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90th Anniversary Commentary: Amino Acid Imbalances: Still in the Balance

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The concept of amino acid imbalance, explored by Alfred Harper in a landmark 1959 *Journal of Nutrition* article (1), is still very relevant today in terms of biology and in a variety of applications. Amino acid imbalances are not only relevant to the efficient feeding of farm animals, for example, with poultry or pig nutrition (2, 3), but also to fundamental biology, where they are implicated in the life span of insects (4). The importance of amino acid imbalance in clinical nutrition continues to be documented with different amino acid supplementation regimens (5–7), as well as in cellular biology (8), and in public health, where amino acids might be fortified into foods (discussed later).

An adequate supply of indispensable amino acids (IAAs), in the right proportions, is required in the diet of animals and humans for adequate growth and maintenance of the metabolically active tissues. In the past decade, the daily requirement and proportions of these IAAs were revisited by an Expert Committee of the WHO/FAO/UN University (9), where the daily requirements for many of the IAAs were revised upward by 2- to 3-fold from the previous 1985 WHO/FAO/UN University Expert Committee (10). These requirements were based on empirical evidence from careful measurements of balance of an IAA such as leucine or phenylalanine, when subjects were fed graded amounts of the IAA under test, and the protein intake was provided at the level of their requirement (11–14). These validated an important theoretical framework proposed by Vernon Young and others (15) on how the pattern of the amino acid requirement in humans could be derived from the obligatory nitrogen loss and the composition of body proteins. The altered scoring pattern of IAAs in the daily protein requirement has also made protein quality an important factor when considering the adequacy of protein intake. With regard to studies that sought to define the amino acid requirement, the amount of intake of protein or other IAA was very important. As Harper states (1), "It would seem advisable for the amino acid balance to be maintained as closely as possible to the ideal if minimum values for amino acid requirements are to be obtained."

Harper engages with the concept of amino acid balance and imbalance in this article (1), which studied the suboptimal growth rate and appetite of animals when consuming proteins that were not nutritionally balanced, or containing the right proportions of IAAs, to meet the daily requirement. Amino acid imbalance is defined in different ways. It is any change in the proportions of the amino acids in a diet that results in an adverse effect, which can be prevented by supplementing the diet with a relatively small amount of the most limiting amino acid or acids. Thus, imbalances can be caused by adding relatively small amounts of 1 or 2 amino acids to a diet—for example, as a relative deficiency of an IAA resulting from an excess of ≥1 other amino acid in the diet, or where there is a small excess of amino acid that causes the relative deficiency (16). It was also important to note that the expected outcome with the intake of the imbalanced protein was associated with adverse effects rather than simply a negative nitrogen balance. Harper points out that amino acid imbalance is specifically where adverse effects (for example, appetite, retarded growth, or fatty liver), beyond the expected fall in the efficiency of protein utilization, are observed. This happens when the dietary protein source, usually one low in protein, has been unbalanced by the addition of amino acids or a quantity of an unbalanced protein, as defined above (1). As documented in the present landmark article, he showed that growth retardation in rats caused by unbalanced diets could be prevented by increasing the diet protein intake, underscoring the complexity of this phenomenon in relation to the basal protein intake and approaches to its correction. The intake of the protein under consideration is clearly important in modulating the outcome, whether it relates to adverse effects, or even biological value in terms of nitrogen homeostasis. For example, the biological value of egg protein, as measured by the nitrogen retention, has been shown to decrease as the intake of the protein increased, underscoring the point that the measurement of protein quality in the frame of biological value was best approached by measuring outcomes at graded amounts of protein intake (17).

Although the basic definition of an amino acid–imbalanced diet did not distinguish between those imbalances caused by adding small amounts of 1 or 2 amino acids to a diet from those imbalances caused by a large excess of protein or a complete lack of an amino acid, Harper's careful distinction of definitions was important because amino acid imbalance was also defined as that occurring by the addition of a small quantity of an IAA, usually the second-limiting amino acid, to a low-protein diet (18). For example, keeping the lysine (first limiting amino acid) content of a rice diet of rats constant and increasing its threonine (second-limiting amino acid) content by small amounts lead to a point when the growth of rats on the threonine-supplemented

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diet is retarded, unless its lysine content is also increased (19). Harper and colleagues subsequently also pointed out that if several amino acids are equally limiting in the diet, less specificity was observed and several different amino acid supplements could cause growth retardation (20, 21). It is important to point out that an amino acid imbalance, while causing growth retardation and a decrease in the efficiency of nitrogen utilization, can also increase the requirement for the most limiting amino acid (16). In another study published at approximately the same time (and similar to other studies he conducted), Harper et al. (22) showed that the addition of 2% histidine to a diet containing 12% casein in young rats could inhibit their appetite and growth. Harper would term this an amino acid antagonism, distinct from toxicity, caused by the addition of a large amount of a specific amino acid to the diet. In the context of so many definitions, this study is also important, because it is specific about the distinction between imbalance and other concepts such as antagonisms and toxicities, which he addressed in many subsequent reviews (23).

The pathophysiology behind the effects and symptoms of amino acid imbalance has been investigated in some detail. It is logical to infer that an amino acid imbalance reduces the efficiency of utilization of the limiting amino acid (16), and therefore nitrogen. When there is a deficiency of the limiting amino acid, whether in isolation or caused by the excess of other amino acids, it could lead to a reduction in the efficiency of protein synthesis, and the oxidation of excess amino acids, as has been shown in the IAA oxidation method (24). Harper's hypothesis related to the plasma concentrations of amino acids, where digestion and absorption with an imbalanced diet were normal, but there was an altered plasma amino acid pattern, with a surplus of all but one of the IAAs in the portal blood. At first pass, the uptake of the amino acids would be such that there would be a reduced supply of the limiting amino acid for peripheral tissues. If muscle protein synthesis continued at a normal rate, the free amino acid patterns of both muscle and plasma would rapidly become unbalanced. The role of the central nervous system was important, because these changes would affect appetite regulation to depress food intake, such that growth would eventually be retarded. This hypothesis accounted for the large depressions in growth and food intake caused by relatively small supplements of the second most limiting amino acid or acids, and the detection of the dietary imbalance is thought to occur in the anterior pyriform cortex, where the concentration of the limiting amino acid can affect appetite and dietary selection in rats (25). Alterations in dietary selection are another feature of amino acid imbalance, at least in rats, and the sensing of IAAs in the diet seems to be innate. Thus, when offered a choice, rats consume a balanced diet in preference to an imbalanced one, but more remarkably, select a protein-free diet incapable of supporting growth instead of an imbalanced diet that would allow growth, albeit at a low level, dependent on the content of the dietary limiting amino acid in the pyriform cortex (26–28). More recent and careful experiments, however, suggest that a previous deprivation of IAAs, or the creation of an immediate physiologic need, is required for this sensing, although specifically, a lysine-deficient diet appears to be immediately sensed in terms of food preference (29). The total energy requirement and therefore the intake is also important, where a significant increase in energy expenditure and intake due to a cold environment suppressed the effect of an amino acid imbalance on the appetite of rats (30). The role of transcription factors in protein synthesis has also been investigated, where the intracellular accumulation of uncharged transfer RNA binds to transcription factors such as general control nonderepressible 2 (GCN2), leading to a series of events resulting in changes in neural activity and a slowing of global rates of translation (31). However, experiments with GCN2-knockout mice indicate that other mechanisms may be at work (29). Other effects relate to dynamic amino acid concentrations, particularly at the tissue level, where rapid effects occur with the intake of an unbalanced crystalline amino acid–supplemented diet linked to early absorption. Free amino acids are rapidly absorbed in comparison to those in protein, where digestion must first take place and could result in temporal differences in the concentration of amino acids at the site of protein synthesis. This has been observed in pigs fed either free or protein-bound lysine (32), with temporal differences in peak lysine concentrations of a few hours in these treatments, and relates to a body of work that has shown the importance of the frequency of feeding in reducing this temporal disparity (33, 34).

Amino acid imbalances are of concern even in human diets: for example, Gopalan (35) documented the influence of a millet-based diet, with a high leucine content, in inducing pellagra in humans and dogs. This pellagragenic effect of leucine is attributed to an increased hepatic oxidation of tryptophan (36). The phenomenon of diet-induced fatty liver is also linked to an imbalanced intake of sulfur-containing amino acids (37), occurring with both low and high intakes. It is also relevant today in aid-based human nutrition, where diets in many poorer parts of the world are heavily cereal-based and present the risk of being deficient in lysine (38). Although there is no doubt that it is possible to meet the higher IAA, or quality protein, requirements from a habitual mixed diet by using complementary sources of protein, as is apparent in the cultural evolution of diets in human societies, the relevance to poverty and food aid is worth noting. Because the mixing of complementary protein food sources to improve diet protein quality relates to the economics of supply of high-quality animal or plant protein, protein quality in food supplies might be augmented by fortification with specific amino acids—for example, the supplementation of a diet with crystalline amino acids is a consideration in improving the quality of vegetable proteins. This was effective when treating children with severe acute malnutrition with ready-to-use therapeutic food (RUTF) (39), in which a soya, maize, and sorghum mix was enriched with amino acids (the exact proportions of supplementary amino acids are not provided in the article) to have about the same or a slightly greater content of IAAs (the final lysine content was ~20% higher) as a milk and peanut-based RUTF. This careful matching of amino acid content is important. When tested against a milk-based RUTF, the amino acid–fortified, vegetable protein–based RUTF was not inferior in terms of recovery rates and length of stay in the hospital, and no adverse events were reported. Similarly, a complementary cereal-legume food fortified with lysine (0.75%) is being tested for its efficacy in Ghana (40). Harper's landmark study (1) and his body of work provide valuable lessons for the rational use of amino acid fortification in feeding animals and human populations, without the potential ill effects of amino acid imbalances.

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