Serum Amino Acid Concentrations in Infants from Malawi are Associated with Linear Growth

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ABSTRACT

Serum amino acid (AA) concentrations are correlated with childhood stunting, but their relation to linear growth velocity has not been explored. This was a secondary analysis of a clinical trial where Malawian infants aged 6–12 mo were given a legume supplement providing 8.2 g/d of protein; anthropometry was conducted at multiple intervals, and fasted serum AA concentrations were measured at 12 mo of age. Lysine, proline, tryptophan, tyrosine, and valine concentrations were higher in infants with a linear growth velocity *z*-score >0 than those <0. Corrected Spearman correlation coefficients between individual AA concentrations and weight-for-height and length velocity from 6 to 12 mo of age were positively correlated for glycine, isoleucine, proline, serine, threonine, tyrosine, and valine. Additionally, weight-for-height was correlated with arginine, asparagine, glutamine, leucine, lysine, methionine, and phenylalanine. The observed associations suggest that testing the hypothesis that essential AA provision will reduce linear growth faltering is warranted. This trial was registered at clinicaltrials.gov as NCT02472262.
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Introduction

Dietary amino acids (AAs), particularly the essential AAs, are vital for growth and development in young mammals. When mammal milk is not part of the habitual diet in animal husbandry, essential AAs are often added to the feed (1). The protein and AA content of the diet is associated with the serum concentration of AAs (2, 3). However, AA metabolism is modulated by the liver to meet a variety of needs and uses for specific AAs, so the relation is not linear. Consumption of AA-deficient diets in animals results in reduced growth (4); in human epidemiology, protein intake is directly associated with height (5). It has been proposed that lower consumption of essential AAs reduces linear growth because the AAs regulate the mechanistic target of rapamycin (mTOR) signaling pathway (6). Animal and cell culture models have implicated a variety of essential AAs in mTOR regulation, and suggest that tRNA that is not bound to an amino acid could be a regulatory factor (7). Activation of the mTOR pathway is essential for cellular growth and metabolic processes (8). The rate of chondrogenesis and endochondral elongation is regulated by mTORC1 (9).

In contrast to this evidence, the global nutrition community has accepted the notion for the last 35 y that dietary protein deficiency exists only when a diet is also deficient in energy (10). This is largely based on the observation that breast milk is only 0.9% protein and allows for optimal infant growth. Recently this notion has been questioned for older children; serum AA concentrations were associated with height in 2–5-y-old Malawian children (11).

This study tested the hypothesis that among older infants, growth velocity and weight-for-height would be correlated with serum AAs in rural Malawi.

Keywords: amino acids, stunting, linear growth, Africa, global health

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Abbreviations used: AA, amino acid; LVZ, length velocity *z*-score; mTOR, mechanistic target of rapamycin.

Methods

Subjects and participation

Of the 355 Malawian infants participating in a legume supplementation trial (12), all children who attained the age of 12 mo from January to June 2017 had a venous blood sample taken for AA analyses. The participants lived in 1 of 2 village clusters surrounding Limela in Machinga District or Masenjere in the Nsajane District. Anthropometric measurements were taken at ages 6, 7.5, 9, 10.5, and 12 mo for each child. At the time of sampling the children's caretakers denied any report of fever, cough, diarrhea, vomiting, food insecurity, or anorexia. The children did not have weight loss on the basis of their longitudinal anthropometry. Mothers gave informed consent for the sampling. Ethical approval was obtained by the College of Medicine Research Ethics Committee of the University of Malawi, the Human Research Protection Office of Washington University in St Louis, and the Johns Hopkins School of Medicine Institutional Review Board. This trial was registered as clinical trial NCT02472262 at clinicaltrials.gov.

For comparison, AA concentrations from 370 healthy German children from the lifestyle and environmental factors and their influence on newborns allergy risk (LINA) cohort at 1 y of age were compiled (13). The comparison group was measured at a single time point and chosen because no acute medical illnesses were present, no medical conditions were found in the health records nor any congenital abnormalities noted. Participation in the study was voluntary, and informed consent was given by the parents. Ethical approval for the German study was obtained by the Ethics Committee of the University of Leipzig (14). Details of the LINA study are described elsewhere (13).

Trained study nurses drew venous blood from infants who had no food other than breastmilk overnight. The serum samples were centrifuged for 10 minutes at 3000 RPM at ambient conditions, aliquoted into cryovials, and snap frozen in liquid nitrogen within 4 h of blood drawing. Cryovials were stored at −80°C until analysis.

For hypothesis generation sample size is not a major consideration. The sample size obtained for this secondary analysis was sufficient only to detect differences of 20% in serum AA concentration when comparing children having linear growth velocity *z*-scores >0 with those having linear growth *z*-scores <0. This allowed us to hypothesize that serum AA concentration can be used as a potential biomarker of protein intake and to explore what amounts of dietary AAs are necessary to promote linear growth.

AA analyses

In both studies, the AA concentrations were assessed in sera using LC-tandem MS. The AbsoluteIDQ p180 Kit (Biocrates LIFE Science AG), was used to standardize the measurements in both populations (11, 15). The interassay and intraassay coefficients of variation ranged from 1% to 11% for AAs.

Data analyses

Anthropometric indexes in Malawian children were calculated based on the WHO 2006 Child Growth Standards using Anthro version 3.2.2 (16) and length velocity *z*-scores (LVZs) on the WHO 2009 standards (17). The study population was divided into 3 groups on the basis of LVZs: LVZ < −2, LVZ ≥ −2 and <0, and LVZ ≥0.

TABLE 1 Characteristics of rural 12-mo-old Malawian infants[1]

Characteristic (n = 127)	
Age, mo	11.3 ± 0.3
Female	60 (47)
Weight-for-height *z*-score	−0.2 ± 0.8
Length-for-age *z*-score	−1.5 ± 1.0
Length velocity *z*-score 6–12 mo	−1.3 ± 1.2
Weight velocity *z*-score 6–12 mo	−0.8 ± 1.0
Mid-upper arm circumference, cm	14.2 ± 0.9
Breastfed	127 (100)
Diarrhea in last 14 d	18 (14)
Fever in last 14 d	24 (19)
Mother alive	126 (99)
Father alive	126 (99)
Siblings	2.5 ± 1.9
People sleeping in the same room with the child	3.2 ± 0.8
Family owns:	
Metal roof	26 (20)
Radio	47 (37)
Bicycle	70 (55)
Animals sleep in house	34 (27)
Clean water source	93 (73)
Sanitary stool disposal	17 (13)

[1]The data are expressed as mean ± SD or *n* (%).

AA concentrations from infants with LVZs ≥0 were compared with children with LVZs <0 using Student *t* test. Also AA concentrations from Malawian infants segregated by LVZ were compared with an age-matched reference population of German children for values less than the mean of the reference population. Spearman correlation coefficients were calculated to explore associations between anthropometry and AA concentrations (IBM SPSS Statistics, version 24; IBM Corp). *P* values for the correlation coefficients were corrected for multiple comparisons using the Benjamini–Hochberg method.

Results

Of the 127 Malawian children studied, all were currently breastfeeding ad libitum, and 34 (27%) were stunted, defined as length-for-age *z*-score <2 (**Table 1**). Growth velocity *z*-scores were <0 from 6 to 12 mo for length in 109/127 infants and for weight in 102/127 infants.

Weight-for-height *z*-score attained at 12 mo of age was directly correlated with 14 AAs: Arg, Asn, Gln, Gly, Ile, Leu, Lys, Met, Phe, Pro, Thr, Trp, Tyr, and Val (**Table 2**). This list included 8 of the 9 essential AAs (Ile, Leu, Lys, Met, Phe, Thr, Trp, Val) and 4 conditionally essential AAs (Arg, Gln, Pro, Tyr). LVZ from 6 to 12 mo was directly correlated in Malawian children with 7 AAs: Gly, Ile, Pro, Ser, Thr, Tyr, and Val.

The German comparison group was measured at age 11.7 ± 0.3 mo, and the number of female children was 173/370 (47%). Length-for-age *z*-score among German children was 0.75 ± 1.0, and weight-for-height *z*-score was −0.2 ± 0.8.

Serum AAs in Malawian children with LVZs < 0 were lower than the German comparison group for Ala, Gln, Leu, Lys, Met, Pro, Ser, Trp, and Val (all *P* values < 0.05, Table 2), but for Malawian children with LVZs ≥0 only Val was less than the German comparison group.

TABLE 2 Plasma amino acid concentrations in 12-mo-old Malawian infants and their correlation to growth velocities and anthropometric status[1]

Amino acid	Malawian infants LVZ < −2 *n* = 37	Malawian infants LVZ ≥ −2, < 0 *n* = 72	Malawian infants LVZ ≥ 0 *n* = 18	Correlation with LVZ	Correlation with WLZ	German infants *n* = 370
Alanine	448 ± 146†	403 ± 116†	482 ± 176	0.066	0.089	532 ± 167
Arginine	110 ± 47	102 ± 42	105 ± 41	0.05	0.164*	82 ± 29
Asparagine	100 ± 37	90 ± 42	94 ± 33	−0.096	0.172*	59 ± 21
Aspartate	103 ± 60	106 ± 73	99 ± 56	0.001	0.04	61 ± 145
Glutamate	349 ± 112	312 ± 109	338 ± 132	−0.055	0.031	259 ± 187
Glutamine	562 ± 246†	550 ± 194†	647 ± 298	0.107	0.208*	694 ± 147
Glycine	331 ± 126	314 ± 102	361 ± 125	0.173*	0.233**	336 ± 108
Histidine	115 ± 38	125 ± 44	117 ± 32	0.045	0.105	85 ± 11
Isoleucine	70 ± 22	70 ± 17	78 ± 27	0.196*	0.183*	74 ± 16
Leucine	153 ± 46†	154 ± 35†	164 ± 49	0.139	0.218*	183 ± 69
Lysine	202 ± 60†	196 ± 52†	230 ± 82‡	0.06	0.245**	256 ± 84
Methionine	29 ± 9†	29 ± 8†	33 ± 13	0.094	0.228**	32 ± 12
Phenylalanine	91 ± 29	88 ± 18	95 ± 20	0.101	0.129*	78 ± 13
Proline	234 ± 58†	244 ± 60†	269 ± 59‡	0.206*	0.210*	270 ± 84
Serine	181 ± 57†	187 ± 71†	227 ± 69‡	0.248**	−0.034	218 ± 62
Threonine	98 ± 35	105 ± 27	117 ± 49	0.177*	0.275**	98 ± 124
Tryptophan	56 ± 20†	59 ± 16†	70 ± 20‡	0.197*	0.220*	72 ± 14
Tyrosine	78 ± 24	79 ± 18	93 ± 20‡	0.256**	0.351**	71 ± 14
Valine	157 ± 45†	152 ± 37†	176 ± 44†,‡	0.193*	0.171	265 ± 75

[1]The data are expressed as mean ± SD (μmol/L) or Spearman correlation coefficient. LVZ, length velocity *z*-score; WLZ, weight for length *z*-score. *Spearman correlation coefficient significant at the $P < 0.05$ level (2-tailed) and **at the $P < 0.01$ level (2-tailed). *P* values corrected for multiple comparisons using Benjamini–Hochberg method. †denotes values that are less than the corresponding value for the German comparison group, $P < 0.05$. ‡denotes values that are greater than the corresponding value for infants with linear growth velocity < 0 *z*-scores, $P < 0.05$.

Discussion

These data demonstrate that serum AA concentrations are higher in 12-mo-old Malawian infants who have more normal growth trajectories and weight-for-height, supporting the notion that an important biological relation between these parameters exists in this population.

Fasting serum AA concentrations have been shown to increase with increased AA intake in infants, although the 2 are not directly proportional (18, 19). Outside of extraordinary clinical conditions, dietary AA intake is believed to be the primary determinant of serum AA concentration. Age and common illnesses are known to affect serum AA concentrations acutely, and some care was taken in this study population to exclude these sources of variation (20). However, we cannot be completely certain that no occult conditions were present and contributed to the variation of serum AA concentrations.

Limitations of these data are many. The study population was from a limited demographic and thus the data should be extrapolated to other populations with caution and skepticism. AA concentrations are not well characterized in African children; the diurnal variation in AA concentration among the study and comparison populations is unknown, and the differences between groups of children who consume distinctly different diets have not been explored. In particular, the Malawian children were likely to have been breastfed about every 2 h, providing a relatively constant source of protein intake upon which the bolus feedings of meals was superimposed. This might be important in that the samples were not collected in a truly fasted state, and postprandial AA concentrations are known to increase 1.5 fold in older individuals (21). This limits our ability to compare with certainty our numerical data with children who are not breastfed. We acknowledge that there are many confounders we have not considered, such as underlying subclinical gut inflammation and the complexity of the diet.

The clinical data available from the comparison group of German children were anthropometric measurements taken at the time of sampling; no growth velocity data were available. The AA concentration data were included to provide the reader with typical values for serum AA concentrations in a reference population without growth faltering. The environment and diet of the German children were likely different in many respects, which precludes drawing inferences beyond this simple comparison. If longitudinal data were available from a reference population, they might strengthen the inferences made about growth velocity and serum AAs.

This study used an accurate, commercial analytical method for AA concentration that has been standardized across diverse laboratories, which was not previously available (22). This tool enables absolute values of a given AA to be compared with confidence across many diverse populations without requiring the analyses to have been conducted in the same laboratory at the same time. This method has been shown to work on dried blood spots (23), which would allow the studies to be conducted in resource-poor settings. This new tool will allow scientists to better understand the meaning and importance of circulating AAs in terms of nutritional well-being.

Because AAs are not largely interconverted by human metabolic pathways in response to metabolic demand, AAs might best be regarded as individual nutrients. The analytical assay used in this study measures each AA independently, and the knowledge generated could lead to important advances for nutritionally vulnerable populations with sub-optimal AA intake. The concentration of a selected number of essential

AAs—perhaps leucine, lysine, methionine, tryptophan, and valine—might be tested for use as biomarkers of the dietary protein quality.

These data associate a biochemical measurement at a time point with growth over a 6-mo period, which limits inferences of causality. The interpretation of these data is more complex than "Malawian children will benefit from more essential AAs in their diet." The study population consumed a fairly monotonous diet, in which every child was estimated to receive ~7 g of breast milk protein, which is of very high quality. The remainder of the dietary protein largely came from plant sources, particularly cereals and legumes, and total protein intake was measured to be ~15 g/d, which is well above the recommended dietary intake of 9.1 g/d. The correlations between all essential AAs and weight-for-height *z*-score and growth velocity support testing the hypothesis that provision of more dietary essential AAs will improve growth in rural African children.

Provision of AAs to vulnerable populations in the 21st century is possible through routes other than consumption of foods with different protein content. Individual AAs can be made commercially in an economical manner by chemical synthesis and genetic engineering of microorganisms (24), and these individual AAs can be added as supplements to basic foodstuffs, similar to micronutrient supplementation of cereal flours. Altering the populations of the gut microbiota to enhance the synthesis of essential AAs, which would then be absorbed, is another route via which AAs might be delivered to humans outside of food (25).

In spite of these uncertainties surrounding this small observational dataset, we offer the following conclusions. Most of the serum AA and essential AA concentrations correlate with growth in this population of 12-mo-old rural African infants. Growth faltering in this population is a public health problem of the largest dimensions. One intervention to increase AA concentrations is to increase dietary AA intake. Clinical trials that are nuanced in design to elucidate effects of individual AAs, yet operationally conducted in the relevant, vulnerable populations, are needed to understand the human health benefits of AA intake. Assessment of individual AA concentrations could be a key element of such trials.

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