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

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ABSTRACT In disadvantaged populations, recurrent infections lead to a loss of body nitrogen and worsened nutritional status. The resulting malnutrition, in turn, produces a greater susceptibility to infection. This study aimed to examine the ability of a new minimally invasive tracer protocol to measure leucine oxidation, and then to use it to quantify the effect of vaccination on leucine kinetics and oxidation. Undernourished men (n = 5; body mass index 16.3 ± 0.9 kg/m²) and children (n = 9; age 4.1 ± 0.6 y; weight-for-age Z-score −2.3 ± 0.7) underwent metabolic studies 6 d before and 1 d after vaccination with diphtheria, pertussis and tetanus (DPT). The tracer protocol was performed in the fed state and involved two 3-h sequential periods of frequent (20 min) oral doses of NaH13CO3 or [1-13C] leucine. Frequent breath samples and urine collections were made. Blood samples were obtained from the men and used for the determination of the isotopic enrichment of α-ketosocapric acid. The prevaccination oxidation of leucine (percentage of dose ± sd) was 18.1 ± 2.3 (men) and 16.7 ± 3.8 (children). One day after vaccination, these values had risen to 19.9 ± 1.9 (P < 0.05) in the men and to 19.5 ± 4.6 (P < 0.01) in the children. In the adults, vaccination was associated with a rise in whole-body protein breakdown [mg protein/(kg h)] from 200 ± 40 to 240 ± 10 (P < 0.05). A minor simulated infection increases leucine catabolism in undernourished humans and this new, minimally invasive protocol is sufficiently sensitive to measure these changes. J. Nutr. 129: 1537–1544, 1999.

KEY WORDS: infection • protein metabolism • undernourished humans • stable isotopes

Linear growth and weight gain, important indices of child health, are lower in children in underprivileged communities (Berg et al. 1988, Waterlow 1973, Wells et al. 1993, WHO 1986). This reduced growth rate, which leads to stunting in later life, is associated with long-term effects, including decreased productivity and functional deficits (Spurr 1988, Vaz et al. 1996). The cause of this reduction in growth rate is likely multifactorial, reflecting the interactions of a poor diet and a poor environment (Henry 1981) and, in particular, the consequences of bacterial infections and parasitic infestations (Scrimshaw et al. 1959).

In a recent paper on underprivileged children in developing countries, Solomons et al. (1993) proposed that the occurrence of “inapparent” infections in children (no clinical findings, but abnormal erythrocyte sedimentation and white cell count) is a factor that increases the demand for nutrients. They argued that during chronic immunostimulation, there is a partitioning of nutrients toward the support of the immune defenses and an effective reduction in the availability of nutrients for growth.

Among other factors, the immune response involves cytokine-stimulated (Jennings et al. 1992, Keusch 1982, Kushner 1982) increase in the synthesis of so-called positive acute-phase proteins by the liver. This increased liver protein synthesis has to be supported either by amino acids derived from the diet or by breakdown of body protein. However, it is clear that there is a cost to this process because even when there is an apparently adequate supply of dietary protein, there is still a net loss of nitrogen from the body during active infection (Reeds et al. 1994). The loss of body protein after the stress of injury or infection is largely from skeletal muscle (Essen et al. 1991, Souba et al. 1990). It has been estimated that the synthesis of acute phase proteins can peak at 1.2 g/(kg body weight) (Waterlow 1991). Reeds et al. (1994) hypothesized that negative nitrogen balance occurs during an acute-phase response because the amino acid composition of the positive
Acute phase proteins differ from that of muscle protein (Barker 1984 and 1987). This leads to an internal amino acid imbalance, and as a result, 2 g of mixed muscle protein must be broken down to support the synthesis of 1 g of the typical mixture of positive acute phase proteins. The excess amino acids liberated from muscle are then oxidized, leading to an increased N loss from the body.

The quantitative nutritional effect of infection has proved difficult to assess. This paper reports the application of an isotopic protocol that attempts to provide such information. We investigated the utility of a minimally invasive approach (Fjeld 1994) to determine leucine oxidation in young children. The method was of short duration and involved only the administration of an oral tracer and the collection of breath. We also used the approach in an analogous study in adults and compared the results with those obtained using the more conventional, but invasive method of blood sampling. As part of the investigation, we used the noninvasive protocol in both adults and children to quantify the changes in leucine oxidation associated with vaccination.

**SUBJECTS AND METHODS**

The studies were performed at the metabolic research unit in the Department of Physiology at St. John’s Medical College, Bangalore. The studies were approved by the Human Ethical Approval Committee of St. John’s Medical College and the Institutional Review Boards for Human Investigations at Baylor College of Medicine. Written consent was obtained from the adults and from the children’s care givers.

**Study 1**

**Subjects.** Five clinically healthy, but chronically undernourished (by the criteria defined in James et al. 1988, i.e., body mass index < 18.5 kg/m², as well as low socioeconomic status) adult male subjects aged 18–26 y were recruited from a neighboring urban slum. They lived in temporary dwellings, which had neither sanitation nor water supply. The socioeconomic status of the subjects was assessed by an education, occupation and family income scale (Kuppuswamy et al. 1984). A clinical examination along with routine blood and urine analyses was carried out before commencing the experimental protocol.

**Anthropometric measurements.** Anthropometric and skinfold measurements were made on d 1, at the start of the study. Duplicate measurements of the subjects’ weight, to the nearest 0.1 kg, were made using a digital scale (Soehnle GmBH, Hamburg, Germany) with the subject dressed in minimal clothing. The heights of the subjects were recorded without footwear, using a vertically mobile scale (Karrimetre, Uppsala, Sweden) and expressed to the nearest 0.1 cm. Duplicate measurements of the biceps, triceps, subcapular and suprailiac skinfolds were made with the subject in the standing position. All skinfold measurements were standardized (Harrison et al. 1988) and carried out to the nearest 0.2 mm, using skinfold calipers (Holtain, Crymmych, UK). The logarithm of the sum of the four skinfolds was used, in age- and gender-specific equations (Durnin and Womersley 1974) to obtain an estimate of body density, from which percentage body fat was determined (Siri 1961).

**General protocol.** The study lasted for 10 d. On d 1, the subjects were brought in for clinical examination and anthropometric measurement. For the next 2 d (adaptation period), the subjects consumed a standardized diet, and on d 3, they were studied with the tracer protocol (see below). They were then allowed to go home. The subjects returned to the laboratory on d 7 for a further 2 d of controlled feeding. On d 9, they were vaccinated with the DPT (diphtheria, pertussis, and tetanus; Biological Ltd., Hyderabad, India) vaccine intramuscularly into the deltoid muscle. The vaccine contained diphtheria and tetanus toxoids, and killed whole-cell pertussis bacteria, with aluminum phosphate as adjuvant. A blood sample was collected 24 h after the vaccination. The DPT vaccine was administered as a 0.5-mL intramuscular injection with no side effects. On d 10, the subjects went home. On d 7, the subjects were once again subjected to a diet adaptation period until the end of d 8. On d 9, the subjects were vaccinated and studied by the tracer protocol 24 h later (d 10).

**Figure 1** The 10 d protocol. Subjects were admitted to the study on d 1 and followed a run-in period of diet adaptation until the end of d 2. On d 3, the tracer protocol was administered, after which the subjects went home. On d 7, the subjects were once again subjected to a diet adaptation period until the end of d 8. On d 9, the subjects were vaccinated and studied by the tracer protocol 24 h later (d 10).
TABLE 1

Hematological parameters of the undernourished men before and 24 h after vaccination with diphtheria, pertussis and tetanus (DPT)

<table>
<thead>
<tr>
<th></th>
<th>Pre-vaccination</th>
<th>Post-vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/L)</td>
<td>122 ± 11</td>
<td>134 ± 12</td>
</tr>
<tr>
<td>Total white cell count (× 10⁹/L)</td>
<td>6.2 ± 1.6</td>
<td>9.7 ± 2.2*</td>
</tr>
<tr>
<td>Differential count, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>50.6 ± 16.9</td>
<td>73.8 ± 9.8*</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>42.6 ± 17.8</td>
<td>23.0 ± 9.5*</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>6.4 ± 7.7</td>
<td>3.0 ± 1.2</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>7.6 ± 4.4</td>
<td>-</td>
</tr>
</tbody>
</table>

1 Values are means ± SD, n = 5.
2 ESR, erythrocyte sedimentation rate.

* P < 0.05, Wilcoxon signed rank test between pre- and postvaccination means.

10, the subjects were once again studied with the tracer protocol (Fig. 1).

Adaptation diet. The subjects were fed according to their requirements [1.6 × basal metabolic rate (BMR)/d] for 2 d before the tracer study. The BMR was estimated by the use of a prediction equation based on weight, specifically for Indians (Soares et al. 1993). The diet consisted of rice, lentils, vegetable oil and vegetables. On the basis of food tables (Gopalan et al. 1996, McCance and Widdowson 1995), the diet contained 75% carbohydrate, 12% fat and 13% protein and supplied 88 mg (0.67 mmol) leucine/(kg body weight · d).

The isotopic enrichment of breath ¹³CO₂ during sequential periods of oral dosing with H¹³CO₃⁻ or ¹³C-leucine in men. Each data point represents the mean (±SD) breath ¹³CO₂ value of five subjects, at each sampling time point. The upper panel shows data obtained before vaccination; the lower panel data were obtained 1 d after vaccination.

Adaptation. For 2 d before the isotopic study, the children were fed according to their requirements (WHO/FAO/UNU 1985) [−370 kJ/(kg · d)] a diet of rice, lentils, vegetable oil and vegetables. On the basis of food composition tables (Gopalan et al. 1996, McCance and Widdowson 1995), the macronutrient nutrient composition of the diet was carbohydrate 75%, fat 12% and protein 13%. The diet supplied 180 mg (1.37 mmol) leucine/(kg body weight · d).

General protocol. The general protocol followed was exactly similar to the adult protocol described above.

Tracer protocol. The protocol administered to the children pre- and postvaccination was similar in general procedure to the adult protocol; the main exception was that no blood samples were taken. The children were admitted to the Metabolic Unit at 0700 h and acclimated to the laboratory for 2 h. Based on their maintenance requirements (209 kJ/kg), the children were then fed a diet of wheat starch (protein-free) biscuits. These were made into a mush with water and given as small divided meals every half-hour during the 6-h protocol (see Fig. 2). Each meal was given after the tracer dose.

After a basal breath sample was collected, a priming dose of NaH¹³CO₃ (0.5 mg/kg) was given orally followed by intermittent maintenance doses of ¹³C bicarbonate and ¹³C leucine were administered for 3 h each. The first 3 h of ¹³C bicarbonate administration also served to prime the bicarbonate pool for the subsequent ¹³C leucine administration. A 22G cannula (Jelco, Tokyo, Japan) was inserted in an antecubital vein and maintained with a saline block. After the baseline blood and breath samples were collected, a priming dose of 0.5 mg/kg NaH¹³CO₃ (99.9%, Cambridge Isotopes, Andover, MA) was given orally (Fig. 2). After the priming dose, intermittent doses of NaH¹³CO₃ [0.166 mg/(kg · 20 min)] were given every 20 min for a period of 3 h. Breath samples were taken every 20 min starting just before administration of the isotope. After 3 h, the NaH¹³CO₃ was stopped and a priming dose of ¹³C leucine (99.9%, Cambridge Isotopes) (2 mg/kg) was given, followed by intermittent hourly doses of ¹³C leucine [2 mg/(kg · h)] every hour for the next 3 h. Breath samples continued to be taken at 20-min intervals and blood samples were collected every 20 min during the last hour of the experiment. Indirect calorimetry (Shetty et al. 1987) was carried out on all subjects during the 6-h duration of the protocol. The intake over 6 h provided one-quarter of their estimated daily energy requirements in an inactive state (1.3 × BMR) (FAO/WHO/UNU 1985) and was given at half-hour intervals (see Fig. 2). The meal was always given after the tracer dose. The diet supplied 61 mg leucine/(kg · d), thereby ensuring that the leucine intake from diet and tracer was the same as the daily leucine intake during the adaptation period. The protocol consisted of two phases in which ¹³C bicarbonate and ¹³C leucine were administered for 3 h each. The first 3 h of ¹³C bicarbonate administration also served to prime the bicarbonate pool for the subsequent ¹³C leucine administration. A 22G cannula (Jelco, Tokyo, Japan) was inserted in an antecubital vein and maintained with a saline block. After the baseline blood and breath samples were collected, a priming dose of 0.5 mg/kg NaH¹³CO₃ (99.9%, Cambridge Isotopes, Andover, MA) was given orally (Fig. 2). After the priming dose, intermittent doses of NaH¹³CO₃ [0.166 mg/(kg · 20 min)] were given every 20 min for a period of 3 h. Breath samples were taken every 20 min starting just before administration of the isotope. After 3 h, the NaH¹³CO₃ was stopped and a priming dose of ¹³C leucine (99.9%, Cambridge Isotopes) (2 mg/kg) was given, followed by intermittent hourly doses of ¹³C leucine [2 mg/(kg · h)] every hour for the next 3 h. Breath samples continued to be taken at 20-min intervals and blood samples were collected every 20 min during the last hour of the experiment. Indirect calorimetry (Shetty et al. 1987) was carried out on all subjects during the 6 h of the protocol in order to measure the excretion rate of CO₂(VCO₂).

Urine was collected in urine bags every 3 h for the next 24 h. Each void was acidified with a known volume of acid, measured and stored in separate containers for subsequent analysis of total urinary N (Varley 1980). In addition, a blood sample was collected before the start of the protocol and at the end of the 24-h urine collection period to measure plasma urea concentration in order to correct for the effect of the change in the urea pool size on N excretion rate (Kaplan 1965).

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of NaH\(^{13}\)CO\(_3\) (0.166 mg/kg) given every 20 min for a period of 3 h (Fig. 2). Breath samples were also taken every 20 min starting just before administration of the isotope. After 3 h, the NaH\(^{13}\)CO\(_3\) was stopped and intermittent maintenance doses of \(^{13}\)C leucine (3 mg/kg) were given, followed by intermittent maintenance doses of \(^{13}\)C leucine (3 mg/kg) every hour for the next 3 h. Breath samples were collected at 20-min intervals. The analysis, calculations and statistics are common to Study 1 and 2.

**Laboratory analysis.** All clinical laboratory analyses were done by routine procedures at the St. John’s Medical College Hospital Central Laboratory. Urine and fecal samples were sent for routine analysis, calculations and statistics are common to Study 1 and 2. The breath samples were analyzed in duplicate with an isotope ratio mass spectrometer (Europa Scientific, Crewe, UK) by the continuous flow technique. The precision of this technique was determined with a Hewlett Packard (Palo Alto, CA) 9890 quadrupole mass spectrometer by negative chemical ionization gas chromatography/mass spectrometry of the pentafluorobenzyl derivative, monitoring ions at m/z 129 and 130.

**Calculations.**
Percentage of \(^{13}\)C leucine oxidized (R)

\[
100 \times \frac{\text{Breath } ^{13}\text{CO}_2 \text{ enrichment}}{\text{Rate of appearance } (R_A) ^{13}\text{CO}_2} \times \frac{\text{Rate of appearance } (R_A) \text{ CO}_2}{\text{Rate of } ^{13}\text{C-leucine administration}} \tag{1}
\]

where breath \(^{13}\)CO\(_2\) enrichment (atom % excess) was measured during the last hour of \(^{13}\)C leucine administration.

\(R_A \text{CO}_2\) was calculated as

\[
\frac{\text{Dose of } ^{13}\text{C-bicarbonate administered } [\mu \text{mol/(kg} \cdot \text{h})]}{\text{Breath } ^{13}\text{CO}_2 \text{ enrichment during the last hour of the NaH}^{13}\text{CO}_3 \text{ administration}} \tag{2}
\]

Leucine flux (Q) [\(\mu \text{mol/(kg} \cdot \text{h})]\]

\[
\text{Rate of administration of } ^{13}\text{C leucine} = \frac{[\text{MPE } ^{13}\text{C leucine tracer}]}{\text{MPE } ^{13}\text{CaKIC}} - 1 \tag{3}
\]

in which MPE is the tracer:tracee ratio of \(^{13}\)CKIC (mol % excess).

Leucine oxidation (E) [\(\mu \text{mol/(kg} \cdot \text{h})\)]

\[
\frac{\text{Breath } ^{13}\text{CO}_2 \text{ enrichment} \times R_A \text{ CO}_2}{\text{Plasma } \alpha \text{KIC enrichment}} \tag{4}
\]

Leucine into protein synthesis (PS) [\(\mu \text{mol leucine/(kg} \cdot \text{h})\)]

\[
= \text{Equation 3} - \text{Equation 4} \tag{5}
\]

### TABLE 2

<table>
<thead>
<tr>
<th>Subject</th>
<th>(^{13})C-KIC(^1) labeling</th>
<th>Leucine flux</th>
<th>(^{13})CO(_2) from leucine</th>
<th>Leucine to protein synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\text{mol }%)</td>
<td>(\mu \text{mol/(kg} \cdot \text{h}))</td>
<td>(\mu \text{mol/(kg} \cdot \text{h}))</td>
<td>(%) dose</td>
</tr>
<tr>
<td>1</td>
<td>9.24</td>
<td>167</td>
<td>2.5</td>
<td>16.7</td>
</tr>
<tr>
<td>2</td>
<td>8.43</td>
<td>179</td>
<td>3.2</td>
<td>21.0</td>
</tr>
<tr>
<td>3</td>
<td>8.01</td>
<td>189</td>
<td>3.2</td>
<td>21.1</td>
</tr>
<tr>
<td>4</td>
<td>8.45</td>
<td>179</td>
<td>3.2</td>
<td>20.8</td>
</tr>
<tr>
<td>5</td>
<td>8.70</td>
<td>174</td>
<td>3.1</td>
<td>20.0</td>
</tr>
<tr>
<td>Mean ± sd</td>
<td>8.57 ± 0.45*</td>
<td>178 ± 8*</td>
<td>3.0 ± 0.3</td>
<td>19.9 ± 1.9*</td>
</tr>
</tbody>
</table>

Prevaccination

Postvaccination

1 DPT, diphtheria, pertussis and tetanus vaccine; KIC, \(\alpha\)-ketoisocaproic acid.

\(\ast P < 0.05\) (by Wilcoxon rank test) with respect to prevaccination values.

### TABLE 3

A comparison of leucine oxidation in men and children studied with intermittent oral doses of \([1-{^{13}}\text{C}]\)leucine before and 24 h after vaccination with DPT\(^1,2\)

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Prevaccination</th>
<th>Postvaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(R_A \text{ CO}_2, \text{mmol/(kg} \cdot \text{h}))</td>
<td>14.27 ± 2.96</td>
<td>14.67 ± 3.02</td>
</tr>
<tr>
<td>(^{13}\text{CO}_2) production from leucine, (\mu \text{mol/(kg} \cdot \text{h}))</td>
<td>2.7 ± 0.3</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>Dose oxidized, %</td>
<td>18.1 ± 2.5</td>
<td>19.9 ± 1.9*</td>
</tr>
<tr>
<td>Children</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(R_A \text{ CO}_2, \text{mmol/(kg} \cdot \text{h}))</td>
<td>13.83 ± 2.55</td>
<td>15.02 ± 3.04</td>
</tr>
<tr>
<td>(^{13}\text{CO}_2) production from leucine, (\mu \text{mol/(kg} \cdot \text{h}))</td>
<td>3.8 ± 0.1</td>
<td>4.6 ± 1.1</td>
</tr>
<tr>
<td>Dose oxidized, %</td>
<td>16.7 ± 3.8</td>
<td>19.5 ± 4.63</td>
</tr>
</tbody>
</table>

1 DPT, diphtheria, pertussis and tetanus vaccine.

2 Values are means ± sd; \(n = 5\) for men and 9 for children.

3 Percentage of dose oxidized postvaccination was 10 and 17% higher than the prevaccination value in the men and children, respectively.

\(\ast P < 0.05; \ast\ast P < 0.01;\) Wilcoxon signed rank test between pre- and postvaccination means.
Leucine from protein breakdown (by tracer method) [g protein/(kg TBW) = leucine content of body protein (g/kg)] in which 131.17 is the molecular weight of leucine and 0.08 is the oxidation:Flux, % 13.7

Leucine oxidation, μmol/(kg · h) 28 ± 6

Leucine flux, μmol/(kg · h) 154 ± 23

Leucine to protein, μmol/(kg · h) 134 ± 11

Leucine from protein, μmol/(kg · h) 131 ± 20

Protein breakdown, mg/(kg · h) 206 ± 30

Protein synthesis, mg/(kg · h) 233 ± 18

Protein balance, mg/(kg · h) 6 ± 10

Nitrogen excretion method

Corrected N excretion, μg/(kg · h) 8.3 ± 4.0

Amino acid N turnover, μg/(kg · h) 47 ± 24

Protein synthesis, mg/(kg · h) 243 ± 130

Variable Prevaccination Postvaccination χ²

13C-KIC, mol % excess 10.1 ± 1.6 8.6 ± 0.5 —
Leucine flux, μmol/(kg · h) 154 ± 23 178 ± 8* 3
Leucine oxidation, μmol/(kg · h) 28 ± 6 35 ± 5** 4
Oxidation:Flux, % 18.07 ± 2.54 19.81 ± 1.85 —
Leucine to protein, μmol/(kg · h) 126 ± 18 142 ± 4 5
Leucine from protein, μmol/(kg · h) 119 ± 23 143 ± 8 6
Protein synthesis, mg/(kg · h) 206 ± 30 232 ± 10 7
Protein breakdown, mg/(kg · h) 200 ± 40 240 ± 10* 8
Protein balance, mg/(kg · h) 6 ± 10 −8 ± 3 9

where TBW is total body water estimated after Siri (1961), and 0.92 is a correction term for urea concentration in plasma water.

Corrected equivalent protein excretion [g protein/(kg · h)]

Corrected urinary N excretion × 6.25 × 10⁻³ (11)

Total amino-N flux [mg N/(kg · h)]

rate of infusion of 13C leucine

rate of excretion of 1³C in breath CO₂

× rate of N excretion (12)

with the rate of excretion of ¹³C in breath CO₂ from Equation 1.

Protein synthesis [mg/(kg · h)]

= (Equation 12 − rate of N excretion) × 6.25 (13)

Statistical analysis. Because of the small sample size, differences were tested with nonparametric tests (Wilcoxon) for paired comparisons and were considered significant if P < 0.05. Correlation was performed by Pearson’s product-moment method. Results are expressed as means ± SD.

RESULTS

Preliminary studies. Preliminary studies to determine the contribution of the diet to the ¹³C enrichment of CO₂ in the breath showed a negligible change in the isotopic enrichment of expired carbon dioxide (<0.001 atom % excess) after feeding.

Study 1. The subjects’ mean height was 164.7 ± 4.1 cm, their weight was 44.0 ± 2.8 kg and their body mass index was 16.3 ± 0.9 kg/m². There was no change in their weight during the 2.8 kg and their body mass index was 16.3 ± 0.9 kg/m². There was no change in their weight during the 10 d of the study. Two of the subjects had Escherichia coli in their fecal samples, but all other routine laboratory tests were normal. After the vaccination, on the study morning, two of the subjects had a mild pyrexia (39°C), and all complained of pain and stiffness at the injection site. The hematological measurements (Table 1) showed that vaccination caused a significant increase in total count of white blood cells
weight-for-age; and was 119. Examination showed no abnormality. Their hemoglobin level statistics 1990) of malnourished, with Z-scores (National Center for Health Sa-

tively. Their mean mid-upper arm circumference was 14.3 was 4.1 and 49.7 respectively. Their mean mid-upper arm circumference was 14.3 was 4.1 and 49.7 respectively. The isotopic enrichment of plasma KIC was significantly (∼5%) during the infusion of either labeled bicarbonate or leucine. Table 2 shows the individual data for plasma KIC labeling, leucine oxidation and leucine kinetics. The isotopic enrichment of plasma KIC was significantly (P < 0.05) lower and the percentage of dose oxidized and leucine flux were significantly (P < 0.05) higher during the second (postvaccination) study. The increase in oxidation after vaccination was not associated with an increase in the R_A of CO2 (Table 3). There was no significant difference in the plasma urea between the start and the end of the protocol either before (8.6 ± 2.6 vs. 8.2 ± 2.9 mmol/L plasma) or after the vaccination (7.0 ± 3.2 vs. 7.8 ± 3.2 mmol/L plasma).

In Table 4, the results for leucine metabolism from the invasive (tracer method) and the noninvasive breath/urine based method are compared. The rates of protein synthesis calculated by the two methods were not significantly different. In neither case did vaccination have a significant effect on whole-body protein synthesis, although whole-body protein breakdown, calculated by the tracer method, was significantly higher 24 h after vaccination than 7 d before.

The tracer method for measuring the production rate of CO2 was compared with the excretion of CO2 by indirect calorimetry, using a recovery factor of 80%, to estimate CO2 production. Leucine oxidation was then calculated using the different values for R_A CO2. The results obtained with the two methods were very similar (Table 5).

**Study 2.** The mean age, weight and height of the children were 41 ± 0.6 y, 12.3 ± 1.3 kg, and 92.5 ± 3.2 cm, respectively. Their mean mid-upper arm circumference was 14.3 ± 1.0 cm; the mean head and chest circumferences were 48.1 ± 1.5 and 49.7 ± 2.3 cm, respectively. The children were malnourished, with Z-scores (National Center for Health Statistics 1990) of −2.4 ± 0.9 for height-for-age; −2.3 ± 0.7 for weight-for-age; and −1.2 ± 0.8 for weight-for-height. The clinical examination was normal, and routine urine and fecal examination showed no abnormality. Their hemoglobin level was 119 ± 10 g/L, the total WBC count was 9.9 ± 3.8 × 10^9 cells/L, with neutrophils 44.4 ± 17.5%, lymphocytes 46.3 ± 17.6% and eosinophils 6.6 ± 2.6%. The ESR was 20.0 ± 16.0 mm/h. The high SD of the latter was due to one subject who had an ESR of 56 mm/h. There was a mild pyrexia (+1°C) in five of the children after vaccination. This was controlled, for ethical reasons, with 120 mg of paracetamol. All of the subjects complained of stiffness and pain at the site of the injection, along with decreased mobility.

Apparent steady states in the enrichment of CO2 were seen after 2 h of 13C-bicarbonate administration (Fig. 4). The calculated R_A of CO2 in both phases is presented in Table 3. There was a significant (P < 0.01; 17%) increase in the oxidation of 13C-leucine, but no significant change in the R_A of CO2 after vaccination.

**DISCUSSION**

The synergy of infections and poor nutrition can lead to a cycle in which malnutrition increases susceptibility to infections and vice versa; a negative relationship between the frequency of infectious diseases and growth has been reported for many countries, including India (Kielmann et al. 1978).

Under the stimulus of proinflammatory cytokines such as interleukins (Dinarello 1984), endocrine changes that lead to the mobilization of amino acids from the periphery, primarily from skeletal muscle (Essen et al. 1993, Souba et al. 1990), are initiated. The amino acids so released are taken up by the splanchnic tissues (Souba et al. 1990) and used for the synthesis of the positive acute phase proteins (Colley et al. 1983).

It has been argued (Reeds et al. 1997) that the associated loss of body protein reflects an amino acid imbalance that results from the dissimilarity of the amino acid composition of the acute phase proteins (Dinarello 1984). Low in branched-chain amino acids (BCAAs) and muscle protein (low in aromatics and cysteine, high in BCAA). According to this hypothesis, amino acids, notably the BCAAs, that are mobilized in excess of the need for acute phase protein synthesis are "wasted" via excess oxidation. The primary objective of this study was to quantify whether this occurs during an "infection" as simulated by vaccination. A secondary objective was to use the investigation to examine the utility of a minimally invasive isotopic protocol that might be applicable to larger-scale studies in the field.

**Methodological considerations.** In designing the isotopic approach to test our main hypothesis, i.e., that infection is associated with an increased oxidation of leucine, a number of points have to be considered.

First, although studies performed in the postabsorptive or fasting state simplify the interpretation of the isotopic data, the main nutritional issue is whether concurrent infection lowers the ability of the individual to retain dietary amino...
acids. We therefore performed the studies in fed subjects. This poses some limitations. First, because leucine oxidation is closely related to leucine intake in the diet, (El Khoury et al. 1995a and 1995b, Pelleiter et al. 1991), the study should be performed at a relatively low but nutritionally adequate leucine (protein) intake. Second, to infer changes in protein balance from the metabolism of a single tracer amino acid, it is important to use diets in which the contribution of the test amino acid is close to its contribution to body protein.

In addition, we also wished to develop a protocol that involved as little manipulation of the subject as possible and specifically one that did not rely on data from the labeling of circulating leucine or its metabolites. Thus, we used oral tracers and based our main conclusions on data obtained only from the analysis of urine and breath. In the adult study, we were able to compare the results from the noninvasive tracer protocol against those from a more conventional protocol that involved blood sampling and analysis of the labeling of circulating α-keto isocaproic acid (Matthews et al. 1982). The results from the two approaches were very similar. However, although the breath/urine "end product" method has the theoretical advantage of not involving any assumptions with regard to precursor amino acid labeling, the variability of the values for protein synthesis derived from the end product approach was high. This highlights the main limitation to the approach, i.e., the wide variability of urinary N excretion when it is measured over short periods of time. Although the method has been used successfully in animals (Yagi and Walser 1990) and in humans (El Khoury et al. 1995b) under well-controlled conditions, the quantification of true body N loss from urine, as an index of net protein oxidation (Munro 1964), is neither easy nor accurate under field conditions.

An important advantage of the short, noninvasive protocol is that there is no need for indirect calorimetry. Indeed, it can be argued that because the method that we used measures CO2 production (R A CO2), it is a more theoretically appropriate approach to the isotopic study of substrate oxidation, and its use obviates the need to measure or assume a recovery factor for excreted CO2.

Response to vaccination. The main objective of this study was to investigate the changes in leucine metabolism after an experimental or simulated infection. Previous studies (Garlick et al. 1980), using 15 N glycine, found an increase in whole-body protein synthesis of 37% ~12 h after vaccination. This is similar to other reports of increased protein synthesis after severe infection (Long et al. 1977) in humans, and during turpentine-induced stress in rats (Wusteman et al. 1990). In this study, the protein synthesis rate in the men increased by only 15%. However, there was a proportionally greater increase in protein breakdown, and protein balance became more negative. In both studies, vaccination increased leucine oxidation, and the increase in the children was higher than that in the adults. This suggests that the metabolic stress of infection may be greater in children. The administration of paracetamol could have also mitigated this response, although paracetamol acts centrally to inhibit prostaglandin synthesis (Dascombe 1985, Vane 1971), with little peripheral anti-inflammatory effect. The higher response in children may also reflect a booster effect of other recent immunizations.

In conclusion, this 13C leucine tracer protocol appears to be sufficiently sensitive to detect protein metabolic changes imposed by the relatively minor stress of vaccination. For comparisons that do not require estimates of protein turnover rates, the protocol requires only a breath collection and attention to the constancy of prior leucine intake. Estimates of protein synthesis can be obtained by the addition of a complete urine collection, plus two blood samples for correction of changes in urea pool size. We believe that this protocol will prove useful in metabolic studies performed outside a metabolic unit setting.

LITERATURE CITED