Nutritionally Essential Amino Acids

Amino acids (AAs) are defined as organic compounds containing both amino and acid groups (1). Proteinogenic AAs are AAs that serve as substrates for protein synthesis in animal cells, and occur naturally as L-AAs except for glycine. Free AAs (those AAs that are not constituents of peptides or polymers) in physiologic fluid exist primarily in an L-isoform, but some of them may be present in a D-isoform. Based on his findings in 1912 that adult dogs exhibited a negative nitrogen balance when fed a tryptophan-free diet but maintained a positive nitrogen balance when fed a proline-free diet, Abderhalden (2) classified AAs as nutritionally essential (EAAs) or nonessential (NEAAs). Beginning in 1924, WC Rose and coworkers (3) published a series of landmark papers on AA nutrition and metabolism in rats and humans that further defined AAs as EAAs or NEAAs based on nitrogen balance or growth. We published an essay on NEAAs in 2017 (4), and EAAs are the focus of the present article.

To date, EAAs are defined as AAs whose carbon skeletons are not synthesized de novo by animal cells or AAs that are insufficiently synthesized de novo by animal cells relative to metabolic needs (1). EAAs are Cys, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Tyr, and Val for all animals (including cattle, chickens, dogs, fish, goats, humans, mice, pigs, rats, sheep, and shrimp). In birds and some mammals (e.g., cats and ferrets), there is no de novo synthesis of arginine owing to the absence of pyrroline-5-carboxylate synthase in the small-intestinal mucosa; therefore, arginine is an EAA for these species. Although adult humans and rats do not require dietary arginine for maintaining nitrogen balance, they exhibit severe reductions in spermatogenesis and sperm viability (e.g., by 90%) after a 9-d period of consuming an arginine-free diet (1). Thus, functional needs (e.g., optimal growth, fertility, lactation, blood circulation, connective tissue health, and immune response) should also be a criterion for defining dietary requirements for both proteinogenic (e.g., arginine) and nonproteinogenic (e.g., taurine in cats, fish, and human infants) AAs. In birds, the synthesis of proline from arginine is limited because of low arginase activity, and the synthesis of proline from glutamate and glutamine is absent owing to the lack of pyrroline-5-carboxylate synthase. The rate of glycine synthesis is much lower than the rate of glycine utilization in poultry and young pigs; therefore, these animals need dietary glycine for maximum growth. Available evidence shows that many species of fish do not synthesize arginine and may not be able to synthesize sufficient glycine and proline (1, 5). The species differences in AA synthesis mean that the list of EAAs can vary among animals.

The rumen of ruminants harbors many species of bacteria that are capable of synthesizing EAAs from ammonia, α-ketoacids (products of carbohydrate fermentation), and sulfur to support low rates of growth and lactation (1). However, the rates of EAA synthesis by ruminal microbes are not sufficient for high rates of growth, lactation, or reproduction performance in ruminants, including steers and cows (5); therefore, their diets must provide sufficient protein and rumen-protected EAAs, such as lysine, methionine, and histidine.

Deficiencies

Deficiencies of EAAs in humans and other animals occur when their diets do not provide adequate amounts of these AAs (1). When an EAA is deficient in a diet, the oxidation of other AAs is increased progressively with increasing dietary intake of AAs or protein. This is because the short supply of this EAA limits the utilization of other AAs for protein synthesis and, therefore, all excessive AAs are degraded in a tissue-specific manner. Syndromes of EAA deficiencies include low appetite and vomiting; impairments in absorption, transport, and storage of organic and inorganic nutrients; reduced synthesis of neurotransmitters; emotional disorders (e.g., moodiness, severe depression, and anxiety); irritability and insomnia; anemia; and reduced transport of oxygen. Affected subjects also exhibit reduced concentrations of AAs and albumin in plasma; endocrine imbalance (including reduced concentrations of insulin, growth hormone, insulin-like growth factor I, and thyroid hormones in plasma); growth stunting of the young; impaired development (including cognitive development) of the young; reduced whole-body energy expenditure and increased accretion of white adipose tissue; skeletal muscle wasting; and physical fatigue and weakness. In addition, inadequate intakes of EAAs result in libido loss, impaired spermatogenesis, reduced fertility, and embryonic death; intrauterine growth restriction and its lifelong negative consequences on postnatal growth, metabolism, and health (e.g., increased risk for obesity, infection, and cardiovascular abnormalities); and reduced milk production. Furthermore, EAA-deficient individuals are beset with impaired antioxidative reactions, increased oxidative stress, and advanced aging; headache and fainting; impaired immune response, frequent infections, and increased rates of morbidity and mortality from infectious diseases; cardiac failure, cardiovascular abnormalities, and hypertension; and tissue fluid retention, including peripheral and periorbital edema (particularly swelling in the abdomen, leg, hands, and feet). Finally, these subjects exhibit losses of calcium and bones; dental abnormalities; hair breakage and loss; reduced production of pigment; appearance of gray

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hair color; pale, dry, or flaking skin; and skin atrophy. Thus, dietary protein deficiency not only contributes to poor growth, cardiovascular dysfunction, and high risk for infectious disease, but also exacerbates the deficiency of other nutrients (including vitamin A and iron), worsens metabolic profiles (e.g., dyslipidemia and hyperglycemia), and causes death in subjects.

The very reason for the severe problems in humans and other animals with an EAA deficiency is a reduced rate of protein synthesis in cells and tissues, particularly skeletal muscle (1). The organisms need a variety of proteins to: *1)* digest and absorb dietary nutrients via the small intestine, *2)* transport nutrients (including long-chain FAs, vitamin A, and iron) and other molecules (e.g., cholesterol and TGs) in blood, and *3)* oxidize nutrients (including FAs and glucose) to water and carbon dioxide. Thus, deficiencies of EAAs and micronutrients (including vitamin A, iron, zinc, and folate) remain a major nutritional problem in poor regions of the world. More than 50% of home-bound elderly in the United States are deficient in $\geq$1 EAA (6). Inadequate EAA intake during gestation and postnatal periods has far-reaching adverse consequences in offspring through mechanisms involving fetal and neonatal programming. This nutritional disorder results in not only impaired growth of fetuses and infants, but also high risk of metabolic syndrome (including hypertension, obesity, and diabetes) and low quality of life as adults. Of particular interest, stunting in boys and girls will have serious negative effects on society and human physical strength, as well as the health (including reproductive health) of affected individuals and their generations of offspring. In the elderly population, EAA deficiencies will exacerbate sarcopenia and further compromise skeletal-muscle function. Similar situations apply to farm, companion, and wild animals (5).

Dietary Recommendations

The current DRIs provide values for dietary requirements of humans for EAAs (7). For healthy adult humans, dietary requirements for EAAs [mg/(kg body weight · d)] are as follows: His, 14; Ile, 19; Leu, 42; Lys, 38; Met + Cys, 19; Phe + Tyr, 33; Thr, 20; Trp, 5; and Val, 24. For growing swine (100–135 kg body weight), NRC-recommended requirements for dietary EAAs (percentage, grams of total amount/100 g of diet) are as follows: Arg, 0.32; His, 0.25; Ile, 0.39; Leu, 0.71; Lys, 0.71; Met, 0.21; Met + Cys, 0.43; Phe, 0.43; Phe + Tyr, 0.70; Thr, 0.49; Trp, 0.13; and Val, 0.49 (8). For 1- to 21-d-old chickens, recommended requirements for dietary digestible EAAs (percentage of lysine, %) are as follows: Arg, 104; His, 29; Ile, 67; Leu, 100; Lys, 100; Met, 42; Met + Cys, 75; Phe, 60; Phe + Tyr, 112; Thr, 67; Trp, 17; and Val, 75 (5). Thus, except for arginine, the patterns of EAA requirements are similar between animals. In all species, dietary requirements for EAAs generally decrease with increasing postnatal or posthatching age. For example, infants, children, and adolescents have 90%, 25%, and 10% greater requirements for EAAs, respectively, than adults (7).

Food Sources

All fresh plant- and animal-source foods provide protein-bound EAAs and, to a much lesser extent, free EAAs (1). Processed foods contain more protein-bound EAAs but less free EAAs than fresh foods. The content of EAAs varies greatly between foods. Milk is an abundant source of EAAs except for arginine and glycine [e.g., in sow milk on day 14 of lactation (g/L whole milk): Leu, 4.58; Lys, 4.18; Met, 1.05; Thr, 2.28; and Trp, 0.66] (1). This is also true for meat [e.g., in beef cuts (g/100 g dry weight): Leu, 6.45; Lys, 6.97; Met, 2.46; Thr, 3.57; and Trp, 0.97] (5). Compared with animal-source foods, plant-source foods generally contain much less EAAs per gram of dry matter, particularly Lys, Met, Thr, and Trp (1). A proper combination of animal and plant sources of foods can meet dietary requirements for EAAs by humans and other animals. Consumption of different plant-based foods may result in a complementary effect to raise dietary AA quality and quantity, but greater care must be taken to achieve desirable EAA concentrations.

Clinical Uses

EAAs can be used along with other proteinogenic AAs to improve the growth and health of humans and other animals with protein malnutrition (1, 5). Examples of clinical use of EAAs are illustrated as follows. First, BCAAs are often consumed by athletes or supplemented to healthy neonates with a normal birth weight for increasing lean muscle mass. Second, arginine is taken orally to augment the synthesis of NO (the major vasodilator and an inhibitor of platelet adhesion to blood-vessel walls), enhance fertility, and improve metabolic profiles. Third, topical administration of lysine can effectively treat herpes simplex (a virus-infected disease) by inhibiting arginine uptake into the host cell. Fourth, tryptophan is used to prevent insomnia by increasing the synthesis of serotonin and melatonin (neurotransmitters) in the brain. Fifth, threonine is supplemented to weanling mammals (e.g., piglets) for augmenting the intestinal production of mucins and maintaining intestinal mucosal integrity. Finally, oral cysteine can alleviate cobalt, selenium, and inorganic arsenic toxicities through chelation with the minerals in the small intestine. Note that oral cysteine exacerbates organic pentavalent arsenic toxicity by acting as a reducing agent to facilitate the conversion of organic pentavalent arsenicals (e.g., roxarsone and arsanilic acid) into the more toxic trivalent state.

Toxicity

An imbalance of dietary EAAs or antagonism among them often causes toxicity in mammals, birds, fish, and shrimp. Among EAAs, methionine is potentially most toxic owing to its metabolites (homocysteine, H_2S, and H_2SO_4) (1). At present, little information is available regarding the toxicity of excess EAAs in humans and other animals. The DRI does not provide data on the Tolerable Upper Intake Levels of dietary EAA intakes by infants, children, or adults (7). When intakes are equally divided daily in 3 meals, adult humans can tolerate well the amounts of EAAs provided from the ingestion of 2 g

beef protein/(kg body weight · d) (1). This is equivalent to the dietary intake [mg/(kg body weight · d)] of Arg, 140; Cys, 28; His, 65; Ile, 105; Leu, 170; Lys, 185; Met, 65; Phe, 86; Thr, 90; Trp, 26; Tyr, 78; and Val, 120. Growing pigs that consume an adequate ration at the rate of 50 g/(kg body weight · d) can tolerate well dietary intakes (percentage of diet, %) of Arg, 3; Cys, 0.75; His, 1; Ile, 2; Leu, 4; Lys, 1.8; Met, 0.75; Phe, 2; Thr, 1.6; Trp, 0.65; Tyr, 2; and Val, 3 (5). Supplementing 4% (grams per 100 g diet) of any EAA to typical corn- and soybean meal–based diets reduces feed intake and growth performance in swine and poultry.

Recent Research

Methodologies involving stable isotopes, as well as assessments of skeletal muscle and whole-body health are currently used to definitively determine the dietary requirements of humans for all EAAs in the life cycle under normal physiologic conditions (e.g., growth, lactation, and pregnancy) and in diseased states (e.g., diabetes, obesity, infection, burns, and cancer). Identifying optimal ratios and amounts of EAAs in low-protein diets along with those of NEAAs is an active area of research in farm animal nutrition to reduce the cost of feed and the excretion of nitrogen into the environment (5). Much research is being conducted to define optimum dietary requirements of EAAs by aquatic animals (e.g., fish and shrimp) and companion animals in response to physiologic, pathologic, and environmental changes. Criteria for assessing dietary requirements of EAAs include embryonic survival and litter size, fetal growth, milk production, postnatal growth, skeletal muscle gain, reduction of white adipose tissue, digestive function and intestinal integrity, immunity and health status, activations of cell signaling, epigenetic changes, food intake, feed efficiency, and meat quality. Results from these lines of research have important implications for improving the growth and health of humans and other animals, while sustaining the global animal agriculture (including aquaculture).

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Abbreviations used: AA, amino acid; EAA, essential amino acid; NEAA, nonessential amino acid.

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