Biomarkers for optimal requirements of amino acids by animals and humans

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1. ABSTRACT

Amino acids are building blocks of proteins and key regulators of nutrient metabolism in cells. However, excessive intake of amino acids can be toxic to the body. Therefore, it is important to precisely determine amino acid requirements by organisms. To date, none of the methods is completely satisfactory to generate comprehensive data on amino acid requirements of animals or humans. Because of many influencing factors, amino acid requirements remain a complex and controversial issue in nutrition that warrants further investigations. Benefiting from the rapid advances in the emerging omics technologies and bioinformatics, biomarker discovery shows great potential in obtaining in-depth understanding of regulatory networks in protein metabolism. This review summarizes the current approaches to assess amino acid requirements of animals and humans, as well as the recent development of biomarkers as potentially functional parameters for recommending requirements of individual amino acids in health and disease. Identification of biomarkers in plasma or serum, which is a noninvasive approach, holds great promise in rapidly advancing the field of protein nutrition.

2. INTRODUCTION

Amino acids are molecules containing both amino and carboxylic acid groups, which function as chemical messengers and as intermediates in metabolism. Thanks to recent advances in protein nutrition, there is a growing awareness that amino acids are not only the building blocks of proteins but are also regulatory molecules in biological systems. Because of tremendous variations in their side chains, amino acids have a large family, fulfilling numerous biochemical properties and functions. Besides their derivatives and small peptides, only 20 of amino acids are used by cells in polypeptide and protein biosynthesis (1). Besides their function as components in humans and animals, amino acids play important roles in signal transduction, gene expression, and cell physiology (2-5). Therefore, it is very important to supply adequate and balanced amino acids to satisfy the physiological needs of organisms (6). Meanwhile, nutritionists should pay more attention to maximize the efficiency of utilization of dietary amino acids to improve growth, development, and health of animals and humans.

There is a rich history of studies on amino acids and their metabolism over the past century. Although the catabolic pathways of amino acids exhibit a number of common characteristics, it is a difficult task to elucidate their complex interactions as well as the underlying cellular and molecular mechanisms. This has hindered our progress in the accurate determination of amino acid requirements by animals. The current availability of robust analytical techniques and the development of bioinformatics have made it possible to gain a deeper understanding of amino acid metabolism in the body. The major objective of this review is to discuss the current methods to assess amino acid requirements of animals and humans. Additionally, we will highlight the recent development of biomarkers as potentially functional parameters for recommending requirements of individual amino acids in health and disease.

3. SOME CONCEPTS ABOUT AMINO ACID REQUIREMENTS

Amino acids keep the body in a healthy balance. Only when unraveling the complex metabolic fate of amino acids, can we understand the exact physiological needs of the body. Thus, it is necessary to understand some concepts about amino acids before assessing the requirements of these nutrients by animals and humans.

3.1. CLASSIFICATION OF AMINO ACIDS

For more than 70 years, amino acids were conventionally classified as nutritionally indispensable (or essential) and dispensable (or nonessential) for humans and animals based on the nitrogen balance studies (7). Regardless of "indispensable" or "dispensable", essential or nonessential amino acids were initially defined not only in dietary terms but also with respect to their role of in protein accretion and whole-body growth (8). The definition of indispensable amino acids is those amino acids that cannot be synthesized by the body and must be ingested from the diet to maintain a positive nitrogen balance in growing animals. Ten amino acids are now generally regarded as nutritionally essential for mammals (6), fish, and poultry (1): arginine, histidine, lysine, methionine, threonine, tryptophan, phenylalanine, valine, isoleucine and leucine. Additionally, glycine and proline must also be supplied in poultry diets (1). Dispensable amino acids are those can be synthesized in adequate amounts by the body.
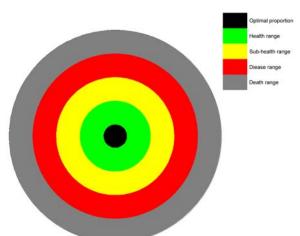
This traditional categorization of amino acids provided a useful guide on protein nutrition in the past decades. However, the concept of functional amino acids, as well as the relationship between indispensable and dispensable amino acids, has received much attention in recent years (6). Some researchers reconsidered the former classification of amino acids in metabolic and functional views and proposed a new concept of conditionally essential amino acids (1). From a metabolic perspective (8), some amino acids (e.g., cysteine and tyrosine) are considered as conditionally essential amino acids, which are not normally required in the diet but must be supplied exogenously to specific populations or under certain physiological conditions where the rate of utilization is greater than the rate of synthesis. In addition, Wu (1, 6) has proposed the concept of functional amino acids which are those amino acids that can regulate key metabolic pathways to improve growth, lactation, reproduction and health in organisms. Functional amino acids may include both essential and nonessential amino acids, and the prototypes are arginine, cysteine, glutamine, leucine, proline, and tryptophan.

3.2. AMINO ACID BALANCE AND IMBALANCE

When the ratio of indispensable and dispensable amino acids in food meets (or closes to) the requirements of animals, we call it "amino acid balance"; otherwise, it is an imbalance. This concept is established on the basis of the relationship between the amino acid composition of a protein and physiological needs (9). Indispensable amino acids are required in specific proportions to satisfy a well-balanced pattern. The amount of these amino acids needed to meet the requirements can be acquired from various ingredients in the diet. Indispensable amino acids are provided in both animal and plant foods. Foods that provide sufficient amounts of both essential and nonessential amino acids relative to requirements of protein synthesis in animals likely contain high quality proteins (or balanced proteins). In contrast, foods that cannot provide a good balance of all indispensable amino acids have low quality proteins (or unbalanced proteins). The more balanced a protein is, the higher efficiency its amino acids will be used for synthesis of tissue proteins. Moreover, if a protein comprises a disproportionately low amount of one or more essential amino acids (e.g., far away from the pattern of amino acid requirements), it will be used inefficiently (10). From this aspect, animal foods are obviously much better than plant foods, due to the low content of many essential amino acids in plants. Though plant foods are often deficient in one or more essential amino acids, they can form complimentarily a large part of the human diet (11). Combined foods from different plant groups, such as "rice and beans", become a good option to make up the amino acid pattern in the right amounts. However, it should be noted that taurine, a physiologically important amino acid for animals and humans, is present only in animal products and absent in all plants.

4. REQUIREMENTS OF AMINO ACIDS BY ANIMALS AND HUMANS

It should be recognized that the metabolic need of amino acids by animals and humans is certainly different from the amount of amino acids in the diet. Therefore, we must ask some pertinent questions before discussing the requirements of amino acids by the organisms. What is the definition of amino acid requirements? What factors can affect the quantity of amino acid requirements? How can we accurately determine the requirements of amino acids by animals and humans?

Figure 1. A proportional model for amino acid requirements by animals. The black spot in the center of circles is the optimal ratio of amino acids required by the body. The circle that is close to the black point corresponds to a healthy range. The next outward circle is defined as sub-healthy range, a state between health and disease and characterized by the deterioration of physiological function. When animals experience a prolonged period of sub-healthy status, they may be inflicted with either metabolic or infectious diseases or both (red circle). When animals are seriously ill and do not respond to medical treatment, they may die (the most outward circle). This model can be applied to all species of animals, but the proportions should be adjusted according to actual situations.

4.1. Definition of amino acid requirements

Following the earlier suggestions (12), one may narrowly focus on only one level of amino acid requirements, namely the minimal requirement based on nitrogen-balance studies, which are the minimum physiological needs for indispensable amino acids by animals to maintain health and participate in biological processes. However, two additional levels of amino acid requirements, namely the operational one and the optimal one, are also considered by some experts (13). The operational requirement based on the net protein utilization (14) considers the fact that a wide range of protein intakes above the minimal values can be achieved (12). The oxidative losses of amino acids were also taken into consideration. Meanwhile, the optimal requirement should be measured by functional terms, e.g. rapid growth, health, and improvement of immunity. The optimal requirement of amino acids is difficult to define as it critically depends on the relationship between amino acid intakes and optimum function of organs (12, 13, 15).

There is a marked difference between amino acids needed by the animal and those that are absorbed from the small intestine. Hence, it is better to establish some separate criteria independent of the level of requirements (12): 1) metabolic needs, defined as the amount of dietary amino acids used for protein accretion and synthesis of non-protein substances; 2) dietary requirements,

corresponding to the amounts of amino acids that must be provided in foods to meet the metabolic needs; 3) recommended dietary intake, equal to the dietary requirement plus 2 standard deviation (SD) that will support the metabolic needs of 97.5% of the population. Here, we propose a simple proportional model of amino acid requirement as shown in Figure 1. The black spot in the center of circles is the optimal ratio of amino acids required by the body and these amino acids must be provided in the diet. It represents an exact number or an extremely narrow range, in which animals or humans can achieve a most balanced condition for maintenance, growth, and function. The circle that is close to the black point corresponds to a healthy range. In this range, the animal or human maintains a dynamic homeostasis via continuous synthesis of protein and continuous degradation of proteins as well as exquisite regulation of nutrient metabolism. The next outward circle is defined as sub-healthy range, a state between health and disease and characterized by the deterioration of physiological function, which can happen in a large number of animals and humans. Within this range, animals or humans do not exhibit obvious pathological changes, but utilize foods at a reduced efficiency and can benefit from nutritional intervention such as supplementation with certain amino acids. When animals or humans experience a prolonged period of sub-healthy status, they may be inflicted with either metabolic or infectious diseases or both (red circle). In a diseased condition, it is hard to repair those parts that have been broken down simply through nutritional intervention. Medical treatments are necessary to restore a new balance. Finally, when animals or humans are seriously sick and do not respond to medical treatment, they may die (the most outward circle). This simple model can be applied to all species of animals and humans, but the proportions should be adjusted according to actual situations. The most important task of constructing this model is to assure the exact optimal ratio of amino acids and the critical value of different ranges. The body is an intricate and complex system that is influenced by many factors. Thus, it is by no means easy in practice to accurately determine the optimal requirements of amino acids by organisms.

4.2. Factors affecting amino acid requirements

Numerous nutritional, biological, and environmental factors can profoundly influence amino acid requirements by animals and humans. Firstly, factors that affect dietary protein digestibility, bioavailability and efficiency should be emphasized. The higher the dietary protein quality is, the more digestible the food is, and the more indispensable amino acids the food provides (10). Recent investigations have also indicated that the quantitative relationship between nitrogen balance and energy intake should be considered under various circumstances (16-18). Secondly, biological factors like gender, age, and health status can strongly influence the requirements of amino acids by animals due to the different metabolic needs (12, 19, 20). Infants, children, sows and boars are different in their stage of development and with distinct metabolic needs, and the genetic makeup of

Table 1. Estimated requirements of indispensable amino acids by adults, growing rats, and lactation sows

Amino acid	Human adults[1]	Growing rats[2]	Lactating sows[3]
	mg/(kg·day)	g/kg diet	g/day
Arginine	0	4.3	25.8
Histidine	10	2.8	19.1
Isoleucine	20	6.2	26.8
Leucine	39	10.7	52.1
Lysine	30	9.2	48.6
Sulfur amino acids (Methionine + cysteine)	15	9.8	23.4
Methionine	10.4	-	12.2
Cysteine	4.1	-	-
Aromatic Amino Acids (Phenylalanine + tyrosine)	25	10.2	53.4
Phenylalanine	-	-	25.9
Threonine	15	6.2	31.1
Tryptophan	4	2.0	8.6
Valine	26	7.4	40.9

[1]Daily amounts of indispensable amino acids are recommended by World Health Organization and Food and Agriculture Organization (28). [2]Data are recommended by National Research Council (29). Requirements of indispensable amino acid are expressed on an as-fed basis for diets containing 10% moisture and 3.8–4.1 kcal metabolizable energy/g. [3]Data are recommended by National Research Council (30). Requirements of total indispensable amino acids are expressed on an as-fed basis for corn–soybean meal diets containing 90% dry matters, energy of 3,400 kcal digestible energy/kg and 17.5% crude protein. It assumes 10 pigs per litter and a 21-day lactation period. Post-farrowing weight of the sow is 175 kg and has no weight change during lactation.

individuals should not be ignored. Thirdly, the role of environmental factors in modulating amino acid requirements has received increasing attention in recent years (12, 19, 21). Of particular interest are effects of thermal stress (high or low ambient temperatures) and air pollution on amino acid metabolism in organisms.

4.3. Methodology of estimating amino acid requirements

Over the past half century, nutritionists have dedicated themselves to define optimal amino acid requirements of humans (22, 23), pigs (24, 25), pets (26, 27) and other animals (28-30) under various nutritional, developmental, environmental, and pathological conditions. Tentative recommendations of amino acid requirements by these species are summarized in Table 1.

The amino acid requirements of animals or humans can be divided into two main parts: a requirement for maintenance and a requirement for protein deposition (e.g., milk production, muscle growth, and fetal growth). In the early days, most of the studies on amino acid requirements focused on indispensable amino acid using nitrogen balance or nitrogen retention as a criterion of adequacy (31). One of the effective methods to assess amino acid requirements of animals is to estimate the requirement of a specific amino acid such as lysine, and then to determine the requirements for other amino acids based on their proportions to lysine (32). These ratios were determined by analysis of amino acids in proteins of a dominant tissue or the whole carcass. Subsequently, the pattern of amino acid requirements was established by directly assessing the regression of daily protein accretion and daily intake for each amino acid in experimental subjects such as growing pigs (33, 34) and fish (35). However, the classic nitrogen-balance studies are not sufficiently sensitive to be suitable for estimating amino acid requirements due to the unresolved problem of the miscellaneous losses of nitrogen even when N-intakes are kept constant (12).

To estimate dynamic amino acid requirements, measurements of plasma amino acids (36, 37) and ^{13}C-labeled amino acid tracer studies (38) were introduced. Due to the obligatory losses of amino acids, the requirements of many indispensable amino acids by healthy adults derived from these techniques turned out to be much higher than those accepted by the nutrition community based on the early nitrogen-balance studies (6, 37). Young *et al.* (38) suggested that tracer studies with ^{13}C-labeled amino acids should only be the interim method to assess amino acid requirements of humans until more convinced techniques are available. Recently, a method named indicator amino acid oxidation (IAAO) has been developed to determine amino acid requirements by adults (39), neonates and children (40), as well as growing pigs (41). The IAAO method has been extensively discussed by Elango *et al.* (42). A central problem with all isotopic studies is the difficulty in determining specific activities or molar enrichments of labeled precursors at the sites of their oxidation.

Although approaches for determination of amino acid requirements by animals and humans were developed over half century ago, there is still no method that can generate comprehensive data on the dynamic aspect of amino acid requirements. As the research on amino acid requirements has become more and more precise and systematic, conventional methods, which studied the influences of individual or a mixture of amino acids, should be integrated with modern techniques to account for the complex metabolic transformations (43). At the same time, some organs such as the small intestine plays an important role in catabolizing and transforming dietary amino acids (44), which make the pattern of amino acids in the portal venous blood markedly different from that in the diet (6). Therefore, the metabolism of amino acids in the small intestine must be taken into consideration when estimating amino acid requirements of animals and humans.

5. BIOMARKERS AS POTENTIAL FUNCTIONAL PARAMETERS FOR AMINO ACID REQUIREMENTS BY ANIMALS AND HUMANS

Although a lot of efforts have been directed to overcoming the weaknesses of the traditional methods, an efficient and effective approach to determine amino acid requirements by animals and humans has not yet been developed. Moreover, the current models of amino acid requirements are based on incomplete understanding of protein metabolism in the body (30). Animals of different species, different developmental stages and pathological conditions, and even different times of the day, will have different patterns of amino acid requirements. It is exciting that there is now a renewed interest in the analysis of the metabolites of amino acids, including ammonia, glucose, ketone bodies, nitric oxide, urea, polyamines, homocysteine, and methylarginines (1). The new developed "omics" technologies provide powerful tools for assays of thousands of intermediary metabolites and produce larger amounts of data than the traditional methods (45). Quantifying the concentrations of appropriate metabolites holds great promise in providing deeper insights into the regulatory networks for protein metabolism, as well as biomarkers to estimate the amino acid requirements by animals and humans. Different methods are required to validate the intermediate metabolites before these substances can be assigned as biomarkers for amino acid requirements. By combining the fast developed molecular-nutrition techniques and bioinformatics with the traditional approaches, it is now possible to gain better understanding of protein metabolism with the goal of assessing the status of amino acid nutrition and providing recommendations of amino acid requirements under various developmental and pathological conditions.

5.1. Biomarkers for the metabolism of amino acids

The lack of in-depth understanding of the cellular and molecular mechanisms responsible for the regulation of amino acid metabolism is a significant barrier that should be overcome before the assays of amino acid requirements by animals and humans reach their full potential. Take arginine for example, which has multiple metabolic pathways and diverse physiological functions in the organisms (46). Growing evidence has shown beneficial effects of arginine in improving growth, development, lactation, reproduction and health (6, 47). For example, arginine supplementation not only improved the growth performance of artificially reared piglets (48), but also enhanced the reproductive performance of primiparous sows (49, 50). Of particular note, the number of live-born piglets increased by two per litter through dietary arginine supplementation (49). This is a great achievement in animal production; however, the mechanisms for arginine's role in regulating embryonic/fetal survival remain unknown. Nevertheless, new data from a metabolomic study offered deeper insight into metabolic changes responsible for the effects of arginine. Combined with conventional biochemical assays for metabolites, He *et al.* (51) determined the effect of arginine supplementation on the metabolome in serum of growing pigs using the NMR-based metabolomic method. Circulating levels of certain biomarkers (such as low density lipoprotein, creatinine, glycerophosphorylcholine, and methylamine) involved in fat deposition, protein synthesis, and microbial metabolism were altered markedly in response to arginine treatment. Some of these metabolites would not have been studied on the basis of traditional methods.

In order to rapidly expand our knowledge of amino acid catabolism and its roles in nutrition and disease, it is necessary to better understand the underlying mechanisms in different organs. For instance, the small intestine is the site where dietary amino acids are ultimately digested and absorbed. Intestinal amino acid catabolism plays a crucial role in modulating the entry of dietary amino acids into extraintestinal tissues. Amino acids, rather than glucose, are the major source of energy for the small intestinal mucosa (1, 2). In addition, most of amino acids in the lumen of the intestine were catabolized by the gut. Data from portal-balance studies show that 30-50% of essential amino acids in the diet were not available for extra-intestinal tissues and less than 10 % of the amino acids utilized by the small intestine were recovered in mucosal protein (44). Although lumenal contents (digesta) of the small intestine cannot be obtained from animals or humans without using invasive surgery, blood samples can be readily obtained from the subjects for the analysis of amino acids as biomarkers for intestinal mass and integrity. Such an amino acid is citrulline, which is virtually absent from the enteral diet and is synthesized from glutamine/glutamate and proline only in the small intestine (1, 6).

5.2. Biomarkers for amino acid requirements under different conditions

Understanding what happens to whole-body metabolism after intake of excess or lack of a single amino acid is a basic way for biomarker discovery. Because dietary excess of some amino acids may cause unpredictable changes such as toxicity in the body (52), the determination of the upper limit of the requirements of these amino acids has important implications for health. Results of a metabolomic study in combination with transcriptomics approach indicate that metabolites in plasma were altered substantially in rats fed excess leucine (53). Urea and alpha-ketoisocaproate were suggested as early markers for the upper limit of leucine requirement (53). Other than leucine, methionine toxicity caused by certain metabolic pathways is more notable than that of other amino acids owing to the quite narrow range of optimal intake (54). Supplementation with too much methionine in rats markedly decreased the ratio of cystathionine to homocysteine which would be the limiting reaction in the disposal of excessive methionine. Additionally, the GC-MS based study identified that homocysteine might be a useful biomarker to help set the upper limit of adequate methionine intake and to serve as a surrogate marker for methionine toxicity (55). As opposed to the excessive increase, a deficiency of amino acids will also lead to adverse consequences. For example, weight loss and hepatic lesions occurred in rats fed with a methionine-deficient diet while proinflammatory and fibrotic genes were up-regulated simultaneously. These adverse outcomes were ameliorated after methionine

administration (56). The findings from these studies greatly strengthen the notion that suitable biomarkers are useful for *in vivo* estimation of amino acid requirements in animals.

There is also growing interest in the role of biomarkers for *in vitro* model of amino acid requirements. Using a comparative functional proteomic approach, the effect of glutamine on epithelial intestinal cell lines was investigated under experimental apoptotic conditions (57). The level of proteins involved in the cell cycle as well as apoptosis signal transduction and cytoskeleton organization varied greatly in response to an increase of glutamine concentration in culture medium. Furthermore, proteomic analysis revealed that proteins related to the cell cycle arrest and apoptosis induced by methionine restriction might be the most plausible biomarkers to inhibit gastric cancer cells growth (58).

5.3. BIomarkers for recommendation of amino acid requirements

Because environmental imprints, genetic makeup, and individual differences affect amino acid metabolism, animals and humans should be ideally fed on the basis of their metabolic needs. For this reason, recommendation of amino acid requirements for individuals is necessary (59, 60) and a major goal of nutrition research. In human nutrition, we can all benefit through learning about our metabolic profiles in response to diet, and putting this knowledge into practice will open a door to optimizing our health.

Inter-individual genetic variations such as single nucleotide polymorphisms (SNP) play a crucial role in determining nutrient requirements (59, 61, 62). Thanks to the human genome project and the subsequent 1000 genomes project (63), potential candidate biomarkers for amino acid requirements can be identified through extensive research. For instance, reducing unfavorable fat deposition and enhancing muscle accretion is not only a critical health objective for people with obesity and type II diabetes, but also an important objective in animal production. Nowadays, some nutritional strategies are available to achieve this goal. For example, dietary supplementation with arginine, alone or in combination with conjugated linoleic acid (64), selectively decreased body white fat mass, while increasing skeletal muscle gain in diabetic rats (65), obese rats (66) and growing-finishing pigs (67). Microarray analysis, which was confirmed by RT-PCR analysis, revealed that arginine supplementation and high fat diet differently modulated gene expression in adipose tissue to influence energy-substrate oxidation, redox state, lipid metabolism, and adipocyte differentiation (68). Distinct from the regulation of high fat diet, arginine supplementation prevented oxidative stress and reduced expression of fatty acid binding protein 1, glycogenin, protein phosphatase 1B, caspases 1 and 2, and hepatic lipase in white adipose tissue. Importantly, arginine supplementation enhanced mRNA levels for peroxisome proliferator-activated receptor gamma, heme oxygenase 3, glutathione synthetase, insulin-like growth factor II, sphingosine-1-phosphate receptor, stress-induced protein in the white adipose tissue of diet-induced obese rats (68), as well as nitric oxide synthase-1, hemeoxygenase-3, AMP-activated protein kinase, and

peroxisome proliferator-activated receptor gamma coactivator 1 alpha in the white adipose tissue of diabetic fatty rats (65). All of these novel findings are expected to elucidate the regulatory role for arginine-nitric oxide pathway in energy metabolism, in support of the prevention and treatment of the metabolic syndrome in obese humans and reduction of excessive fat deposition in animals of agricultural importance (69). In this regard, biomarkers for the arginine-nitric oxide pathway in plasma or serum, such as arginine, citrulline, nitrite/nitrate, biopterin, and methylarginines, may be useful for assessing the patterns of muscle and adipose tissue growth in animals and, therefore, their requirements for amino acids.

Proteomics, which analyzes the composition of proteins in plasma, cells, or tissues (70, 71), holds great promise in identifying biomarkers for accurate requirements of amino acids and recommendation of their intakes by animals (72). As reported for C57BL/6L and C3H/HeJ mice fed a high-fat diet (73), animals may also exhibit different requirements for amino acids among individuals. Biomarkers discovered through proteomics will aid in the development of personalized programs to formulate specific diets for animals. An example is the altered expression of intestinal proteins related to glutathione synthesis, as well as the altered radio of oxidized glutathione to reduced glutathione in tissues of postweaning pigs (1). This would call for special needs of cysteine, glycine, and glutamine/glutamate in the diet for weanling swine.

Growing evidence has shown that age (74), gender (75), body mass index (BMI) (75), diurnal variation (76), physical exercise (77), and diet (78) can influence the metabolic profiles of animals and humans. Branched-chain amino acids (BCAA), which are not synthesized in the body but play a crucial role in activating the mTOR signaling pathway (79), have attracted much attention from life scientists. Leucine acts as a nutrient signal to stimulate protein synthesis in skeletal muscle of infants (80) and neonatal pigs (6). Interestingly, a recent study identified a BCAA-related metabolite signature that distinguished obese from lean men (81). Work with rats demonstrated that BCAA made a direct contribution to the development of obesity-associated insulin resistance (81). Another metabolomic study using UPLC-Q-TOF MS confirmed the abnormal metabolism of BCAA and aromatic amino acids, as well as fatty acid synthesis and oxidation in obese humans compared with lean subjects (82). Thus, BCAA may be useful biomarkers for the metabolic syndrome as well as for altered protein nutrition in obesity and diabetes.

6. SUMMARY AND FUTURE DIRECTIONS

Despite improvements in methodologies for assessing requirements of amino acids by animals and humans (42), many problems remain to be solved before more accurate data can be obtained (5). An important issue to be considered is the extensive catabolism of dietary amino acids by microorganisms in the gastrointestinal tract (83). Other issues would include the molecular mechanisms responsible for the regulation of intestinal mucosal function

(84), muscle protein turnover (85), interactions between amino acids and other dietary factors (86, 87), protein intake (88), nitric oxide-dependent angiogenesis and blood flow (89), and amino acid transport (90). Based on valid biomarkers, development of a dynamic model simulating practical conditions of livestock production would be ideal to increase the efficiency of utilization of nutrients in the body. This concept can also apply to human nutrition, although human subjects can choose the foods they like to eat. We are hopeful that, with the fast development of the emerging omics platforms and bioinformatics, biomarker discovery will greatly advance knowledge about the metabolism and requirements of amino acids in mammals, birds, and fish.

7. ACKNOWLEDGEMENTS

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Abbreviations: AAs: amino acids, BCAAs: branched-chain amino acids, BMI: body mass index, FAO: food and agriculture organization, GC-MS: gas chromatography-mass spectrometry, IAAO: indicator amino acid oxidation, NRC: national research council, RT-PCR: real-time polymerase chain reaction, SD: standard deviation, SNP: single nucleotide polymorphism, UPLC-Q-TOF MS: ultra performance liquid chromatography and quadruple-time of flight mass spectrometry, WHO: world health organization

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