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## Increased plasma Gln and Leu $R_a$ and inappropriately low muscle protein synthesis rate in AIDS wasting

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### Abstract

Muscle protein wasting occurs in human immunodeficiency virus (HIV)-infected individuals and is often the initial indication of acquired immunodeficiency syndrome (AIDS). Little is known about the alterations in muscle protein metabolism that occur with HIV infection. Nine subjects with AIDS wasting ( $CD4 < 200/mm^3$ ), chronic stable opportunistic infections (OI), and  $\geq 10\%$  weight loss, fourteen HIV-infected men and one woman ( $CD4 > 200/mm^3$ ) without wasting or OI (asymptomatic), and six HIV-seronegative lean men (control) received a constant intravenous infusion of [ $1-^{13}C$ ]leucine (Leu) and [ $2-^{15}N$ ]glutamine (Gln). Plasma Leu and Gln rate of appearance ( $R_a$ ), whole body Leu turnover, disposal and oxidation rates, and [ $^{13}C$ ]Leu incorporation rate into mixed muscle protein were assessed. Total body muscle mass/fat-free mass was greater in controls (53%) than in AIDS wasting (43%;  $P = 0.04$ ). Fasting whole body proteolysis and synthesis rates were increased above control in the HIV+ asymptomatic group and in the AIDS-wasting group ( $P = 0.009$ ). Whole body Leu oxidation rate was greater in the HIV+ asymptomatic group than in the control and AIDS-wasting groups ( $P < 0.05$ ). Fasting mixed muscle protein synthesis rate was increased in the asymptomatic subjects (0.048%/h;  $P = 0.01$ ) but was similar in AIDS-wasting and control subjects (0.035 vs. 0.037%/h). Plasma Gln  $R_a$  was increased in AIDS-wasting subjects but was similar in control and HIV+ asymptomatic subjects ( $P < 0.001$ ). These findings suggest that AIDS wasting results from 1) a preferential reduction in muscle protein, 2) a failure to sustain an elevated rate of mixed muscle protein synthesis while whole body protein synthesis is increased, and 3) a significant increase in Gln release into the circulation, probably from muscle. Several interesting explanations for the increased Gln  $R_a$  in AIDS wasting exist.

### Keywords

acquired immunodeficiency syndrome-wasting syndrome; immune cell function; amino acid metabolism; metabolic complications; stable isotopes; mass spectrometry; leucine; glutamine

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Infection with the human immunodeficiency virus (HIV-1) typically progresses to involuntary weight loss characterized by a preferential reduction in lean tissue mass (8, 9, 15, 20). The cause of HIV-associated wasting is important to understand because weight loss contributes to reduced immunocompetence in acquired immunodeficiency syndrome (AIDS), and mortality from AIDS wasting is correlated with the magnitude of lean tissue

depletion (8, 14, 20, 30). Clarifying the mechanism for AIDS wasting will advance the development of anticatabolic and anabolic interventions.

The pathogenesis of AIDS wasting, in particular muscle protein wasting, is unknown. Mulligan et al. (24) reported that resting energy expenditure is directly related to the amount of HIV-1 mRNA in the circulation (viral burden). This suggests that a high viral load may actuate substrate metabolism. However, the specific metabolic perturbations and/or dysregulations responsible for AIDS-associated muscle protein wasting are unknown, especially with respect to muscle amino acid metabolism.

Macallan et al. (19) reported that the rate of leucine release from whole body protein was increased in AIDS wasting in both fasting and fed subjects. Selberg et al. (32) studied HIV-infected subjects with weight loss and poor nutritional intake and found an increased rate of whole body protein turnover using the oral [<sup>15</sup>N]glycine approach, but HIV-seronegative controls were not studied. These reports suggest that the rate of whole body protein turnover is increased in AIDS wasting. The rates of muscle protein synthesis in HIV-infected, AIDS-without weight loss, and AIDS-wasting subjects were determined by McNurlan et al. (23) using the [<sup>2</sup>H]phenylalanine flooding-dose technique. They reported that the fractional rate of muscle protein synthesis was not different between seronegative control subjects and among the three groups of HIV-infected subjects. However, the absolute rate of muscle protein synthesis (g/day) was not reported. If AIDS wasting is characterized by a loss of total body muscle mass, the absolute rate of muscle protein synthesis should be reduced in AIDS wasting. We hypothesized that whole body proteolysis would be increased, whereas muscle mass and muscle protein synthesis rate would be decreased in AIDS wasting. A constant intravenous infusion of [1-<sup>13</sup>C]leucine was used to trace whole body protein metabolism because it is an essential amino acid, is not synthesized de novo, and has traditionally been used to determine the rate of muscle protein synthesis in vivo (2, 3, 25, 36–38).

Skeletal muscle plays a primary role in maintaining nitrogen homeostasis during critical illness by releasing glutamine into the circulation. Whether this is true in AIDS wasting is unknown. Glutamine is the most abundant amino acid in the circulation and skeletal muscle free amino acid pool (10, 16). It is a primary transporter of amino nitrogen from skeletal muscle to other tissues as a precursor for urinary ammonia, nucleic acid, cyclic nucleotide synthesis, as an oxidative fuel and as a constituent of cellular proteins (16). About 70% of plasma glutamine rate of appearance ( $R_a$ ) derives from skeletal muscle glutamine release (29), and about 40% of plasma glutamine  $R_a$  is due to direct release of glutamine from muscle protein breakdown (6, 10, 21, 27, 29). Plasma glutamine  $R_a$  reflects the rate of release of glutamine from protein (particularly muscle and liver) and the rate of de novo glutamine synthesis from other amino acid precursors, a portion of which are released during muscle proteolysis (29). Skeletal muscle glutamine release increases after trauma, surgery, sepsis, and catabolic hormone excess (4, 10, 16). In addition, glutamine is essential for the normal function of rapidly proliferating cells of the immune system (e.g., lymphocytes, macrophages, enterocytes, and thymocytes; Ref. 4). In HIV infection, the CD4 T lymphocytes become infected at a rapid rate ( $10^8$ – $10^9$  cells/day). CD4 and CD8 T lymphocytes must proliferate at an equally rapid rate to defend against invading pathogens and to replace infected CD4 T cells. Glutamine is a primary substrate for these T cells (16, 26–28). On the basis of these observations, we hypothesized that plasma glutamine  $R_a$  would be elevated in AIDS wasting.

We have determined plasma leucine turnover rate and glutamine  $R_a$  in subjects with AIDS wasting, HIV-infected subjects without wasting, and HIV-seronegative control subjects. We

found a disproportionate loss of muscle mass, a higher plasma glutamine  $R_a$ , and an inappropriately low rate of muscle protein synthesis in subjects with AIDS wasting.

## METHODS

### Subjects

HIV-infected volunteers were recruited through the AIDS Clinical Trials Unit at Washington University School of Medicine and the Infectious Disease Clinic at Barnes Hospital (Table 1). All HIV-infected subjects were treated with at least one standard antiretroviral agent [azidothymidine (AZT or zidovudine), dideoxyinosine, dideoxycytidine, stavudine, and/or lamivudine] and were clinically stable on this therapy for at least 1 mo before study. On the basis of the severity of disease and wasting, the HIV-infected subjects were assigned to one of two groups: 1) asymptomatic subjects with <10% body weight loss within the previous year, CD4 lymphocyte counts >200 cells/mm<sup>3</sup> (range 204–634 cells/mm<sup>3</sup>) measured within 30 days of study, and no history of opportunistic infection ( $n = 14$  men, 1 woman), and 2) symptomatic subjects with involuntary, unexplained weight loss of at least 10% body weight within the previous year and CD4 lymphocyte counts <200 cells/mm<sup>3</sup> (0–174 cells/mm<sup>3</sup>). All of these men ( $n = 9$ ) had <2 watery stools/day, suggesting that malabsorption and gastrointestinal dysfunction were not the cause of wasting. All of these men had an active, stable, non-life-threatening AIDS-defining opportunistic infection (*mycobacterium avium* complex, cytomegalovirus retinitis, or esophagitis) at the time of study. Six healthy, HIV-seronegative (confirmed by ELISA and HIV antibody test) control subjects, matched for the body mass index of the asymptomatic subjects, were also studied as a comparison group. None of the subjects were taking anabolic agents (testosterone, dihydroepiandrosterone, or growth hormone) or appetite stimulants (Megace or marinol) for at least 1 mo before study. The Human Studies Committee at Washington University School of Medicine approved the study, and all subjects provided informed consent before participating.

### Dietary control

Subjects were admitted to the General Clinical Research Center (GCRC) for a 48-h period. During this time, they consumed a flesh-free diet containing defined amounts of protein and calories. Flesh-free meals were employed to reduce the effects of dietary creatinine on 24-h urinary creatinine excretion measurements used to estimate whole body muscle mass (35). Before admission, a research dietitian surveyed each participant's typical eating habits and designed the 2-day meal plan to provide 1.5 g protein · kg<sup>-1</sup> · day<sup>-1</sup> and 130–190 kJ (31–45 kcal) · kg body wt<sup>-1</sup> · day<sup>-1</sup>. Of the total calories, ~20% derived from protein, ~50% from carbohydrate, and 30% from fat. The controlled intake did not differ in composition from the subject's habitual intake (Table 2). Diet survey analyses of habitual intake indicated that the subjects in the three groups consumed similar and adequate amounts of macronutrients before study (Table 2). The meals were prepared in the Research Kitchen and served to the subjects on the GCRC. The subjects were instructed to eat all the food provided and no other food. Any small amount not consumed was weighed, and the daily intake record was corrected accordingly.

### Whole body glutamine and leucine rates of appearance and muscle protein synthesis rate

After the evening meal on *day 2* (at 1800), whole body and skeletal muscle amino acid kinetics were determined during an overnight (14-h) intravenous infusion of [1-<sup>13</sup>C]leucine and [2-<sup>15</sup>N]glutamine. The plasma glutamine  $R_a$  was determined using a constant intravenous infusion of [2-<sup>15</sup>N]glutamine (6.8 μmol · kg<sup>-1</sup> · h<sup>-1</sup>, ~99 atom%; MassTrace, Woburn, MA) (6, 21, 29). A primed (7.58 μmol/kg) constant intravenous infusion (7.58 μmol · kg<sup>-1</sup> · h<sup>-1</sup>) of [1-<sup>13</sup>C]leucine (~99 atom%; MassTrace) was used to estimate the rates

of whole body protein breakdown, synthesis, and amino acid oxidation, using the reciprocal pool approach (2, 3, 22, 25, 31, 37), and to determine the in vivo rate of incorporation of [ $^{13}\text{C}$ ]leucine into skeletal muscle protein (2, 25, 36–38). Whole body and skeletal muscle amino acid kinetics were measured during an overnight fast (1900–0800).

In blood samples taken before and at 30-min intervals during the last 2.5 h of the amino acid tracer infusion, plasma  $\alpha$ -ketoisocaproic acid ( $\alpha$ -KIC) was isolated, derivatized (33), and analyzed by gas chromatography (GC)-electron impact-mass spectrometry (MS) to determine  $^{13}\text{C}$  enrichment. These measurements were performed on a Hewlett-Packard 5940 GC/MS system with selected ion monitoring of ions at  $m/z$  232 and 233 at the appropriate retention times for the KIC-QTMS derivative (25, 31, 36–38). The plasma [ $\alpha$ - $^{13}\text{C}$ ]KIC enrichment value was used to calculate the rate of whole body protein breakdown (2, 3, 20, 22, 37). This value was also used as the precursor pool enrichment for the calculation of the fractional rate of mixed muscle protein synthesis (25, 33, 36–38).

In the same blood samples, plasma glutamine was isolated by using a modification of a described anion-exchange chromatographic method (5). [ $\text{U-}^{13}\text{C}_5$ ]glutamine was added to the plasma before isolation and used as an internal standard for the quantification of plasma glutamine concentration (Cambridge Isotope Laboratories, Andover, MA). The glutamine was converted to the heptafluorobutylpropyl ester derivative and analyzed by GC-MS in negative ion chemical ionization mode.  $^{15}\text{N}$  enrichment was determined by selected ion monitoring of ions at  $m/z$  407 and 408, and glutamine concentration was determined by monitoring ions at  $m/z$  407 and 412 at the appropriate retention time.

Exhaled breath samples were collected into 20-ml evacuated tubes (Becton-Dickinson Vacutainer) before and at the end of the amino acid tracer infusion. Breath samples were analyzed for  $^{13}\text{CO}_2$  enrichment ( $m/z$  45 and 44 ions) by use of isotope ratio MS (IRMS). The  $^{13}\text{C}$  enrichment values were used in conjunction with 15-min measurements of  $\text{CO}_2$  production (ml/min) made at the same time points with a Delta-Trac indirect calorimeter (Sensormedics, Yorba Linda, CA) to determine the rate of whole body leucine oxidation (2, 22). The difference between the measured whole body protein breakdown rate and leucine oxidation rate is the nonoxidative disposal rate of leucine, which represents the whole body protein synthesis rate (3, 22, 37).

To determine the in vivo rate of incorporation of leucine into mixed muscle protein, muscle protein [ $^{13}\text{C}$ ]leucine enrichment was measured using GC-combustion-IRMS (38) on two samples (~10–20 mg wet wt) removed from the vastus lateralis muscle. One sample was removed 90 min after the tracer amino acid infusion began, and a second muscle sample was removed from the contralateral vastus lateralis at the end of the infusion (14 h). Samples were prepared for GC-combustion-IRMS analysis as described (38).

### Body composition

After an overnight fast, body fat and fat-free mass (FFM) were determined using a Hologic QDR-2000 enhanced-array whole body dual-energy X-ray absorptiometer (Waltham, MA). Whole body images were processed using Hologic software (version 5.64A). Estimates of total body muscle mass were derived by averaging two 24-h urine creatinine excretion values (35). Urinary creatinine was determined colorimetrically using a commercially available automated analyzer (Kodak Ektachem 700XR).

### Statistics

All parameters were compared in the three groups by ANOVA. When this indicated a significant difference ( $P < 0.05$ ), a Student-Newman-Keuls post hoc test was used to identify which of the three groups differed. Means  $\pm$  SE are reported.

## RESULTS

### Body composition

Total body muscle mass was disproportionately low in the AIDS-wasting group ( $23 \pm 2$  kg; 36% body weight and 43% of FFM; Table 1). Muscle mass was higher in the HIV+ asymptomatic group ( $27 \pm 2$  kg; 38% body weight and 52% FFM), and highest in the HIV-seronegative control group ( $31 \pm 1$  kg; 42% body weight and 53% FFM;  $P = 0.04$ ). When expressed per kilogram of FFM, there was a preferential loss of total body muscle mass in the AIDS-wasting group. There was a significant correlation between total body muscle mass and FFM ( $P < 0.002$ ,  $r = 0.72$ ). By convention, we expressed all amino acid kinetic parameters per kilogram of FFM. Expressing them per kilogram of total body muscle mass augmented the group differences observed.

### Glutamine kinetics

The plasma glutamine  $R_a$  was higher in the AIDS-wasting group than in the other two groups ( $P < 0.001$ ; Fig. 1). Plasma glutamine  $R_a$  was similar in HIV-seronegative controls and HIV+ asymptomatic subjects and within the normal range reported by others using a [ $^{15}\text{N}$ ]glutamine tracer (5, 6, 29). During the final 2 h of the 14-h infusion, the [ $2\text{-}^{15}\text{N}$ ]glutamine tracer had equilibrated in the plasma pool, and a plateau [ $^{15}\text{N}$ ]glutamine enrichment (mol% excess) in the plasma was achieved (Table 3). The coefficients of variation for the [ $^{15}\text{N}$ ]glutamine enrichments determined during the final 2 h of the infusion were 4.8, 2.8, and 5.6% for the control, HIV+ asymptomatic, and AIDS-wasting groups, respectively. Plasma glutamine concentration tended to be greater in the AIDS-wasting group ( $385 \pm 29$   $\mu\text{M}$ ) and HIV+ asymptomatic group ( $407 \pm 43$   $\mu\text{M}$ ) than in the HIV-seronegative controls ( $350 \pm 31$   $\mu\text{M}$ ;  $P > 0.05$ , NS).

### Leucine kinetics

The whole body protein breakdown rate and nonoxidative disposal rate of leucine were greater ( $P = 0.009$ ) in the HIV-infected subjects than in control subjects (Fig. 2). Similar findings have been reported by Macallan et al. (19). The rate of [ $^{13}\text{C}$ ] leucine oxidation was greater in the HIV+ asymptomatic group ( $P < 0.05$ ) than it was in the AIDS-wasting and control groups. These kinetic measurements were made during an overnight fast, so whole body net leucine balance (synthesis minus breakdown) was negative in all three groups but most negative in the HIV+ asymptomatic group ( $-37 \pm 1.7$  vs.  $-30 \pm 2.6$   $\mu\text{mol} \cdot \text{kg FFM}^{-1} \cdot \text{h}^{-1}$  in AIDS wasting;  $P < 0.05$ ).

The fractional rate of mixed muscle protein synthesis was greater ( $P = 0.01$ ) in the HIV+ asymptomatic group than in the AIDS-wasting group and the control group ( $0.048 \pm 0.003\%/h$ ; Fig. 3). The fractional rate of mixed muscle protein synthesis was similar in the control and AIDS-wasting subjects (AIDS wasting =  $0.035 \pm 0.004\%/h$ ; control =  $0.037 \pm 0.002\%/h$ ). Compared with previous studies that used the [ $1\text{-}^{13}\text{C}$ ]leucine constant-infusion technique (2, 25, 33, 38), the rate of mixed muscle protein synthesis in the control group was on the low end of the normal range. This may be due to the change in diet imposed by the controlled-protein, flesh-free diet, or the 2 days of physical inactivity imposed by the GCRC admission. Regardless, the HIV-seronegative group was included in the study design to control for these effects, so that comparisons between these controls and the HIV-infected subjects are valid.

The absolute rate of mixed muscle protein synthesis (g protein/h) was calculated from whole body muscle mass, the fractional rate of synthesis, and the assumption that muscle is 19% protein (2, 25). The absolute rate of mixed muscle protein synthesis was greater in the HIV+ asymptomatic group ( $2.4 \pm 0.2$  g protein/h) than in the AIDS-wasting group ( $1.5 \pm 0.2$  g

protein/h;  $P = 0.01$ ) and similar to that in the control group ( $2.2 \pm 0.2$  g protein/h). When the absolute rate of mixed muscle protein synthesis was expressed as a percentage of the whole body protein synthesis rate, this percentage was within the normal range (2, 25) in control ( $24 \pm 2\%$ ) and HIV+ asymptomatic subjects ( $25 \pm 2\%$ ) but was decreased in the AIDS-wasting subjects ( $15 \pm 1\%$ ;  $P < 0.001$ ). This indicates that muscle protein synthesis contributed less to the elevated rate of whole body protein synthesis in the AIDS-wasting subjects. Conversely, the synthesis of other body proteins (e.g., liver, enterocytes, and lymphocytes) must have contributed more to the increased rate of whole body protein synthesis in the AIDS-wasting group.

## DISCUSSION

Our findings suggest the following. 1) AIDS wasting is associated with a preferential reduction in total body muscle mass. In the AIDS-wasting group, muscle mass represented a disproportionately small percentage of FFM and body weight than in the control group (Table 1). 2) HIV infection accelerated the rates of whole body proteolysis and synthesis when examined after an overnight fast and compared with HIV-seronegative subjects. This indicates that some body proteins are synthesized and some degraded at faster rates in HIV-infected subjects. This agrees with earlier reports and suggests that whole body protein turnover is accelerated in HIV-infected subjects when measured in the overnight-fasted condition (19, 20, 32). The increased rate of whole body leucine turnover requires additional energy expenditure and supports the finding that HIV infection is associated with a hypermetabolic state (24), especially with respect to amino acid metabolism. 3) The increased plasma glutamine  $R_a$  in AIDS wasting may reflect an increased rate of de novo glutamine synthesis in muscle and liver. This is an energy-requiring process and may contribute to the hypermetabolic condition.

These findings suggest that muscle amino acid kinetics may be different in patients with AIDS wasting and HIV+ asymptomatic subjects. In AIDS wasting, fasting whole body leucine balance was similar to that of HIV-seronegative subjects, but muscle protein synthesis contributed less to the rate of whole body protein synthesis. This may indicate that more amino acids are utilized for the synthesis of other proteins in the whole body and fewer are available for the synthesis of muscle protein. In HIV+ asymptomatic subjects, muscle protein synthesis rate was elevated and represented a normal proportion of the whole body protein synthesis rate. In this group, a tendency to lose muscle protein may be associated with the increased rate of whole body leucine oxidation. This may result in a more negative whole body leucine balance (Fig. 2).

The measurement of whole body leucine turnover rate using the [ $^{13}\text{C}$ ]leucine constant-infusion method reflects amino acid metabolism in all proteins in the body (2, 3, 22, 37), not one protein in particular. To better understand muscle amino acid metabolism in HIV disease, we examined in vivo amino acid incorporation into skeletal muscle protein. We found that the fractional rate of mixed muscle protein synthesis was greater in HIV+ asymptomatic subjects than in HIV-seronegative controls and AIDS-wasting subjects. Likewise, the absolute rate of muscle protein synthesis was greater in the HIV+ asymptomatic subjects than in the AIDS-wasting subjects. The increased rate of muscle protein synthesis may protect the HIV+ asymptomatic subjects from amino acid losses that may occur through an increased amino acid oxidation rate. The AIDS-wasting subjects appear to have a reduced capacity to utilize amino acids in muscle protein synthetic pathways. Instead, other rapidly synthesized proteins (e.g., immune cells, enterocytes, and acute-phase proteins) may utilize circulating amino acids at the expense of muscle protein. Taken collectively, the disproportionate reduction in total body muscle mass and the reduced rate of muscle protein synthesis relative to whole body protein synthesis suggest that AIDS

muscle protein wasting results from an inability to sustain an elevated rate of muscle protein synthesis when whole body proteolysis and protein synthesis are increased.

These findings do not support an earlier study that reported reduced rates of whole body protein breakdown and synthesis in subjects infected with HIV (34). This discrepancy may be explained by the use of a different amino acid tracer methodology. The subjects received an oral [<sup>15</sup>N]glycine bolus dose with Ensure, and urine <sup>15</sup>N excretion was measured while small quantities of Ensure were consumed throughout the 9-h study. The HIV-infected subjects' dietary intake before this quasi-fed metabolic study was not controlled, and this may have influenced the measured rates of whole body protein metabolism. Not all of the HIV-infected subjects in the earlier studies were receiving antiretroviral therapy (i.e., AZT). The influence of antiviral medications on human muscle amino acid metabolism has not been reported. The possibility exists that these nucleoside analogs block mitochondrial DNA synthesis and uncouple muscle mitochondrial respiration, causing a myopathy similar to that characterized in AZT-treated rodents (17, 18).

We found that the fractional and absolute rates of muscle protein synthesis were decreased in AIDS-wasting subjects compared with HIV+ asymptomatic subjects. This does not agree with a recent report that HIV-seronegative control, HIV-infected, AIDS-no weight loss, and AIDS-wasting subjects have a similar rate of muscle protein synthesis measured by the amino acid flooding-dose technique. In this study, a large amount of [<sup>2</sup>H]phenylalanine (45 mg/kg) and unenriched phenylalanine was administered over a 10-min period, and the amount of [<sup>2</sup>H]phenylalanine incorporated into mixed muscle protein was determined 90 min later. Typically, the flooding-dose technique results in measured values for muscle protein synthesis rates that are approximately two times higher than the constant-infusion approach (33). As expected, our constant-infusion measurements of muscle protein synthesis rate in HIV-infected subjects are one-half those reported by McNurlan et al. (23). This, as well as imposed changes in dietary and physical activity patterns, may explain why we found differences in muscle protein synthesis not previously observed in HIV-infected subjects (23).

Muscle protein synthesis rate was determined by incorporation of [1-<sup>13</sup>C]leucine into a mixture of structural, contractile, mitochondrial, and enzymatic proteins in the muscle sample. Even though contractile proteins constitute 70–75% of the mixed muscle proteins, it is of interest to isolate individual muscle proteins (e.g., mitochondrial, sarcoplasmic, myosin heavy chain, and actin) from the muscle specimens and determine [<sup>13</sup>C]leucine incorporation into individual proteins with different synthesis rates (2). The expression of individual muscle proteins is regulated differently, and it is possible that they are affected differently in AIDS wasting. Identifying the muscle protein(s) most affected in AIDS wasting will assist in designing appropriate and efficacious anabolic and anticatabolic interventions.

It is important to note that we did not directly determine the rate of muscle proteolysis in the current study. The balance between the rates of muscle protein synthesis and proteolysis accounts for the magnitude of muscle protein wasting. A direct measure of muscle proteolysis would require the determination of amino acid balance (e.g., phenylalanine, tyrosine, and 3-meth-ylhistidine) across a large muscle group (e.g., thigh muscles) using arterial and venous cannulation and blood flow rate determinations. This procedure would have adversely affected subject recruitment into the current study.

The plasma leucine and glutamine kinetic measures were made after an overnight fast. In AIDS wasting, the disproportionately low muscle protein synthesis rate and increased rate of whole body leucine turnover may reflect a lack of sufficient amino acid availability to

support muscle protein synthesis. Although unprecedented, proteolytic processes may be amplified by fasting in HIV-infected individuals more than they are in seronegative controls. We have not addressed the possibility that AIDS muscle wasting results from an inadequate increase in the rate of muscle protein synthesis during feeding. Experiments to determine how the rate of muscle protein synthesis is affected by feeding in AIDS wasting are of interest.

The reasons for the increased plasma glutamine  $R_a$  in AIDS wasting are unclear. We have previously reported that plasma glutamine-glutamate concentration in AIDS patients is not greater than in HIV-seronegative controls (13). We have confirmed this observation in the current study using an isotope-dilution MS determination of plasma glutamine concentration. Because glutamine did not accumulate in the circulation, glutamine uptake by some other tissue(s) must be increased to match the elevated plasma glutamine  $R_a$  in AIDS wasting. Possibilities include the following: 1) to provide glutamine as a fuel for colonocytes, enterocytes, and mucosal cells of the intestinal tract to maintain structural integrity, absorptive function and smooth muscle protein synthesis (10, 21) (In our studies, volunteers with obvious gastrointestinal dysfunction were excluded, but subclinical enteropathies might have existed); 2) to transfer excess nitrogen formed by proteolysis to the liver for urea synthesis to prevent excessive ammoniogenesis (6, 10, 21, 29); 3) to provide a gluconeogenic substrate to the kidneys (29) or to facilitate ammonia excretion to maintain acid-base balance (16); 4) to provide glutamine for hepatic glutathione synthesis to replenish levels that may be depleted by chronic illness [Several (7, 11, 12) but not all investigations (1) have supported administration of the antioxidant and glutathione precursor *N*-acetylcysteine to reduce oxidative damage in HIV disease]; and 5) to provide a substrate or a precursor for nucleic acid synthesis in quiescent and activated, rapidly proliferating T and B lymphocytes and macrophages known to avidly utilize glutamine (4, 26–28). Newsholme et al. (26–28) have reported that activated human lymphocytes have a 50% greater rate of utilization of glutamine than quiescent human lymphocytes *in vitro*. We found a 25% greater plasma glutamine  $R_a$  in AIDS-wasting subjects with advanced disease and 0–174 CD4 cells/mm<sup>3</sup>. It is conceivable that rapidly proliferating lymphocytes and macrophages utilize a portion of the elevated glutamine  $R_a$  in AIDS wasting. These possibilities need further investigation.

In summary, our findings suggest that AIDS wasting is associated with a preferential and disproportionate reduction in muscle mass. This may be caused by 1) a failure to sustain an increased rate of mixed muscle protein synthesis while whole body protein turnover is increased, 2) a dysregulation of endogenous amino acid metabolism away from muscle protein synthetic pathways and toward other pathways of amino acid metabolism (oxidation), and 3) a significant increase in glutamine release into the circulation. This may reduce the rate of muscle protein synthesis. The increased plasma glutamine  $R_a$  may reflect muscle injury and accelerated protein degradation. It may also reflect a regulated process whereby *de novo* synthesis of glutamine is increased to satisfy an elevated glutamine requirement by other tissues. These findings may indicate that glutamine is a conditionally essential nutrient in people living with HIV or AIDS.

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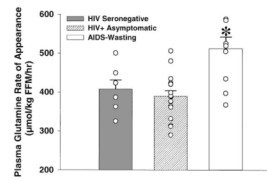
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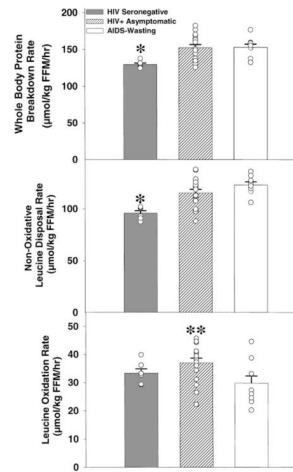
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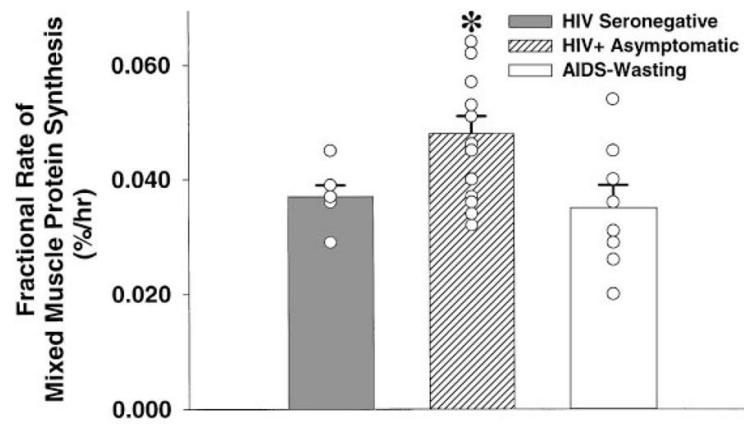
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**Fig. 1.** Plasma glutamine rate of appearance [ $R_a$ ,  $\mu\text{mol glutamine} \cdot \text{kg fat-free mass (FFM)}^{-1} \cdot \text{h}^{-1}$ ] in AIDS-wasting, HIV+ asymptomatic, and HIV-seronegative control subjects.  $\circ$ , Individual subjects. \* $P < 0.001$  vs. HIV+ asymptomatic and seronegative control groups.



**Fig. 2.** Fasting whole body leucine turnover rate ( $\mu\text{mol leucine} \cdot \text{kg FFM}^{-1} \cdot \text{h}^{-1}$ ) in AIDS-wasting, HIV+ asymptomatic, and HIV-seronegative control subjects. Whole body protein breakdown rate and nonoxidative leucine disposal rate (whole body protein synthesis rate) were elevated in HIV+ subjects compared with control.  $*P = 0.009$  vs. HIV+ and AIDS groups. Leucine oxidation rate was greater in HIV+ asymptomatic.  $**P < 0.05$  vs. AIDS wasting).



**Fig. 3.** Fractional rate of mixed skeletal muscle protein synthesis in AIDS-wasting, HIV+ asymptomatic, and HIV-seronegative control subjects. \* $P = 0.01$  vs. HIV-seronegative and AIDS-wasting groups.

Table 1

Descriptive characteristics

Group	n	Age, yr	Ht, cm	Wt, kg	Body Mass Index, kg/m <sup>2</sup>	Fat-Free Mass, kg	Muscle Mass, kg	CD4 Count, cells/mm <sup>3</sup>	Years Known HIV+
HIV seronegative	6	30±3	182±2	73.6±3	22±1	58.6±1.8	31±1	NA	NA
HIV+ asymptomatic	15	37±2	174±2	70.6±4	23±1	52.0±2.7	27±2	426±37	5±1
AIDS wasting	9	40±2	181±2	63.4±3	19±1	52.9±1.5	23±2*	78±34†	7±1

Values are means ± SE. NA, not applicable.

\*  $P = 0.04$  vs. control.†  $P < 0.001$  vs. HIV+ asymptomatic.

**Table 2**

Participants' habitual dietary intake

Group	Dietary Intake, kcal · kg body wt <sup>-1</sup> · day <sup>-1</sup>			
	Total	Protein	Carbohydrate	Fat Alcohol
HIV seronegative	42±1	9.0±0.8	18±1	15±1 0.0±0.0
HIV+ asymptomatic	44±4	8.5±0.7	22±3	13±2 0.4±0.4
AIDS wasting	42±7	9.8±1.7	20±3	12±3 0.6±0.6

Values are means ± SE. No significant differences in habitual intake were observed between groups.

**Table 3**  
 Plasma [<sup>15</sup>N]glutamine enrichment during final 2 h of [2-<sup>15</sup>N]glutamine infusion

Group	[ <sup>15</sup> N]glutamine Enrichment, mol % excess				
	12 h	12.5 h	13 h	13.5 h	14 h
HIV seronegative	2.28±0.15	2.60±0.22	2.50±0.21	2.48±0.17	2.51±0.27
HIV + asymptomatic	2.73±0.15	2.83±0.13	2.88±0.16	2.69±0.15	2.73±0.10
AIDS wasting	1.67±0.13	1.56±0.17	1.79±0.13	1.78±0.08	1.76±0.14

Values are means ± SE.