Intestinal parasites increase the dietary lysine requirement in chronically undernourished Indian men[1–3]

Anura V Kurpad, Meredith M Regan, Dilip Nazareth, Savita Nagaraj, Justin Gnanou, and Vernon R Young

ABSTRACT

Background: We previously used the 24-h indicator amino acid balance method to show that the lysine requirement in undernourished Indian men from low socioeconomic and unsanitary environments is ≈50% higher than the mean requirement of 30 mg lysine · kg^{-1} · d^{-1} in well-nourished men.

Objective: It is possible that this higher lysine requirement in persons with chronic undernutrition is due to environmental influences, including the presence of intestinal parasites. We assessed this possibility by using 24-h indicator amino acid balance (with leucine) at both the "normal" requirement for lysine intake and the higher requirement, before and after successful treatment to eradicate intestinal parasites in affected, undernourished men.

Design: Fourteen chronically undernourished men were studied before and after treatment for intestinal parasites, during each of two 7-d (6-d dietary adaptation plus 1-d tracer experiment) diet periods supplying either 30 ($n = 7$) or 45 ($n = 7$) mg lysine · kg^{-1} · d^{-1} from an L-amino acid diet. Twenty-four–hour indicator amino acid balance was estimated on day 6 by [^{13}C]leucine tracer infusion.

Results: Before the parasite treatment, subjects were in neutral 24-h leucine balance at both lysine intakes. After the eradication of intestinal parasites, there was a significant ($P < 0.001$) improvement in 24-h leucine balances, which were positive at both lysine intakes.

Conclusions: On the basis of the 24-h indicator amino acid balance approach, it appears that intestinal infestation with parasites increases the requirement for lysine and that this may be one factor responsible for the higher lysine requirement observed in persons with chronic undernutrition. *Am J Clin Nutr* 2003;78:1145–51.

KEY WORDS Chronically undernourished Indian men, lysine requirement, indicator amino acid oxidation, indicator amino acid balance, intestinal parasites

INTRODUCTION

We earlier measured the lysine requirement in chronically undernourished adult South Asian men (1) with the use of the 24-h indicator amino acid oxidation and balance methods (2). Although the subjects were chronically undernourished, came from poor socioeconomic backgrounds, and had low habitual protein and lysine intakes, we found their lysine requirements to be ≈50% higher, when expressed per kilogram of body weight, than the requirements in otherwise similar, but healthy, well-nourished South Asian (Indian) men (3, 4). This was in contrast to the possibility that the daily lysine requirement would decrease in adaptation or accommodation to habitually low lysine intakes.

We proposed that the higher lysine requirement in the undernourished subjects was linked to their body composition, because they had less muscle mass (and, therefore, a relatively greater visceral mass) than did healthy, well-nourished subjects (1). If the daily lysine requirement were partitioned into a muscle and visceral compartment requirement, and if the visceral or splanchnic need for lysine (and possibly other amino acids) were higher than the need of muscle, then the muscle-to-visceral organ ratio of the fat-free mass would be an important factor in the determination of amino acid requirements. By extrapolation from this hypothesis, it is also possible that the visceral or splanchnic demand for amino acids is independently increased because of other environmental factors, such as injuries (5) or immunostimulation (6).

The prevalence of intestinal infestation in rural and urban communities in India is reported to be high. A study of rural South Indian subjects found that 97.4% excreted parasite eggs, and 74.3% had multiple parasitic infestations (7). It is even more likely that subjects from urban slums would have intestinal parasites, because sanitation is very poor or nonexistent in such environments. In other studies in South India, the prevalence of nemathelminth, hookworm, and pinworm infestations was also high, ranging from 30% to 60% for different parasites and areas (8–10). Similar results have also been reported in South Asian refugees arriving in Stockholm, where one-half of the subjects had parasitic infestations (11). The effect of chronic intestinal parasitic infestations on nutrient requirements in chronic undernutrition, specifically with regard to the requirement of essential amino acids, is not known, and it is possible that this environmental influence could, through direct (diversion of nutrients) or indirect (hyperplastic intestinal epithelial response) means, increase these requirements. There-

TABLE 1
Characteristics of the study subjects[1]

Characteristic	Value
Age (y)	20.1 ± 1.3
Weight (kg)	43.3 ± 3.4
Height (m)	1.60 ± 0.03
BMI (kg/m^2)	16.8 ± 1.1
Midupper arm circumference (cm)	22.8 ± 1.8
Percentage body fat (%)	10.5 ± 1.6
Fat-free mass (kg)	38.8 ± 2.9

[1] $\bar{x} \pm$ SD; $n = 14$.

fore, this study was designed to assess the effect of antiparasite treatment on the leucine balance at lysine intakes that approximated the requirement for normal well-nourished and healthy adults (3, 4, 12, 13) and at the higher requirement that we have ascertained for chronically undernourished adults (1).

SUBJECTS AND METHODS

Subjects and anthropometry

Fourteen undernourished but otherwise healthy adult male subjects participated in this experiment. The subjects were selected after screening to confirm the presence of intestinal parasites. Their chronic undernutrition status was determined on the basis of a body mass index (in kg/m^2) < 18.5 and low socioeconomic status (14, 15). Fecal samples from the recruited subjects were tested (*see* below) to detect the presence of intestinal parasites such as protozoa, nematodes, and helminths. The subjects were weighed to the nearest 0.1 kg, and their height was measured to the nearest 1.0 cm. The logarithm of the sum of 4 (biceps, triceps, subscapular, and suprailiac) skinfold thicknesses was used in age- and sex-specific equations (16) to obtain an estimate of body density, from which percentage body fat and fat-free mass were estimated (17; **Table 1**). The purpose of the study and the potential risks involved were explained to each subject, and the Human Ethical Review Board of St John's Medical College approved the research protocol.

Fecal sample analysis for parasites

Fecal samples were screened for intestinal helminthic ova and cysts of intestinal protozoa within 1 h of collection (18) with the use of the semiquantitative Kato-Katz thick-smear technique (19, 20). Briefly, a portion of the fecal sample was sieved to remove fiber and coarse debris, and care was taken not to disperse fecal aerosols. A 50-mg portion of the sieved fecal sample was emulsified and placed on a clean glass slide, after which helminthic ova and protozoan cysts were enumerated in the entire smear. A similar smear, prepared as above, was stained with freshly prepared Lugol's iodine and examined for ova and cysts. This procedure was performed on all the fecal samples collected for screening and on those collected after the treatment for intestinal parasites. Samples that were found to be negative for ova and cysts after antiparasite treatment were concentrated by the formol ether concentration technique to rule out presence of a small number of ova or cysts (19).

TABLE 2
Composition of the L-amino acid mixtures used to supply 2 daily amounts of lysine

Amino acid	Lysine intake	
	$30 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$	$45 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$
	mg/g mixture	
L-Tryptophan	18.43	19.22
L-Threonine	55.63	58.03
L-Isoleucine	74.22	77.42
L-Leucine[1]	36.09	35.70
L-Lysine HCl	33.34	49.47
L-Methionine	35.06	36.57
L-Cystine	26.00	27.12
L-Phenylalanine	64.57	67.35
L-Tyrosine	48.12	50.19
L-Valine	83.00	86.57
L-Histidine HCl	36.23	37.79
L-Arginine HCl	89.28	93.13
L-Alanine	101.12	80.65
L-Aspartic acid	14.09	14.70
L-Glutamic acid	34.82	36.36
Glycine	107.14	80.71
L-Proline	47.62	49.67
L-Serine	95.24	99.34
Total[2]	1000.00	1000.00

[1] Leucine (9.44 mg · kg^{-1} · d^{-1}) was added to each mixture every day, except on the infusion day, when the same amount of leucine was infused as tracer.

[2] Subjects received $\approx$ 1.13 g mixture · kg^{-1} · d^{-1}, which provided 160 mg N · kg^{-1} · d^{-1}.

Treatment for intestinal parasites

Treatment for parasites consisted of a single 400-mg dose of albendazole, which has been shown to be effective in eradicating mixed helminthic infection to $\approx$98% in mild to moderate infestations (21). Giardiasis and amebiasis were treated by the administration of a single 2-g dose of tinidazole (22). The efficacy of the treatment had been verified in an earlier pilot experiment in which subjects with parasites were treated by the regimen described above, and egg counts were performed on 3 consecutive stool specimens (23) as described above (21), both before the treatment and 4 d after the treatment. The treatment was successful in that no parasite eggs were detected 4 d after the administration of the drugs.

Diet and experimental design

Fourteen chronically undernourished subjects were studied during two 6-d diet adaptation periods, one before treatment for intestinal parasites and one after treatment. They consumed a weight-maintaining diet based on an L-amino acid mixture as previously described (1; **Table 2**) and were randomly assigned to receive a diet supplying either 30 ($n = 7$) or 45 ($n = 7$) mg lysine · kg^{-1} · d^{-1}. The diet periods were followed by a 24-h [^{13}C]leucine tracer study.

The subjects were studied under metabolic ward conditions and supplied daily with amounts of energy designed to maintain body weight during the diet period; the subjects were also encouraged to maintain their customary levels of physical activity by walking, using an exercise bicycle, or both, according to an activity schedule that was drawn up when the subjects

were admitted to the ward. The major energy supply was given in the form of a sugar-oil formula and as protein-free, wheat-starch cookies, and all other nutrients were provided in adequate amounts as described previously (2). During this 6-d dietary adaptation period, diets were supplied to the subjects in 3 meals/d. On the day of the tracer experiment, the diet was supplied in the form of 10 small hourly meals. The diet periods were separated by 2–4 wk; during this interval, subjects consumed their free-choice diets.

After the first diet period, the subjects were readmitted to the metabolic ward for a period of 10 d, and they were immediately put on an antiparasite treatment regimen as described above. During this period, the subjects were provided with diets that were similar to their habitual intake. The success of the antiparasite treatment was confirmed by fecal sample collections on day 3 after the treatment to ensure that the subjects were free of parasites. If parasites were detected, the dose was repeated, and fecal samples were checked until it was confirmed that there were no parasites in the sample. Immediately after this evaluation, the subjects continued into the second experimental diet period for a further 6 d, after which the second tracer study was done. Plasma samples were collected from the subjects before the tracer infusion in each phase to measure the plasma concentrations of immunoglobulin E (IgE; Immuno-Biological Laboratories, Hamburg, Germany) and C-reactive protein (DSL Laboratories, Webster, TX) by using an enzymatically amplified "2-step" sandwich-type immunoassay on an automated immunoassay system (Alpha Prime; SFRI Laboratoire, Saint Jean d'Illac, France).

24-h tracer-infusion protocol and sample collection

The primed 24-h intravenous [^{13}C]leucine approach was used, with indirect calorimetry and blood and breath sampling as previously described (1–4). Briefly, 1-[^{13}C]leucine (99.3 atom%; MassTrace, Woburn, MA) was given as a primed, constant intravenous infusion at a known rate of $\approx 2.8 \ \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ (the prime was $\approx 4.2 \ \mu\text{mol/kg}$) into an antecubital vein. The bicarbonate pool was primed with 0.8 μmol sodium [^{13}C]bicarbonate (99.9 atom%; MassTrace)/kg. The analyses of breath for $^{13}CO_2$ enrichment with the use of isotope ratio mass spectrometry (Europa Scientific Ltd, Crewe, United Kingdom) and blood samples for ^{13}C enrichments of plasma α-ketoisocaproic acid and leucine with the use of gas chromatography–mass spectrometry (Varian, Palo Alto, CA) were as previously described (4, 22).

Leucine kinetics and balance

Leucine oxidation ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot 30 \ \text{min}^{-1}$) was computed for consecutive half-hour intervals (4, 12) as the ratio of the $^{13}CO_2$ production rate ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot 30 \ \text{min}^{-1}$) to the plasma [^{13}C]$\alpha$-ketoisocaproic acid enrichment (mol percent excess) at that time, and the leucine flux was calculated as the ratio of the tracer infusion rate ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot 30 \ \text{min}^{-1}$) to the plasma [^{13}C]$\alpha$-ketoisocaproic acid enrichment (mol percent excess) at that time. Leucine balance ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) was computed as the leucine input (dietary leucine + intravenous tracer) minus the leucine output (sum of leucine oxidation at half-hourly intervals).

Statistical methods and data evaluation

Plasma concentrations of IgE and C-reactive protein were transformed by taking the natural log before analysis. Values are summarized as geometric means ± SDs and are compared, among all subjects, before and after treatment for parasites by using paired t tests.

The weight change and metabolic variables are presented as arithmetic means ± SDs, and they were analyzed by using mixed-models analysis of variance. The model for weight change over the 6-d experimental diet periods included a factor for treatment phase (pretreatment or posttreatment). The models for 12-h leucine oxidation and flux included treatment phase, metabolic phase (fasted or fed), lysine intake, and interactions among the 3 factors. Model contrasts were used to make pairwise comparisons of interest, as appropriate, based on the significance of the interactions and main effects. The model for 24-h indicator amino acid balance (leucine) included treatment phase, lysine intake, and the interaction; comparisons with zero balance were made by using the model. For each model, two-sided P values of 0.05 indicated significance for tests of interactions and main effects, and P values of pairwise comparisons were adjusted by using Tukey's method. The data were analyzed by using SAS software (version 8.2; SAS Institute Inc, Cary, NC).

RESULTS

Subjects

Of 51 subjects screened for intestinal parasites, 23 (45%) were positive. Fourteen subjects were selected from this group, of whom 7 had *Ascaris lumbricoides*, 2 each had *Giardia lamblia* and *Ankylostoma duodenale*, and 5 had *Trichuris trichura* infestations. One subject had *Entamoeba* sp, but all subjects were asymptomatic. Three of the subjects had mixed infestations. During the 6-d experimental diet periods, subjects experienced a small but significant ($P = 0.03$) average weight loss of 0.18 ± 0.26 kg across diet periods; there was no significant difference in weight loss between diet periods ($P = 0.94$).

Inflammatory response

Plasma total IgE concentrations were high before parasite treatment, with a mean value for all subjects of 1.8 ± 2.2 kIU/mL (range: 0.2–10.1 kIU/mL), and they did not show any significant decrease 10 d after the antiparasite treatment, with a mean of 1.7 ± 2.1 kIU/mL (range: 0.2–9.6 kIU/mL). Plasma C-reactive protein concentrations were not elevated and did not change significantly, with geometric means for all subjects of 0.29 ± 0.38 mg/L (range: 0.05–4.20 mg/L) and 0.35 ± 0.39 mg/L (range: 0.07–2.99 mg/L), before and after treatment, respectively. The differences between medians was 0.014, and the interquartile range (25th, 75th percentiles) was −0.22, 0.15.

Leucine oxidation, balance, and flux

For leucine oxidation, there were no significant interactions between treatment phase, metabolic phase, and lysine intake, but there were significant main effects (**Table 3**). Without regard to metabolic phase and lysine intake, leucine oxidation

TABLE 3
Summary of leucine oxidation, balance, and flux at 2 lysine intakes in chronically undernourished Indian men before and after treatment for intestinal parasites[1]

	Lysine intake			
	30 mg · kg^{-1} · d^{-1}		45 mg · kg^{-1} · d^{-1}	
Leucine index	Before treatment	After treatment	Before treatment	After treatment
Oxidation (mg leucine · kg^{-1} · d^{-1})[2]				
12-h Fasted	23.6 ± 3.7	21.2 ± 2.6	21.3 ± 1.4	20.1 ± 1.9
12-h Fed	28.5 ± 4.0	26.4 ± 2.7	28.8 ± 4.3	25.2 ± 2.3
Total (24 h)	52.1 ± 6.1	47.7 ± 4.6	50.1 ± 4.6	45.4 ± 2.6
Total intake (diet + tracer) (mg leucine · kg^{-1} · d^{-1})	49.6 ± 0.4	49.6 ± 0.6	49.6 ± 0.5	49.6 ± 0.5
24-h Balance[3]				
Intake − oxidation (mg leucine · kg^{-1} · d^{-1})	−2.5 ± 6.0	1.9 ± 4.4	−0.5 ± 4.5	4.2 ± 2.7
Percentage of intake (%)	−5.1 ± 12.1	3.9 ± 8.9	−1.0 ± 9.0	8.5 ± 5.5
Flux (μmol · kg^{-1} · 30 min^{-1})				
12-h Fasted	56.3 ± 5.9	55.5 ± 4.5	55.0 ± 6.6	55.2 ± 4.2
12-h Fed	56.6 ± 4.2	53.8 ± 3.9	55.8 ± 6.9	53.6 ± 5.3

[1] $\bar{x}$ ± SD; $n = 7$ per intake.

[2] Significant effects of treatment period (before treatment compared with after treatment) and metabolic phase (fasted compared with fed), each $P <$ 0.001; no interactions with or main effect of lysine intake (mixed-models ANOVA).

[3] Significant effect of treatment period, $P < 0.001$; no interaction with or main effect of lysine intake; significantly different from zero leucine balance after treatment ($P < 0.01$) but not before treatment (mixed-models ANOVA).

was significantly ($P = 0.0002$) higher before treatment than after treatment. Across treatment phases and lysine intakes, leucine oxidation was significantly ($P < 0.0001$) lower during fasting than during feeding. Across metabolic phases and treatment phases, there was no effect of lysine intake on leucine oxidation ($P = 0.22$).

With respect to leucine balance (Table 3), the results were essentially the same whether expressed as an absolute balance or as a percentage of leucine intake. There was no significant interaction between treatment phase and lysine intake, which indicated that the changes in daily leucine balance from before treatment to after treatment were similar for both lysine intakes. Daily leucine balance increased significantly after treatment ($P < 0.001$), regardless of lysine intake; the balance was not significantly different from the zero balance before treatment ($P = 0.29$), but it was significantly higher than the zero balance after treatment ($P < 0.01$). The leucine balances tended to be more negative or less positive at lysine intakes of 30 mg · kg^{-1} · d^{-1} than at those of 45 mg · kg^{-1} · d^{-1}, but these differences were not significant ($P = 0.26$). There were no effects of lysine intake, metabolic phase, or treatment phase on leucine flux.

DISCUSSION

The findings in the present study support our hypothesis that the higher requirement for lysine observed in adults with chronic undernutrition (1) is related to their living environment. The present subjects were similar to the subjects in our earlier study in terms of their anthropometry, socioeconomic condition, and antecedent dietary intake. We had proposed both that the higher lysine need, expressed per kilogram of body weight, of undernourished subjects was due to a relatively higher splanchnic requirement that resulted from a relatively lower muscle mass and the fact that chronic immunostimulation could increase the splanchnic fate of lysine and possibly the requirement independently, by increasing the synthesis and

turnover of acute phase proteins (1). This increase in requirement results from the fact that the immune response involves a cytokine-stimulated (24–26) increase in the synthesis of so-called positive acute phase proteins by the liver, which has to be supported either by amino acids derived from the diet or by the breakdown of body protein. An enhanced acute phase response has been observed in a study on otherwise clinically normal Indian slum dwellers, whose plasma concentrations of interleukin 6 and tumor necrosis factor α were higher than those of urban middle-class subjects (27). The increased requirement for amino acids also has important negative consequences during linear growth and weight gain, which are found to be less in children in underprivileged communities (28–31). The cause of this lower growth rate is likely to be multifactorial, reflecting the interactions of a poor diet and a poor environment, particularly the consequences of bacterial infections and parasitic infestations (32). During chronic immunostimulation, there is a partitioning of nutrients toward the support of the immune defenses as well as an effective reduction in the availability of nutrients for growth and other maintenance requirements (33).

The prevalence of parasitic infestation in our subjects was ≈45%, which is higher than the value of 12.5% that was reported for urban adults from north India (34) but similar to other such values reported earlier (35, 36). The subjects in the present study were all asymptomatic, and a similarly high asymptomatic positivity was also reported earlier (34). Most of the subjects had a high plasma IgE concentration, which did not decline significantly after treatment within the study period. Helminthic infections stimulate the interleukin 4–dependent polyclonal synthesis of IgE (37). It is thought that malnutrition (37) and poverty (38) potentiate the polyclonal stimulation of IgE synthesis, and, because the polyclonal stimulus diminishes the specific IgE antibody response, it has been implicated in immune evasion (39) by these parasites. We found a similar picture in our subjects, in whom the plasma IgE

concentration was some 10-fold higher than normal, which is suggestive of parasitic infestation rather than of atopy (40). Although we did not look for eosinophilia, which would have been indicative of allergic disease in our subjects, it is clear that some of the body's protein transactions were shifted toward this immune response.

After treatment for intestinal parasites, the leucine balance of the subjects rose from an approximate equilibrium (Table 3) to a distinctly positive balance at both intakes of lysine. This suggests that the physiologic requirement for lysine, in noninfected undernourished persons, is in the range of 30 mg $\cdot$ kg^{-1} $\cdot$ d^{-1} or possibly less, and that noninfected undernourished subjects will accrete tissue at this and the higher lysine intake. The present leucine balance data for the untreated groups are comparable to those reported earlier, in which there was considerable variation around the mean balance values at lysine intakes $\geq$ 30 mg $\cdot$ kg^{-1} $\cdot$ d^{-1} (1). In that study, linear regression analysis best summarized the leucine balance-lysine intake relation, estimating the mean intake of lysine for leucine equilibrium to be 44.4 mg $\cdot$ kg^{-1} $\cdot$ d^{-1}.

Studies in neonatal pigs (41) showed that intestinal lysine oxidation is significant and that it accounts for the utilization of a disproportionately large amount of dietary supply when protein intake is restricted. Thus, the lower efficiency of lysine utilization or a higher lysine requirement may be a consequence of the anatomic changes in the intestinal wall that accompany chronic intestinal parasitic infestations (42). Specifically, morphologic changes such as a reversible (43) villous flattening and lowering of the villus to crypt ratio are associated with Giardia infection (44), whereas tapeworm infestations are associated with villous damage at the point of attachment of the worm to the intestinal wall (45). Impaired nutrient absorption and increased losses from the gastrointestinal tract are considered to be causal factors in the nutritional disturbance created by these intestinal infections (46). If the intestinal absorption of amino acids improved after treatment, resulting in lower fecal losses, the improved leucine balances observed after treatment might have improved even further, but this would not alter the findings of this experiment.

As pointed out in the 1985 FAO/WHO/UNU report on energy and protein requirements (47), the prevalence in developing countries of malnutrition and common infections, particularly those of the gastrointestinal and respiratory tracts, is such that those conditions can be regarded as an ordinary part of life; this fact cannot be ignored in the assessment of requirements. However, although there is a considerable body of data on the qualitative effect of intestinal parasites and infections on protein and amino acid metabolism (48), there are essentially no quantitative estimations of the effect of parasitism on the physiologic requirement. We believe that the present study offers the opportunity to provide such a quantitative value. Thus, mean leucine balance was improved by $\approx$4.6 mg $\cdot$ kg^{-1} $\cdot$ d^{-1} after treatment for parasites at both levels of lysine intake. Assuming that the body ratio (by wt) of lysine:leucine in mixed protein is $\approx$1.0 (49), then lysine retention was also increased by 4.6 mg $\cdot$ kg^{-1} $\cdot$ d^{-1} as a result of the treatment. Furthermore, if the lysine intake is retained with an efficiency of $\approx$34%, based on our studies at submaintenance intakes of lysine in undernourished Indian men (1), that implies that the parasitic infection reduced the utilization of dietary lysine for protein synthesis by $\approx$14 mg $\cdot$ kg^{-1} $\cdot$ d^{-1}. This amount approximates 50% of the lysine requirement in healthy adults, presuming that

the leucine intake provided to these subjects was adequate, at $\approx$1.25 times the leucine requirement in undernourished subjects (50). In addition, that amount is quantitatively equivalent to the difference that we have identified (1, 3, 4) in the estimated mean requirements for lysine between healthy subjects and undernourished subjects who were infected with intestinal parasites but who apparently were otherwise clinically well. Therefore, our various studies provide a coherent picture, and it is suggested from these different experiments that parasitic infection increases the requirement for lysine by 50% above that for normal healthy subjects.

It might be of interest to point out that, in contrast to the higher lysine requirement established for untreated undernourished subjects (1) than for well-nourished subjects (3, 4), we recently ascertained that the leucine requirement for untreated, undernourished subjects (50) is essentially identical to that for well-nourished subjects (51). These contrasting findings were not due to the study of different subject populations in the different experiments. The undernourished subjects in the present study and those in the previous studies on lysine and leucine requirement were very similar in terms of socioeconomic background because they were recruited from the same slums in Bangalore, and 4 of the 14 subjects in the present study had participated in the earlier studies (1, 50). In addition, the subjects in all these studies were similar in terms of anthropometry, age, and body composition. There were no significant differences in age, anthropometry, and body composition between the subjects in the lysine (1) and leucine (50) requirement studies. Furthermore, the difference between the mean body mass index values of the subjects in the present study and those of the pooled subjects of the previous studies (1, 50) was just 1%, and the difference in percentage body fat and fat-free mass (kg) was 0.3% and 2%, respectively. None of these differences was significant, which suggested that the pools of subjects in whom these different studies were conducted were homogeneous in terms of environment, socioeconomic condition, nutritional status, and clinical well-being. However, we did not characterize the subjects in the earlier studies (1, 50) in terms of intestinal parasites.

It is difficult to offer a further interpretation of the difference between our findings for lysine and leucine, but it might be due to an interaction of factors. With respect to leucine, for example, there may be increased oxidative losses in the intestinal tissues that are balanced by lower rates of leucine oxidation in peripheral tissues that are related to a diminished muscle mass, which is an important determinant of leucine catabolism (52). This is speculative but emphasizes the need for further studies on comparative aspects of amino acid metabolism and requirements in different pathophysiological states.

Our previous studies (1–4, 12, 13, 53) on the adult requirements for amino acids suggested that the requirement for some of the indispensable amino acids is 2–3 times higher than that suggested in the 1985 WHO/FAO/UNU report (46). This research has provided a major basis for the newly recommended patterns of amino acid requirements by an Expert Working Consultation on Protein and Amino Acid Requirements, meeting in Rome in July 2001 and in Geneva in April 2002, under the auspices of FAO/WHO/UNU (Internet: http://www.fao.org/es/esn/require/upcoming.htm; accessed December 2001). However, the application of these amino acid requirements to populations on a global basis has been questioned, because it is thought that

there may be adaptive reductions in the requirement for amino acids with habitual diets that are low in protein, amino acids, or both. On the other hand, there may be increased requirements that are due to other factors, such as chronic but subclinical immunostimulation. In summary, the present 24-h indicator amino acid balance investigation, which used a [^{13}C]leucine tracer, in chronically undernourished Indian subjects suggests that the higher-than-normal requirement for lysine that we reported previously (1) is due largely to the intestinal parasite load of these subjects.

REFERENCES

1. Kurpad AV, Regan MM, Raj T, et al. Lysine requirements of chronically undernourished adult Indian men, measured by a 24-h indicator amino acid oxidation and balance technique. Am J Clin Nutr 2003; 77:101–8.
2. Kurpad AV, El-Khoury AE, Beaumier L, et al. An initial assessment, using 24-h [^{13}C]leucine kinetics, of the lysine requirements of healthy adult Indian subjects. Am J Clin Nutr 1998;67:58–66.
3. Kurpad AV, Raj T, El-Khoury AE, et al. Lysine requirements of healthy adult Indian subjects, measured by an indicator amino acid balance technique. Am J Clin Nutr 2000;73:900–7.
4. Kurpad AV, Regan MM, Raj T, et al. Lysine requirements of healthy adult Indian subjects receiving long-term feeding, measured with a 24-h indicator amino acid oxidation and balance technique. Am J Clin Nutr 2002;76:404–12.
5. Reeds PJ, Fjeld CR, Jahoor F. Do the differences in the amino acid composition of acute phase and muscle proteins have a bearing on nitrogen loss in traumatic states? J Nutr 1994;124:906–10.
6. Kurpad AV, Jahoor F, Borgonha S, et al. A minimally invasive tracer protocol is effective for assessing the response of leucine kinetics and oxidation to vaccination in chronically energy-deficient adult males and children. J Nutr 1999;129:1537–44.
7. Kang G, Mathew MS, Rajan DP, et al. Prevalence of intestinal parasites in rural Southern Indians. Trop Med Int Health 1998;3:70–5.
8. Haswell-Elkins MR, Elkins DB, Manjula K, Michael E, Anderson RM. The distribution and abundance of Enterobius vermicularis in a South Indian fishing community. Parasitology 1987;95:339–54.
9. Haswell-Elkins MR, Elkins DB, Manjula K, Michael E, Anderson RM. An investigation of hookworm infection and reinfection following mass anthelmintic treatment in the south Indian fishing community of Vairavankuppam. Parasitology 1988;96:565–77.
10. Estibeiro S, Pinto MJW, Rodrigues S, Verenkar MP. Prevalence of intestinal taeniasis in three areas of Goa. Indian J Med Microbiol 2000;18:116–8.
11. Persson A, Rombo L. Intestinal parasites in refugees and asylum seekers entering the Stockholm area, 1987–88: evaluation of routine stool screening. Scand J Infect Dis 1994;26:199–207.
12. El-Khoury AE, Basile A, Beaumier L, et al. Twenty-four-hour intravenous and oral tracer studies with L-[1-^{13}C]-2-α-aminoadipic acid and L-[1-^{13}C]lysine as tracers at generous nitrogen and lysine intakes in healthy adults. Am J Clin Nutr 1998;86:827–39.
13. El-Khoury AE, Pereira PCM, Borgonha S, et al. Twenty-four-hour oral tracer studies with L-[1-^{13}C]lysine at a low (15 mg. kg^{-1}d^{-1}) and intermediate (29 mg. kg^{-1}d^{-1}) lysine intake in healthy adults. Am J Clin Nutr 2000;72:122–30.
14. James WPT, Ferro-Luzzi A, Waterlow JC. Definition of chronic energy deficiency in adults. Report of a working party of the IDECG. Eur J Clin Nutr 1988;42:969–81.
15. Kuppusawamy B. Socio-economic status scale (urban). New Delhi: Manasayan, 1984.
16. Durnin JVGA, Womersley J. Body fat assessed by total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br J Nutr 1974;32:77–97.
17. Siri WE. Body composition from the fluid spaces and density: analysis of methods. In: J Brozek, A Henschel, eds. Techniques for measuring body composition. Washington, DC: National Academy of Science, 1961:223-44.
18. Scolari C, Torti C, Beltrame A, et al. Prevalence and distribution of soil transmitted helminthic infections in urban and indigenous school children in Ortiguueira, state of Parana, Brasil: implications for control. Eur J Trop Med Int Health 2000;5:302–7.
19. Gillespie SH, Hawkey PM, eds. Medical parasitology—a practical approach. Oxford, United Kingdom: IRL Press, 1995.
20. Montresor A, Crompton DWT, Bundy DAP, Hall A, Savioli L. Guidelines for evaluation of soil transmitted helminths and schistosomiasis at community level. WHO/CTC/SIP/98.1. Geneva: World Health Organization, 1998.
21. Reynolds JEF, ed. Martindale. The extra pharmacopeia. 31st edition. London: The Royal Pharmaceutical Society, 1996:103-27.
22. Zaat JOM, Mank ThG, Assendelft WJJ. Drugs for treating giardiasis (Cochrane Review). In: *The Cochrane Library,* 3, 2003. Oxford: Update Software. Internet: www.cochrane.org (accessed 15 May 2003).
23. Parasites of the intestinal tract. Atlanta: CDC, 2003. Internet: www.dpd.cdc.gov (accessed 15 May 2003).
24. Jennings G, Bourgeois C, Elia M. The magnitude of the acute phase protein response is attenuated by protein deficiency in rats. J Nutr 1992;122:1325–31.
25. Kushner I. The phenomenon of the acute phase response. Ann N Y Acad Sci 1982;389:39–48.
26. Keusch GT. Immune function in the malnourished host. Pediatr Ann 1982;11:1004–14.
27. Yudkin JS, Yajnik CS, Mohammed Ali V, Bulmer K. High levels of circulating proinflammatory cytokines and leptin in urban, but not rural, Asian Indians. Diabetes Care 1999;22:363–4.
28. Berg KJ, Jalil F, Lindblad BS. Longitudinal analysis of infantile growth in an urban area of Lahore, Pakistan. Acta Pediatr Scand 1988;77:392–401.
29. Wells JCK, Davis PSW, Lee PC. Patterns of infant weight gain in developing countries. J Trop Pediatr 1993;39:214–8.
30. WHO Working Group. Use and interpretation of anthropometric indicators of nutritional status. Bull WHO 1986;64:929–41.
31. Joseph B, Rebello A, Kullu P, Raj VD. Prevalence of malnutrition in rural Karnataka, South India: a comparison of anthropometric indicators. J Health Popul Nutr 2002;20:239–44.
32. Scrimshaw NS, Taylor CE, Gordon JE. Interactions of nutrition and infection. Am J Med Sci 1959;237:367–403.
33. Solomons NW, Mazariegos M, Brown KH, Klasing K. The underprivileged, developing country child: environmental contamination and growth failure revisited. Nutr Rev 1993;51:1–6.
34. Ramesh GN, Malla N, Raju GS, et al. Epidemiological study of parasitic infestations in lower socioeconomic group in Chandigarh (North India). Indian J Med Res [A] 1991;93:47–50.
35. Sen R. Pathogenic intestinal parasites and bacteria in slum dwellers in Calcutta. Indian J Med Res 1968;56:1371–80.
36. Srivastava VK, Rai SN. Parasitic infestations in slum dwellers. Indian J Parasitol 1986;10:209–11.
37. Hagel I, Lynch NR, Di Prisco MC, Sanchez J, Perez M. Nutritional status and the IgE response against *Ascaris lumbricoides* in children from a tropical slum. Trans R Soc Trop Med Hyg 1995;89:562–5.
38. Hagel I, Lynch NR, Perez M, Di Prisco MC, Lopez R, Rojas E. Relationship between the degree of poverty and the IgE response to Ascaris infection in slum children. Trans R Soc Trop Med Hyg 1993;87:16–8.
39. Lynch NR, Hagel IA, Palenque ME, et al. Relationship between helminthic infection and IgE response in atopic and nonatopic children in a tropical environment. J Allergy Clin Immunol 1998;101:217–21.
40. Thakur BK, Murali MR, New D, Smith TH, Volkman D. Hypereosinophilia and markedly elevated immunoglobulin E in a 3-year-old child. Ann Allergy Asthma Immunol 1998;80:371–6.
41. van Goodoever JG, Stoll B, Henry JF, Burrin DG, Reeds PJ. Adaptive regulation of intestinal lysine metabolism. Proc Natl Acad Sci U S A 2000;97:11620–5.
42. Huby F, Hoste H, Mallet S, Fournel S, Nano JL. Effects of the excretory/secretory products of six nematode species, parasites of the digestive tract, on the proliferation of HT29-D4 and HGT-1 cell lines. Epithelial Cell Biol 1995;4:156–62.

43. Ruest N, Couture Y, Faubert GM, Girard C. Morphological changes in the jejunum of calves naturally infected with Giardia spp. and Cryptosporidium spp. Vet Parasitol 1997;69:177–86.

44. Khanna R, Joshi K, Kum K, Malik AK, Vinayak VK. An ultrastructural analysis of changes in surface architecture of intestinal mucosa following *Giardia lamblia* infection in mice. Gastroenterol Jpn 1990; 25:649–58.

45. Merchant MT, Aguilar L, Avila G, Robert L, Flisser A, Willms K. *Taenia solium*: description of the intestinal implantation sites in experimental hamster infections. J Parasitol 1998;84:681–5.

46. Crompton DWT, Nesheim MC. Nutritional impact of intestinal helminthiasis during the human life cycle. Annu Rev Nutr 2002;22:35–59.

47. World Health Organization. FAO/WHO/UNU expert consultation. Energy and protein requirements. World Health Organ Tech Rep Ser 1985:724:145–50.

48. Scrimshaw NS, Bistrian BR, Brunser O, et al. Effects of disease on desirable protein/energy ratios. In: Scrimshaw NS, Schürch B, eds. Protein-energy interactions. Lausanne, Switzerland: IDECG, Nestlé Foundation, 1992:385-98.

49. Reeds PJ. Dispensable and indispensable amino acids for humans. J Nutr 2000;130:1835S–40S.

50. Kurpad AV, Regan MM, Raj T, et al. Leucine requirement and splanchnic uptake of leucine in chronically undernourished adult Indian subjects. Am J Clin Nutr 2003;77:861–7.

51. Kurpad AV, Raj T, El-Khoury AE, et al. Daily requirement for and splanchnic uptake of leucine in healthy adult Indians. Am J Clin Nutr 2001;74:747–55.

52. Tessari P, Garibotto G, Zanetta M, et al. Splanchnic amino acid and protein turnover in humans: insight from isotope dilution and catheterization studies. In: Tessari P, Soeters PB, Pittoni G, Tiengo A, eds. Amino acid and protein metabolism in healthy and disease: nutritional implications. London: Smith-Gordon Co, Ltd: 1997:31-44.

53. Young VR, Borgonha S. Nitrogen and amino acid requirements: the Massachusetts Institute of Technology amino acid requirement pattern. J Nutr 2000;130:1841S–9S.