets (25, 26). Accordingly, BCAA stimulation of the IIS/TOR pathways is greater in younger, not older, animals (81). Unsurprisingly, early onset DR extends rodent life span significantly (139), but late onset DR is less effective (6). The protein source during early life also affects health during aging. Although milk protein can chronically overstimulate IIS and contribute to insulin resistance (**Table 1**), restriction of milk protein only during weaning can significantly increase mouse life span (140). Requirements for AAs may also change qualitatively as an animal physically matures. Some evidence in mice suggests subtle changes in the body's AA composition with development (141). In humans, a low protein intake appears to benefit groups of 50–65 years of age but may be detrimental when applied to older ages (11). Therefore, it is clear that age and life stage can affect both the requirement for AAs and the response to AA intake levels.

The EAA requirements of individual cells or tissues can vary depending on tissue-specific AA metabolism. For example, enterocytes secrete threonine-rich proteins and so require a higher threonine intake than other cell types (47, 55). Hepatocytes require high levels of methionine to serve many transsulfation, transmethylation, and folate metabolism reactions (55). The type and abundance of AA transporters also determines which AAs enter readily into which cells. These aspects require further study in conjunction with more systematic analysis of tissue-specific usage of individual AAs.

In humans, gender defines AA requirements as males require more AAs than nonpregnant females, which reflects body size differences to an extent (4, 24). Beyond inborn errors of metabolism, the maintenance of health requires adequate AA supply, as multiple immunological processes depend on AAs and an imbalanced AA intake can suppress the immune system (20). The efficiency of the immune response declines and the susceptibility to infections increases upon low AA intake (4); thus, infectious or disease conditions that increase the function of immunological processes may raise dietary AA requirements (20). In the future, more work will be required to understand how specific disease states increase the requirement for specific AAs, as an imbalanced AA intake can suppress the immune system (20).

In addition, healthy physical activity increases the metabolic rate and promotes protein degradation, AA oxidation, and depression of protein synthesis in humans, thereby increasing AA requirements (4). The metabolic rate of individuals can also be modulated by environmental conditions, as lower temperatures can increase the metabolic rate in endothermic animals (4). Similarly, seasonal increases in day cycle duration can promote physical activity, thereby increasing the metabolic rate particularly at younger ages (4). Such increases in metabolic rate also translate to increases in AA requirements.

In summary, numerous findings from different branches of nutritional research clearly indicate that both environmental and internal state factors must be considered when estimating AA requirements. Such considerations make possible the prospect of defining individualized AA requirements. In turn, individualized AA patterns hold a promising potential for promoting health.

CONCLUSIONS

Identifying beneficial AA intake levels can lead to improvements in human nutrition. In human populations, health benefits for older age groups mirror most mortality gains, and late life dietary interventions based on AA intake are beneficial (11). As AA intake is critical to the DR response,

dietary AAs provide a powerful intervention strategy for human health. Indeed, recent evidence shows that a fasting mimicking diet based on a limited plant-based AA intake benefits human health (18). Such dietary manipulations comprise a drug-free intervention for healthy aging. Moreover, nutritional efficiency can have diverse applications within our societies, as it can help to end starvation, devise tools against obesity and disease, enhance produce yield in the food industry, and assist patients in numerous clinical applications including cancer.

SUMMARY POINTS

1. Even with adequate intake of macronutrients, the protein and AA content of the diet are critical for health during aging.
2. An imbalanced supply of AAs occurs when the requirement for dietary AA, usually determined by their effects on anabolic traits, is not matched by their intake.
3. This dietary AA requirement is affected by multiple factors, including genetic diversity, gender, age, and health status.
4. AA absorption and availability is determined by the gut microbiota, the AA's identity, and first pass metabolism.
5. Ingested and systemically available AAs are sensed by various mechanisms, involving TOR, GCN2, GPCRs, and other sensors.
6. Excess AA intake levels can overstimulate growth signaling, which can be chronically detrimental and decrease longevity.
7. Limited or imbalanced AA intake levels can downregulate growth signaling, inducing a maintenance mode.
8. Experimental animal models can inform human nutrition, increasing our understanding of how AA intake affects human health and aging.

FUTURE ISSUES

1. Further dissection of AA sensing by the CNS coupled to characterization of AA transporters along the blood-brain barrier is needed; AA transporters across other tissues should also be characterized.
2. Interactions among transceptors and intracellular AA sensors across tissues warrant further elucidation.
3. Interactions between systemic (e.g., neuronal, hormonal) and local (e.g., GCN2, TOR) responses to ingested AAs should be fully described.
4. The metabolic role of circulating AAs and of the energy efficiency and metabolic flux of different AA sources requires characterization.
5. TOR activation by specific AAs requires further clarification to include all EAAs and NEAAs.
6. Uncharged tRNA abundance and consequent GCN2 activation, both with free AA diets and protein diets, requires detailed quantification.
7. TOR-independent and GCN2-independent sensing of AAs should be elucidated.

8. The role of IIS and TOR overstimulation in health deterioration and age-related pathology also requires clarification.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

1. Keenan KP, Wallig MA, Haschek WM. 2013. Nature via nurture: effect of diet on health, obesity, and safety assessment. *Toxicol. Pathol.* 41:190–209
2. Simpson SJ, Raubenheimer D. 2012. *The Nature of Nutrition: A Unifying Framework from Animal Adaptation to Human Obesity*. Princeton NJ: Princeton Univ. Press
3. Solon-Biet SM, McMahon AC, Ballard WO, Ruohonen K, Wu LE, et al. 2014.The ratio of macronutrients, not caloric intake, dictates cardiometabolic health, aging, and longevity in ad libitum-fed mice. *Cell Metab.* 19:418–30

(4) Review of the methodological and biological factors affecting human AA requirements.

4. **Food and Nutrition Board, Institute of Medicine. 2001. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. Washington, DC: The National Academies Press**

(5) An account of the effects of dietary interventions upon health and life span across species.

5. **Fontana L, Partridge L. 2015. Promoting health and longevity through diet: from model organisms to humans. *Cell* 161:106–18**
6. Weindruch R, Walford RL. 1988. *The Retardation of Aging and Disease by Dietary Restriction*. Springfield, IL: Thomas.
7. Mair W, Piper MDW, Partridge L. 2005. Calories do not explain extension of life span by dietary restriction in *Drosophila*. *PLOS Biol.* 3:e223
8. Grandison RC, Piper MDW, Partridge L. 2009. Amino-acid imbalance explains extension of lifespan by dietary restriction in *Drosophila*. *Nature* 462:1061–64
9. Horáková M, Deyl Z, Hausmann J, Macek K. 1988. The effect of low protein-high dextrin diet and subsequent food restriction upon life prolongation in Fischer 344 male rats. *Mech. Ageing Dev.* 45:1–7
10. Nakagawa S, Lagisz M, Hector KL, Spencer HG. 2012. Comparative and meta-analytic insights into life extension via dietary restriction. *Aging Cell* 11:401–9
11. Mirzaei H, Suarez JA, Longo VD. 2014. Protein and amino acid restriction, aging and disease: from yeast to humans. *Trends Endocrinol. Metab.* 25(11):558–66
12. Douris N, Melman T, Pecherer JM, Pissios P, Flier JS, et al. 2015. Adaptive changes in amino acid metabolism permit normal longevity in mice consuming a low-carbohydrate ketogenic diet. *Biochim. Biophys. Acta* 10:2056–65
13. Orgeron ML, Stone KP, Wanders D, Cortez CC, Van NT, Gettys TW. 2014. The impact of dietary methionine restriction on biomarkers of metabolic health. *Prog. Mol. Biol. Transl. Sci.* 121:351–76

14. Zimmerman JA, Malloy V, Krajcik R, Orentreich N. 2003. Nutritional control of ageing. *Exp. Gerontol.* 38:47–52
15. Shin AC, Fasshauer M, Filatova N, Grundell LA, Zielinski E, et al. 2014. Brain insulin lowers circulating BCAA levels by inducing hepatic BCAA catabolism. *Cell Metab.* 20:898–909
16. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. 2013. The hallmarks of aging. *Cell* 153:1194–217
17. Rizza W, Veronese N, Fontana L. 2014. What are the roles of calorie restriction and diet quality in promoting healthy longevity? *Ageing Res. Rev.* 13:38–45
18. Brandhorst S, Choi IY, Wei M, Cheng CW, Sedrakyan S, et al. 2015. A periodic diet that mimics fasting promotes multi-system regeneration, enhanced cognitive performance, and healthspan. *Cell Metab.* 22:86–99
19. Hine C, Harputlugil E, Zhang Y, Ruckenstuhl C, Lee BC, et al. 2015. Endogenous hydrogen sulfide production is essential for dietary restriction benefits. *Cell* 160:132–44
20. Li P, Yin Y-L, Li D, Kim SW, Wu G. 2007. Amino acids and immune function. *Br. J. Nutr.* 98:237–52
21. Parrella E, Maxim T, Maialetti F, Zhang L, Wan J, et al. 2013. Protein restriction cycles reduce IGF-1 and phosphorylated Tau, and improve behavioral performance in an Alzheimer's disease mouse model. *Aging Cell* 12:257–68
22. **Westerterp-Plantenga MS, Nieuwenhuizen A, Tomé D, Soenen S, Westerterp KR. 2009. Dietary protein, weight loss, and weight maintenance. *Annu. Rev. Nutr.* 29:21–41**
23. Tremblay F, Lavigne C, Jacques H, Marette A. 2007. Role of dietary proteins and amino acids in the pathogenesis of insulin resistance. *Annu. Rev. Nutr.* 27:293–310
24. **World Health Organ. 2007. *Protein and amino acid requirements in human nutrition*. Geneva: United Nations University**
25. **Harper AE, Benevenga NJ, Wohlhueter RM. 1970. Effects of ingestion of disproportionate amounts of amino acids. *Physiol. Rev.* 50:428–558**
26. Park B-C. 2006. Amino acid imbalance-biochemical mechanism and nutritional aspects. *Asian-Australas. J. Anim. Sci.* 19:1361–68
27. **Board on Agriculture, National Research Council. 1995. *Nutrient Requirements of Laboratory Animals*. Washington, DC: National Academy Press. 4th ed.**
28. Piper MDW, Blanc E, Leitão-Gonçalves R, Yang M, He X, et al. 2014. A holidic medium for *Drosophila melanogaster*. *Nat. Methods* 11:100–5
29. Tucker LA, Erickson A, LeCheminant JD, Bailey BW. 2015. Dairy consumption and insulin resistance: the role of body fat, physical activity, and energy intake. *J. Diabetes Res.* 2015:206959
30. Brody S. 1945. *Bioenergetics and growth; with special reference to the efficiency complex in domestic animals*. New York: Reinhold
31. Almquist HJ. 1953. Interpretation of amino acid requirement data according to the Law of Diminishing Returns. *Arch. Biochem. Biophys.* 44:245–47
32. McCay CM, Crowell MF, Maynard LA. 1935. The effect of retarded growth upon the length of life span and upon the ultimate body size. *J. Nutr.* 10:63–79
33. Jünger MA, Rintelen F, Stocker H, Wasserman JD, Végh M, et al. 2003. The *Drosophila* Forkhead transcription factor FOXO mediates the reduction in cell number associated with reduced insulin signaling. *J. Biol.* 2:20
34. Enesco HE, Samborsky J. 1986. Influence of dietary protein restriction on cell number, cell size and growth of mouse organs during the course of aging. *Arch. Gerontol. Geriatr.* 5:221–33
35. Miller RA, Harper JM, Galecki A, Burke DT. 2002. Big mice die young: Early life body weight predicts longevity in genetically heterogeneous mice. *Aging Cell* 1:22–29
36. Selman C, Nussey DH, Monaghan P. 2013. Ageing: It's a dog's life. *Curr. Biol.* 23:R451–53
37. Rollo CD. 2002. Growth negatively impacts the life span of mammals. *Evol. Dev.* 4:55–61
38. Bartke A. 2012. Healthy aging: Is smaller better? *Gerontology* 58:337–43
39. Partridge L, Gems D, Withers DJ. 2005. Sex and death: What is the connection? *Cell* 120:461–72
40. O'Brien DM, Kyung-Jin M, Larsen T, Tatar M. 2008. Use of stable isotopes to examine how dietary restriction extends *Drosophila* lifespan. *Curr. Biol.* 18:R155–56

(22) Discusses the role of protein intake in the modulation of metabolism and obesity in humans.

(24) Lists methodological and biological factors affecting AA requirements and provides AA requirements across ages and genders.

(25) Review of how imbalanced AA intakes can affect health and physiology in rodents.

(27) Official AA recommendations for laboratory animals based on thorough review of the literature and empirical data.

41. Hoffman JM, Creevy KE, Promislow DEL. 2013. Reproductive capability is associated with lifespan and cause of death in companion dogs. *PLOS ONE* 8:e61082
42. Tabatabaie V, Atzmon G, Rajpathak SN, Freeman R, Barzilai N, Crandall J. 2011. Exceptional longevity is associated with decreased reproduction. *Aging* 3:1202–5
43. Jahan-Mihan A, Luhovyy BL, El Khoury D, Anderson GH. 2011. Dietary proteins as determinants of metabolic and physiologic functions of the gastrointestinal tract. *Nutrients* 3:574–603
44. Rutherfurd SM, Moughan PJ. 2012. Available versus digestible dietary amino acids. *Br. J. Nutr.* 108:S298–305
45. Bröer S. 2008. Amino acid transport across mammalian intestinal and renal epithelia. *Physiol. Rev.* 88:249–86
46. Zhang Z, Adelman AS, Rai D, Boettcher J, Lőnnerdal B. 2013. Amino acid profiles in term and preterm human milk through lactation: a systematic review. *Nutrients* 5:4800–21
47. Davila A-M, Blachier F, Gotteland M, Andriamihaja M, Benetti PH, et al. 2013. Intestinal luminal nitrogen metabolism: role of the gut microbiota and consequences for the host. *Pharmacol. Res.* 68:95–107
48. Metges CC, Petzke KJ. 2007. Utilization of essential amino acids synthesized in the intestinal microbiota of monogastric mammals. *Br. J. Nutr.* 94:621–22
49. Wikoff WR, Anfora AT, Liu J, Schultz PG, Lesley SA, et al. 2009. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *PNAS* 106:3698–703
50. McDonald P, Edwards RA, Greenhalgh JFD, Morgan CA, Sinclair LA. 2010. *Animal Nutrition*. London: Pearson
51. Zhang C, Li S, Yang L, Huang P, Li W, et al. 2013. Structural modulation of gut microbiota in life-long calorie-restricted mice. *Nat. Commun.* 4:1–10
52. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, et al. 2011. Linking long-term dietary patterns with gut microbial enterotypes. *Science* 334:105–8
53. Erkosar B, Storelli G, Defaye A, Leulier F. 2013. Host-intestinal microbiota mutualism: "learning on the fly." *Cell Host Microbe* 13:8–14
54. Neuman H, Debelius JW, Knight R, Koren O. 2015. Microbial endocrinology: the interplay between the microbiota and the endocrine system. *FEMS Microbiol. Rev.* 39(4):509–21
55. Stoll B, Burrin DG. 2006. Measuring splanchnic amino acid metabolism in vivo using stable isotopic tracers. *J. Anim. Sci.* 84:E60–72
56. Hinton T, Noyes DT, Ellis J. 1951. Amino acids and growth factors in a chemically defined medium for *Drosophila*. *Physiol. Zool.* 24:335–53
57. Ball RO, Courtney-Martin G, Pencharz PB. 2006. The in vivo sparing of methionine by cysteine in sulfur amino acid requirements in animal models and adult humans. *J. Nutr.* 136:1682S–93S
58. Sarup P, Pedersen SMM, Nielsen NC, Malmendal A, Loeschcke V. 2012. The metabolic profile of long-lived *Drosophila melanogaster*. *PLOS ONE* 7:e47461
59. Tomás-Loba A, Bernardes de Jesus B, Mato JM, Blasco MA. 2012. A metabolic signature predicts biological age in mice. *Aging Cell* 12(1):93–101
60. Selman C, Kerrison ND, Corray A, Piper MD, Lingard SJ, et al. 2006. Coordinated multitissue transcriptional and plasma metabonomic profiles following acute caloric restriction in mice. *Physiol. Genom.* 27(3):187–200
61. Richards SE, Wang Y, Claus SP, Lawler D, Kochhar S, et al. 2013. Metabolic phenotype modulation by caloric restriction in a lifelong dog study. *J. Proteome Res.* 12:3117–27
62. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, et al. 2011. Metabolite profiles and the risk of developing diabetes. *Nat. Med.* 17:448–53
63. Wijeyesekera A, Selman C, Barton RH, Holmes E, Nicholson JK, Withers DJ. 2012. Metabotyping of long-lived mice using ^{1}H NMR spectroscopy. *J. Proteome Res.* 11:2224–35
64. Kilberg MS, Pan Y-X, Chen H, Leung-Pineda V. 2005. Nutritional control of gene expression: how mammalian cells respond to amino acid limitation. *Annu. Rev. Nutr.* 25:59–85
65. Wauson EM, Lorente-Rodríguez A, Cobb MH. 2013. Minireview: nutrient sensing by G protein-coupled receptors. *Mol. Endocrinol.* 27:1188–97

66. Pochini L, Scalise M, Galluccio M, Indiveri C. 2014. Membrane transporters for the special amino acid glutamine: structure/function relationships and relevance to human health. *Front. Chem.* 2:61
67. Nicklin P, Bergman P, Zhang B, Triantafellow E, Wang H, et al. 2009. Bidirectional transport of amino acids regulates mTOR and autophagy. *Cell* 136:521–34
68. Lemaitre B, Miguel-Aliaga I. 2013. The digestive tract of *Drosophila melanogaster*. *Annu. Rev. Genet.* 47:377–404
69. Libert S, Pletcher SD. 2007. Modulation of longevity by environmental sensing. *Cell* 131:1231–34
70. Bjordal M, Arquier N, Kniazeff J, Pin JP, Leopold P. 2014. Sensing of amino acids in a dopaminergic circuitry promotes rejection of an incomplete diet in *Drosophila*. *Cell* 156:510–21
71. **Gietzen DW, Hao S, Anthony TG. 2007. Mechanisms of food intake repression in indispensable amino acid deficiency. *Annu. Rev. Nutr.* 27:63–78**
72. **Chaveroux C, Lambert-Langlais S, Cherasse Y, Averous J, Parry L, et al. 2010. Molecular mechanisms involved in the adaptation to amino acid limitation in mammals. *Biochimie* 92:736–45**
73. Alves VS, Motta FL, Roffé M, Delamano A, Pesquero JB, Castilho BA. 2009. GCN2 activation and eIF2 α phosphorylation in the maturation of mouse oocytes. *Biochem. Biophys. Res. Commun.* 378:41–44
74. Efeyan A, Comb WC, Sabatini DM. 2015. Nutrient-sensing mechanisms and pathways. *Nature* 517:302–10
75. Dang Do AN, Kimball SR, Cavener DR, Jefferson LS. 2009. eIF2 α kinases GCN2 and PERK modulate transcription and translation of distinct sets of mRNAs in mouse liver. *Physiol. Genomics* 38:328–41
76. De Sousa-Coelho AL, Relat J, Hondares E, Pérez-Martí A, Ribas F, et al. 2013. FGF21 mediates the lipid metabolism response to amino acid starvation. *J. Lipid Res.* 54:1786–97
77. Leib DE, Zachary AK. 2015. Re-examination of dietary amino acid sensing reveals a GCN2-independent mechanism. *Cell Rep.* 13:1081–89
78. Karnani MM, Apergis-Schoute J, Adamantidis A, Jensen LT, de Lecea L, et al. 2011. Activation of central orexin/hypocretin neurons by dietary amino acids. *Neuron* 72(4):616–29
79. Blouet C, Schwartz GJ. 2012. Brainstem nutrient sensing in the nucleus of the solitary tract inhibits feeding. *Cell Metab.* 16:579–87
80. Deval C, Chaveroux C, Maurin AC, Cherasse Y, Parry L, et al. 2009. Amino acid limitation regulates the expression of genes involved in several specific biological processes through GCN2-dependent and GCN2-independent pathways. *FEBS J.* 276:707–18
81. Suryawan A, O'Connor PMJ, Bush JA, Nguyen HV, Davis TA. 2009. Differential regulation of protein synthesis by amino acids and insulin in peripheral and visceral tissues of neonatal pigs. *Amino Acids* 37:97–104
82. Stenesen D, Suh JM, Seo J, Yu K, Lee KS, et al. 2013. Adenosine nucleotide biosynthesis and AMPK regulate adult life span and mediate the longevity benefit of caloric restriction in flies. *Cell Metab.* 17:101–12
83. Chotechuang N, Azzout-Marniche D, Bos C, Chaumontet C, Gausserès N, et al. 2009. mTOR, AMPK, and GCN2 coordinate the adaptation of hepatic energy metabolic pathways in response to protein intake in the rat. *Am. J. Physiol. Endocrinol. Metab.* 297:E1313–23
84. Tews JK, Kim YW, Harper AE. 1980. Induction of threonine imbalance by dispensable amino acids: relationships between tissue amino acids and diet in rats. *J. Nutr.* 110:394–408
85. Davis AJ, Austic RE. 1994. Dietary threonine imbalance alters threonine dehydrogenase activity in isolated hepatic mitochondria of chicks and rats. *J. Nutr.* 124:1667–77
86. Palii SS, Kays CE, Deval C, Bruhat A, Fafournoux P, Kilberg MS. 2009. Specificity of amino acid regulated gene expression: analysis of genes subjected to either complete or single amino acid deprivation. *Amino Acids* 37:79–88
87. Takenaka A, Oki N, Takahashi SI, Noguchi T. 2000. Dietary restriction of single essential amino acids reduces plasma insulin-like growth factor-I (IGF-I) but does not affect plasma IGF-binding protein-1 in rats. *J. Nutr.* 130:2910–14
88. Liao C-Y, Rikke BA, Johnson TE, Gelfond JA, Diaz V, Nelson JF. 2011. Fat maintenance is a predictor of the murine lifespan response to dietary restriction. *Aging Cell* 10:629–39
89. Linford NJ, Beyer RP, Gollahon K, Krajcik RA, Malloy VL, et al. 2007. Transcriptional response to aging and caloric restriction in heart and adipose tissue. *Aging Cell* 6:673–88

(71) Report on GCN2-mediated molecular responses to AA limitations.

(72) Revision of transcriptional and translational adaptations to limiting AAs.

90. Demetriades C, Doumpas N, Teleman AA. 2014. Regulation of TORC1 in response to amino acid starvation via lysosomal recruitment of TSC2. *Cell* 156:786–99
91. Kim J, Guan K-L. 2011. Amino acid signaling in TOR activation. *Annu. Rev. Biochem.* 80:1001–32
92. Chantranupong L, Wolfson RL, Sabatini DM. Nutrient-sensing mechanisms across evolution. *Cell* 161:67–83
93. Wolfson RL, Chantranupong L, Saxton RA, Shen K, Scaria SM, et al. 2015. Sestrin2 is a leucine sensor for the mTORC1 pathway. *Science* 351:43–48
94. Wang S, Tsun Z-Y, Wolfson RL, Shen K, Wyant GA, et al. 2015. Lysosomal amino acid transporter SLC38A9 signals arginine sufficiency to mTORC1. *Science* 347:188–94
95. Tsun Z-Y, Bar-Peled L, Chantranupong L, Zoncu R, Wang T, et al. 2013. The folliculin tumor suppressor is a GAP for the RagC/D GTPases that signal amino acid levels to mTORC1. *Mol. Cell* 52:495–505
96. Li L, Kim E, Yuan H, Inok K, Goraksha-Hicks P, et al. 2010. Regulation of mTORC1 by the Rab and Arf GTPases. *J. Biol. Chem.* 285:19705–9
97. Jewell JL, Kim YC, Russell RC, Yu F-X, Park WH, et al. 2015. Differential regulation of mTORC1 by leucine and glutamine. *Science* 347:194–98
98. Lamming DW, Ye L, Karajisto P, Golcalves MD, Saitoh M, et al. 2012. Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. *Science* 335:1638–43
99. Saha AK, Xu XJ, Balon TW, Brandon A, Kraegen EW, Ruderman NB. 2011. Insulin resistance due to nutrient excess: Is it a consequence of AMPK downregulation? *Cell Cycle* 10:3447–51
100. Han JM, Jeong SJ, Park MC, Kim G, Kwon NH, et al. 2012. Leucyl-tRNA synthetase is an intracellular leucine sensor for the mTORC1-signaling pathway. *Cell* 149:410–24
101. Saxton RA, Knockenhauer KE, Wolfson RL, Chantranupong L, Pacold ME, et al. 2015. Structural basis for leucine sensing by the Sestrin2-mTORC1 pathway. *Science* 351:53–58
102. Norheim F, Gjelstad IMF, Hjorth M, Vinknes KJ, Langleite TM, et al. 2012. Molecular nutrition research: the modern way of performing nutritional science. *Nutrients* 4:1898–944
103. Journel M, Chaumontet C, Darcel N, Fromentin G, Tomé D. 2012. Brain responses to high-protein diets. *Adv. Nutr.* 3:322–29
104. Kohl S, Behrens M, Dunkel A, Hofmann T, Meyerhof W. 2013. Amino acids and peptides activate at least five members of the human bitter taste receptor family. *J. Agric. Food Chem.* 61:53–60
105. Lindgren O, Pacini G, Tura A, Holst JJ, Deacon CF, Ahren B. 2015. Incretin effect after oral amino acid ingestion in humans. *J. Clin. Endocrinol. Metab.* 100:1172–76
106. Alfa RW, Park S, Skelly KR, Poffenberger G, Jain N, et al. 2015. Suppression of insulin production and secretion by a decretin hormone. *Cell Metab.* 21:323–33
107. Jiang Y, Rose AJ, Sijmonsma TP, Broer A, Pfenninger A, et al. 2015. Mice lacking neutral amino acid transporter B^0AT1 (Slc6a19) have elevated levels of FGF21 and GLP-1 and improved glycaemic control. *Mol. Metab.* 4:406–17
108. Crespo CS, Cachero AP, Jiménez LP, Barrios V, Ferreiro EA. 2014. Peptides and food intake. *Front. Endocrinol.* 5:58
109. Jordi J, Herzog B, Camargo SMR, Boyle CN, Lutz TA, Verrey F. 2013. Specific amino acids inhibit food intake via the area postrema or vagal afferents. *J. Physiol.* 591:5611–21
110. McCormick MA, Tsai SY, Kennedy BK. 2010. TOR and ageing: a complex pathway for a complex process. *Philos. Trans. R. Soc. B* 366:17–27
111. Edwards C, Canfield J, Copes N, Brito A, Rehan M, et al. 2015. Mechanisms of amino acid-mediated lifespan extension in *Caenorhabditis elegans*. *BMC Genet.* 16:8
112. Santos J, Leao C, Sousa MJ. 2012. Growth culture conditions and nutrient signaling modulating yeast chronological longevity. *Oxidative Med. Cell. Longev.* 2012:10
113. Cai Z, Zhou Y, Xiao M, Yan L-J, He W. 2015. Activation of mTOR: a culprit of Alzheimer's disease? *Neuropsychiatr. Dis. Treat.* 11:1015–30
114. Kimball SR, Jefferson LS. 2006. New functions for amino acids: effects on gene transcription and translation. *Am. J. Clin. Nutr.* 83:500S–7S
115. Bunpo P, Dudley A, Cundiff JK, Cavender DR, Wek RC, Anthony TG. 2009. GCN2 protein kinase is required to activate amino acid deprivation responses in mice treated with the anti-cancer agent L-asparaginase. *J. Biol. Chem.* 284:32742–49

116. Rousakis A, Vlassis A, Vlanti A, Patera S, Thireos G, Syntichaki P. 2013. The general control nonderepressible-2 kinase mediates stress response and longevity induced by target of rapamycin inactivation in *Caenorhabditis elegans*. *Aging Cell* 12:742–51
117. Ye J, Palm W, Peng M, King B, Lindsten T, et al. 2015. GCN2 sustains mTORC1 suppression upon amino acid deprivation by inducing Sestrin2. *Genes Dev.* 29:2331–36
118. Pal S, Ellis V. 2010. The acute effects of four protein meals on insulin, glucose, appetite and energy intake in lean men. *Br. J. Nutr.* 104:1241–48
119. Hoffman JR, Falvo MJ. 2004. Protein—which is best? *J. Sports Sci. Med.* 3:118–30
120. van Milgen J. 2002. Modeling biochemical aspects of energy metabolism in mammals. *J. Nutr.* 132:3195–202
121. Chin RM, Fu X, Pai MY, Vergnes L, Hwang H, et al. 2014. The metabolite α-ketoglutarate extends lifespan by inhibiting ATP synthase and TOR. *Nature* 510(7505):397–401
122. D'Antona G, Ragni M, Cardile A, Tedesco L, Dossena M, et al. 2010. Branched-chain amino acid supplementation promotes survival and supports cardiac and skeletal muscle mitochondrial biogenesis in middle-aged mice. *Cell Metab.* 12:362–72
123. Poff AM, Ari C, Arnold P, Seyfried TN, D'Agostino DP. 2014. Ketone supplementation decreases tumor cell viability and prolongs survival of mice with metastatic cancer. *Int. J. Cancer* 135:1711–20
124. Gibson AA, Seimon RV, Lee CMY, Ayre J, Franklin J, et al. 2014. Do ketogenic diets really suppress appetite? A systematic review and meta-analysis. *Obes. Res. Clin. Pract.* 8:36
125. Pedroso JAB, Zampieri TT, Donato J. 2015. Reviewing the effects of l-leucine supplementation in the regulation of food intake, energy balance, and glucose homeostasis. *Nutrients* 7:3914–37
126. Komar B, Schwingshackl L, Hoffmann G. 2015. Effects of leucine-rich protein supplements on anthropometric parameter and muscle strength in the elderly: a systematic review and meta-analysis. *J. Nutr. Health Aging* 19:437–46
127. Jakubowicz D, Froy O. 2013. Biochemical and metabolic mechanisms by which dietary whey protein may combat obesity and type 2 diabetes. *J. Nutr. Biochem.* 24:1–5
128. McCarty MF, Barroso-Aranda J, Contreras F. 2009. The low-methionine content of vegan diets may make methionine restriction feasible as a life extension strategy. *Med. Hypotheses* 72:125–28
129. Melnik BC, John SM, Schmitz G. 2013. Milk is not just food but most likely a genetic transfection system activating mTORC1 signaling for postnatal growth. *Nutr. J.* 12:103
130. Newgard CB. 2012. Interplay between lipids and branched-chain amino acids in development of insulin resistance. *Cell Metab.* 15:606–14
131. Poulain M, Herm A, Pes G. 2013. The Blue Zones: areas of exceptional longevity around the world. *Vienna Yearb. Popul. Res.* 11:87–180
132. Trichopoulou A, Vasilopoulou E. 2000. Mediterranean diet and longevity. *Br. J. Nutr.* 84:S205–9
133. Buckland G, Agudo A, Travier N, Huerta JM, Cirera L, et al. 2011. Adherence to the Mediterranean diet reduces mortality in the Spanish cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC-Spain). *Br. J. Nutr.* 106:1581–91
134. Metaxakis A, Partridge L. 2013. Dietary restriction extends lifespan in wild-derived populations of *Drosophila melanogaster*. *PLOS ONE* 8:e74681
135. Liao C-Y, Rikke BA, Johnson TE, Diaz V, Nelson JF. 2010. Genetic variation in the murine lifespan response to dietary restriction: from life extension to life shortening. *Aging Cell* 9:92–95
136. Ram R, Mehta M, Balmer L, Gatti DM, Morahan G. 2014. Rapid identification of major-effect genes using the collaborative cross. *Genetics* 198:75–86
137. Miller DS, Payne PR. 1968. Longevity and protein intake. *Exp. Gerontol.* 3:231–34
138. Gilani GS, Sepehr E. 2003. Protein digestibility and quality in products containing antinutritional factors are adversely affected by old age in rats. *J. Nutr.* 133:220–25
139. Sun L, Sadighi Akha AA, Miller RA, Harper JM. 2009. Life-span extension in mice by preweaning food restriction and by methionine restriction in middle age. *J. Gerontol. A* 64A:711–22
140. Ozanne SE, Hales CN. 2004. Lifespan: catch-up growth and obesity in male mice. *Nature* 427:411–12
141. John AM, Bell JM. 1976. Amino acid requirements of the growing mouse. *J. Nutr.* 106:1361–67
142. Zhang X, Beynen AC. 1993. Lowering effect of dietary milk-whey protein v. casein on plasma and liver cholesterol concentrations in rats. *Br. J. Nutr.* 70:139–46

143. Kalu DN, Masoro EJ, Yu BP, Hardin RR, Hollis BW. 1988. Modulation of age-related hyperparathyroidism and senile bone loss in Fischer rats by soy protein and food restriction. *Endocrinology* 122:1847–54
144. Shertzer HG, Woods SE, Krishan M, Genter MB, Pearson KJ. 2011. Dietary whey protein lowers the risk for metabolic disease in mice fed a high-fat diet. *J. Nutr.* 141:582–87
145. Bounous G, Gervais F, Amer V, Batist G, Gold P. 1989. The influence of dietary whey protein on tissue glutathione and the diseases of aging. *Clin. Investig. Med.* 12:343–49
146. Crowe FL, Key TJ, Allen NE, Appleby PN, Roddam A, et al. 2009. The association between diet and serum concentrations of IGF-1, IGFBP-1, IGFBP-2, and IGFBP-3 in the European Prospective Investigation into cancer and nutrition. *Cancer Epidemiol. Biomark. Prev.* 18:1333–40
147. Coker RH, Miller S, Schutzler S, Deutz N, Wolfe RR. 2012. Whey protein and essential amino acids promote the reduction of adipose tissue and increased muscle protein synthesis during caloric restriction-induced weight loss in elderly, obese individuals. *Nutr. J.* 11:105
148. Noguchi T. 2000. Protein nutrition and insulin-like growth factor system. *Br. J. Nutr.* 84:S241–44
149. O'Neill B. 2010. A scientific review of the reported effects of vegan nutrition on the occurrence and prevalence of cancer and cardiovascular disease. *Biosci. Horiz.* 3:197–212
150. Berg JM, Tymoczko JL, Stryer L. 2006. *Biochemistry*. New York: Freeman