

Effects of tuberculosis and HIV infection on whole-body protein metabolism during feeding, measured by the [¹⁵N]glycine method¹⁻³

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ABSTRACT

Background: Tuberculosis (TB) and HIV infection are wasting diseases that frequently occur together and have severe consequences on nutritional status.

Objective: The objective was to determine the effects of TB and HIV, separately and together, on protein metabolism.

Design: Protein metabolism was determined in the fed state in 11 healthy control subjects, in 10 patients with HIV infection without TB or other active infection (HIV group), in 10 patients with active TB without HIV infection (TB group), and in 8 patients with HIV infection and active TB (HIVTB group) with the use of oral [¹⁵N]glycine and measurement of enrichment in urinary urea and ammonia.

Results: Whole-body protein flux and degradation were lower in the HIV group than in the control group (mean flux: 3.53 ± 0.40 compared with 4.75 ± 0.97 g · kg lean body mass⁻¹ · 12 h⁻¹; $P = 0.002$). Protein flux, synthesis, and degradation were not significantly different between the control group and the TB and HIVTB groups. Net protein balance was strongly anabolic in the control, HIV, and TB groups but was neutral in the HIVTB group ($P < 0.001$ for comparison between groups).

Conclusions: HIV infection was associated with a significant down-regulation of whole-body protein flux. TB alone was not associated with abnormal protein metabolism, but net anabolism in the fed state was impaired in the HIVTB group. *Am J Clin Nutr* 2003;78:319–25.

KEY WORDS HIV, tuberculosis, infection, protein metabolism, glycine, wasting

INTRODUCTION

Chronic infections such as HIV and tuberculosis (TB) are often associated with a deterioration in nutritional status and sometimes profound wasting. In many developing countries, TB is the most frequent opportunistic infection in patients with HIV infection, and coinfecting patients appear to be at much greater risk of profound wasting. Postmortem examination of persons who died from slim disease suggested that infection with TB exacerbates the wasting process of HIV-infected patients in Africa (1). Wasting usually reflects the loss of fat mass and body cell mass, and significant tissue loss can persist for months after the introduction of effective therapy for TB (2). Mortality is increased in HIV (3–5) and in TB (6, 7) patients who have significant wasting. One important question is whether nutritional intervention is effective in preventing or correcting wasting, thereby leading to an improved prognosis and greater efficacy of anti-infective drugs.

For any infection there is a complex interaction between the host response and the virulence of the organism, which modulates the overall metabolic response and the degree and pattern of tissue loss. In patients with HIV infection and TB, a reduction in appetite—which leads to decreases in energy, protein, and nutrient intakes—may interact with increased losses and altered metabolism as part of the inflammatory and immune responses (2, 8, 9). A clear understanding of the underlying metabolic changes may help to ensure effective nutritional intervention in wasted patients. In the presence of ongoing infection, it is possible that supplemental feeding will increase fat mass but not lean tissue mass, a change that is likely to be of little clinical benefit (10). Clinically stable patients with HIV appear to have a normal response to feeding (11), although the response appears blunted in those with an acute opportunistic infection (12). Patients with TB may have some attenuation of the protein anabolic response to food (11). No study has compared protein metabolism between patients with HIV infection and TB with the same experimental methods or has measured the effects of the co-occurrence of the 2 infections in the same patient.

We therefore investigated the effects of HIV and TB infections, both independently and together, on protein metabolism. Our hypothesis was that TB would be associated with reduced net anabolism during feeding and that this would be further impaired in patients coinfecting with TB and HIV.

SUBJECTS AND METHODS

Patients

The study was conducted at the Communicable Disease Centre, Tan Tock Seng Hospital, Singapore, between October 1999 and June 2000. Male patients aged 18–60 y were recruited from 4 clinical groups according to the specific inclusion criteria described

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below. We excluded subjects from all groups if they had type 1 diabetes, diarrhea (defined as >3 loose stools/d at any time during the previous week), symptoms of an upper respiratory tract infection, or clinically apparent jaundice.

Subjects in the control group were healthy members of hospital staff who were considered to be at low risk of HIV infection or healthy blood donors who were receiving counseling for asymptomatic hepatitis B virus infection. (These subjects were negative for hepatitis B e antigen and HIV antibodies and had normal liver function.) Patients with HIV infection only (HIV group) were required to have confirmed HIV infection (by enzyme-linked immunosorbent assay and Western Blot analysis) and to be free of opportunistic infection for ≥ 1 mo before the study began. Patients infected with tuberculosis only (TB group) were required to have a clinical diagnosis of tuberculosis, to have been receiving antituberculosis chemotherapy for <2 wk at the time of the study, and to have a negative result from an HIV antibody test or to be free of HIV risk factors, which was ascertained by specific questioning by the primary physician and study staff. Patients infected with both HIV and TB (HIVTB group) were required to have confirmed HIV infection, to have a clinical diagnosis of TB, to have been receiving antituberculosis therapy for <2 wk at the time of study, and to be free of other HIV-related opportunistic infections for ≥ 1 mo before the study began. The protocol was explained to the subjects before participation, and all subjects gave written informed consent. The study was approved by the Ethics Committee of Tan Tock Seng Hospital.

Methods

Protein metabolism

Whole-body protein metabolism was measured by using the [^{15}N]glycine end product method as previously reported (13, 14) and as outlined below. Subjects were studied in the morning after an overnight fast, during which time only water was permitted. A single oral dose of 100 mg [^{15}N]glycine (99% atoms excess; Icon Services Inc, Summit, NJ) was dissolved in 100 mL sterile water and administered at 0800. Subsequently, at 3-h intervals thereafter until the completion of the 12-h protocol, subjects were given drinks containing a powdered nutritional supplement (2 g/kg body wt, Ensure; Ross Laboratories, Columbus, OH) made up in sterile water to a volume of ≈ 200 mL. Subjects were allowed to drink additional water as desired during the 12-h study protocol but no other drinks or food orally. This regimen was designed to provide the daily food requirement over the 12-h period. The total protein intake was equivalent to 1.27 g/kg body wt, and the total energy intake was 151 kJ/kg body wt.

A blood sample (20 mL) was collected into heparin-containing tubes before and 12 h after the administration of [^{15}N]glycine. The plasma was separated and stored at -70°C until analyzed. A urine sample was collected into a 200-mL container with 0.1 mL chlorhexidine and 1.5 mL of 7 mol HCl/L before the administration of [^{15}N]glycine. After the administration of the isotope, urine was collected continuously for 12 h into a container with 2 mL chlorhexidine and 20 mL of 7 mol HCl/L. The total volume of urine was measured, and aliquots were stored at -30°C until analyzed.

The enrichments of urea nitrogen and ammonia nitrogen in urine and the enrichment of urea nitrogen in blood were measured as previously described (14). The rate of nitrogen flux was calculated by using the following equation (13):

$$Q = d \times E_x / e_x \quad (1)$$

where Q is the rate of nitrogen flux (grams of nitrogen over 12 h), d is the amount of isotope administered (grams of ^{15}N), E_x is the amount of ammonia or urea excreted (grams of nitrogen over 12 h), and e_x is the amount of isotope excreted in urine as ammonia or the amount of isotope excreted in urine as urea and retained in the urea pool of the body (grams of ^{15}N over 12 h). Rates of flux were estimated on the basis of the recovery of label in either ammonia or urea as the end product and also by using the arithmetic average for the 2 end products. A factor of 6.25 was used to convert the flux values from grams of nitrogen to grams of protein.

Whole-body rates of protein synthesis and degradation were calculated by using the following equation:

$$Q = E_t + S = I + D \quad (2)$$

where E_t is the corrected rate of protein excretion (calculated from total nitrogen in urine), S is the rate of protein synthesis, I is the rate of protein intake from the diet, and D is the rate of whole-body protein degradation (13). The calculation assumes that 100% of the administered food is absorbed. Rates were expressed as absolute rates of protein turnover, rates relative to body weight, and rates relative to total lean body mass.

Dietary, anthropometric, body-composition, and laboratory measurements

The habitual food consumption of all subjects was estimated by using a 3-d dietary recall method. Commercial computer software (NUTRITIONIST IV; The Hearst Corporation, San Bruno, CA) was used for the analysis.

Height was measured to the nearest 1 mm with a portable stadiometer, and body weight was measured to the nearest 0.1 kg with the use of calibrated electronic scales. Body mass index was calculated as weight (in kg) divided by the square of the height (in m). Body composition was measured by dual-energy X-ray absorptiometry with a Hologic QDR 2000+ scanner with software version 5.71 (Hologic Inc, Bedford, MA). The scan was performed between 0900 and 1000 on the morning of the study day. The subjects wore light indoor clothing, and the measurements were made in the fed state. Appendicular lean tissue mass was obtained from the sum of lean tissue in the arms and legs, and visceral lean tissue mass was considered to be the trunk lean tissue measurement (ie, the remaining lean tissue, other than from the head region).

Blood was also collected on the study day for measurement of the erythrocyte sedimentation rate and C-reactive protein and albumin concentrations with the use of standard laboratory methods. Case sheets of patients were reviewed for details of HIV and TB disease and treatment.

Statistical analysis

A comparison of variables between the 4 clinical disease groups was made by analysis of variance with a post hoc Scheffe's test. Pearson's test was used to assess the correlation between flux measurements and regional body composition. All statistical tests were two sided, and a P value <0.05 was regarded as significant. The analysis was performed by using SPSS version 9.0 (SPSS Inc, Chicago).

RESULTS

A total of 45 subjects were studied. Of these, 4 failed to complete the protocol adequately (incomplete urine collection and



TABLE 1

Comparison of anthropometric, body-composition, and laboratory variables between the 4 groups of subjects¹

	Group				P ²
	Control (n = 11)	HIV (n = 10)	TB (n = 10)	HIVTB (n = 8)	
Age (y)	40.8 ± 8.4	37.1 ± 4.1	40.3 ± 9.9	36.3 ± 4.8	0.45
Height (cm)	167.4 ± 4.7	169.6 ± 5.9	167.9 ± 4.6	168.6 ± 8.5	0.85
Weight (kg)	61.2 ± 8.9	63.0 ± 3.3	50.8 ± 6.2 ^{3,4}	51.0 ± 7.6 ^{3,4}	<0.001
BMI (kg/m ²)	21.8 ± 3.0	22.0 ± 1.5	18.0 ± 2.3 ^{3,4}	17.9 ± 2.3 ^{3,4}	<0.001
Fat (kg)	13.7 ± 5.4	11.3 ± 2.9	7.4 ± 3.5 ³	7.0 ± 3.7 ³	0.002
Lean tissue mass (kg)	44.1 ± 4.6	47.5 ± 3.9	40.1 ± 2.8 ⁴	41.0 ± 5.3 ⁴	0.001
Appendicular lean tissue mass (kg)	18.7 ± 2.0	19.6 ± 18.7	15.7 ± 2.0 ^{3,4}	15.8 ± 2.4 ^{3,4}	<0.001
Visceral lean tissue mass (kg)	22.1 ± 2.2	24.5 ± 2.0	21.2 ± 1.3 ⁴	22.1 ± 2.8	0.009
Visceral:appendicular lean tissue mass	1.19 ± 0.06	1.25 ± 0.06	1.36 ± 0.15 ³	1.41 ± 0.10 ^{3,4}	<0.001
Hemoglobin (g/dL)	14.6 ± 1.3	15.4 ± 1.1	13.1 ± 1.6 ⁴	10.2 ± 1.7 ³⁻⁵	<0.001
White cells (× 10 ⁹ /L)	6.39 ± 1.15	5.07 ± 1.99	9.66 ± 5.75 ⁴	4.54 ± 2.47 ⁵	0.009
Lymphocytes (%)	32.0 ± 7.3	30.2 ± 5.7	20.1 ± 7.6 ^{3,4}	21.2 ± 8.9 ³	0.001
Total protein (g/L)	76.4 ± 4.5	79.3 ± 3.3	79.8 ± 4.4	76.1 ± 12.1	0.71
Albumin (g/L)	44.9 ± 1.4	44.4 ± 2.3	38.5 ± 4.0 ^{3,4}	29.6 ± 3.5 ³⁻⁵	<0.001
Globulin (g/L)	30.9 ± 3.9	34.9 ± 4.5	41.3 ± 4.8 ³	46.5 ± 9.0 ^{3,4}	<0.001
ESR (mm/h)	7.6 ± 9.1	8.9 ± 11.8	81.8 ± 39.0 ^{3,4}	79.7 ± 36.2 ^{3,4}	<0.001
CRP (mg/L)	5.7 ± 4.8	3.9 ± 0.9	51.8 ± 45.4 ^{3,4}	23.4 ± 14.3	<0.001

¹ $\bar{x} \pm$ SD. Body composition was measured by dual-energy X-ray absorptiometry. ESR, erythrocyte sedimentation rate; CRP, C-reactive protein. TB, tuberculosis; HIVTB, coinfection with HIV and TB.

²ANOVA with post hoc Scheffe's test.

³Significantly different from the control group, $P < 0.05$.

⁴Significantly different from the HIV group, $P < 0.05$.

⁵Significantly different from the TB group, $P < 0.05$.

noncompliance with the feeding schedule) and 2 others were excluded from the analysis because of later evidence that contradicted the initial clinical diagnosis of TB. Thus, 39 subjects were available for the analysis: 11 control subjects (8 hospital staff and 3 carriers of hepatitis B), 10 in the HIV group, 10 in the TB group, and 8 in the HIVTB group. The HIV group had a mean CD4⁺ count of 259 cells/mm³ and a mean HIV viral load of 4.4 log₁₀ copies/mL; 4 patients in this group were receiving antiretroviral treatment at the time of the study (dual or single nucleosides only). In the TB group, all subjects had pulmonary disease (one had lymphadenopathy also) and all but one (in whom the diagnosis was confirmed by clinical and radiologic response to antituberculosis treatment) had *Mycobacterium tuberculosis* cultured in their sputum. The mean duration of TB treatment at the time of the study was 6 d (range: 1–12 d), and none of the patients had taken corticosteroids. The HIVTB group had a mean CD4⁺ cell count of 40 cells/mm³, and a mean HIV viral load of 5.3 log₁₀ copies/mL; only 1 patient was receiving antiretroviral treatment (dual nucleosides) at the time of the study. All subjects in this group had disseminated tuberculosis (including pulmonary disease in all but one) involving a variety of sites (lymph nodes, liver, spleen, prostate, and blood) and all had *M. tuberculosis* cultured from one or more sites. The mean duration of TB treatment at the time of the study was 9 d (range: 5–13 d), and one patient had taken adjunctive corticosteroids. Of the 39 subjects, 30 (77%) were Chinese, and the racial distribution (Chinese compared with other Asian races) was not significantly different between the 4 groups.

The characteristics of the groups of subjects are shown in **Table 1**. There was no difference in age or height between the groups. Whereas no significant differences in weight or body mass index were observed between the HIV and the control groups, both the TB and HIVTB groups were severely wasted. This

wasting was characterized by a significant loss of fat mass and appendicular lean tissue mass; visceral lean tissue mass was relatively preserved. The HIV group had hematologic values that did not differ significantly from those of the control group, but hemoglobin concentrations and lymphocyte counts (%) were significantly lower in the TB and HIVTB groups than in the control and HIV groups. The TB and HIVTB groups had clear evidence of ongoing inflammation on the basis of elevated erythrocyte sedimentation rates and C-reactive protein concentrations, which were not evident in the HIV and control groups. Total plasma proteins were not significantly different between the 4 groups, but plasma albumin was significantly lower and plasma globulin was significantly higher in the TB and HIVTB groups than in the control and HIV groups.

The habitual energy and protein intakes of the subjects are shown in **Table 2**. No significant difference in total energy consumption or energy consumption relative to body weight was observed between the 4 groups. The HIV group had a significantly higher consumption of protein, and the proportion of total energy derived from protein was greater in this group.

The results of the protein metabolism measurements are given in **Table 3**. Estimates of nitrogen flux based on the excretion of isotope in either urea or ammonia are shown, as are values of protein flux derived from the average of the end products. Absolute values and values normalized by body weight or lean body mass are all shown. The pattern and direction of differences between the patient groups was similar regardless of the end product used for the estimate of flux or of the method of expression, although these differences were not significant when calculations were based on the ammonia end product alone. Protein flux calculated from the average end product was 26% lower and the rate of protein degradation was 35% lower in the HIV group than in the control group, but protein synthesis and

TABLE 2
Comparison of habitual energy and protein intakes between the 4 groups¹

	Group				<i>P</i> ²
	Control (<i>n</i> = 11)	HIV (<i>n</i> = 10)	TB (<i>n</i> = 10)	HIVTB (<i>n</i> = 8)	
Energy					
(kcal)	1733 ± 465	1966 ± 270	1616 ± 380	1820 ± 534	0.308
(kcal/kg body wt)	28.6 ± 7.1	31.2 ± 4.3	32.3 ± 9.0	36.6 ± 12.9	0.70
Protein					
(g)	63.6 ± 14.5	91.1 ± 19.5 ³	66.0 ± 18.3 ⁴	69.1 ± 23.4	0.008
(g/kg LBM)	1.5 ± 0.3	1.9 ± 0.4	1.6 ± 0.5	1.7 ± 0.6	0.15
Protein:energy (%)	15.0 ± 2.1	18.5 ± 2.8 ³	15.0 ± 1.8 ⁴	16.3 ± 2.2	0.004

¹ $\bar{x} \pm$ SD. LBM, lean body mass measured by dual-energy X-ray absorptiometry. TB, tuberculosis; HIVTB, coinfection with HIV and TB.

²ANOVA with post hoc Scheffe's test.

³Significantly different from the control group, *P* < 0.05.

⁴Significantly different from the HIV group, *P* < 0.05.

net balance were not significantly different from the control group. Rates of protein flux, synthesis, degradation, and net balance were not significantly different between the TB and control groups. The HIVTB group had values of protein flux, synthesis, and degradation that were not significantly different from those of the control group. However, the net protein balance was significantly lower in the HIVTB group than in the control group and was close to zero. There was no significant correlation in either the TB or HIVTB group between the duration of TB treatment before the study and any of the protein metabolism variables.

The ratio of urea flux to ammonia flux did not differ significantly between the HIV, TB, and control groups but showed a trend to higher values in the HIVTB group than in the other groups

(*P* = 0.063 for between-group difference). There was no significant correlation between the ratio of urea to ammonia flux and the ratio of visceral to appendicular lean tissue mass (*r* = 0.157).

DISCUSSION

In the current study we found that whole-body protein flux and protein degradation were significantly lower in the HIV-infected patients than in the other 3 groups. Because the HIV patients reported a good dietary intake and were not wasted, it is likely that the difference was due to a direct effect of HIV infection on protein homeostasis. The near normal values of protein flux in the HIVTB group indicate that the down-regulation of protein turnover can be reversed when there is sufficient inflammatory

TABLE 3
Comparison of protein metabolism measurements between the 4 groups¹

	Group				<i>P</i> ²
	Control (<i>n</i> = 11)	HIV (<i>n</i> = 10)	TB (<i>n</i> = 10)	HIVTB (<i>n</i> = 8)	
Protein flux					
<i>Q</i> _u (g N/12 h)	26.3 ± 4.0	22.3 ± 4.7	24.9 ± 5.6	30.4 ± 5.0 ³	0.011
<i>Q</i> _a (g N/12 h)	40.2 ± 11.0	31.5 ± 6.1	37.5 ± 8.6	33.6 ± 10.7	0.166
<i>Q</i> _u / <i>Q</i> _a	0.70 ± 0.21	0.72 ± 0.17	0.70 ± 0.29	1.0 ± 0.36	0.063
<i>Q</i> (g/12 h)	207.7 ± 38.0	168.1 ± 26.3 ⁴	194.8 ± 27.1	200.1 ± 27.2	0.033
<i>Q</i> (g · kg body wt ⁻¹ · 12 h ⁻¹)	3.47 ± 0.83	2.66 ± 0.33	3.92 ± 0.88 ³	4.02 ± 0.97 ³	0.002
<i>Q</i> (g · kg LBM ⁻¹ · 12 h ⁻¹)	4.75 ± 0.97	3.53 ± 0.40 ⁴	4.89 ± 0.91 ³	4.97 ± 1.11 ³	0.002
Protein synthesis					
(g/12 h ⁻¹)	169.7 ± 36.4	136.4 ± 21.5	164.9 ± 31.4	147.3 ± 38.9	0.093
(g · kg body wt ⁻¹ · 12 h ⁻¹)	2.84 ± 0.76	2.16 ± 0.27	3.33 ± 0.93 ³	2.97 ± 1.05	0.019
(g · kg LBM ⁻¹ · 12 h ⁻¹)	3.88 ± 0.92	2.86 ± 0.34	4.15 ± 1.00 ³	3.69 ± 1.29	0.023
Protein degradation					
(g/12 h ⁻¹)	146.6 ± 39.7	105.1 ± 24.2 ⁴	144.1 ± 30.5	149.1 ± 28.3	0.011
(g · kg body wt ⁻¹ · 12 h ⁻¹)	2.47 ± 0.83	1.66 ± 0.33	2.92 ± 0.88 ³	3.02 ± 0.97 ³	0.002
(g · kg LBM ⁻¹ · 12 h ⁻¹)	3.37 ± 1.02	2.20 ± 0.41 ⁴	3.63 ± 0.95 ³	3.73 ± 1.11 ³	0.002
Protein intake, excretion, and balance					
Intake (g · kg LBM ⁻¹ · 12 h ⁻¹)	1.39 ± 0.11	1.33 ± 0.07	1.31 ± 0.11 ⁴	1.24 ± 0.10 ⁴	0.007
Excretion (g · kg LBM ⁻¹ · 12 h ⁻¹)	0.87 ± 0.12	0.66 ± 0.11	0.74 ± 0.27	1.28 ± 0.52 ³⁻⁵	<0.001
Balance (g · kg LBM ⁻¹ · 12 h ⁻¹)	0.52 ± 0.19	0.67 ± 0.13	0.52 ± 0.26	-0.04 ± 0.57 ³⁻⁵	<0.001

¹ $\bar{x} \pm$ SD. All protein metabolism values were calculated from averages of end products and are expressed as grams of protein, except where stated. LBM, lean body mass measured by dual-energy X-ray absorptiometry; *Q*_u, flux calculated from the urea end product only; *Q*_a, flux calculated from the ammonia end product only; *Q*, flux calculated from the average of *Q*_u and *Q*_a; TB, tuberculosis; HIVTB, coinfection with HIV and TB.

²ANOVA with post hoc Scheffe's test.

³Significantly different from the HIV group, *P* < 0.05.

⁴Significantly different from the control group, *P* < 0.05.

⁵Significantly different from the TB group, *P* < 0.05.

stimulus, which suggests that the block is not absolute but more likely represents a selective response to some feature of the chronic infection.

It is likely that the protein kinetic response to any infection will vary with dietary intake, body composition, nutritional-metabolic state, and intensity of the response to the infective agent. The varied findings in previous studies of protein turnover in HIV-infected patients may reflect differences in these variables or differences in standardization approaches. When oral [¹⁵N]glycine and the end product method were used in 9 asymptomatic subjects with AIDS, whole-body protein flux was reduced and there was a reduction in the fractional synthesis rate for fibrinogen (15). This finding, together with an inversion in the ratio of plasma essential to nonessential amino acids, led the authors to argue that the decrease in protein flux represented a starvation response. A similar pattern of change in the present study cannot be explained on this basis because the HIV patients had a good nutritional intake. It is also unlikely that the significantly higher ratio of protein to energy in the diet of the HIV patients (possibly a deliberate modification that was perceived to be advantageous) explained the observed down-regulation of protein flux. In contrast with these results, when the same method was used, whole-body protein flux was found to be high and variable in a group of AIDS patients who were receiving an adequate food intake but who were experiencing weight loss (16). The authors report an association between protein flux and body cell mass as a proportion of body weight, which raises the possibility that the apparent increase in flux might be due in part to changes in body composition that were not fully accounted for when body weight was used as the denominator (17, 18). The results of protein-turnover studies in HIV-infected children emphasize the need to standardize for body composition (19). The possible differences in the overall rates of protein metabolism between our group of Asian patients and in white cohorts from other studies, because of racial differences in body composition, does not explain the between-group differences we observed. Initial reports in which [¹³C]leucine was used to assess changes in protein metabolism suggest that both protein synthesis and degradation increased (20). Later studies indicated that, in wasted patients, muscle protein synthesis might be down-regulated, even when there is an overall increase in whole-body synthesis (21, 22) and that the extent of change might be responsive to the level of activity or to nutritional or hormonal modulation (12, 23, 24). In addition to differences in body composition and nutritional intake, the contrasting results between studies may also reflect the marked clinical heterogeneity of patients with HIV disease. Measurements of resting energy expenditure in HIV-infected patients suggest the occurrence of hypometabolism in some patients (25, 26) and marked hypermetabolism in others, which are usually associated with secondary infections (27). The higher rates of protein flux in the HIVTB than in the HIV group in this study support the suggestion that protein flux is elevated in patients with secondary infection and more advanced disease, possibly reflecting the direct effects of the infection or the effects of the associated wasting (20).

We found no significant differences in protein metabolism variables between the TB and control groups. This finding is in general agreement with the findings of the only previously published study of protein metabolism in TB (11). Given the marked inflammatory stimulus evoked by TB, the lack of a difference may appear somewhat surprising, but the pattern at the whole-body level may mask more complex alterations in protein metabolic


rates in specific tissues during an active inflammatory process. Nevertheless, the findings contrast with the observation of a 36% increase in protein flux in patients with melioidosis (a chronic bacterial infection similar to TB) (28) and with the increased flux documented in patients with surgical sepsis (29, 30), measles (31), and malaria (32). It may be that the degree of malnutrition offset the stimulus of TB on protein metabolism in our patients (31) or that our patients had a particularly effective adaptive response of protein metabolism to the chronic inflammatory state (28, 33). The TB patients achieved a state of net anabolism during feeding that is consistent with the results of studies of nitrogen balance in TB patients (34), the results of a previous study of protein metabolism in TB patients (ie, a blunted but still strongly anabolic response) (11), and the results of studies in other chronic infections (20, 28). Taken together, the evidence supports the idea that patients with TB should be fed to achieve net protein accretion.

The patients with concomitant HIV and TB infections (HIVTB group) had protein flux rates that were not significantly different from those of the control group. However, although net protein balance varied widely in the HIVTB group (probably reflecting the greater clinical heterogeneity of the patients in this group), net anabolism was significantly lower in the HIVTB group than in all other groups. The absence or attenuation of net anabolism in fed patients is consistent with studies in patients with acute severe infections such as malaria and surgical sepsis and in HIV patients with opportunistic infections (12, 29, 30, 32) but contrasts with the findings of most studies of chronic infection (11, 20, 28). This may reflect the fact, which is confirmed by our observations in the current study, that TB is a more fulminant and disseminated disease in patients with advanced HIV disease than it is in patients without HIV infection, who usually have localized pulmonary TB. Thus, in HIV-infected patients, coinfection with TB may evoke a response that more resembles acute, severe septicemia rather than a chronic infection. It is worth noting that the HIVTB group had more advanced HIV disease than did patients in the HIV group, as evidenced by lower CD4⁺ counts and higher viral loads. Higher levels of HIV replication per se may also contribute to the metabolic disturbances that we observed in the HIVTB group. The absence of net anabolism that we observed may also reflect a failure of adaptive nitrogen-conserving responses, which are ordinarily associated with chronic infection (28, 33). Although the mechanism of these responses is unknown, they may well be linked to the maturing immune response to infection. Thus, in the context of an HIV-induced deficiency of the immune response to chronic TB, there may be a corresponding deficiency of adaptive nitrogen-conserving responses. The findings may explain the marked adverse effect of TB on body cell mass in patients coinfecting with TB and HIV, which remains even after a period of effective treatment (2), and indicate that administration of an anabolic agent concomitant with feeding might be of benefit in the restoration of good nutritional status.

Whole-body protein turnover measurement with the use of [¹⁵N]glycine provides a summary of complex exchange reactions within the body. However, its application is limited and the basic assumption that there is a single homogenous metabolic pool of body protein without compartmentation of processes is of questionable validity (35). Nevertheless, under standard conditions, useful information can be obtained with the end product method (14, 17, 18, 35), and it gives similar results to the alternative [¹³C]leucine method (35). Although the concept remains to be validated, it has been suggested that in addition to the use of the end



product average, calculation of rates based on the individual end products—either urea or ammonia—might provide an understanding of the changes taking place in different compartments of the body. The ammonia end product may more closely reflect events in muscle, whereas the urea end product may weight toward visceral or hepatic activity (13, 36, 37). We found that the patients in the HIVTB group had a trend toward an elevated ratio of urea to ammonia flux ($P = 0.06$); however, in the other 2 groups the ratio was not significantly different from that of the control group. This elevated ratio cannot simply be explained by the higher ratio of visceral to appendicular lean tissue mass in the HIVTB group because the TB group also had an elevated lean tissue ratio but no elevated flux ratio.

Furthermore, we did not find a strong correlation between the flux ratio and the lean tissue ratio, as was observed in previous studies (17, 18). Therefore, the observed elevation in the ratio of urea to ammonia may reflect changes in compartmental protein metabolism induced by infection. The results agree with the findings in rat studies, which showed a decrease in muscle protein synthesis and an increase in liver protein synthesis in response to sepsis (38). Although we did not measure changes in the metabolism of sulfur amino acids in the current study, such changes have been documented in HIV infection and may modulate the balance of protein synthesis in liver and muscle (39, 40). Given the worldwide importance of TB and HIV coinfection and the frequency of wasting and its adverse effect on prognosis, additional studies are warranted to further define the metabolic response of protein to these infections and to investigate ways of restoring the response toward normality. 

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NIP wrote the draft of the manuscript and helped design the study, recruit the patients, and collect and analyze the data. Y-MN performed the protein metabolism and other clinical measurements. CBEC helped design the study and recruit the patients. CP analyzed the urine and blood samples for protein flux measurement. AAJ helped design the study, analyze the data, and draft the manuscript. All authors contributed to the writing of the final manuscript. The authors had no conflicts of interest in relation to this research.

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