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INVITED REVIEW

Protein and amino acid supplementation in older humans

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Abstract The aging process is a continuum throughout life and often associated with deterioration of body function as well as accumulation of chronic disabilities and of disease. The impact of nutritional status on morbidity and mortality is unquestioned. Malnutrition increases the risk for frailty and nutritional deficits can influence immune status, response to medical treatments and recovery from acute illnesses, including surgery. Health-promoting interventions implemented individually, such as exercise programs, preventive home visits, comprehensive geriatric evaluation and management, and attention to adequate nutrition with or without nutritional supplements, have been shown in separate studies to be both feasible and effective in reducing age-related deterioration. Protein and its constituent amino acids (AA) are key components of any healthy diet. Sarcopenia, the slow but progressive loss of lean muscle mass associated with advancing age, has been the focus of many studies but there is no clear-cut answer to the question of how to restrain the process. The more general question of how the requirements for protein and specific AA change with age continues to be investigated. A shift towards studying the efficacy and safety of specific AA or combination of AA that may sustain and/or enhance physiologic processes, ranging from specific tissue metabolism to overall function (e.g. exercise performance, immune function, cognition, and chronic disease development) has occurred. This review focuses on recent studies examining the use of specific AA or mixtures as supplements in the elderly and whether/how AA may assist in the maintenance of health and independence.

Keywords Aging · Protein · Amino acids · Human

Introduction

Significant advances in science and medicine have led to increased life expectancy globally, but with it has come the challenge of balancing the burden of morbidity, quality of life, and access to affordable healthcare. Aging is an inevitable process, and as the proportion of older individuals continues to rise worldwide, identifying and implementing health-promoting interventions becomes ever more important. Good nutritional status is as important for the very young as it is for the growing child, the teen, the young adult, the middle aged, and the aged.[1] Protein and its constituent amino acids are key components of any healthy diet; maintenance of body protein stores is key to survival, especially when the individual is stressed by injury or disease.

Sarcopenia, the slow but progressive loss of lean muscle mass associated with advancing age, contributes to the

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[1] There is no general agreement on the age that a person becomes old. Most developed countries have accepted the chronological age of 65 years as a definition of an older or elderly person. The United Nations has agreed to the cutoff of 60^{+} years for the older population. Geriatrics is the branch of medicine dealing with the diagnosis and treatment of disease of the "elderly," usually patients over 65 years. The World Health Organization defined "middle-age" as 45–59 years, "elderly" as 60–74 years, and "aged" as over 75 years. This review uses "elderly" and "older" interchangeably because of the evolution of investigators' definitions of the terms. However, the mean age or age range of the study participants are provided to permit the reader to evaluate the data in the appropriate context.

vulnerability of the older person (Dutta 1997; Evans 1997) but to date, there is no clear consensus on how to restrain the process. In addition to sarcopenia, bone health, cognitive function, immune status, and noncommunicable diseases (obesity, diabetes mellitus, hypertension, atherosclerosis, etc.) are concerns for the elderly. Increasing knowledge over the past decade about the role of specific amino acids (AA) in sustaining or enhancing physiologic processes, ranging from specific tissue metabolism to overall function, has stimulated interest in whether specific AA-containing supplements may help to mitigate or treat age-related disorders.

Decades of research have contributed to the debate about whether human protein requirements change with advancing age in adults. Table 1 shows the Recommended Dietary Allowances (RDA) for different age groups reported by the Institute of Medicine in 2005 and the "safe levels" reported by the World Health Organization/Food and Agriculture Organization/United Nations University (WHO/FAO/UNU) in 2007. Based on analysis of available nitrogen balance studies, these reports state that the RDA or safe level of protein intake for both adult men and adult women is ~0.8 g of good quality protein per kg body weight per day and that the Acceptable Macronutrient Distribution Range (AMDR) for protein is 10–35 % of energy for adults. Since protein availability and intake are often considered adequate, if not surfeit in many countries, much of nutrition research has focused on the other two macronutrients: carbohydrate and fat. Interest in determining the optimal intakes of carbohydrate and fat has also been driven by concern about the common age-related disorders affected by excess intakes of carbohydrate and fat (e.g. diabetes mellitus, obesity, cardiovascular disease) rather than inadequate intakes of these macronutrients. However, as the population ages, conditions that affect function and ability to live independently have garnered more attention and led to a shift in research focus back to the maintenance of body protein nutriture because it is well accepted that loss of body protein is associated with increased morbidity and mortality. Loss of skeletal muscle, osteopenia, reduced immunity, and impaired wound healing are all affected by the aging process as well as body protein status and AA availability. Common social and physical issues such as being homebound, institutionalized, or poor, and changes in taste, ability to prepare, consume or absorb food can impact the access of elders to the necessary AA available in high-quality protein. These issues have recently been reviewed by Wolfe (Wolfe 2012) and Volpi et al. (2012).

Protein requirements and use of protein supplements

Studies aimed at determining protein requirements have classically relied on the nitrogen balance technique that has been considered the "gold standard." In 2003, Rand, Pellet and Young (Rand et al. 2003) published a meta-analysis of nitrogen balance studies conducted between 1972 and 1994 for estimating the protein requirements of healthy adults. They concluded on the basis of nitrogen balance responses of 235 subjects in 19 different studies that the mean protein requirement (EAR) is 0.65 g good-quality protein/kg/d and that the RDA is 0.83 g/kg/d. These values have remained the basis for many of the recommendations today (Table 1). More recently, Conley et al. (2012) reported on a comparison between the nitrogen balance approach and the use of isotopically labeled leucine to determine optimal protein intakes and concluded that both methods resulted in similar estimates and that the protein requirement is comparable between young and old adults. Nevertheless, there continues to be debate about whether protein intakes above the RDA have additional benefits for older adults, especially with respect to sarcopenia and osteoporosis (Millward 2008). Although investigators continue to try to refine the actual requirement of protein per se, the actual differences between the various studies are small and the recommendations do not appear to differ significantly with advancing age, independent of co-morbid conditions. In a 10-day study of healthy younger (24 ± 1 years) and older (70 ± 2 years) men, Walrand et al. (2008) found that high protein intake (3 g/kg fat-free mass/day) increased net daily nitrogen (N) balance but had no "beneficial" effects on muscle protein synthesis (MPS[2]) in both groups. However, high protein widened the differences in glomerular filtration rates between young and old, raising concern about potential adverse effects on renal function in the elderly. Another consideration is the reported differences in the first-pass splanchnic extraction of AA between younger and older individuals (Boirie et al. 1997; Volpi et al. 1999). In these early studies, higher splanchnic extraction was found in the healthy elderly volunteers (average age 68.2 and 71 years, respectively). Despite the differences, however, age per se did not appear to influence the responsiveness of parameters of leucine turnover or MPS.

Perhaps not unexpected, there has been an explosion of studies focused on the use of protein supplements in the elderly and several are summarized in Table 2. Many of the studies used isolates from whey, casein or soy although a few employed intact protein in its natural "matrix" that included other naturally occurring nutrients (e.g. beef patty). Although the AA composition of the proteins used was reported in some of the studies and attempts made to mimic the AA composition with AA mixtures to identify the key AA(s) responsible for the physiological effects, variability in source as well as study design and approach

[2] For simplicity, MPS will be used to denote fractional synthesis rates of muscle protein assessed with stable isotope tracers.

Table 1 Dietary protein allowances and amino acid requirements

Protein (19–70+ years)	WHO/FAO/UNU "safe levels"[a] g/kg/d	IOM RDA[b] g/day	
Women	0.83	46	
Men	0.83	56	
Essential Amino Acids (EAA)	WHO/FAO/UNU mg/kg/day[a]	IOM RDA[b]	IOM EAR[b]
Leucine	39	42	34
Isoleucine	20	19	15
Valine	26	24	19
Methionine + cysteine (methionine)	15 (10)	19	15
Phenylalanine + tyrosine	25	33	27
Threonine	15	20	16
Tryptophan	4	5	4
Histidine	10	14	11
Lysine	30	38	31

[a] World Health Organization et al. (2007)

[b] Institute of Medicine (2002/2005) This report may be accessed via www.nap.edu

makes it difficult to attempt a meta-analysis. Nevertheless, general conclusions that could be drawn from studies investigating the effects of protein supplements on MPS or N balance in the elderly as surrogates for maintenance of skeletal muscle mass or body protein nutriture are as follows:

1. MPS increases 3–5 h after a bolus of protein or AA mixture to a similar degree in young and old individuals.
2. The type of protein appears to exert different patterns and magnitude of stimulation of MPS with a more consistent, positive effect with whey compared to soy or casein proteins.
3. A beef patty containing approximately 30 g of protein induced a ~50 % increase in MPS, similar to the magnitude of response to two patties (~60 g protein).
4. Resistance exercise before or after ingestion of protein may be synergistic in the stimulation of MPS.
5. Potential age-associated differences in splanchnic extraction of orally administered protein or AA may impact peripheral availability of AA as substrate for protein synthesis or as precursors for bioactive compounds (e.g. neurotransmitters, glutathione, polyamines, creatine, nitric oxide, etc.).

Unfortunately, studies examining the effects of protein supplementation in groups of elders with specific diseases are difficult to assess because of the variability in the patient population and the selected outcomes. In addition, supplements used in studies of patient populations were often a mixture of protein and energy sources rather than protein or AAs alone, confounding interpretation as energy intake influences utilization of protein. Although the use of general nutritional supplements in the older age groups is beyond the scope of this review, one can say that the likely key to good health in older age is a combination of appropriate physical activity and a healthy diet providing adequate intakes of protein and other important nutrients. Practically speaking, because there is little harm associated with higher protein intakes within a reasonable range, intakes above the RDA may be appropriate for most older adults (Millward 2008; Wolfe 2012; Volpi et al. 2012) while remaining cognizant of the balance between meeting minimal requirements and supplementing with protein or AA to protect against loss or to induce other biological effects.

Since specific AA are reported to have unique physiologic effects beyond being constituents of protein, it seems prudent to begin to shift the research focus beyond protein per se and consider the efficacy of individual AA or AA combinations that may further enhance function and quality of life in older age. The reported requirements for the essential amino acids (EAA) are shown in Table 1 and dietary supplements consisting of AA mixtures are often designed to mimic the AA pattern (or composition) seen in an "ideal" protein, most often egg. An interesting perspective presented by Stein and Blanc (2011) questions the efficiency of AA countermeasures for prevention of body protein loss during bed rest, space flight or other clinical conditions associated with physical inactivity. The rest of this review will focus on studies in older humans examining supplementation with the branched chain amino acids (BCAA: leucine, isoleucine, valine), glutamine/glutamate,

Table 2 Selected studies on protein supplementation in healthy elderly volunteers

Reference	Participants/design	Protein AA composition	Outcomes/conclusions
Paddon-Jones et al. (2006)	Whey: 3F/4M (69 ± 2 years) Essential Amino Acids (EAA): 4F/3M (67 ± 2 years) 15 g Whey protein isolate or EAA given as a bolus after baseline samples with assessment of muscle protein synthesis rates as the primary outcome	Whey (0.26 g histidine 0.7 g isoleucine; 1.75 g leucine; 1.40 g lysine; 0.33 g methionine; 0.44 g phenylalanine; 0.68 g threonine; 0.19 g tryptophan; 0.70 g valine; 7.09 g Non-essential Amino Acids, NEAA) EAA (1.64 g histidine 1.56 g isoleucine; 2.79 leucine; 2.30 g lysine; 0,46 g methionine; 2.30 g phenylalanine; 2.20 g threonine; 0.10 g tryptophan; 1.73 g valine)	Both EAA and whey supplements stimulated MPS 3.5 h after ingestion but the increase appeared higher in EAA group; no differences in insulin concentrations. Conclusion: Both whey and EAA stimulated MPS with EAA possibly more "efficient" but less practical. Differences in composition may affect responses.
Katsanos et al. (2008)	Whey: 2F/3M (65 ± 2 years) EAA: 1F/4M (68 ± 3 years) NEAA: 3F/2M (64 ± 2 years) Three groups of men and women administered a bolus of 15 g whey, 6.72 g EAA or 7.57 g NEAA after baseline samples followed by phenylalanine balance assessed by arteriovenous differences 3.5 h after ingestion	Whey (EAA 0.31 g histidine; 0.8 g isoleucine; 1.88 g leucine; 1.50 g lysine; 0.33 g methionine; 0.51 g phenylalanine; 0.72 g threonine; 0.30 g tryptophan; 0.73 g valine; NEAA 0.74 g alanine; 0.35 g arginine; 1.725 g asparagine/aspartate; 0.45 g cysteine; 2.46 g glutamate/glutamine; 0.27 g glycine; 0.66 g proline; 0.55 g tyrosine) Total AA 14.86 g EAA (0.3 g histidine 0.78 g isoleucine; 1.72 g leucine; 1.36 g lysine; 0.36 g methionine; 0.51 g phenylalanine; 0.95 g threonine; 0.10 g tryptophan; 0.74 g valine; no tryptophan) Total AA 6.72 g NEAA (0.76 g alanine; 0.40 g arginine; 1.19 g asparagine/aspartate; 0.40 g cysteine; 2.52 g glutamate/glutamine; 0.28 g glycine; 0.76 g proline; 0.78 g serine; 0.48 g tyrosine) Total AA 7.57 g	Phenylalanine balance increased after whey but not in EAA or NEAA groups. Insulin response was higher after whey. Conclusion: Whey improves leg muscle protein accrual beyond those attributable to its EAA content.
Hays et al. (2009)	11F (age 65–85 years); 2 withdrew for final $n = 9$ Double-blind, crossover 15 day trials with 1 week washout. Protein intake 0.8 g/kg/d with supplement providing ~half total protein	Whey (Resource Beneprotein Instant Protein Powder, Nestle Healthcare) Hydrolyzed collagen fortified with tryptophan (Pro-Stat 101, Medical Nutrition USA)	No difference in nitrogen balance between two trials; both supplements supported N balance. Conclusion: Collagen-based protein supplement may be equivalent to whey protein as a supplement.
Symons et al. (2009)	7F/10M (68 ± 2 years) 9F/8M (35 ± 3 years) 4 separate groups receiving 113 or 340 g beef after baseline samples followed by 5 h postabsorptive samples to assess muscle protein synthesis (MPS)	113 g beef patty: 220 kcal; 30 g protein, 11 g fat	Muscle protein synthesis increased similarly in both age groups with both servings associated with ~50 % increase in MPS. Conclusion: Ingestion of more than 30 g protein in single meal does not further enhance stimulation of MPS in young and old.
Symons et al. (2011)	4F/3M (67 ± 2 years) 4F/3M (29 ± 13 years) Muscle protein synthesis assessed at baseline and 5 h after 340 g beef and bout of resistance exercise	340 g beef patty: 660 kcal; 90 g protein, 33 g fat	MPS increased similarly in young and old. Conclusion: Aging does not diminish increase in MPS after high-quality protein meal and bout of resistance exercise.

Table 2 continued

Reference	Participants/design	Protein AA composition	Outcomes/conclusions
Burd et al. (2012)	14M (72 ± 1 years) divided into 2 groups. Acute bout of unilateral resistance exercise with one leg followed by isotope infusion and muscle biopsy at 4 h. Protein ingested after exercise and before infusion.	Group 1: 20 g micellar casein (MCN-85, AMCO, Burlington, NJ: 8.2 g EAA, 4.0 g BCAA and 1.6 g leucine) Group 2: 20 g whey protein isolate (Alacen 352, Fronterra, Palmerston North, New Zealand: 10.2 g EAA, 5.2 g BCAA and 2.8 g leucine)	Whey increased plasma insulin transiently whereas casein did not; glucose stable throughout. Both proteins induced increases in EAA and Leucine levels with different patterns. Feeding increased MPS at rest and after resistance exercise; whey increased MPS to a greater degree in both conditions. Conclusion: Stimulation of MPS is likely dependent on amplitude and rate/duration of rise in AA levels after a meal.
Yang et al. (2012a)	37M (71 ± 4 years) divided into 4 groups; healthy and light-moderate activity level Acute bout of unilateral resistance exercise with one leg followed by isotope infusion and muscle biopsy study. 4 levels of whey protein administered after exercise bout and before isotope infusion. MPS assessed before and 4 h after protein bolus in both exercised and non-exercised legs.	W0- no whey protein: $n = 10$ W10–10 g whey protein: $n = 7$ W20–20 g whey protein: $n = 9$ W40–40 g whey protein: $n = 10$ Whey PGP International, IWPI 9500, California W20 AA profile: 1.4 g isoleucine; 2.0 g leucine; 1.8 g lysine; 0.4 g methionine; 1.4 g threonine; 0.6 g phenylalanine; 0.4 g tryptophan; 1.2 g valine; 0.4 g histidine 1.0 g alanine; 0.4 g arginine; 2.2 g asparagine/aspartate; 0.4 g cysteine; 3.4 g glutamic acid; 0.4 g glycine; 0.8 g serine; 0.6 g tyrosine. Total AA-20 g; Total EAA 9.2 g; Total BCAA 5.2 g	MPS increased after W20 and W40 with only W40 inducing greater MPS than W10. Higher MPS in exercised vs non-exercised leg. Conclusion: 20 g whey appears to maximally stimulate MPS with synergistic effects of exercise.
Yang et al. (2012b)	30M (71 ± 5 years) divided into 3 groups; healthy and light-moderate activity level Acute bout of unilateral resistance exercise with one leg followed by isotope infusion and muscle biopsy study. Levels of soy protein ingested after bout of exercise and before isotope infusion. Results compared with responses to whey (Yang et al. 2012a).	S0–no soy protein: $n = 10$ (taken from above) S20–20 g soy protein: $n = 10$ S40–40 g soy protein: $n = 10$ Soy Solae Co, SUPRO 660-IP, St. Louis, MO S20 AA profile: 1.0 g isoleucine; 1.6 g leucine; 1.3 g lysine; 0.3 g methionine; 0.7 g threonine; 1.0 g phenylalanine; 0.2 g tryptophan; 1.0 g valine; 0.5 g histidine 0.9 g alanine; 1.5 g arginine; 2.3 g asparagine/aspartate; 0.2 g cysteine; 3.8 g glutamic acid; 0.8 g glycine; 1.1 g serine; 0.8 g tyrosine. Total AA-20 g; Total EAA 7.1 g; Total BCAA 3.9 g	Both doses of soy did not induce MPS in rested leg whereas whey had in study above. In exercised leg, MPS did not differ with S20 but increased with S40 compared with S0 group. Conclusion: Soy appears to be less effective than whey in the stimulation of MPS 4 h after ingestion in older men.

arginine, the sulfur amino acids (SAA, methionine and cysteine), EAA mixtures, and other nitrogen-containing compounds such as taurine, citrulline, and creatine.

Branched chain amino acids (leucine, isoleucine, valine)

BCAA are perhaps the most studied group of essential AA with respect to human health. The unique role of leucine in metabolism is well known (e.g. Millward 2012; Valerio et al. 2011; Nicastro et al. 2012), but there are few studies examining the individual effects of valine and isoleucine per se. A mixture of the BCAA is often administered because of concern for the effects of an imbalanced intake (Shinnick and Harper 1977). To try to isolate the responses to leucine per se, investigators often manipulate the amount of leucine relative to valine and isoleucine, relative to total protein intake or relative to a mixture of EAA.

Despite differences in study design, outcome measures, and relatively small sample sizes, the body of available data suggests that both acute and chronic supplementation with leucine do not significantly and consistently increase muscle mass or strength despite slight increases in indices of MPS assessed several hours after ingestion of the supplement (Leenders and van Loon 2011). However, because of the regulatory role that leucine plays in MPS and as a nitrogen donor for alanine and glutamine production as well as an insulin secretagogue, leucine remains heavily investigated as a potential pharmaconutrient (Leenders and van Loon 2011; Jonker et al. 2012; Valerio et al. 2011). Koopman et al. (2006) reported that co-ingestion of whey protein and 13 g leucine after 30 min of standardized physical activity resulted in similar increases in MPS and body protein balance in 8 young (~20 years) and 8 older (~75 years) men. This was followed by a study in 8 men (~73 years) receiving whey protein with or without 12.8 g of additional leucine that showed no apparent difference in MPS (Koopman et al. 2008). A more recent study from the same laboratory administered 20 g intrinsically L-[1-^{13}C]phenylalanine-labeled casein protein to 24 healthy older men (age 74.2 ± 1 years), half of whom also co-ingested 2.5 g crystalline leucine and reported that those who received the additional leucine exhibited a greater increase in MPS during the 6-h postprandial period (Wall et al. 2013).

Another approach used to determine the acute effects of leucine supplementation involves the administration of a mixture of other EAA in addition to leucine (usually histidine, isoleucine, lysine, methionine, phenylalanine, threonine and valine) ranging in amounts totaling 6.7–15 g EAA and corresponding to leucine intakes of 1.7–2.8 g/day. A series of studies led investigators to conclude that the dose of EAA, especially leucine, was important in the stimulation of MPS in the elderly with a threshold dose of ~3 g leucine or the equivalent of 20–30 g of high-quality protein (Paddon-Jones and Rasmussen 2009; Volpi et al. 2012; Wolfe 2012). However, it is still unclear as to what extent acute stimulation of MPS by AA mixtures translates into the maintenance of or increase in muscle mass. Many of these published studies highlight key factors that influence outcomes of in vivo studies in humans designed to determine the acute effects of manipulating AA content of test meals: (1) dose of the AA; (2) type and content of the accompanying protein, AA mixture and/or carbohydrate in the test meal; (3) cross-over vs two-group design that may differ in statistical power; (4) physical activity state (rested vs. post-exercise); and (5) time after ingestion and/or exercise bout. A few selected studies are detailed in Table 3 and recently reviewed by Breen and Phillips (2011).

In longer-term studies focused on older individuals, it appears that additional leucine intake ranging from ~2.8 to 7.9 g/day may influence parameters of protein status (e.g. lean body mass or MPS) but the presence of common age-related co-morbid conditions, such as sarcopenia or glucose intolerance/diabetes mellitus, affects the outcomes of interest. A 3-month intervention study in 30 men (~71 years) receiving 7.5 g/d of supplemental leucine revealed no differences in muscle mass nor glucose and lipid concentrations (Verhoeven et al. 2009). Similar conclusions were reached in a study of 60 men with type 2 diabetes mellitus (~71 years) who received 7.5 g/day leucine for 6 months. The investigators found no significant effects on muscle mass or glycemic control but suggested that differences may have not been detectable because the individuals consumed adequate amounts of protein daily (Leenders et al. 2011). In a shorter 2-week study, eight healthy sedentary men and women were supplemented with 12 g/d leucine together with 7 g EAA and exhibited increased MPS and anabolic signaling (Casperson et al. 2012). Overall, results from the longer-term studies suggest that, as in studies employing acute ingestion of AA supplements, additional leucine is associated with variable and often marginal changes in muscle protein metabolism, but this has not changed the belief that there are anabolic effects of leucine supplementation when administered together with high-quality protein that could be harnessed in preventive or therapeutic modalities. It seems timely to begin to investigate the effects of leucine supplementation on systems beyond skeletal muscle.

In contrast to leucine, isoleucine and valine have not been extensively studied individually. Because leucine-induced amino acid antagonism has been reported in animal studies (May et al. 1991; Shinnick and Harper 1977) and high intakes of leucine induce lower plasma levels of valine and isoleucine in humans (Matthews 2005), most

Table 3 Selected studies of skeletal muscle responses to amino acids in healthy volunteers

Reference	Participants/Design	Amino acid mixture	Outcomes
Volpi et al. (1999)	3 F/4M young (30 ± 2 years); 2 F/6M elderly (71 ± 2 years Stable isotope study before and during 3 h of oral AA supplementation	40 g AA with composition based on meat protein given as 30 ml bolus every 10 min	Higher splanchnic AA uptake in elderly; similar AA delivery to leg, increase in MPS, and net AA balance in young and old. Conclusion: Muscle protein anabolism can be stimulated by oral AA in young and old to a similar extent.
Volpi et al. (2003)	EAA group: 1 F/5M (69 ± 2 years) Balanced AA group: 2 F/6M (71 ± 2 years) Stable isotope study before and during 3 h of oral AA supplementation (30 ml every 10 min)	EAA-18 g (2 g histidine; 1.9 g isoleucine; 3.2 g leucine; 3.9 g lysine; 1.0 g methionine; 1.7 g phenylalanine; 1.9 g threonine; 0.5 g tryptophan; 2.0 g valine) ~74 kcal Balanced AA-40 g (18 g EAA; 2.5 g alanine; 2.8 g arginine; 3.7 g aspartate; 0.5 g cysteine;5.8 g glutamine; 1.9 g glycine; 1.9 g proline; 1.7 g serine; 1.5 g tyrosine) ~166 kcal	MPS increased significantly in both groups; no change in muscle protein breakdown; no difference between EAA and Balanced AA groups. Conclusion: EAA are primarily responsible for stimulation of muscle protein anabolism in the elderly.
Paddon-Jones et al. (2004)	4 F/3M (67 ± 2 years sd); 4 F/2M (34 ± 4 years sd) Stable sotope study before and 4 h after bolus EAA ingestion	15 g EAA (1.64 g histidine; 1.56 g isoleucine; 2.79 g leucine; 2.33 g lysine; 0.46 g methionine; 2.33 g phenylalanine; 2.20 g threonine; 1.73 g valine)	15 g EAA acutely stimulated MPS similarly in young and old but elders did not have increase in plasma insulin after EAA and experienced slower rise in AA concentrations and net phenylalanine balance across the leg. Conclusion: EAA ingestion can acutely stimulate MPS in young and old to a similar extent.
Katsanos et al. (2006)	26 % Leucine: 3 F/7M (67 ± 2 years) 41 % Leucine: 5 F/5M (66 ± 2 years) 26 % Leucine: 4 F/4M (31 ± 2 years) 41 % Leucine: 4 F/4M (29 ± 3 years) Stable isotope study before and 3.5 h after oral 6.7 g AA supplement without tryptophan. 26 % leucine in AA mixture based on EAA content of 15 g whey protein	26 % Leucine mixture (0.304 g histidine; 0.781 g isoleucine; 1.721 g leucine; 1.36 g lysine; 0.362 g methionine; 0.506 g phenylalanine; 0.955 g threonine; 0.738 g valine); 41 % Leucine mixture (0.239 histidine; 0.614 isoleucine; 2.79 leucine; 1.069 lysine; 0.284 methionine; 0.398 phenylalanine; 0.751 threonine; 0.580 valine)	Older group receiving 41 % leu mixture increased MPS whereas the 26 % groups had a marginal increase. Young groups increased MPS after both mixtures. Conclusion: Increasing the proportion of leucine in an EAA mixture appears to reverse attenuated MPS response in the elderly with no further stimulation in the young.
Dillon et al. (2009)	Placebo: 7F (69 ± 3 years) EAA: 7F (67 ± 1 years) 3 months of EAA or placebo supplementation with stable isotope study before and at the end of the 3 months. Samples at baseline and 6 h after ingestion of 7.5 g EAA	7.5 g EAA supplement twice/d for 3 months (0.82 g histidine; 0.78 g isoleucine; 1.39 g leucine; 1.17 g lysine; 0.23 g methionine; 1.17 g phenylalanine; 1.10 g threonine; 0.86 g valine). No tryptophan. Total 15 g/day 7.5 g EAA bolus during isotope and balance studies	Bolus of EAA acutely stimulated MPS in both groups at 0 months. At 3 mos, MPS and lean body mass increased only in EAA supplemented group. Strength did not change in both groups. Basal expression of IGF-1 increased in muscle of EAA group. Conclusion: EAA improved LBM and MPS in older women after 3 months of supplementation.

investigators administer BCAA or EAA mixtures. However, there was a recent case report of the use of 3 g valine in a patient with hepatitis C (HCV)-related cirrhosis being associated with improvement of fatigue and reduction in viral load and indices of hepatic fibrosis (Kawaguchi et al. 2012).

Higher intakes of BCAA mixtures have been reported to exert beneficial effects on body weight and body fat in middle-aged individuals from Western and East Asian countries (Qin et al. 2011). This population-based study used data collected in the International Study of Macro-/Micronutrients and Blood Pressure (INTERMAP) Study to determine the association between BCAA intake assessed by dietary recall and overweight/obesity status of the multiethnic group. The investigators found an inverse relationship between BCAA intake and prevalence of obesity/overweight status. Undoubtedly, the interplay between BCAA and metabolic health is complex, and the issue has recently been the topic of several reviews (Newgard 2012; Adams 2011; Zanchi et al. 2012). Others have reported on the use of BCAA in chronic liver disease with improvement in insulin resistance and as an adjunct in the treatment of HCV (Kawaguchi et al. 2011; Nagao et al. 2012; Holecek 2010). The therapeutic use of BCAA and its keto acids in hepatic and renal diseases and other clinical conditions has also been extensively reviewed (Kawaguchi et al. 2011; Cano et al. 2006; Jonker et al. 2012; Adams 2011). Other aspects of BCAA supplementation that are beyond the scope of this review relate to the effects on the brain and on physiologic responses to acute exercise as recently reviewed by Fernstrom (2012). The potential effects of BCAA ingestion on mood, perceived exertion, and mental fatigue have important implications for the overall management of older individuals, especially because of current recommendations that good nutrition and physical activity go hand in hand. Unfortunately, there are few studies examining the changes in mood or behavior in elders consuming AA supplements. However, delayed muscle soreness and muscle fatigue induced by squat exercise were reduced with BCAA supplementation (Shimomura et al. 2006). Jackman et al. (2010) reported that a BCAA mixture consumed before, during, and up to 3 days after acute eccentric exercise was associated with reduced muscle soreness but did not attenuate exercise-induced decrements in muscle function or indices of muscle injury associated with intense eccentric exercise. Although effects of BCAA supplementation on physical performance are variable (Fernstrom 2012; Jackman et al. 2010), ratings of perceived exertion and mental fatigue may be affected by the supplements (Blomstrand 2006), suggesting that BCAA facilitate adherence to exercise regimens. Hence, the overall conclusion supported by a variety of studies is that BCAA appear to exert generally small but positive effects similar in magnitude to EAA mixtures in individuals affected by conditions commonly associated with aging (DM, renal disease, hepatic disease, overweight/obesity) and that BCAA may exert positive influences on mood and possibly behavior. However, causality and mechanisms have yet to be established.

Sulfur amino acids (methionine, cysteine)

Methionine is an essential amino acid necessary for normal growth and development, playing key roles in protein synthesis, methylation reactions, polyamine synthesis, and as a precursor for the dispensable AA, cysteine. The dietary requirement for methionine, based on early nitrogen balance studies, is usually reported as part of the requirement for total sulfur amino acids (SAA) (Table 1) and ranges between 10 and 19 mg/kg/d (Fukagawa and Galbraith 2004; Fukagawa 2006; World Health Organization et al. 2007; Institute of Medicine 2002/2005). Although described as one of the most toxic AA in relation to animal growth, there is little evidence in humans for serious toxicity except at very high levels of intake (Garlick 2006). Despite the importance of methionine in human metabolism, dietary restriction of methionine has been put forth as a strategy to control growth of certain cancers, to extend the lifespan and to influence obesity and insulin resistance (Cavuoto and Fenech 2012; Ables et al. 2012). In a clinical evaluation of the effects of 16 weeks of dietary methionine restriction (2 mg methionine/kg body weight) in humans with the metabolic syndrome, Plaisance et al. (2011) reported increased fat oxidation and a reduction in intrahepatic lipid content, but no differences in insulin sensitivity, weight loss or adiposity. The authors acknowledged the challenges associated with designing diets with restricted methionine content and that there was poor compliance and a high withdrawal rate. Although animal models support the positive effects of methionine restriction, this would be very difficult to achieve in humans even if many of age-associated diseases may be mitigated by methionine restriction (e.g. certain cancers, insulin resistance, hypertension, obesity) because of palatability and because few naturally occurring proteins are sufficiently low in methionine.

Cysteine, in its role as the rate-limiting AA for glutathione (GSH) synthesis, may be conditionally essential when specific tissues or individuals experience oxidative stress. Recently, Sekhar et al. (2011) reported that older individuals (70.3 $\pm$ 2.4 years) had lower red blood cell concentrations of GSH and two precursor AA, glycine and cysteine, higher indices of oxidative stress, and lower GSH synthesis rates compared with younger control subjects (39.8 $\pm$ 1.0 years). Of interest is that these differences

were mitigated by 2 weeks of precursor supplementation (cysteine as *N*-acetylcysteine at 0.81 mmol/kg/d and glycine at 1.33 mmol/kg/day). The importance of dietary cysteine has been highlighted by its designation as a "nutraceutical" (McPherson and Hardy 2011, 2012). However, as noted by the authors, there are concerns related to the stability, toxicity, and absorption of both GSH and cysteine when administered orally. Cysteine is easily oxidized to its insoluble dimeric form, cystine, and both are toxic at high concentrations (Baker 2006). Cysteine precursors such as *N*-acetylcysteine (NAC) and L-oxothiazolidine-4-carboxylic acid (OTZ) (Fukagawa et al. 2000) have been studied, but the efficacy of both relative to possible side effects associated with each limit their widespread use (McPherson and Hardy 2011). The use of cysteine-rich proteins as a means of boosting cysteine availability has been described in animal models (Blouet et al. 2007; Mariotti et al. 2004), but studies in humans are limited (McPherson and Hardy 2011). Because oxidative stress is implicated in the aging process as well in the pathogenesis of many age-related disorders, focusing on ways to enhance antioxidant capacity by increasing GSH stores seems worthwhile. GSH is the body's major endogenous antioxidant and important in the maintenance of redox status, including the balance of reduced and oxidized forms of dietary antioxidants such as vitamins E and C, and the metabolism of xenobiotics. A major challenge still to be overcome is identification of appropriate biomarkers that will permit evaluation of targeted nutritional intervention that is both palatable and easy to administer.

Taurine (2-aminoethanesulfonic acid), one of the few amino acids not incorporated into proteins, is formed from cysteine and involved in the conjugation of bile acids, osmoregulation, retinal and neurological development, regulation of cellular calcium levels, and immune function (Shao and Hathcock 2008; Wojcik et al. 2010; Ripps and Shen 2012; Marcinkiewicz and Kontny 2012). It is abundant in the brain, retina, muscle, and other organs. Despite the fact that the health effects of taurine supplementation are largely unknown (Ripps and Shen 2012; Wojcik et al. 2010), several therapeutic benefits have been proposed for disorders ranging from diabetes mellitus to cardiovascular diseases, retinal degeneration, and skeletal muscle dysfunction (Ripps and Shen 2012; Wojcik et al. 2010; Shao and Hathcock 2008; Imae et al. 2012; Menzie et al. 2012; Abebe and Mozaffari 2011; Marcinkiewicz and Kontny 2012). Because there has been little evidence for adverse effects resulting from oral administration of taurine (Shao and Hathcock 2008; Brosnan and Brosnan 2006; Baker 2006), it is a common additive in energy drinks that have increased in popularity amongst adolescents, young adults, and athletes. Humans obtain most of their taurine from foods (Wojcik et al. 2010), but there has been recent interest in the regulation of taurine production (Stipanuk and Ueki 2011) and whether taurine supplementation may be indicated under certain conditions (van Stijn et al. 2012; Imae et al. 2012; Ito et al. 2012). Existing human studies suggest that taurine may be beneficial in reducing CVD risk and lowering blood pressure (Wojcik et al. 2010; Satoh and Kang 2009). In middle- to older-aged men and women participating in a double-blind, crossover study, 28 days of a multi-nutrient supplement containing 500 mg taurine, 2 g leucine, 500 mg isoleucine, and 500 mg valine together with B vitamins and plant extracts resulted in a reduction in inflammatory markers and improved markers of pain, strength, and power in men and improved balance and reduced anxiety in women (Dunn-Lewis et al. 2011). Finally, in a recent review, Kim et al. (2007) described animal and human studies suggesting a beneficial role of taurine in protection against diabetes and some of its complications. Intakes of 3–6 g/d have been used in humans studies (Abebe and Mozaffari 2011; Wojcik et al. 2010), but clearly further studies of efficacy and safety in humans are warranted, especially as one component of supplements containing other bioactive compounds.

Creatine is a guanidino compound synthesized endogenously from methionine, arginine and glycine in the liver, pancreas, and kidney (Brosnan and Brosnan 2007) and obtained in the diet from meats. It is non-enzymatically converted to creatinine, which is then excreted in the urine. Supplementation of creatine has been used to increase intracellular phosphocreatine, which is key to maintaining ATP homeostasis. Since advancing age is associated with a reduction in muscle mass and consequently muscle weakness and functional limitations, there has been great interest in identifying ergogenic compounds that can be used in the treatment of sarcopenia. Recent reviews detail creatine metabolism and summarize the ergogenic and therapeutic effects of creatine supplementation (Gualano et al. 2012; Brosnan et al. 2011; Candow 2011). Creatine supplementation has minimal side effects (Jager et al. 2011) and is inexpensive, but whether it is the answer to retarding sarcopenia is still unclear (Candow 2011). One animal study of interest reported that creatine supplementation increased skeletal muscle content of carnosine in male senescence-accelerated mice that was associated with improved resistance to contractile fatigue (Derave et al. 2008). Consideration of novel combinations of compounds that may influence muscle function in older individuals would be another fruitful area of research.

Glutamine/glutamate

Glutamine is one of the most abundant AA in the body with most being produced in skeletal muscle and metabolized by the intestine, kidney, and liver (Holecek 2012). In both human and animal studies, glutamine has been reported to

have beneficial effects on nitrogen balance, immunity, and gut integrity (Bollhalder et al. 2013; Holecek 2012), and glutamine supplementation has been used in critically ill patients, those with muscle-wasting disorders, infected with HIV, undergoing cancer chemotherapy or suffering from inflammatory bowel disease. Intravenous glutamine supplementation is standard care when parenteral nutrition is administered in the intensive care unit. A recent systematic review and meta-analysis of randomized clinical trials confirmed that in severely ill patients, glutamine supplementation reduced infections and length of stay but the meta-analysis did not demonstrate a significant reduction in mortality (Bollhalder et al. 2013). The benefits of enterally administered glutamine remain controversial. Healthy individuals also consume large quantities of glutamine believing that it would aide in muscle building, enhance athletic performance, boost immunity or improve memory. In his recent review, Holecek concluded that short-term intake of high amounts of glutamine may be safe but that there are still unanswered questions about the chronic consumption of a glutamine-enriched diet (Holecek 2012). Nevertheless, investigators have recently reported that in vitro and in mouse models of Alzheimer's disease, glutamine supplementation may protect neurons against DNA damage, beta-amyloid protein, and oxidative stress (Chen and Herrup 2012). In a randomized, crossover, double-blind human study designed to assess the safety of oral administration of L-glutamine (0.5 g/kg/d) in middle-aged and elderly residents of a long-term-care facility, increases in serum urea nitrogen and creatinine and a decrease in estimated glomerular filtration rate were reported with the conclusion that although the differences were not clinically significant, careful monitoring of renal function during supplementation was warranted (Galera et al. 2010).

Glutamate, another abundant AA in the body, elicits a unique taste known as umami when present in food and believed to serve as a signal for protein ingestion. Recent reviews highlight the importance of glutamate in gut and brain function (Kitamura et al. 2012) and the numerous physiological pathways affected by free glutamate (Brosnan and Brosnan 2012). Zahr et al. (2013) recently reported that older age was associated with lower glutamate levels in the striatum of the brain assessed with magnetic resonance spectroscopy, which was also associated with higher systolic blood pressure and poorer performance on complex visuomotor tasks, suggesting that changes in brain glutamate may mediate some of the behavioral changes found in "normal" aging. Others reported reduced glutamate levels in the hippocampus of patients with Alzheimer's disease (Rupsingh et al. 2011). On the flip side, glutamate acts not only as a neurotransmitter but may also induce neuronal cell death during periods of cerebral ischemia, another common age-associated phenomenon (Kostandy 2012). Another potentially important role for glutamate as a signaling molecule relates to bone health as discussed in the recent review by Cowan et al. (2012).

In summary, although there is still debate about how and when to use glutamine and/or glutamate supplementation in older individuals, there is general agreement that intravenous glutamine is indicated for critically ill patients receiving parenteral nutrition (Bollhalder et al. 2013) and that umami plays a key role in mediating taste and appetite responses to protein-rich foods.

Arginine/citrulliine

A number of reviews on arginine and citrulline have been recently published (e.g. (Luiking et al. 2012; Bahri et al. 2012; Cynober et al. 2010), so details will not be presented here. Arginine is considered to be a conditionally essential AA and has been a "hot" topic since it was identified as the precursor for nitric oxide (NO), which is ubiquitous and serves in a range of critical roles in the regulation of the function of many different organs in the body. Sources of arginine include the diet, endogenous proteolysis and de novo synthesis, the latter of which is dependent on the availability of citrulline (Luiking et al. 2012). The typical Western diet supplies a $\sim$3–6 g of L-arginine/d. Arginine is not only a component of protein but also participates in the regulation of body protein via the mTOR signaling pathway (Boger 2007; Luiking et al. 2012). It is also an important urea cycle intermediate and contributes to the biosynthesis of polyamines (spermidine, putrescine, spermine), proline, and creatine. By virtue of its role as the precursor for NO, much of the research has been directed towards its relationship to vascular function and responses to acute stressors such as trauma, sepsis, hepatic failure, and other illnesses requiring intensive care treatments. The effects of L-arginine and the underlying mechanisms for the effects differ according to the concentration range achieved in plasma with oral supplementation at doses of 3–8 g/day associated with few untoward effects but with higher intakes reportedly associated with gastrointestinal symptoms (Boger 2007; Luiking et al. 2012). Acute hemodynamic effects of intravenous or intraarterial arginine at higher doses are reported to be related to the endocrine secretagogue effects (insulin and human growth hormone) and unspecific vasodilation through osmotic/pH effects (Boger 2007). Unfortunately, the beneficial effects of arginine supplementation have not been uniform and there remains controversy about whether long-term supplementation would be helpful. Reasons for the uncertainty are likely related to the complex interactions between the synthesis and catabolism of arginine and NO production,

and the challenges placed on investigators by the compartmentalization of arginine metabolism in vivo, as well-described by Luiking et al. (2012).

Because aging per se is associated with impairment of the cardiovascular system, there has been significant interest in the possible use of L-arginine prophylactically against age-related endothelial dysfunction (Khan et al. 2012; Heffernan et al. 2010; Schutte et al. 2010). In 30 older (~71 years) versus 36 younger (~26 years) individuals, Yavuz et al. (2008) found significantly lower flow-mediated dilatation (FMD) of the brachial artery via ultrasound in the elderly and concluded that endothelial dysfunction assessed by FMD was impaired even in the absence of disease. Endothelial dysfunction has been implicated in age-related changes in cognitive function, reduced performance of activities of daily living, and in the pathogenesis of age-associated diseases such as hypertension, stroke, erectile dysfunction and renal impairment (Khan et al. 2012; Heffernan et al. 2010). In addition, since L-arginine plays a role in immune function and inflammation (Heffernan et al. 2010; Luiking et al. 2012), there has been interest in the addition of arginine to immunomodulating diets administered peri- or postoperatively to decrease the risk of postoperative complications, especially in elders at high risk. With respect to arginine supplementation per se, the conclusion drawn by Heffernan et al. (2010) is that L-arginine supplementation as a "general vascular panacea for all aging persons is not warranted at this time."

An important consideration in evaluating the pros and cons of therapies aimed at modulating the L-arginine-NO axis is that the relationship between arginine availability and NO synthesis is not one of simple precursor availability and that compartmentalization plays a role in the "arginine paradox" reviewed in detail by Luiking et al. (2012). Arginine catabolism by arginase or competition with dimethylarginines leads to uncoupling of NO synthase (NOS), and NO synthesis may be further influenced by cofactor availability (e.g. tetrahydrobiopterin). The situation is further complicated by the interaction with the availability of S-adenosylmethionine (SAM), which acts as the donor for the synthesis of the dimethylarginines, thus linking arginine with SAA metabolism.

Citrulline, a non-protein AA synthesized from arginine in the urea cycle, has emerged as a promising pharmaconutrient in strategies to increase arginine availability to endothelial and immune cells and modulate protein synthesis (Bahri et al. 2012; Cynober et al. 2010), but unfortunately, most of the data have been generated in animal models. In a recent randomized, crossover study in healthy men ($n = 5$) and women ($n = 7$) receiving 7 days of 0.18 g/kg/d citrulline or an iso-nitrogenous placebo, Thibault et al. (2011) found increased plasma citrulline, arginine, and ornithine concentrations but no differences in albumin, transthyretin, insulin, and IGF-1 levels, urinary nitrate excretion, or nitrogen balance after citrulline supplementation compared with placebo. Moreover, citrulline supplementation did not alter whole body protein kinetics assessed in the postaborptive state with L-[1-^{13}C]leucine infusions. Other studies in humans focused on the effects of citrulline supplementation on cardiovascular parameters. Four weeks of oral citrulline (6 g/day) given to healthy young men attenuated the blood pressure response to the cold pressor test used to assess sympathetic-mediated vasoconstriction leading the authors to conclude that citrulline supplementation may be used to modulate aortic blood pressure (Figueroa et al. 2010). In a 7-day study of middle-aged men, citrulline supplementation (5.6 g/d) improved an index of arterial stiffness independent of blood pressure (Ochiai et al. 2012). More recently, patients with congestive heart failure (HF) were assigned to placebo or 3 g citrulline per day for 4 months in addition to their conventional HF treatments (Balderas-Munoz et al. 2012). There was improvement in HF functional class and left ventricular ejection fraction in those receiving citrulline, suggesting that it may be an important adjuvant in the treatment of patients with compensated systolic HF. Hence, it does appear that there may potential benefits of citrulline supplementation in selected individuals as recently discussed (Cynober et al. 2010; Bahri et al. 2012; Jonker et al. 2012; Luiking et al. 2012).

Essential AA mixtures

Despite interest in elucidating the mechanisms of action of supplementation with single AA, the human body requires the availability of the full complement of AA through dietary protein or endogenous proteolysis. Since loss of body protein is one of the hallmarks of aging, significant effort has gone into determining the optimal blend of AA that could prevent, attenuate or restore the loss. A series of studies in the 1990s and early 2000s recently reviewed (Wolfe 2012; Jonker et al. 2012) demonstrated that increased availability of AA stimulated muscle protein synthesis. Moreover, the availability of EAA was key to the stimulation of protein synthesis (Wolfe 2012; Volpi et al. 2003). As noted for the BCAA, the response is affected by the dose, the profile of the mixture, and the status of the individual (health as well as activity level). Although leucine alone may have similar effects, a mixture of EAA appears to most consistently enhance MPS and improve function (Table 3). Dillon et al. (2009) were able to improve lean body mass in older women after 3 months of supplementation but the group sizes were small.

Some of the effects of AA supplementation in individuals with common age-related disorders are presented in

Table 4 Effects of amino acid(s) or protein supplementation in elders with type 2 diabetes mellitus, sarcopenia or after bedrest

Reference	Design/participants	Supplement	Outcome/conclusion
Borsheim et al. (2008)	16 weeks of supplementation. Total AA intake: 22 g/d 7 F/5 M (67 ± 6 years, sd); Glucose intolerant based on oral 75 g glucose tolerance test	11 g EAA + arginine mixture (0.36 g histidine; 0.94 g isoleucine; 3.95 g leucine; 1.88 g lysine; 0.39 g methionine; 0.51 g phenylalanine; 1.05 g threonine; 0.82 g valine; 1.1 g arginine). No tryptophan.	At end of 16 weeks, increased lean body mass and increased leg lean mass by DEXA. Improved physical function (walking speed; 5-step test; floor-transfer test; leg strength). Conclusion: EAA mixture with arginine improved lean body mass and indices of physical function but did not affect glucose tolerance in glucose intolerant elderly men.
Solerte et al. (2008a)	16 weeks of AA or placebo; 2 week washout; crossover for 16 week; 26 week "maintenance" AA 34 type 2 diabetics (age range 65–83 years); HbA1c range 7.2–10.5 %; randomized, open-label crossover comparing AA mixture vs placebo as 2 snacks/d	Commercial mixture (Big One; Professional Dietetics, Milan, Italy), 70.6 kcal/d with 8 g/d AA (0.3 g histidine; 1.25 g isoleucine; 2.5 g leucine; 1.3 g lysine; 0.1 g methionine; 0.2 g phenylalanine; 0.7 g threonine; 1.25 g valine; 0.3 g cysteine; 0.06 g tyrosine; 0.04 g tryptophan)	AA supplementation was associated with a reduction in fasting glucose and insulin levels and HOMA-IR (homeostatic model assessment of insulin resistance). Conclusion: AA supplementation improved metabolic control and insulin sensitivity in older diabetics.
Solerte et al. (2008b)	Randomized, open-label crossover comparing AA mixture vs placebo. Length of treatments: 4 months AA or placebo; 2 week washout; crossover for 4 months. 8 months "maintenance" AA 41 sarcopenic outpatients (age 66–84 years); normal blood glucose but HOMA-IR consistent with moderate insulin resistance and hyperinsulinemia	As described above-70.6 kcal/d with 8 g/d AA provided as 2 snacks/d	AA supplementation associated with increases in whole-body lean mass after 6 and 18 months. Fasting glucose and insulin levels and HOMA-IR were reduced during AA treatments. Slight reduction in serum tumor necrosis factor-alpha (TNF-α) and significant increase in serum insulin-like growth factor-1 (IGF-1). Conclusion: AA's more effective than placebo in improving glucose homeostasis and lean body mass with reduction in index of inflammation in sarcopenic elders.
Ferrando et al. (2010)	Assessment of MPS before and after 10 days bedrest. Supplemented group received 45 g EAA/d Control: 6 F/6M (68 ± 5 years, sd) EAA: 9 F/1M (71 ± 6 years, sd); moderately active and healthy	15 g EAA + arginine supplement 3 times/d between meals (0.488 g histidine; 1.286 g isoleucine; 5.382 g leucine; 2.561 g lysine; 0.538 g methionine; 0.698 g phenylalanine; 1.435 g threonine; 1.116 g valine; 1.495 g arginine). No tryptophan	MPS was decreased in control and maintained in EAA groups. No effect of EAA on maintenance of total or leg lean mass. Muscle function outcomes (floor transfer impaired in control; trend towards maintenance in stair ascent power and standing plantar flexion with EAA). Conclusion: AA supplementation attenuated effects of bedrest on MPS and indices of muscle function.
Rondanelli et al. (2011)	41 nursing home residents (age 75–95 years) randomized to EAA supplemented (11 F/9M) or control groups (13 F/8M); length of intervention 8 wks	4 g/d EAA twice/d (0.15 g histidine; 0.625 g isoleucine; 1.25 g leucine; 0.65 g lysine; 0.05 g methionine; 0.1 g phenylalanine; 0.35 g threonine; 0.625 g valine; 0.03 g tyrosine; 0.02 g tryptophan; 0.15 g cysteine); 35.3 kcal	EAA group had improved nutritional panel (Mini Nutritional Assessment scores, serum albumin levels), less depressive symptoms (Geriatric Depression Scale), greater hand grip strength, improved activities of daily living and quality of life scores. Conclusion: EAA improved quality of life in institutionalized elders.

Table 4 continued

Reference	Design/participants	Supplement	Outcome/conclusion
Kim et al. (2012)	155 sarcopenic, community-dwelling women (age ≥ 75 years) randomized to 1) exercise plus AA supplement ($n = 38$), 2) exercise alone ($n = 39$), 3) AA alone ($n = 39$) or 4) health education alone ($n = 39$) Exercise = 60 min session twice a week (strengthening, balance and gait training) Health education = monthly class focused on cognitive function, osteoporosis and oral hygiene. No specific instructions on diet or physical activity	3 g AA supplement twice a day (0.32 g isoleucine; 1.26 g leucine; 0.42 g lysine; 0.21 g phenylalanine; 0.32 g threonine; 0.32 g valine; 0.165 g "other") for 3 months	Overall compliance ~ 70 % for all groups. Walking speed increased in exercise alone and exercise + AA groups; Exercise + AA increased knee extension strength and leg muscle mass. Conclusion: Exercise is effective in improvement of muscle mass and functional fitness but not sufficient to increase strength. Combination of exercise and AA can improve muscle strength and mass and indices of function.

Table 4. As pointed out by the authors, one advantage of an EAA drink is that it does not affect satiety and does not alter the metabolic effects of subsequent meals. Insulin resistance and type 2 diabetes mellitus are common in older individuals. The encouraging results in 12 older glucose-intolerant men and women (67 ± 6 years), who received 22 g EAA plus arginine (Table 4) for 16 weeks, included increased lean body mass, muscle strength, and physical function without significant changes in either dietary intake or physical activity (Borsheim et al. 2008). In a longer, crossover study, 34 diabetics received 8 g/d of EAA or placebo for an 8-week period followed by 26 weeks of maintenance EAA intake for a total of 60 weeks (Solerte et al. 2008a). Results showed improved glucose control, reduced hemoglobin A1c levels, and increased insulin sensitivity. The same investigators also studied 41 sarcopenic individuals (age range 66–84 years) with the same supplement for 4 months and demonstrated increased lean mass, improved insulin sensitivity, reduction in TNF-alpha, and increased IGF-1 levels (Solerte et al. 2008b). More recently, Rondanelli et al. (2011) reported that twice daily supplementation with 4 g EAA improved the quality of life of 41 nursing home residents between the ages of 75 and 95 years (Table 4). Furthermore, in sarcopenic, community-dwelling women of age over 75 years, Kim et al. (2012) found that the combination of exercise and 3 g AA twice a day improved muscle strength and mass and indices of function (Table 4). These data are supportive of a potential role for EAA mixtures, especially in conjunction with physical activity, in preventing common age-related disorders or treating certain disease states. In addition to effects on protein status, EAA, including BCAA, also reduce inflammation (Jonker et al. 2012; Nicastro et al. 2012) and has been shown to be effective in preventing the decrease in MPS and in maintaining muscle function in elderly people placed on 10 days of bed rest despite not having an effect on lean mass (Ferrando et al. 2010).

Dietary protein and AA are also important in the maintenance of bone health (Tome 2012; Levis and Lagari 2012) but there is no clear level of intake deemed to be optimal. Bone mineral density is positively associated with protein intake but the relationship to fracture risk remains controversial (Tome 2012).

Safety of AA supplementation

Establishing the upper limits of safety for AA intakes has long been of scientific interest but with the growing use of food supplements globally, consideration of safety becomes paramount. In the 2005 IOM report on Dietary Reference Intakes, tolerable upper intake levels (UL) could not be established for any of the AA's due to insufficient

data on dose–response relationships (Institute of Medicine 2002/2005). Elango et al. (2012) recently published results from a study in healthy young men designed to determine the UL for leucine under acute dietary conditions and concluded that on the basis of plasma and urinary variables, the UL for leucine may be 500 mg/kg/d or ~35 g/d. UL for isoleucine and valine have not been determined, but it is generally believed that single doses as high as 9.5 g of the BCAA acutely are not associated with adverse effects (Institute of Medicine 2002/2005). Galera et al. (2010) examined the effects of 0.5 g/kg/d of L-glutamine supplementation in middle-aged and elderly men and women and concluded that although elevations in serum urea nitrogen and creatinine were not clinically significant, renal function should be monitored in those receiving oral glutamine up to these doses. In his review, Watford concluded that glutamine intakes of up to 0.57–0.75 g/kg/d (~40–55 g/person/day) by any route were not associated with deleterious effects (Watford 2008). Despite the large number of studies of glutamate toxicity in animals and humans, the general view is that there are few adverse effects of L-glutamate consumption (Institute of Medicine 2002/2005; Manzo et al. 2012). Studies on the UL of the SAA are also sparse and as discussed above, issues of stability make it difficult to administer cysteine per se whereas methionine is tolerated relatively well but consideration must be given to palatablity and to its role in overall metabolism. As previously noted, there are few studies focused on the upper limits of safety for elders for any of the AA but there is no indication that the UL's are different between young and old.

Summary and conclusions

Unfortunately, significant variability in the composition of the different solutions and in the selected outcome measures complicate interpretation of the results of published studies, but overall, it appears that mixtures of AA between 6 and 15 g/d are associated with positive effects on indices of function and insulin/glucose homeostasis. Since the matrix of whole foods is likely to influence absorption and bioavailability of nutrients as well as interact in the induction of biological effects, it would be important to determine the synergistic effects of single AA and AA mixtures when ingested as part of meals. More importantly, longer-term, multi-center studies with measureable outcomes and standardized doses and mixtures should be conducted to establish true efficacy and feasibility of the interventions. Data also need to be generated systematically to identify the synergism between physical activity regimens and supplement intake, especially to determine the optimal dose and timing of administration. It is time to expand research to encompass new paradigms for studying the efficacy of specific foods and/or supplements on age-related conditions. An important component of future research is the establishment of biomarkers that are useful and consistent in the assessment of outcomes of interest. Since part of the standard of care for older people is the assessment of bone density, perhaps the use of DEXA can be expanded to assess muscle mass. Other anthropometric measures such as mid-thigh circumference may also be useful.

If one were to summarize the gaps in our knowledge about specific AA supplements in aging humans in one sentence, it would be that new research paradigms are needed to enable an integrated, systems evaluation of efficacy rather than the reductionist evaluation of a single compound at different doses. Investigators will need to agree upon standard doses and mixtures, experimental design, and outcome measures in multi-center collaborative studies. Patients with specific conditions also need to be studied with standardized protocols to maximize the evaluation of the data to determine real efficacy. All of this will require large numbers of volunteers and large resources, including possibly centralized laboratories for sample processing and analyses.

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