Leucine requirement and splanchnic uptake of leucine in chronically undernourished adult Indian subjects[1–3]

Anura V Kurpad, Meredith M Regan, Tony Raj, Sureka Varalakshmi, Justin Gnanou, Prashanth Thankachan, and Vernon R Young

ABSTRACT

Background: We showed previously by the 24-h direct amino acid balance (DAAB) method that the leucine requirement of well-nourished Western and South Asian subjects is ≈40 mg · kg^{-1} · d^{-1}.

Objective: It is not known whether this estimated leucine requirement is applicable in chronic undernutrition; therefore, we assessed the leucine requirement in Indian men with chronic, but stable, undernutrition.

Design: We studied 26 chronically undernourished men during 2 randomly assigned 7-d diet periods consisting of an L-amino acid diet (n = 20) and supplying either 14 and 30 (n = 10) or 22 and 40 (n = 10) mg leucine · kg^{-1} · d^{-1} or consisting of the subjects' habitual cereal-and-lentil-based diets (n = 6). The 24-h DAAB was estimated on day 6 by using a [^{13}C]leucine tracer infusion. The splanchnic uptake of leucine was determined at an intake of 40 mg · kg^{-1} · d^{-1} by administering [$^{2}H_3$]leucine orally.

Results: By using mixed-models linear regression of leucine balance against leucine intake, we estimated a zero leucine balance at a leucine intake of 39.6 mg · kg^{-1} · d^{-1}. The splanchnic first-pass uptake of [$^{2}H_3$]leucine was 22.7% and 11.5% of the intake in the fasted and fed phases, respectively. The subjects were in neutral leucine balance with their habitual cereal-and-lentil-based diets.

Conclusion: On the basis of the 24-h DAAB approach, a mean leucine requirement of 40 mg · kg^{-1} · d^{-1} is proposed for healthy and for chronically undernourished Indian adults. *Am J Clin Nutr* 2003;77:861–7.

KEY WORDS India, chronic undernutrition, leucine requirement, amino acid oxidation, amino acid balance, splanchnic uptake

INTRODUCTION

In a previous study, we measured the lysine requirement of chronically undernourished South Asian men (1) with use of the 24-h indicator amino acid oxidation and balance method (2). We found that the requirement was ≈50% higher, when expressed per kg body weight, than was the requirement in similar, but healthy and well-nourished, South Asian (Indian) men (3, 4). We proposed that the higher lysine requirement in the undernourished subjects was linked to their body composition, because they had a lower muscle mass and therefore a relatively higher visceral mass than did the healthy, well-nourished subjects (1). This hypothesis suggests a possibly higher visceral or splanchnic need for lysine (and perhaps other amino acids), implying that the muscle-to-visceral organ ratio of the fat-free mass (FFM) is an important factor in determining amino acid requirements. Thus, it is important to determine whether this increased requirement is representative of the requirements for all of the indispensable amino acids, or whether it is specific to those amino acids that are preferentially utilized and catabolized in the splanchnic region. Hence, it was of interest to determine the leucine requirement in chronically undernourished subjects, with their relatively low muscle mass (5). The leucine requirement is defined operationally as the minimum intake of dietary leucine needed to maintain leucine balance.

The leucine requirement in normal, well-nourished Western and Indian men has been determined to be ≈40 mg · kg^{-1} · d^{-1} by the obligatory amino acid loss method (6) and by short-term direct amino acid oxidation (7–9) and 24-h direct amino acid balance (DAAB) techniques (10–12). These findings for the leucine requirement were ≈2.5 times higher than the 1985 FAO/WHO/UNU recommendation (13). However, an argument against the global acceptability of these estimates for the leucine requirement (7–12) is that they were made in healthy, young Western and Indian males.

Therefore, the current study was designed to assess the leucine requirement of chronically undernourished South Asian young men (n = 20) with the 24-h DAAB technique using L-amino acid mixtures supplying graded levels of leucine intake and given for an adaptation period of 1 wk. Because we had previously observed a small but significant weight loss in these subjects during the experimental diet period, and had attributed this to a negative carbohydrate balance created by the experimental diet (1), we also assessed the leucine balance and body-weight response in a group of similar subjects (n = 6) consuming their habitual cereal-and-lentil-based diets for 1 wk. Therefore, this second set of subjects also underwent the same experimental protocol, except that their usual cereal-and-lentil-based diets, supplying ≈70 mg

[1] From the Department of Physiology and Division of Nutrition (AVK, TR, SV, and PT) and the Department of Biochemistry (JG), St John's Medical College, Bangalore, India, and the Laboratory of Human Nutrition, Massachusetts Institute of Technology, Cambridge, MA (MMR and VRY).

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[3] Reprints not available. Address correspondence to VR Young, Laboratory of Human Nutrition, Massachusetts Institute of Technology, Cambridge, MA 02139. E-mail: vryoung@mit.edu.

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TABLE 1
Characteristics of chronically undernourished Indian men studied to determine their leucine balance and requirements[1]

Characteristic	Graded leucine intake (n = 20)	Normal diet (n = 6)
Age (y)	21.6 ± 2.3	21.3 ± 1.5
Weight (kg)	44.0 ± 2.9	44.4 ± 3.8
Height (m)	1.6 ± 0.0	1.6 ± 0.1
BMI (kg/m^2)	17.1 ± 0.9	17.1 ± 0.8
MUAC (cm)	22.9 ± 0.9	22.6 ± 1.2
Body fat (%)	11.6 ± 3.03	9.5 ± 2.0
Fat-free mass (kg)	38.9 ± 2.2	40.1 ± 2.9

[1] $\bar{x}$ ± SD. MUAC, midupper arm circumference.

leucine · kg^{-1} · d^{-1}, were given for 1 wk before the leucine 24-h DAAB experiment.

Because the splanchnic region is quantitatively important in overall body amino acid utilization (14), we have estimated the splanchnic uptake of leucine in chronically undernourished subjects at a leucine intake of 40 mg · kg^{-1} · d^{-1}, with simultaneous administration of leucine tracers by the oral and intravenous routes.

SUBJECTS AND METHODS

Subjects and anthropometry

A total of 26 undernourished men participated in this experiment. The subjects were weighed to the nearest 0.1 kg and their height was measured to the nearest 0.1 cm. The logarithm of the sum of 4 skinfold thicknesses (biceps, triceps, subscapular, and suprailiac) was used (1) in age- and sex-specific equations (15) to obtain an estimate of body density. From this estimate, the percentage body fat and FFM were determined (16) (**Table 1**). The purpose of the study and the potential risks involved were explained to the subjects, who gave their written informed consent. The Human Ethical Review Board of St John's Medical College approved the research protocol.

Diet and experimental design

Two experiments were conducted. In the first experiment, the effect of a graded leucine intake on 24-h DAAB was studied. Two groups of 10 subjects each were randomly assigned to 2 separate 6-d diet periods during which they received a weight-maintaining diet containing an L-amino acid mixture, as described previously (12). The 2 test amounts of daily leucine intake during the respective diet periods were either 14 and 30 or 22 and 40 mg · kg^{-1} · d^{-1} (**Table 2**).

In the second experiment, 6 subjects received diets typical of their usual diets; the meals were planned on the basis of a 3-d dietary recall. The protein content of the diet was 9% of energy intake, and nonprotein energy was provided as fat (≈23%) and carbohydrate (≈68%). The main source of protein was vegetarian and cereal-based (≈75%), and the carbohydrate was provided in the form of rice and beet sugar. The diet was analyzed for its nutrient content by using a nutrient database (17). The leucine intake (diet plus tracer) was 73.1 mg · kg^{-1} · d^{-1}.

The 24-h tracer-infusion protocol, sample collection, and calculations

The primed 24-h intravenous [^{13}C]leucine and oral [$^2H_3$]leucine (for splanchnic uptake measurements) approach was used, with indirect calorimetry and blood and breath sampling as described previously (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 ≈2.8 μmol · kg^{-1} · h^{-1} (the prime was ≈4.2 μmol/kg) into an antecubital vein. The bicarbonate pool was primed with 0.8 μmol · L^{-1} · kg^{-1} of [^{13}C]sodium bicarbonate (99.9 atom%; MassTrace). In subjects who received the highest amount of leucine intake (40 mg · kg^{-1} · d^{-1}; n = 10) in the graded-intake experiment, the splanchnic uptake of leucine was studied by using a primed, intermittent (hourly) oral administration of [$^2H_3$]leucine in a dose of 2.8 μmol · kg^{-1} · h^{-1}, with a prime of ≈4.2 μmol/kg.

We have described previously (4, 12) the breath analyses for $^{13}CO_2$ enrichment by isotope ratio mass spectrometry (Europa Scientific Ltd, Crewe, United Kingdom) and blood analyses for ^{2}H- and ^{13}C-enrichments of plasma α-ketoisocaproic and leucine by gas chromatography–mass spectrometry (Varian, Palo Alto, CA).

TABLE 2
Composition of amino acid mixtures used to supply 4 different leucine intakes

Amino acid	Amount of leucine (mg · kg^{-1} · d^{-1})			
	14	22	30	40
	mg/g mixture			
L-Tryptophan	15.77	15.74	15.70	15.66
L-Threonine	47.61	47.51	47.41	47.28
L-Isoleucine	63.52	63.39	63.25	63.08
L-Leucine[1]	4.30	11.59	18.67	27.62
L-Lysine HCl	84.56	84.38	84.20	83.98
L-Methionine	30.00	29.94	29.88	29.80
L-Cystine	22.25	22.20	22.16	22.10
L-Phenylalanine	55.26	55.14	55.03	54.88
L-Tyrosine	41.18	41.09	41.01	40.90
L-Valine	71.03	70.88	70.73	70.54
L-Histidine HCl	31.00	30.94	30.87	30.79
L-Arginine HCl	76.41	76.25	76.09	75.88
L-Alanine	193.58	193.16	192.75	192.24
L-Aspartic acid	12.06	12.04	12.01	11.98
L-Glutamic acid	29.83	29.77	29.70	29.62
Glycine	99.37	94.02	88.82	82.25
L-Proline	40.75	40.66	40.58	40.47
L-Serine	81.51	81.33	81.16	80.94
Total[2]	1000.00	1000.00	1000.00	1000.00

[1] 9.25 mg leucine · kg^{-1} · d^{-1} was added to each mixture every day, except on the infusion day, when this amount of leucine was infused as a tracer. If 2 tracers, ie, [$^2H_3$] and [^{13}C]leucine, were infused, appropriate reductions in unlabeled leucine intake were made in the diet.

[2] A total of 1.11 g · kg^{-1} · d^{-1} was given to subjects; this provided 160 mg N · kg^{-1} · d^{-1}.

Leucine oxidation, balance, and splanchnic uptake

Leucine oxidation (μmol · L^{-1} · kg^{-1} per 30 min) was computed for consecutive half-hourly intervals (4, 12) as the ratio of the $^{13}CO_2$ production rate (μmol · L^{-1} · kg^{-1} per 30 min) to the plasma [^{13}C]α-ketoisocaproic acid (KIC) enrichment (mole percent excess) at that time.

Leucine balance (mg · kg^{-1} · d^{-1}) was computed as leucine input (dietary leucine + intravenous tracer) minus leucine output (sum of leucine oxidation at half-hourly intervals). For the calculation of leucine balance, we included in the effective daily leucine intake that amount of tracer given during both the fasted

TABLE 3
Summary of leucine oxidation and flux with 4 different leucine intakes in chronically undernourished Indian men[1]

	Leucine intake (mg·kg^{-1}·d^{-1})			
Leucine index	14	22	30	40
Oxidation (mg leucine·kg^{-1}·d^{-1})[2]				
12-h Fasted	12.5 ± 3.6	15.0 ± 4.0	16.8 ± 3.2	20.7 ± 3.0
12-h Fed	11.1 ± 3.8	15.1 ± 4.1	16.2 ± 4.3	20.1 ± 4.3
Total (24 h)	23.6 ± 6.9[3,4]	30.1 ± 7.4[3]	33.0 ± 6.3[3]	40.7 ± 5.8
Total intake (mg leucine·kg^{-1}·d^{-1})	14.2 ± 0.7	22.6 ± 0.5	30.5 ± 0.6	40.7 ± 0.6
24-h Balance[5]				
Intake − oxidation (mg leucine·kg^{-1}·d^{-1})	−9.4 ± 6.6[3,6]	−7.6 ± 6.9[3,6]	−2.5 ± 6.5	0.0 ± 5.6
(% of intake)	−66.0 ± 45.8[3,6]	−33.2 ± 30.9[3,6]	−8.4 ± 21.5	0.0 ± 13.9
Flux (μmol·kg^{-1}·30 min^{-1})[7]				
12-h Fasted	59.8 ± 4.7	62.6 ± 6.8	64.2 ± 4.7	65.6 ± 7.5
12-h Fed	56.3 ± 6.2	59.4 ± 5.2	60.0 ± 5.7	65.1 ± 6.9
Total (24 h)	58.1 ± 4.9[3]	61.0 ± 4.7[3]	62.1 ± 4.8	65.4 ± 5.4

[1] $\bar{x}$ ± SD; n = 10.
[2] Significant effect of leucine intake ($P < 0.0001$), but no interaction with or main effect of metabolic phase (fasted compared with fed) by mixed-models ANOVA.
[3] Significantly different from intake of 40 mg·kg^{-1}·d^{-1}, $P < 0.05$ (mixed-models ANOVA and Tukey's method).
[4] Significantly different from intake of 30 mg·kg^{-1}·d^{-1}, $P < 0.05$ (mixed-models ANOVA and Tukey's method).
[5] Significant effect of leucine intake, $P < 0.01$ (mixed-models ANOVA).
[6] Significantly different from 0, $P < 0.05$ (mixed-models ANOVA).
[7] Significant effects of leucine intake and metabolic phase, $P \leq 0.01$ (mixed-models ANOVA).

phase and fed phase (12 h each) of the day. We included the tracer delivered during the fasted phase because we recently showed that the tracer is retained, presumably within the free leucine pool of the body, during the fasted phase (18).

The splanchnic uptake of leucine was calculated for the 12-h fasted and 12-h fed phases, as follows (12):

$$\text{Splanchnic uptake (\%)} = [1 - ([^2H_3]\text{leucine enrichment}/[^2H_3]\text{leucine infusion rate})/([^{13}C]\text{leucine enrichment}/[^{13}C]\text{leucine infusion rate})] \times 100 \quad (1)$$

where leucine enrichment refers to the mean plasma enrichment in the 12-h fasted phase and 12-h fed phase, corrected for the tracer infusion rates.

The respiratory gas exchange data obtained during the 24-h tracer studies on day 7 were used to assess substrate (fat and carbohydrate) oxidation rates. Leucine oxidation was used as a surrogate for protein oxidation on the basis of a leucine content in mixed body protein of 8% (19). The heat equivalents of body protein and fat (for the fasted phase) and of the amino acid mixture and other components of the diet (for the fed phase) were calculated according to the method of Livesey and Elia (20). On the basis of this approach, the 24-h oxidation rates for carbohydrate and fat and their balances were also calculated as the difference between their daily intakes and oxidation.

Statistical methods and data evaluation

The data are presented as means ± SDs. The metabolic variables were analyzed by using mixed-models analysis of variance. The models for 12-h leucine oxidation and flux included diet period, metabolic phase (fasted or fed), leucine intake, and the intake-by-phase interaction. If the intake-by-phase interaction was significant, then model contrasts were used to make pairwise comparisons of interest and comparisons of 24-h values between different leucine intakes. If the interaction was not significant and the main effect of leucine intake was significant, then model contrasts were used for comparisons between intakes without regard to metabolic phase.

The model for 24-h DAAB (leucine) included diet period and leucine intake; comparisons against zero balance were made by using the model, and model contrasts were used for comparisons between intakes if the main effect was significant. The zero leucine balance was determined from the best-fitting model (linear or curvilinear) of balance on leucine intake, and the 95% CI was determined by using Fieller's method.

Plasma enrichments and splanchnic uptake of leucine were estimated only at the 40- mg·kg^{-1}·d^{-1} intake of leucine. The model for enrichments included factors for tracer (^{13}C or $^2H_3$), molecular form (leucine or KIC), metabolic phase, and the interactions; model contrasts were used to make pairwise comparisons of interest, as appropriate from the significance of interactions. The model for splanchnic uptake of leucine included a factor for metabolic phase. The model for body weight included diet period, day (screening or infusion), and the interaction of diet period by day.

A two-sided P value of 0.05 indicated significance for all tests of interaction and main effects; P values of pairwise comparisons were adjusted by using Tukey's method. The data were analyzed by using SAS version 8.2 (SAS Institute Inc, Cary, NC).

RESULTS

Anthropometry

During the experimental 6-d diet period, the subjects experienced a small, statistically nonsignificant weight loss of 0.07 ± 0.20 kg on average, regardless of diet period ($P = 0.12$). In contrast to this small weight loss, there was a small, nonsignificant weight gain of 0.13 ± 0.2 kg on average when the subjects consumed their normal diets during the 6-d adaptation period.

Leucine oxidation

There was no significant interaction between intake and metabolic phase, indicating that the effects of leucine intake on leucine oxidation were similar across both metabolic phases (**Table 3**).

TABLE 4
Summary of tracer enrichments in plasma and splanchnic uptake, in the fasted and fed phases, in subjects who were administered [^{13}C]leucine intravenously and [$^{2}H_3$]leucine orally at a leucine intake of 40 mg·kg^{-1}·d^{-1} in the graded leucine intake experiment[1]

	Metabolic phase		
Tracer and route	Fasted	Fed	24-h period
Tracer enrichments in plasma			
[^{13}C]Leucine, IV (mol % excess)	3.5 ± 0.5	3.8 ± 0.3[2]	3.7 ± 0.3
[$^{2}H_3$]Leucine, oral (mol % excess)	2.7 ± 0.2	3.4 ± 0.3[3]	3.1 ± 0.2
[^{13}C]KIC (mol % excess)	2.4 ± 0.3	2.4 ± 0.2[2]	2.4 ± 0.1
[$^{2}H_3$]KIC (mol % excess)	1.7 ± 0.4	2.5 ± 0.8[3]	2.1 ± 0.5
Splanchnic uptake (%)	22.7 ± 9.7	11.5 ± 7.4[4]	16.5 ± 7.5

[1] $\bar{x}$ ± SD; n = 10. KIC, α-ketoisocaproic acid; IV, intravenous. There was a significant interaction between the combination of tracer and route (^{13}C compared with $^{2}H_3$) and metabolic phase ($P < 0.01$) and a significant main effect of molecular form (leucine compared with KIC; $P < 0.0001$) (mixed-models ANOVA).

[2,3] Significantly different from fasted without regard to molecular form (mixed-models ANOVA): [2] $P < 0.01$, [3] $P < 0.0001$.

[4] Significantly different from fasted, $P < 0.01$ (mixed-models ANOVA).

There was a significant effect of leucine intake on leucine oxidation ($P < 0.0001$); it was lower at the 14, 22, and 30 mg·kg^{-1}·d^{-1} intakes than at the 40 mg·kg^{-1}·d^{-1} intake ($P < 0.05$). The oxidation at the 14 mg·kg^{-1}·d^{-1} intake was significantly lower than that at the 30 mg·kg^{-1}·d^{-1} intake ($P < 0.05$), but the oxidation at the 22 and 30 mg·kg^{-1}·d^{-1} intakes and the 14 and 22 mg·kg^{-1}·d^{-1} intakes were not significantly different. There was no effect of metabolic phase on leucine oxidation.

Leucine balance

With respect to leucine balance, the results were essentially the same whether expressed as an absolute balance or as a percentage of leucine intake (Table 3). Daily leucine balance was affected by leucine intake ($P < 0.01$) and was lower at the 14 and 22 mg·kg^{-1}·d^{-1} intakes than at the 40 mg·kg^{-1}·d^{-1} intake ($P < 0.05$); the 30 and 40 mg·kg^{-1}·d^{-1} intakes did not differ significantly. The 14, 22, and 30 mg·kg^{-1}·d^{-1} intakes did not differ significantly from one another. Both the 14 and 22 mg·kg^{-1}·d^{-1} intakes were significantly different from zero balance ($P < 0.05$). A linear relation between leucine intake and leucine balance resulted in the equation:

$$\text{Leucine balance} = -15.6 + 0.39 \times \text{leucine intake} \quad (2)$$

The slope has a 95% CI of 0.20, 0.59. The resulting zero-balance intercept was 39.6 mg·kg^{-1}·d^{-1} (95% CI: 32, 56).

Leucine flux

There was no significant interaction between intake and metabolic phase (Table 3). There was a significant effect of leucine intake on leucine flux ($P < 0.01$). The flux was lower at the 14 and 22 mg·kg^{-1}·d^{-1} intakes than at the 40 mg·kg^{-1}·d^{-1} intake ($P < 0.05$); the 30 and 40 mg·kg^{-1}·d^{-1} intakes did not differ significantly. The 14, 22, and 30 mg·kg^{-1}·d^{-1} intakes did not differ significantly from one another. There was a significant effect of metabolic phase on leucine flux ($P = 0.01$) in that leucine flux was significantly higher in the fasted phase without regard to leucine intake.

Normal-diet experiment: leucine oxidation, balance, and flux

In the normal-diet experiment, the mean 24-h leucine oxidation rate was 71.5 ± 8.9 mg·kg^{-1}·d^{-1}, which gave a mean 24-h leucine balance of 1.6 ± 12.6 mg·kg^{-1}·d^{-1}. The 12-h fasted and fed leucine oxidation rates were 30.5 ± 5.1 and 41.0 ± 7.9 mg/kg per 12 h, respectively, giving a fasted-to-fed oxidation ratio of 0.8.

Substrate oxidation and balance

In the graded leucine intake experiment, although there were no differences between the different leucine intake amounts, the mean substrate balance (intake − oxidation) across all amounts of leucine intake for both dietary phases on day 7 was −1.0 ± 0.8 and 0.7 ± 0.4 g·kg^{-1}·d^{-1} for carbohydrate and fat, respectively. The negative carbohydrate balance was −1.0 ± 0.7 and −0.9 ± 1.0 g·kg^{-1}·d^{-1} for the first and second diet periods, respectively. Similarly, there was a positive fat balance of ≈0.7 ± 0.4 g·kg^{-1}·d^{-1} across all leucine intakes for both diet periods. Although there would be no net effect on body energy balance, these energy substrate alterations suggest that over the 1-wk dietary adaptation period, there was a carbohydrate (glycogen) loss from the body of ≈45 g/d. This glycogen loss, along with a concomitant loss of water associated with glycogen (21), might amount to a weight loss of ≈0.13–0.18 kg at the end of the week's experimental feeding.

When the subjects were given their habitual diets, the mean carbohydrate balance was −0.33 g·kg^{-1}·d^{-1} on day 7 during the 24-h tracer infusion, while their fat balance was positive at 1.16 g·kg^{-1}·d^{-1}. This is concordant with the trend of a small weight gain experienced by the subjects at the end of the 1-wk dietary adaptation period.

Splanchnic uptake of leucine

The plasma enrichments of [^{13}C] and [$^{2}H_3$]leucine and of [^{13}C] and [$^{2}H_3$]KIC, in the fasted and fed phases, are shown in **Table 4**. For this analysis, an average enrichment over each 12-h period (fed or fasted) was calculated from the half-hourly measurements. There was a significant interaction of metabolic phase with the combination of tracer and route ($P < 0.01$). With the $^{2}H_3$ tracer, regardless of molecular form (KIC or leucine), enrichment was significantly higher in the fed phase than in the fasted phase ($P < 0.0001$), and with the ^{13}C tracer, the difference was small but statistically significant ($P < 0.01$). The plasma KIC enrichment was significantly lower than was the plasma leucine enrichment, without regard to tracer and route or metabolic phase ($P < 0.0001$), and the average ratio between [^{13}C]KIC and [^{13}C]leucine was 0.7.

The splanchnic uptake of leucine, calculated from the plasma leucine enrichments for the 2 tracers, was 22.7 ± 9.7% and

11.5 ± 7.4% in the fasted and fed phases, respectively. The difference between the fasted and fed phases was statistically significant ($P < 0.01$). Averaged over the 24-h period, splanchnic uptake of leucine was 16.5 ± 7.5%. When compared with the splanchnic leucine uptake of 10 well-nourished subjects (12; individual data were not reported), the chronically undernourished subjects had a significantly lower uptake regardless of metabolic phase ($P = 0.001$). The well-nourished subjects had a fasted splanchnic leucine uptake of 45.7 ± 17.8% and a fed uptake of 33.9 ± 19.2%.

DISCUSSION

The findings in the present study add to the growing body of tracer-derived leucine balance data that we have generated to quantify adult requirements for leucine (7–12) and other amino acids (2–4, 22, 23) in South Asian (Indian) and Western subjects. This pattern of amino acid requirement (for leucine, lysine, and threonine) is similar to the proposed Massachusetts Institute of Technology amino acid requirement pattern (6), which we have recommended for use in the assessment of dietary amino acid adequacy or in the planning of adequate intakes of indispensable amino acids. This pattern has provided a major basis for the amino acid requirement pattern recently accepted at the Working Group Meeting on Protein and Amino Acid Requirements, held in Rome in July 2001, under the auspices of FAO/WHO/UNU (http://www.fao.org/es/esn/require/upcoming.htm; accessed 1 December 2001). However, the application of these amino acid requirements to populations on a global scale has been questioned, because it is thought that there may be adaptive reductions in the requirements for amino acids when people habitually consume diets that are low in protein, amino acids, or both. On the other hand, requirements may be increased because of other factors, such as chronic but subclinical immunostimulation. Thus, there is a real need for data on requirements for specific indispensable amino acids in populations other than normal, healthy, affluent, well-nourished persons in the Western world or India.

In an earlier study that we conducted on the lysine requirements of a chronically undernourished group of men, we found that the lysine requirement was ≈50% higher than that of normal, well-nourished men (1). It was not clear whether this increased requirement reflected high splanchnic sequestration or an increased visceral requirement resulting from a high viscera-to-muscle ratio, which in turn resulted from the low muscle mass component of the FFM. In contrast to the higher lysine requirement in chronic undernutrition (1), the chronically undernourished men in the present study had a leucine requirement similar to that of well-nourished men (12). Given the lower muscle mass in the undernourished subjects (1) and the relatively higher degree of leucine oxidation in muscle, this seems reasonable. It was shown in dogs that splanchnic oxidation accounted for only 13% and 41% of total-body leucine oxidation in the fasted and fed conditions, respectively (24). Further, in fasted humans, muscle accounted for the largest part of whole-body leucine oxidation (5), whereas during mixed amino acid infusions, ≈70% of infused branched chain amino acids were removed from the circulation by muscle (25).

The leucine flux in the present experiment (average of ≈62 μmol/kg per 30 min) was higher at all amounts of leucine intake when compared with the flux measured in normal-weight subjects (≈46 μmol/kg per 30 min) in a similar experiment in which the subjects received the same amount of protein for 7 d before the experiment (12). It is possible that the higher leucine and protein turnover, when expressed per unit of body weight, is simply a function of body composition differences, in that chronically undernourished subjects have a relatively higher preservation of visceral tissue (with a higher rate of protein turnover) in comparison with skeletal muscle (1). Other possible reasons for high leucine (and protein) turnover include an adaptation to the presence of subclinical infections (26, 27) or a response to the adequate protein intake in the experimental diet of the undernourished subjects (28). However, it was shown in healthy subjects that rates of protein synthesis change little with protein intake over a wide range of intakes (29, 30). The finding of a relatively higher leucine flux (per unit body weight) in the undernourished subjects of the present study is also consistent with the results of previous studies carried out in similar subjects (31–33).

The normal leucine requirement in the present study also can be functionally related to the body tissue compartments rather than the body weight, for example, either to the FFM or the muscle mass, which accounted for ≈52% of these subjects' FFM [compared with ≈62% of the FFM in well-nourished control subjects (1)]. Thus, the similar values for leucine requirements per unit body weight in well-nourished subjects (12) and undernourished subjects (present study), become lower in the undernourished subjects when the requirement is expressed per unit FFM (48 and 44 $mg \cdot kg\ FFM^{-1} \cdot d^{-1}$ in well-nourished and undernourished subjects, respectively), but become higher when expressed per unit muscle mass (77 and 85 $mg \cdot kg\ muscle^{-1} \cdot d^{-1}$ in well-nourished and undernourished subjects, respectively), assuming the ratio of muscle to FFM stated above (1). These numerical transformations are indicative of the importance of viewing amino acid requirements from a functional perspective and a metabolic-tissue perspective; however, the practical prescription of a global requirement is best expressed in terms of an easily measurable unit, such as body weight.

Another aim of the present study was to measure the splanchnic uptake of leucine in undernourished subjects and to compare these estimates with those for well-nourished subjects. The quantity of leucine taken up by the splanchnic region was reported to be in the range of ≈10% to 30% of intake in the postabsorptive and postprandial states (34–38); in our previous study on well-nourished subjects, it was ≈46% (12). In the present study, splanchnic leucine uptake in undernourished subjects (≈26%) was significantly lower than that of well-nourished Indians in the fasted and fed phases. We do not know why the splanchnic uptake was lower, because we had anticipated a possibly higher uptake in our chronically undernourished and presumably immunostimulated subjects. This low value may be a reflection of the interindividual variability, or a consequence of the villous atrophy that can accompany chronic intestinal parasitic infestations, or both (39). In addition to these possibilities, there is the intriguing finding of Boirie et al (40) that the splanchnic uptake was positively correlated with body mass index (BMI). For the combined data from undernourished and well-nourished subjects, with well-nourished subjects having a mean BMI of ≈21 (12), the Pearson's product-moment correlation coefficient between BMI and 24-h splanchnic uptake of leucine was 0.57. Thus, if the relation suggested by Boirie et al (40) is true, and this is similar in our subjects, this may partly account for the lower splanchnic uptake seen in the chronically undernourished subjects with low BMI.

In summary, the present investigation of 24-h [^{13}C]leucine tracer kinetics in chronically undernourished Indian men studied with 4 test amounts of leucine, including the 1985 FAO/WHO/UNU

amount of 14 mg · kg^{-1} · d^{-1} (13), indicates that this international requirement value of 14 mg · kg^{-1} · d^{-1} is not adequate for this Indian population. We conclude that our proposed tentative leucine requirement of 40 mg · kg^{-1} · d^{-1}, determined on the basis of [^{13}C]leucine tracer studies in healthy, well-nourished Western and Indian subjects (1, 2), applies similarly to chronically undernourished adults in South Asia.

AVK was involved in the study design, data collection, sample and data analyses, and writing of the manuscript. TR, SV, and PT were involved in the data collection and analyses. JG was involved in the data collection and sample analyses. VRY and MMR were involved in the study design, data analyses, and writing of the manuscript. The authors had no conflicts of interest.

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