The protein metabolic response to HIV infection in young children

Farook Jahoor, Stuart Abramson, and William C Heird

ABSTRACT
Background: Growth failure often precedes secondary infections in HIV-infected infants and children, suggesting that inadequate protein deposition may be an early manifestation of infection by the virus. However, the protein metabolic response elicited by the virus in young children is unknown.

Objective: We compared children with HIV infection and age-matched children without HIV infection with regard to whole-body and splanchnic protein kinetics and synthesis of acute phase proteins (APPs).

Design: Whole-body and splanchnic leucine kinetics and fractional and absolute synthesis rates of 2 positive and 4 negative APPs were measured in 6 asymptomatic, HIV-infected children (4 males and 2 females) aged 6–17 mo and 4 uninfected children (3 females and 1 male) aged 7–9 mo who were in the fed state.

Results: Compared with the control children, the HIV-infected children had significantly lower dietary energy and protein intakes and leucine balance and significantly faster leucine flux and fractional splanchnic leucine extraction; there was no significant difference between the groups in leucine oxidation rates. The HIV-infected children also had significantly higher plasma concentrations of fibrinogen. The concentrations of 2 of the 4 negative APPs, albumin and HDL apolipoprotein A-I, were significantly lower in the HIV-infected children but not in adults. However, little is known about protein metabolism in asymptomatic, HIV-infected infants and young children.

Conclusions: Children with HIV infection but without secondary infection have reduced protein balance because of an inability to down-regulate protein catabolism. Furthermore, the acute phase protein response elicited by HIV infection is characterized by higher concentrations and synthesis rates of positive APPs without lower concentrations of some negative APPs.

KEY WORDS Leucine kinetics, acute phase proteins, HIV infection, children, stable isotope

INTRODUCTION
Loss of lean body mass is a common finding in adults with AIDS, and in these patients, loss of lean body mass is a major predictor of both morbidity and mortality (1–3). For example, a lean body mass <66% of the expected value is a strong predictor of death (2). Reduced food intake, nutrient malabsorption, elevated resting energy expenditure, and altered protein and lipid metabolism are mechanisms that appear to contribute to this loss of lean body mass (4–8).

Interestingly, loss of lean body mass is not a common finding in asymptomatic, HIV-infected adults, ie, those without secondary infections (8, 9). Studies in such patients, like those in patients with other stressed states, showed faster rates of whole-body protein turnover in those patients than in healthy control subjects. However, dietary protein intake and net rates of protein catabolism are similar to those observed in healthy control subjects. Hence, protein balance is positive (8, 9).

In contrast, growth failure secondary to low rates of lean tissue deposition is common in infants and young children with HIV infection, including those without secondary infections (10–12). Moreover, early growth failure is an important predictor of a poor prognosis (13, 14). Thus, it appears that HIV infection, per se, induces a disturbance in protein metabolism that leads to reduced protein deposition in infants and young children but not in adults. However, little is known about protein metabolism in asymptomatic, HIV-infected infants and young children.

This study was designed to determine the effect of HIV infection in the absence of secondary infections on the whole-body protein kinetics of infants and young children. The subjects were studied in the fed state to determine the effect of the virus on splanchnic uptake and utilization of dietary protein as well as on whole-body protein kinetics. Finally, the rates of synthesis of positive and negative acute phase proteins (APPs) were measured to determine whether HIV infection without secondary infection elicits an APP response.

SUBJECTS AND METHODS

Subjects
Five infants (3 males and 2 females) and 1 young male child with perinatally transmitted HIV infection and 4 uninfected infants (1 male and 3 females) who were also born to HIV-infected mothers participated in the study. The HIV-positive children were diagnosed with the use of HIV culture and polymerase

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TABLE 1
Clinical characteristics of the HIV-infected subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>CD4 count$^2$ (cells/µL (% of total lymphocytes)</th>
<th>CDC classification</th>
<th>Medications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>514 (10)</td>
<td>C3</td>
<td>AZT, sulfamethoxazole:trimethoprim, nystatin</td>
</tr>
<tr>
<td>2</td>
<td>315 (30)</td>
<td>C3</td>
<td>Nystatin, erythromycin:sulfisoxazole</td>
</tr>
<tr>
<td>3</td>
<td>2011 (33)</td>
<td>B1</td>
<td>AZT, sulfamethoxazole:trimethoprim</td>
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<tr>
<td>4</td>
<td>1197 (40)</td>
<td>A1</td>
<td>Sulfamethoxazole:trimethoprim</td>
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<td>5</td>
<td>1242 (29)</td>
<td>N2</td>
<td>AZT, sulfamethoxazole:trimethoprim, nystatin</td>
</tr>
<tr>
<td>6</td>
<td>864 (30)</td>
<td>N1</td>
<td>Stavudine, sulfamethoxazole:trimethoprim</td>
</tr>
</tbody>
</table>

$^1$CDC, Centers for Disease Control and Prevention; AZT, azydothymidine.
$^2$Normal value: ≥500 cells/µL.

The HIV-infected children had a mean age of 9 mo (Table 2). According to the Wellcome Classification (16), 3 of the 6 HIV-infected children with body weight–for–age < 80% of the expected value were wasted. On the basis of their weight-for-length, 2 of the 3 undernourished children were also stunted. Four uninfected children with a socioeconomic background similar to that of the HIV-infected children were recruited as control subjects. The control subjects had a mean age of 8 mo and a mean body weight–for–age of 91% of the expected value. They were deemed to be in good health on the basis of a complete medical history and physical examination. On the basis of 3-d dietary intake records, the mean (± SD) habitual energy and protein intakes of the HIV-infected subjects (70 ± 10 kcal · kg$^{-1}$ · d$^{-1}$ and 1.5 ± 0.4 g protein · kg$^{-1}$ · d$^{-1}$, respectively) were significantly lower than those of the uninfected subjects (112 ± 10 kcal · kg$^{-1}$ · d$^{-1}$ and 2.25 ± 0.2 g protein · kg$^{-1}$ · d$^{-1}$, respectively).

The study was approved by the Baylor Affiliates Review Board for Human Subject Research of the Baylor College of Medicine. Written informed consent was obtained from ≥1 parent or guardian of each child enrolled.

**Experimental protocol**

The study was performed in the Pediatric General Clinical Research Center of Texas Children’s Hospital. The children were admitted to the Pediatric General Clinical Research Center the day before the experiment was performed. The experiment commenced the following morning. During the experiment, the subjects received hourly feeds equivalent to their habitual intakes for that portion of the day to ensure that the same amount of energy and protein were given during the course of the isotope infusions. The feeds were started 2 h before the isotope infusions commenced. The mean energy and protein intakes of the HIV-infected group during the study period were 4.2 ± 0.5 kcal · kg$^{-1}$ · h$^{-1}$ and 96 ± 21 mg · kg$^{-1}$ · h$^{-1}$, respectively; the mean intakes of the

TABLE 2
Physical characteristics of the subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Sex</th>
<th>Age</th>
<th>Weight (kg)</th>
<th>Length (cm)</th>
<th>Length-for-age (%)</th>
<th>Weight-for-age (%)</th>
<th>Weight-for-length (%)</th>
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<tbody>
<tr>
<td>HIV infected</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>6</td>
<td>4.8</td>
<td>51.0</td>
<td>77</td>
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<td>126</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>10</td>
<td>7.2</td>
<td>70.5</td>
<td>98</td>
<td>77</td>
<td>80</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>6</td>
<td>8.0</td>
<td>70.2</td>
<td>105</td>
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<td>92</td>
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<tr>
<td>4</td>
<td>M</td>
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<td>10.8</td>
<td>73.8</td>
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<td>57.2</td>
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<td>68</td>
<td>114</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>9</td>
<td>8.2</td>
<td>68.3</td>
<td>97</td>
<td>92</td>
<td>100</td>
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<tr>
<td>$\bar{x} \pm SE$</td>
<td>9 ± 2</td>
<td>7.5 ± 1</td>
<td>62 ± 8</td>
<td>92 ± 4</td>
<td>84 ± 6</td>
<td>104 ± 7</td>
<td></td>
</tr>
<tr>
<td>Normal value</td>
<td>8.9</td>
<td>70.7</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Uninfected |     |     |             |             |                    |                    |                       |
| 1          | F   | 7   | 6.5         | 66.6        | 99                 | 81                 | 83                    |
| 2          | M   | 9   | 9.2         | 70.6        | 100                | 103                | 100                   |
| 3          | F   | 8   | 7.3         | 64.3        | 93                 | 87                 | 106                   |
| 4          | F   | 9   | 8.3         | 70          | 99                 | 93                 | 95                    |
| $\bar{x} \pm SE$ | 8 ± 0.5 | 7.6 ± 1 | 67 ± 3 | 98 ± 2 | 91 ± 5 | 96 ± 5 |
| Normal value | 8.4 | 69.2 | 100 | 100 | 100 |

$^1$There were no significant differences between the HIV-infected subjects and the uninfected subjects.
Whole-body and splanchnic leucine kinetics and the rates of synthesis of positive and negative APPs were determined by the simultaneous infusion of $[^2\text{H}_3]\text{leucine}$ and $[^1\text{C}]\text{leucine}$ as shown in Figure 1. Two intravenous access sites were established in opposite arms by the insertion of 24G catheters. One intravenous catheter was used for infusion of the labeled compounds and the other for blood sampling. Sterile solutions of $[^2\text{H}_3]\text{leucine}$, $[^1\text{C}]\text{leucine}$, and NaH$^{13}\text{CO}_3$ (99.9%; Cambridge Isotope Laboratories, Woburn, MA) were prepared in a 9-g NaCl/L solution. After baseline blood and breath samples were collected, a primed intermittent oral (constant intragastric for subject 1) administration of $[^2\text{H}_3]\text{leucine}$ (priming dose = 20 μmol/kg, intermittent dosage = 20 μmol·kg$^{-1}$·h$^{-1}$) was started and maintained for 6 h. The oral dose of isotope was given at 30-min intervals. A primed constant intravenous infusion of NaH$^{13}\text{CO}_3$ (priming dose = 4.5 μmol/kg, intravenous infusion at 6 μmol·kg$^{-1}$·h$^{-1}$) also was started and maintained for 2 h. After 1 h, 4 breath samples were collected at 20-min intervals. A primed constant intravenous infusion of NaH$^{13}\text{CO}_3$ (priming dose = 4.5 μmol/kg, intravenous infusion at 6 μmol·kg$^{-1}$·h$^{-1}$) also was started and maintained for 2 h. After 1 h, 4 breath samples were collected at 20-min intervals to the end of the NaH$^{13}\text{CO}_3$ infusion. A primed constant intravenous infusion of $[^1\text{C}]\text{leucine}$ (priming dose = 13 μmol/kg, intravenous infusion at 13 μmol·kg$^{-1}$·h$^{-1}$) was then started and maintained for 4 h. Additional 1.5-mL blood samples were obtained at 3, 4, and 6 h, and 0.5-mL blood samples were obtained at 5.25, 5.5, and 5.75 h. Breath samples were also collected at the latter times, as well as at 6 h.

Sample analyses

The blood samples were drawn into prechilled tubes containing sodium EDTA and a cocktail of sodium azide, merthiolate, and soybean trypsin inhibitor. They were centrifuged immediately at 1000 × g and 4°C for 10 min, and the plasma was removed and stored immediately at −70°C until analyzed.

Plasma concentrations of 6 proteins [albumin, HDL apolipoprotein (apo) A-I, retinol-binding protein (RBP), transthrethin, α-$\text{1}$-antitrypsin, and fibrinogen] were measured by using radial immunodiffusion with NL RID kits (The Binding Site, San Diego). Albumin, fibrinogen, VLDL apo B-100, and HDL apo A-I were extracted from plasma as previously described (17). Transthrethin and α-$\text{1}$-antitrypsin were isolated from plasma by sequential immunoprecipitation with anti-human transthrethin and α-$\text{1}$-antitrypsin (Behring, Somerville, NJ) as previously described (18). The immunoprecipitates and protein precipitates were subjected to sodium dodecyl sulfate–gel electrophoresis to separate the particular protein from its specific antibody and to separate apo A-I from HDL. A pure standard of the protein (Sigma, St Louis) and low-molecular-weight standards (Biorad Laboratories, Richmond, CA) were also included in the gel (18). After staining with Coomassie brilliant blue dye, the bands corresponding to the protein standard were cut out and washed several times. The dried protein precipitates and gel bands were hydrolyzed in a 6-mol HCl/L solution at 110°C for 12 h. Amino acids released from hydrolysis of the proteins and plasma amino acids were extracted by cation-exchange chromatography and converted to the $n$-propyl ester heptafluorobutyramide derivative. The tracer-tracee ratio of leucine was measured by negative chemical ionization gas chromatography–mass spectrometric analysis on a Hewlett-Packard 5890 quadrupole mass spectrometer (Hewlett-Packard, Palo Alto, CA) with selective monitoring of ions having mass-to-charge ratios of 349–352. The tracer-tracee ratio of plasma α-$\text{ketoisocaproic}$ acid (α-KICA) were measured by negative chemical ionization gas chromatography–mass spectrometric analysis of its pentafluorobenzyl derivative with selective monitoring of ions having mass-to-charge ratios of 129–132. The breath samples were analyzed in duplicate for $^{13}\text{C}$ abundance in carbon dioxide by gas isotope ratio mass spectrometry (Europa Scientific, Crewe, United Kingdom) with selective monitoring of ions having mass-to-charge ratios of 44 and 45.
**Calculations**

Carbon dioxide flux (RaCO₂) was calculated from the steady state equation:

\[ RaCO₂ = [(E_{\text{inf}}/E_{\text{a}}) - 1] \times i \]  

where \( E_{\text{inf}} \) and \( E_{\text{a}} \) are the isotopic enrichments (atom percent excess) of bicarbonate in the infusate and of carbon dioxide in expired breath, respectively, at isotopic steady state, and \( i \) is the rate of infusion of the tracer in \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \). Whole-body and splanchnic leucine kinetics were calculated as previously described (19). At isotopic steady state, the fraction of the intragastric tracer that enters the systemic circulation (sampled compartment) is given by the ratio of the plasma tracer-tracee ratio of the intragastric tracer to the tracer-tracee ratio of the intravenous tracer, normalized for the infusion rates of the tracers. This fraction is given by

\[ \%\text{Leu}_{\text{pl}} = \frac{1 - [(E_{\text{inf}}/E_{\text{iv}}) \times (i_{\text{iv}}/i_{\text{ig}})]}{100} \]  

Splanchnic leucine use (Leu_{splan}) was calculated as the product of the fraction of leucine tracer extracted by the splanchnic tissues and enteral leucine intake:

\[ \text{Leu}_{\text{splan}} (\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}) = (1 - [(E_{\text{inf}}/E_{\text{iv}}) \times (i_{\text{iv}}/i_{\text{ig}})]) \times \text{enteral Leu intake} \]

Total leucine flux (\( Q \)) was calculated as

\[ Q (\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}) = i_{\text{iv}}/E_{\text{ig}}^{13\text{C}} - \text{KICA} \]

where \( E_{\text{ig}}^{13\text{C}} - \text{KICA} \) is the plateau enrichment of KICA derived from the [13C]leucine tracer. Leucine oxidation (Leu_{ox}) was calculated as follows:

\[ \text{Leu}_{\text{ox}} (\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}) = \text{Ra}^{13\text{CO}_2}/E_{\text{ig}}^{13\text{C}} - \text{KICA} \]

where Ra^{13CO₂} is the rate of production of labeled carbon dioxide obtained from the product of RaCO₂ and the plateau isotopic enrichment of expired carbon dioxide during the [13C]leucine infusion.

Leucine used for protein synthesis (Leu_{syn}) was calculated as leucine flux minus leucine oxidation:

\[ \text{Leu}_{\text{syn}} (\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}) = Q - \text{Leu}_{\text{ox}} \]

Leucine derived from protein breakdown (Leu_{bkd}) was calculated as the difference between leucine flux and all sources of leucine intake:

\[ \text{Leu}_{\text{bkd}} (\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}) = Q - (\text{diet Leu} + \text{IG Leu} + \text{IV Leu}) \]

Leucine balance (Leu_{bal}) was calculated as the difference between leucine intake and leucine oxidation:

\[ \text{Leu}_{\text{bal}} (\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}) = (\text{diet Leu} + \text{IG Leu} + \text{IV Leu}) - \text{Leu}_{\text{ox}} \]

The fractional synthesis rate (FSR) of each protein was calculated by using the precursor-product equation:

\[ \text{FSR} (%/d) = \frac{[(E_{\sigma} - E_{\bar{\sigma}})/E_{\text{ig}}] \times 2400}{(t_{\text{ig}} - t_{\text{a}})} \]

where \( E_{\sigma} - E_{\bar{\sigma}} \) is the increase in isotopic enrichment of albumin (or transthyretin, RBP, HDL apo A-I, fibrinogen, or \( \alpha_1 \)-antitrypsin)-bound leucine over the period \( t_{\text{ig}} - t_{\text{a}} \) of the infusion, and \( E_{\text{ig}} \) is the plateau isotopic enrichment of VLDL apo B-100–bound leucine. In this calculation, the plateau enrichment of VLDL apo B-100–bound leucine in plasma is assumed to represent the enrichment of the intrahepatic leucine pool from which the APPs are synthesized (17).

The absolute intravascular absolute synthesis rate (IV ASR) of albumin (or RBP, transthyretin, HDL apo A-I, fibrinogen, or \( \alpha_1 \)-antitrypsin) was estimated as the product of FSR and the intravascular mass of the protein:

\[ \text{IV ASR} (\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}) = \text{IV protein mass} \times \text{FSR} \]

where the intravascular mass of a protein is the product of the plasma volume and the plasma concentration of the protein. A plasma volume of 60 mL/kg was used on the basis of measurements done with the dye dilution technique of Gibson and Evans (20) in a comparable group of children after recovery from severe malnutrition (21).

**Statistics**

Data are expressed as means ± SEMs. Differences between groups were detected by the unpaired t test. Statistical significance was set at \( P < 0.05 \). Pearson correlations between outcome variables were performed with the use of GRAPHPAD INSTAT version 3 (GraphPad Software Inc, San Diego).

**RESULTS**

**Leucine kinetics**

Although the HIV-infected children had significantly lower leucine intakes (\( P < 0.01 \)) than did the uninfected children, leucine oxidation did not differ significantly between the 2 groups because the HIV-infected group oxidized 81 ± 10% of total leucine intake whereas the control group oxidized only 58 ± 5% (Figure 2). As a consequence, leucine balance was significantly lower (\( P < 0.01 \)) in the HIV-infected subjects than in the uninfected subjects. Whole-body endogenous leucine flux, i.e., leucine flux derived from protein breakdown, was significantly faster (\( P < 0.05 \)) in the HIV-infected group. However, there was no significant difference in nonoxidative leucine flux, i.e., leucine used for protein synthesis, between the 2 groups (Figure 3). In the HIV-infected group there was a significant correlation between energy intake and leucine balance (\( r = 0.98, P < 0.004 \)). There were also tendencies for positive and negative correlations between energy intake and nonoxidative leucine flux (\( r = 0.78, P = 0.11 \)) and endogenous leucine flux (\( r = -0.70, P = 0.18 \), respectively).

Despite the significantly lower (\( P < 0.01 \)) enteral leucine intake of the HIV-infected group (Figure 4), leucine uptakes by splanchnic tissue were not significantly different between the 2 groups because the fraction of enteral leucine extracted by the splanchnic tissues of the HIV-infected children was significantly greater (\( P < 0.05 \)).

**Positive acute phase proteins**

The HIV-infected group had significantly higher plasma fibrinogen (\( P < 0.01 \)) and \( \alpha_1 \)-antitrypsin (\( P < 0.001 \)) concentrations than did the uninfected group (Table 3). The larger plasma pools
FIGURE 2. Mean (± SEM) total leucine intake, oxidation, and balance in 6 asymptomatic, HIV-infected children and 4 uninfected children who were in the fed state. *Significantly different from uninfected, P < 0.01 (unpaired t test).

FIGURE 3. Mean (± SEM) endogenous and nonoxidative leucine flux in 6 asymptomatic, HIV-infected children and 4 uninfected children who were in the fed state. *Significantly different from uninfected, P < 0.05 (unpaired t test).

of fibrinogen and α1-antitrypsin in the HIV-infected group were associated with ASRs that were significantly faster than those of the uninfected group.

Negative acute phase proteins

Plasma concentrations of albumin and HDL apo A-I were significantly lower (P < 0.05) in the HIV-infected group than in the uninfected group. The lower plasma HDL apo A-I concentration of the HIV-infected group was associated with a significantly faster FSR (P < 0.01), and there was a trend toward a faster ASR (P = 0.09). There were no significant differences between the 2 groups in either the plasma concentrations or the FSRs and ASRs of transthyretin and RBP (Table 3).

DISCUSSION

Studies of infants who were born to HIV-infected mothers and subsequently became HIV positive showed that the infants’ birth weights were lower than those of infants who remained HIV negative and that the HIV-positive infants grew less rapidly than did HIV-negative infants (10–13). These observations suggest that growth retardation may have begun in utero and continued during the asymptomatic phase of the infection. The observation that unexplained weight loss often precedes secondary infection further suggests that a decrease in protein deposition may be an early manifestation of infection by the virus (10, 12). The present finding of a lower leucine balance in the asymptomatic, HIV-infected children than in the uninfected children supports such a suggestion.

Two factors could contribute to the lower protein balance, a lower dietary intake of energy and protein and a greater rate of protein oxidation relative to intake. Whereas the control group, whose mean protein intake (2.25 ± 0.2 g · kg⁻¹ · d⁻¹) was 45% greater than the recommended dietary allowance (RDA; 22), oxidized only 58% of their leucine intake, the HIV-infected group, whose protein intake (1.5 g · kg⁻¹ · d⁻¹) was equal to the RDA, oxidized 81% of their leucine intake. In other words, the uninfected group used ≈42% of their leucine intake to synthesize new protein, but the HIV-infected group used only ≈19%. This lower availability of dietary leucine for protein synthesis in the HIV-infected children, combined with their faster endogenous leucine flux, which is indicative of greater whole-body proteolysis, suggests that infection by the virus per se elicits the same changes in whole-body protein metabolism as do other conditions of stress (23, 24). On the other hand, our present finding of a significant correlation between energy intake and leucine balance and the finding of others that a low energy intake has negative effects on protein balance (25) suggest that the lower leucine (hence, protein) balance of the HIV-infected children is due to their lower energy intakes (70 kcal · kg⁻¹ · d⁻¹, or 26% lower than the RDA for energy, compared with 112 kcal · kg⁻¹ · d⁻¹, or 18% higher than the RDA, in the control children). If this suggestion is true, then increasing the energy intakes of HIV-infected children may improve their protein balance and thereby prevent growth failure.
FIGURE 4. Mean (± SEM) enteral (Ent) leucine intake and fractional and absolute splanchnic (Spl) leucine extraction (ext) in 6 asymptomatic, HIV-infected children and 4 uninfected children who were in the fed state. *Significantly different from uninfected, P < 0.01 (unpaired t test).

We are not aware of other studies of protein metabolism in asymptomatic, HIV-infected children. There are, however, 2 reports of similar studies in adults that used the same tracer technique that we used in our study (8, 9). Both studies, however, reported no differences in the habitual dietary intakes of energy and protein between asymptomatic, HIV-infected adults and uninfected control subjects; hence, their findings cannot be directly compared with those of the present study. Nevertheless, in agreement with our present findings, both studies reported faster rates of protein breakdown in HIV-infected subjects than in control subjects (8, 9). Also in agreement with our present findings, 1 of the 2 studies, which was performed with subjects in the fed state, reported no differences in leucine oxidation rate and leucine utilized for protein synthesis (9). Unlike our present finding of a reduced leucine balance in the HIV-infected subjects, however, the 2 studies reported no difference in balance between the asymptomatic, HIV-infected adults and the control subjects. These findings further support the thesis that the deficient energy intakes of the HIV-infected children in the present study may have been the underlying cause of their lower protein balance.

Interestingly, splanchnic leucine uptakes (≈30 μmol·kg⁻¹·h⁻¹) did not differ significantly between the 2 groups of children despite the HIV-infected children having an enteral leucine intake that was 44 μmol·kg⁻¹·h⁻¹ lower than that of the uninfected children. This comparable leucine uptake was achieved by a higher fractional rate of extraction of enteral leucine by the HIV-infected group. A similar finding has been reported for the splanchnic uptake of lysine in piglets fed either a high-protein (23%) or a low-protein (9.2%) diet (26). These findings suggest that the amino acid requirement and, hence, the protein requirement of organs constituting the splanchnic bed are the same in the 2 groups of children and that this requirement is satisfied by first-pass splanchnic uptake regardless of dietary protein intake.

In an earlier study in asymptomatic, HIV-infected adults, we found that infection by the virus alone elicits a different APP response than that elicited by bacterial infections: the higher concentrations and faster synthesis rates of the positive APPs were not accompanied by lower concentrations and slower synthesis rates of most of the negative APPs (17). Only plasma HDL apo A-I concentrations were significantly lower in the HIV-infected group than in the uninfected group despite a significantly faster FSR in the HIV-infected group and no significant difference between the 2 groups in the ASR of the protein. Our present findings in the asymptomatic, HIV-infected children are almost identical to our earlier findings in the asymptomatic, HIV-infected adults. Both the plasma concentrations and the ASRs of the positive APPs were considerably higher in the asymptomatic, HIV-infected children than in the uninfected control children. Although the plasma concentrations and fractional and absolute synthesis rates of positive and negative acute phase proteins (APPs) in HIV-infected (n = 6) and uninfected children (n = 4)

### TABLE 3

<table>
<thead>
<tr>
<th>Protein</th>
<th>Concentration</th>
<th>Fractional synthesis rate</th>
<th>Absolute synthesis rate</th>
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<td>g/L</td>
<td>%/d</td>
<td>mg·kg⁻¹·d⁻¹</td>
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<tr>
<td></td>
<td>Uninfected</td>
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<tr>
<td>Positive APPs</td>
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<td>35 ± 9.5</td>
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<td>Albumin</td>
<td>34 ± 2.4</td>
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<td>16 ± 3.3</td>
</tr>
<tr>
<td>HDL apo A-I</td>
<td>1.4 ± 0.1</td>
<td>0.8 ± 0.2²</td>
<td>37 ± 6.6</td>
</tr>
<tr>
<td>Transferrin</td>
<td>0.12 ± 0.02</td>
<td>0.11 ± 0.01</td>
<td>61 ± 11</td>
</tr>
<tr>
<td>RBP</td>
<td>0.024 ± 0.003</td>
<td>0.031 ± 0.004</td>
<td>210 ± 46</td>
</tr>
</tbody>
</table>

² ± SEM; apo, apolipoprotein; RBP, retinol-binding protein.

²Significantly different from uninfected, P < 0.05 (Student’s unpaired t test).
concentrations of 2 of the 4 negative APPs, albumin and HDL apo A-I, were significantly lower in the HIV-infected children, these were not associated with slower synthesis rates. These results suggest that HIV infection does elicit an APP response. However, the response is atypical because the higher concentrations and faster synthesis rates of the positive APPs are not accompanied by lower concentrations of all the negative APPs.

In agreement with our previous findings in HIV-infected adults (17), plasma HDL apo A-I concentrations were significantly lower in the HIV-infected children than in the uninfected children despite a faster FSR and a trend toward a faster ASR (P = 0.09). Because the pool size of a plasma protein is determined by the balance between its rates of synthesis and catabolism (or loss from the intravascular space), our findings suggest that the lower HDL apo A-I and albumin concentrations in the asymptomatic, HIV-infected children result from faster rates of catabolism or of loss from the intravascular space relative to the rate of synthesis. The lower plasma HDL apo A-I concentrations also suggest that cholesterol transport and, hence, metabolism may be impaired in HIV-infected patients even in the absence of secondary infections. This suggestion is supported by the lower plasma concentrations of total and HDL cholesterol, as well as of HDL apo A-I, reported by Grunfeld et al (5) in symptom-free, HIV-infected adults before the onset of hypertriglyceridemia.

Our present and previous findings (17) of similar or faster FSRs and ASRs of the negative APPs in asymptomatic, HIV-infected subjects refutes the belief that the lower plasma concentrations of negative APPs induced by stressed states are due to reduced synthesis of these proteins (27). These findings in HIV-infected subjects are further supported by our finding that the lower albumin concentrations in marasmic children were accompanied by a faster albumin FSR when the children were also stressed by infections than when their infections were cleared (28). Similarly, Mansoor et al (29) reported that the hypoalbuminemia of head trauma subjects was accompanied by a 60% increase in the rate of albumin synthesis during the acute response to head trauma. Together, these findings suggest that the stress of infection, inflammation, and injury does not suppress the synthesis rate of negative APPs. In all likelihood, concentrations of albumin and of other negative APPs decrease precipitously in response to infections, severe trauma, and inflammation because of an increased transcapillary escape rate and an increased catabolic rate (30, 31).

Finally, it can be argued that the present findings should be interpreted with caution because of the small sample size. However, the data reported for both leucine kinetics and the APPs in the HIV-infected children are in close agreement with the findings of larger studies in HIV-infected adults (5, 8, 9, 17), which suggests that the findings are valid despite the small number of subjects studied.

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FJ contributed to all aspects of the production of this manuscript. He was involved in the design of the study and in the collection, analysis, and interpretation of the data. He was also the primary writer of the manuscript. SA was involved in the execution of the experiments, the care of the research subjects, data collection, and manuscript writing. WH was involved in the design of the study, interpretation of the data, and writing of the manuscript. None of the authors had any conflicts of interest with the funding agencies.

REFERENCES