Lysine Requirements of Moderately Undernourished School-Aged Indian Children Are Reduced by Treatment for Intestinal Parasites as Measured by the Indicator Amino Acid Oxidation Technique

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Abstract

Background: Lysine requirements of well-nourished children from developing regions have been found to be similar to those of children from developed regions (33.5 mg·kg⁻¹·d⁻¹). However, intestinal parasites have been shown to increase lysine requirements in undernourished adults, and it is not known if a similar phenomenon occurs in undernourished children from poor and unsanitary environments.

Objective: Our objective was to measure the lysine requirement of moderately undernourished school-aged Indian children by the indicator amino acid oxidation technique before and after successful treatment for intestinal parasites.

Methods: Twenty-one undernourished school-aged children (8-12 y of age) with z scores between −2 SD and −3 SD for height-for-age or weight-for-age, who tested positive for intestinal parasites, were studied before and after successful antiparasitic treatment. Children were fed any 2 of 7 levels of lysine intakes (5, 15, 25, 35, 50, 65, and 80 mg·kg⁻¹·d⁻¹) in random order. The lysine requirement was determined by applying a 2-phase linear regression crossover analysis on the fractional oxidation rate of the tracer L-[1-¹³C] phenylalanine in response to the graded lysine intakes.

Results: The lysine requirement of undernourished children with intestinal parasite infestations was determined to be 42.8 mg·kg⁻¹·d⁻¹ (95% CI: 32.6, 53.1 mg·kg⁻¹·d⁻¹), and after successful antiparasitic treatment it was determined to be 35.5 mg·kg⁻¹·d⁻¹ (95% CI: 25.5, 45.5 mg·kg⁻¹·d⁻¹). The results were significantly different (P < 0.05), although the 95% CIs overlapped.

Conclusions: The lysine requirement in undernourished children is similar to that of well-nourished children, and intestinal parasitic infestation increased the lysine requirement by ~20%.

Keywords: lysine, requirement, undernourished children, intestinal parasites, IAAO

Introduction

Requirements for lysine have important implications on the assessment of the nutritional protein quality of diets, especially in poor, vulnerable populations that subsist on cereal-based diets that supply a major portion of their energy and indispensable amino acid intake (1, 2). Using the minimally invasive indicator amino acid oxidation (IAAO) method, we recently determined the mean daily lysine requirement of well-nourished Indian school-aged children to be 33.5 mg·kg⁻¹·d⁻¹ (3), which was similar to the requirement of western (Canadian) children (35 mg·kg⁻¹·d⁻¹) (4). This similarity of requirements was observed in adults as well (5, 6). According to WHO estimates, malnutrition, as measured by stunting, affects 32.5% of children in developing countries (7). The prevalence of underweight children in India is among the highest in the world; ~47% of the children are malnourished (8), and ~25% of them suffer chronic undernourishment as reflected by stunting or low height-for-age. In addition, infections have an adverse effect on metabolism of most nutrients, including protein and amino acids, and the actual requirement of indispensable amino acids may be increased because of catch-up growth (9), subclinical infections caused by chronic immunostimulation, parasitic infestations, and poor hygiene (10, 11).
Among all infestations, intestinal parasitic infection is commonly found in developing countries. It was reported that rural South Indians have very high rates of intestinal parasitic infestations (12–14). The prevalence rate of single and/or multiple intestinal parasitic infestations among Indian school-aged children ranges from 12% to 70% (12). Intestinal parasites alter net nutrient absorption mainly by diverting nutrients for their own need, therefore, less is available for absorption by the host (15). Furthermore, parasite-induced mucosal inflammation and villous atrophy over time impair nutrient absorption (15). Previously, intestinal parasites have been shown to increase the lysine requirement by ~50% in chronically undernourished adult men (16, 17). However, this phenomenon has not been demonstrated in chronically undernourished growing children.

Therefore, the objective of the current study was to determine the lysine requirements of moderately undernourished (otherwise apparently healthy) school-aged children from low/middle-class socioeconomic status, who tested positive for intestinal parasites with use of the IAAO method (18, 19). The effect of antiparasite treatment on the lysine requirements in children was determined in the same set of children by measuring the rate of phenylalanine oxidation at lysine intake levels that were above and below the requirements previously ascertained for well-nourished children (3, 4).

Methods

Participants and anthropometry. Children were recruited from local schools situated within 20 km from St. John’s Research Institute, and all were from low- to middle-class socioeconomic status (20). Ninety-five children were screened for study recruitment. Twenty-one moderately undernourished school-aged children (13 boys and 8 girls) were recruited for study participation based on their z scores: between −2 SD and −3 SD for height-for-age and/or weight-for-age (Table 1). In addition, normal serum albumin concentration (≥4 g/dL) and testing positive for intestinal parasites were used as selection markers to identify study participants. At the time of recruitment, children were apparently healthy and free from infections, and had no recent history of illness.

All experiments were conducted at the metabolic ward of the Division of Nutrition, St. John’s Medical College and Research Institute, Bangalore, India. Subject weight was measured in minimal clothing to the nearest 0.1 kg with a digital scale (Morgen Lab Ltd.). Subject height was recorded to the nearest 0.1 cm (Raven). The skinfold from 4 sites (biceps, triceps, subscapula, and suprailium) was measured in duplicate to the nearest 0.2 mm with a skinfold caliper (Holtain), and body fat composition was calculated with use of standard equations (21) (Table 1). The institutional ethical review board at St. John’s Medical College and Hospital and the research ethics board at The Hospital for Sick Children (SickKids), Toronto, Canada, approved all study procedures. Permission was obtained from the school authority or principal before approaching the parents, and written informed consent from parents with an oral assent of the child were obtained at study enrollment.

Fecal sample collection, analysis for parasites, and treatment. Fecal samples were collected at 2 time points, at the time of screening and after successful antiparasitic treatment. Field staff was trained in proper fecal sample collection procedures (22, 23); however, at the time of consent each child’s mother was also provided instructions on how to collect the samples, if they preferred to do the collection at home. In this case, mothers or caregivers were provided a sterilized plastic container labeled with the child’s name and identification number. Once the sample was collected it was delivered within 3 h to the microbiology laboratory at St. John’s Medical College Hospital for parasitic analysis.

Three consecutive day fecal samples were collected for intestinal parasite analysis including cysts, ova, worms of intestinal helminths, and protozoa. Samples were analyzed with use of the semiquantitative Kato-Katz thick-smear technique (22, 23) similar to the previous study in adult men (17). After completion of the first set of studies, children were provided a single dose of albendazole (400 mg) and a twice-daily dose of tinidazole (50 mg · kg⁻¹ · d⁻¹) for 3 d for the eradication of helminths, giardiasis, and amebiasis infection. Effectiveness of the treatment was ensured with subsequent fecal samples testing negative for ova and cysts (17). In some cases, the treatment was extended to 7 d to achieve a complete eradication of parasitic infestations, and then subjects participated in the post-treatment experiments.

Experimental design and study diets. Based on the screening criteria for anthropometry (−2 SD to −3 SD for height-for-age and/or weight-for-age), biochemical measure (normal serum albumin concentration), and testing positive for intestinal parasitic infections, children were recruited for the study. After an overnight fast, resting energy expenditure and body composition analysis were measured as previously described (3). During the pre-parasitic treatment tracer studies, each child was assigned to any 2-test lysine intakes (5, 15, 25, 35, 50, 65, and 33.5–35 mg lysine d⁻¹ b a s e d o n3 - df o o dr e c o r d s . A l lc h i l d r e nc o n s u m e da)

| TABLE 1 Anthropometric, metabolic, and dietary characteristics of undernourished Indian school-aged children (n = 21) |
|-----------------|-----------------|
| General characteristics | Values |
| Age, y | 8.3 ± 0.6 |
| Weight, kg | 19 ± 1.4 |
| Height, cm | 119.1 ± 5.3 |
| Weight-for-age z scores | −2.65 ± 0.3 |
| Height-for-age z scores | −2.04 ± 0.6 |
| BMJ, kg/m² | 13.4 ± 0.7 |
| REE, kcal/d | 968 ± 96 |
| Energy intake, kcal/d | 1646 ± 163 |
| FFN, kg | 16.7 ± 1.7 |
| Body fat, % | 14.9 ± 3.5 |
| Albumin, g/dL | 4.4 ± 0.2 |

1 Values are means ± SDs. FFM, fat-free mass; REE, resting energy expenditure.
phenylalanine (indicator amino acid) and NaH\textsuperscript{13}CO\textsubscript{3} (99%; Cambridge Isotope Laboratories) were provided orally starting with the fifth meal, as a prime dose (0.176 mg/kg of NaH\textsuperscript{13}CO\textsubscript{3} and 1.09 mg/kg of L-[\textsuperscript{1-13C}] phenylalanine) and subsequent continuous doses of L-[\textsuperscript{1-13C}] phenylalanine (1.958 mg/kg) hourly. The amount of phenylalanine (25 mg · kg\textsuperscript{-1} · d\textsuperscript{-1}) and tyrosine (61 mg · kg\textsuperscript{-1} · d\textsuperscript{-1}) was kept constant throughout (3). The rate of CO\textsubscript{2} production was also measured by indirect calorimetry immediately after the fifth meal.

Collection and analysis of samples. Breath samples were collected with use of a breath bag designed for children (Quintron; Terumo Medical), and urine samples were collected before and after oral isotope protocol as described previously (3). Breath and urine samples were stored and analyzed as described previously (3). Isotopic enrichment of breath was expressed as atom percent excess, and urinary enrichment was expressed as moles percent excess.

Tracer kinetics. The rate of L-[\textsuperscript{1-13C}] phenylalanine oxidation was calculated as the fraction of tracer dose of phenylalanine oxidized (F\textsuperscript{13CO\textsubscript{2}}) (3, 4). This is represented by the rate of \textsuperscript{13}CO\textsubscript{2} released by tracer phenylalanine administration (\mu mol · kg\textsuperscript{-1} · h\textsuperscript{-1}) expressed as a fraction of the rate of tracer phenylalanine administration. This was calculated by the following equation (24):

\[ F_{13CO2} = (VCO2 \times 13CO2 \times R)/d \]

where the rate of \textsuperscript{CO2} production is the carbon dioxide production rate (\mu mol · kg\textsuperscript{-1} · h\textsuperscript{-1}), \textsuperscript{13}CO\textsubscript{2} is the breath enrichment in atoms percent excess, R is the recovery factor for carbon dioxide, which was assumed to be 0.82, and d is the dosing rate of tracer phenylalanine (\mu mol · kg\textsuperscript{-1} · h\textsuperscript{-1}).

Whole-body phenylalanine flux was calculated from the dilution of L-[\textsuperscript{1-13C}] phenylalanine in the body amino acid pool at isotopic steady state with use of the following equation (3, 4):

\[ Q = d \times \left[ \frac{E1/Eu - 1}{S2} \right] \]

where Q is the phenylalanine flux (\mu mol · kg\textsuperscript{-1} · h\textsuperscript{-1}), d is the rate of L-[\textsuperscript{1-13C}] phenylalanine dosed (\mu mol · kg\textsuperscript{-1} · h\textsuperscript{-1}), and E\textsubscript{1} and E\textsubscript{u} are the isotopic enrichments as mole fractions (mole percent excess) of the infused and urinary phenylalanine, respectively, at isotopic plateau.

Statistical analysis. Results are expressed as means ± SDs. A mixed linear model with subject as a random variable using PROC MIXED (SAS/STAT, version 9.0; SAS Institute) was used to analyze the effect of lysine intake on the isotopic enrichments as mole fractions (mole percent excess) of the rate of tracer phenylalanine administration. This was calculated by the following equation (24):

\[ F_{13CO2} = (VCO2 \times 13CO2 \times R)/d \]

where the rate of \textsuperscript{CO2} production is the carbon dioxide production rate (\mu mol · kg\textsuperscript{-1} · h\textsuperscript{-1}), \textsuperscript{13}CO\textsubscript{2} is the breath enrichment in atoms percent excess, R is the recovery factor for carbon dioxide, which was assumed to be 0.82, and d is the dosing rate of tracer phenylalanine (\mu mol · kg\textsuperscript{-1} · h\textsuperscript{-1}).

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Results

Subject characteristics. Twenty-one moderately undernourished school-aged children, 13 boys and 8 girls, completed the study (Table 1). All children remained in apparent good health throughout and no adverse events were reported. From the initial 95 selected undernourished children, 21 screened positive for intestinal parasites; thus, the overall parasite prevalence rate was 22.1%. The most common parasites found in the study children were Giardia lamblia (cytis) infections with 33.3% prevalence and Entamoeba histolytica and Entamoeba coli (cytis) with a 33.2% prevalence rate (Table 2); nevertheless, all children were asymptomatic.

Indicator amino acid (L-[\textsuperscript{1-13C}] phenylalanine) oxidation and flux. The oxidation of L-[\textsuperscript{1-13C}] phenylalanine was measured by breath (\textsuperscript{13}CO\textsubscript{2}) isotope enrichment, which decreased substantially in response to the graded test lysine intakes similar to our earlier studies (3, 4) (Figure 1). According to the principle of the IAAO method, when the test indispensable (lysin) intake was low, increased oxidation rate of L-[\textsuperscript{1-13C}] phenylalanine (indicator amino acid) was observed in both pre- and post-antiparasite-treated children, with a linear decline until lysine intake was between 35 and 50 mg · kg\textsuperscript{-1} · d\textsuperscript{-1}. Further increases in test lysine intake had no effect on phenylalanine oxidation.

Two-phase linear regression crossover analysis of \textsuperscript{13}CO\textsubscript{2} was used to identify the inflection point—breakpoint, which represents the mean (estimated average requirement) lysine requirement. Before treatment the breakpoint in children was identified at 42.8 mg · kg\textsuperscript{-1} · d\textsuperscript{-1} (95% CI: 32.6, 53.1; r\textsuperscript{2} = 0.60) (Figure 1), and after treatment the breakpoint was identified at 35.5 mg · kg\textsuperscript{-1} · d\textsuperscript{-1} (95% CI: 25.5, 45.5; r\textsuperscript{2} = 0.55) (Figure 1). The upper 95% CI represents the RDA and was 53.1 mg · kg\textsuperscript{-1} · d\textsuperscript{-1} (before treatment) and 45.5 mg · kg\textsuperscript{-1} · d\textsuperscript{-1} (after treatment). Comparison of the mean lysine requirements of children before and after treatment revealed significant differences (P < 0.05), although the 95% CIs overlapped.

Phenylalanine flux was not affected by different lysine intakes at both times of pre- and postparasite treatment (Table 3), which is necessary for the IAAO method (7, 26).

Discussion

This study determined the mean and upper 95% CI lysine requirements in moderately undernourished school-aged Indian children from low socioeconomic environments carrying intestinal

\[ \text{TABLE 2} \]

Prevalence of intestinal parasitic infestations in undernourished Indian school-aged children (n = 21)

<table>
<thead>
<tr>
<th>Intestinal parasites</th>
<th>Values, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entamoeba histolytica and Entamoeba coli (cytis)</td>
<td>33.2</td>
</tr>
<tr>
<td>Giardia lamblia (cytis)</td>
<td>33.3</td>
</tr>
<tr>
<td>Enterobius vermicularis (adult worm and ova)</td>
<td>9.5</td>
</tr>
<tr>
<td>Hymenolepis nana (eggs)</td>
<td>4.8</td>
</tr>
<tr>
<td>Giardia lamblia and Entamoeba histolytica (cytis)</td>
<td>4.8</td>
</tr>
<tr>
<td>Entamoeba histolytica, Hymenolepis nana, and Trichuris trichiura (cytis and eggs)</td>
<td>4.8</td>
</tr>
<tr>
<td>Ascaris lumbricoides, Entamoeba coli, and Trichuris trichiura (ova and cysts)</td>
<td>4.8</td>
</tr>
<tr>
<td>Ascaris lumbricoides and Giardia lamblia (ova)</td>
<td>4.8</td>
</tr>
</tbody>
</table>

\footnote{All subjects were asymptomatic.}

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parasites of 42.8 and 55.5 mg·kg\(^{-1}\)·d\(^{-1}\), respectively. The mean lysine requirement estimate is \(\sim 20\%\) higher than the earlier estimates of lysine requirements of 33.5 mg·kg\(^{-1}\)·d\(^{-1}\) in healthy Indian children (3) and 35 mg·kg\(^{-1}\)·d\(^{-1}\) in Canadian (4) children determined with use of the same IAAO method. However, treatment for intestinal parasite infestation resulted in a significant \((P < 0.05)\) reduction in the mean daily requirement of lysine in the same children to 35.5 mg·kg\(^{-1}\)·d\(^{-1}\). The post-treatment lysine requirement estimate is in line with the current WHO/FAO/United Nations University recommendation of 35 mg·kg\(^{-1}\)·d\(^{-1}\) (9) for this age group and also compares well to that of the mean daily lysine requirement of well-nourished Indian and Canadian children (3, 4). Previously, a similar phenomenon was demonstrated where higher lysine requirements of 44 mg·kg\(^{-1}\)·d\(^{-1}\) in chronically undernourished (otherwise apparently healthy) Indian adults normalized to 30 mg·kg\(^{-1}\)·d\(^{-1}\) after antiparasitic treatment (16, 17). To the best of our knowledge, this is the first empirical data on the lysine requirement of undernourished school-aged children who are dependent on predominantly cereal-based diets.

The prevalence of intestinal parasitic infestations among Indian school-aged children in various parts of India has been reported to be \(\sim 21–22\%\) (27–30), which is similar to our present study prevalence of 22.1%. Generally, children from low socioeconomic backgrounds are prone to chronic parasitic infections because of the interaction between poor diet, poor quality of drinking water, and in general, a poor environment (29). Previous reports have suggested that infections can potentially increase nutrient requirements (10, 26), although, to our knowledge, this study is the first to demonstrate that intestinal parasites increase the lysine requirement by \(\sim 20\%\) in undernourished children. This increase is comparatively less than the 50% increase in lysine requirements observed in adult undernourished Indian men (17). The likely explanation, as suggested by Crompton and Nesheim (26), is that the nutritional impact of parasites is directly related to the intensity of the infection. The prevalence of parasitic infestation was \(\sim 45\%\) in the adult men (17) compared to \(\sim 21\%\) in school-aged children. Furthermore, in the adult men \(\sim 30–50\%\) of the type of infestation was found to be due to helminthics (Ascaris lumbricoides and Trichuris trichiura), whereas in the current study the children were mostly found to have protozoan parasites (G. lambia and E. histolytica). This is important because intestinal morphologic changes have been reported to be different with Giardia infection with villous flattening and lowering of the villous-to-crypt ratio, which are reversible (31–34). Trichuris and other tapeworm infections, on the other hand, tend to damage the villous at the point of attachment to the intestinal wall, and concomitant bacterial infections are established because of the damage in the intestinal wall (26). Nevertheless, in both studies it is important to note that all subjects were asymptomatic, and the increase in lysine requirements was substantial in the presence of intestinal parasite infestation when compared with the treated subjects.

The splanchnic use, primarily the small intestine, of lysine was reported to be \(\sim 30\%\) of whole-body lysine oxidized and may affect the systemic availability of lysine for protein synthesis and other functions (35–37). Indeed, lysine requirements in neonates who are enterally fed were found to be \(\sim 23\%\) higher, 130 mg·kg\(^{-1}\)·d\(^{-1}\), when compared to 105 mg/kg/d in parenterally fed neonates (38, 39). In addition, parasite infections induce chronic immunostimulation (40), which would increase the splanchnic use of lysine for synthesis of acute phase proteins, which in turn would reduce the availability of lysine for growth. Helminth infections have been shown to stimulate IL 4–dependent polyclonal synthesis of IgE, and it was hypothesized that undernourishment diminishes the specific IgE antibody response (40), which was implicated in immune evasion by the parasites (41). In the adult study, plasma IgE concentrations were found to be 10-fold higher in infected subjects compared...
with normal subjects (16, 17), although this was not measured in the current study’s children. Thus, intestinal parasites exacerbate small intestinal demand for lysine and increase the lysine requirements. Threonine is an amino acid that plays a key role in gut mucin synthesis (42), especially during sepsis (43). Intestinal threonine use was shown to be very high in neonatal piglets (36) and infants (44). It is possible that threonine requirements might be affected during childhood malnourishment and should be explored in future studies.

Lysine is the first limiting amino acid in cereal-based diets (45); thus, lysine supplementation and fortification was attempted as a strategy to improve the protein quality of such diets (46–49) and positively affect child health. Lysine supplementation at 1 g/d for 16 wk in a double-blind, randomized trial was shown to reduce diarrheal episodes and the total number of ill days in children in Ghana (46). Similarly, lysine fortification of wheat flour to families living in China, Pakistan, and Syria for 3 mo improved some aspects of nutritional status (serum transferrin, prealbumin) and immune function (CD4, CD8, and complement C3) when compared with controls (47–49). No such studies have been published specifically targeting young children who are chronically undernourished with subclinical parasite infestation, and future studies need to be conducted. Particularly in India, where the average diet provides ~80% of the protein from cereal/grain-based sources (45, 50, 51), lysine supplementation may have potential benefits. We estimated previously that the mean lysine intake of 7-year-old, lower socioeconomic class, Indian children, weighing ~20 kg, was ~48 mg · kg⁻¹ · d⁻¹ with an interindividual variability of 20% (3). This would mean that ~10% of children would be at risk of dietary lysine deficiency. In the presence of intestinal parasites and an increase of 20% in the lysine requirements, this would likely make the risk of deficiency considerably higher.

In summary, the lysine requirement in moderately undernourished Indian children with parasites was determined to be 42.8 mg · kg⁻¹ · d⁻¹, and the mean lysine requirement after parasitic treatment was determined to be 35.5 mg · kg⁻¹ · d⁻¹. The lysine requirement of treated children is not different from that estimated in well-nourished Indian children (3) and Canadian children (4) of 33.5 and 35 mg · kg⁻¹ · d⁻¹, respectively. Thus, there is no difference in lysine requirements in school-aged children with different ethnicity and healthy or moderately undernourished nutritional status. However, intestinal parasite infestation increases the lysine requirement by ~7 mg · kg⁻¹ · d⁻¹ (~20%) and is evidence that subclinical infections negatively affect lysine needs and potentially affect growth in children living in poor and unsanitary conditions.

Acknowledgments

RRP conducted the research, analyzed the data, and wrote the paper; RE, ROB, AKV, and PBP designed the research (project conception, development of overall research plan, and study oversight), analyzed the data, and wrote the paper; and RE had primary responsibility for final content. All authors read and approved the final manuscript.

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