You might find this additional information useful...

This article cites 43 articles, 21 of which you can access free at:
http://ajpendo.physiology.org/cgi/content/full/288/1/E278#BIBL.

This article has been cited by 5 other HighWire hosted articles:

Hormonal regulators of muscle and metabolism in aging (HORMA): design and conduct of a complex, double masked multicenter trial
Clinical Trials, October 1, 2007; 4 (5): 560-571.
[Abstract] [PDF]

Nutrient Ingestion, Protein Intake, and Sex, but Not Age, Affect the Albumin Synthesis Rate in Humans
A. E. Thalacker-Mercer, C. A. Johnson, K. E. Yarasheski, N. S. Carnell and W. W. Campbell
[Abstract] [Full Text] [PDF]

Modifying muscle mass - the endocrine perspective.
A M Solomon and P M G Bouloux
[Abstract] [Full Text] [PDF]

Whole-Body Proteolysis Rate Is Elevated in HIV-Associated Insulin Resistance.
D. N. Reeds, W. T. Cade, B. W. Patterson, W. G. Powderly, S. Klein and K. E. Yarasheski
Diabetes, October 1, 2006; 55 (10): 2849-2855.
[Abstract] [Full Text] [PDF]

Whole body leucine flux in HIV-infected patients treated with or without protease inhibitors
M. Prod'homme, C. Rochon, M. Balage, H. Laurichesse, I. Tauveron, C. Champredon, P. Thieblot, J. Beytout and J. Grizard
Am J Physiol Endocrinol Metab, April 1, 2006; 290 (4): E685-E693.
[Abstract] [Full Text] [PDF]

Updated information and services including high-resolution figures, can be found at:
http://ajpendo.physiology.org/cgi/content/full/288/1/E278

Additional material and information about AJP - Endocrinology and Metabolism can be found at:
http://www.the-aps.org/publications/ajpendo

This information is current as of February 2, 2008.
Reducing plasma HIV RNA improves muscle amino acid metabolism

Kevin E. Yarasheski, Samuel R. Smith, and William G. Powderly
Department of Internal Medicine, Division of Metabolism, Endocrinology and Lipid Research, and Division of Infectious Diseases, Washington University School of Medicine, St. Louis, Missouri
Submitted 6 August 2004; accepted in final form 7 September 2004

Yarasheski, Kevin E., Samuel R. Smith, and William G. Powderly. Reducing plasma HIV RNA improves muscle amino acid metabolism. Am J Physiol Endocrinol Metab 288: E278–E284, 2005. First published September 14, 2004; doi:10.1152/ajpendo.00359.2004.—We reported (Yarasheski KE, Zachwieja J, Gischler J, Crowley J, Horgan MM, and Powderly WG. Am J Physiol Endocrinol Metab 275: E577–E583, 1998) that AIDS muscle wasting was associated with an inappropriately low rate of muscle protein synthesis and an elevated glutamine rate of appearance (Ra Gln). We hypothesized that high plasma HIV RNA caused dysregulation of muscle amino acid metabolism. We determined whether a reduction in HIV RNA (≥1 log) increased muscle protein synthesis rate and reduced Ra, Gln and muscle proteasome activity in 10 men and 1 woman (22–57 yr, 60–108 kg, 17–33 kg muscle) with advanced HIV (CD4 = 0–311 cells/μl; HIV RNA = 10–375 × 10^3 copies/ml). We utilized stable isotope tracer methodologies ([13]C[Leu] and [15]N[Gln]) to measure the fractional rate of mixed muscle protein synthesis and plasma Ra, Gln in these subjects before and 4 mo after initiating their first or a salvage antiretroviral therapy regimen. After treatment, median CD4 increased (98 vs. 139 cells/μl, P = 0.009) and median HIV RNA was reduced (155,828 vs. 100 copies/ml, P = 0.003). Mixed muscle protein synthesis rate increased (0.062 ± 0.005 vs. 0.078 ± 0.006%/h, P = 0.01), Rm, Gln decreased (387 ± 33 vs. 323 ± 15 μmol·kg fat-free mass^−1·h^−1, P = 0.04), and muscle proteasome chymotrypsin-like catalytic activity was reduced 14% (P = 0.03). Muscle mass was only modestly increased (1 kg, P = not significant). We estimated that, for each 10,000 copies/ml reduction in HIV RNA, ~3 g of additional muscle protein are synthesized per day. These findings suggest that reducing HIV RNA increases muscle protein synthesis and reduces muscle proteolysis, but muscle protein synthesis relative to whole body protein synthesis rate is not restored to normal, so muscle mass is not substantially increased.

human immunodeficiency virus; metabolic complications; body composition; mass spectrometry; antiretroviral medications; cachexia; lentivirus

BEFORE THE AVAILABILITY of highly active antiretroviral therapy (HAART), human immunodeficiency virus (HIV)-associated muscle protein wasting and weight loss were pervasive problems associated with high rates of morbidity and mortality (for review see Ref. 11). In developing countries where HAART is not currently available, muscle protein wasting and weight loss persist as life-threatening problems.

In the current era of HAART, HIV-associated muscle protein wasting continues to be a clinical problem (11, 36, 38). Morbidity and mortality rates are low in effectively treated patients with undetectable plasma viremia (HIV-1 RNA), and patients are gaining weight. But the weight gain is primarily visceral adipose tissue, sometimes associated with peripheral lipodystrophy. Longitudinal and cross-sectional studies indicate that muscle or lean mass is not being restored to normal levels (22, 24). Failure to maintain/restore lean mass appears to be particularly problematic in HIV-infected people with initially low (<15%) body fat content (26).

The pathogenesis for HIV-associated muscle wasting is only partially understood. Nutritional, hormonal, biochemical, inflammatory, viral, and behavioral factors play a role (21, 25, 26, 33, 38). It appears that protein synthetic and proteolytic pathways are both involved. We have reported that, under controlled nutritional conditions, acquired immunodeficiency syndrome (AIDS) muscle wasting is associated with a dysregulation of muscle amino acid metabolism compared with asymptomatic HIV-infected people and seronegative control subjects (44). In AIDS wasting, the portion of whole body protein synthesis that is devoted to the synthesis of muscle proteins is lower, and glutamine rate of appearance into the circulation (Rm, Gln) is higher than in HIV-asymptomatic and control subjects. Lloreta et al. (20) reported greater expression of the genes encoding for the ubiquitin-ATP-dependent proteolytic system in muscle samples obtained from AIDS-wasting patients than in seronegative control subjects. Transgenic mice expressing HIV-1 develop muscle wasting, leukocyte infiltration into muscle, and increased mRNA expression for ubiquitin and proteasome core proteins (13). Interestingly, when these mice were treated with a chemical inhibitor of IκB kinase 2, nuclear factor-κB-dependent gene expression was suppressed, muscle mass was preserved, and life span was increased. In humans, it is not clear whether HIV infection per se signals the dysregulation of muscle amino acid metabolism.

We hypothesized that HIV, or some viral factor associated with chronic HIV infection, is responsible for the observed dysregulation of muscle amino acid/protein metabolism. To test this, we determined whether a reduction in plasma HIV-1 RNA (≥1 log) increases muscle protein synthesis rate and reduces Rm, Gl at and muscle proteasome activity in people living with advanced HIV infection.

METHODS

Subjects. HIV-infected volunteers were recruited from the AIDS Clinical Trials Unit and the Infectious Disease Clinics at Washington University School of Medicine (Table 1). Ten HIV-positive men and
one HIV-positive woman [22–57 yr, 19–36 kg/m²], median HIV RNA 155,828 (range 10–353 × 10⁴) copies/ml, median CD4 98 (0–311) cells/µl were enrolled. All subjects were weight stable (±5%) for 1 mo before enrollment. Two of these subjects had no history of weight loss, were initially naive to all anti-HIV medications, and were scheduled to initiate HAART (Table 2). Nine of the subjects had lost weight in the past and were experienced patients who were clinically stable on HAART for ≥1 mo before enrollment but were scheduled to change to a “salvage” HAART regimen because they were developing resistance to their current HAART regimen. Muscle amino acid metabolism was examined at baseline (High HIV RNA) and 4.2 ± 1.8 mo after the medication change when plasma HIV RNA was reduced (Low HIV RNA).

Before enrollment, volunteers received a physical examination, including a medical/medication history, a blood chemistry profile, serum endocrine profile [testosterone, IGF-I, insulin, C-peptide, glucaagon, leptin, cortisol, TSH, and thyroxine], complete blood cell count (including CD4 count), and plasma HIV RNA quantitation (Roche Amplicor HIV-1 Monitor; Roche Diagnostics, Indianapolis, IN). Volunteers with plasma HIV-1 RNA >10,000 copies/ml were included. Volunteers were excluded if they were taking prescription medications that might affect muscle amino acid metabolism (β-adrenergic blockers, β-agonists, Ca²⁺ channel blockers, corticosteroids) or if they had a metabolic (e.g., diabetes), neuromuscular (moderate to severe peripheral neuropathy), or other disorder that might affect muscle amino acid metabolism. None of the subjects took anabolic agents or appetite stimulants during the course of the study and for ≥1 mo before the baseline study. None of the subjects was exercise trained or participated regularly in exercise activities that would constitute exercise training (as defined in Ref. 29). 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 that time, they consumed a flesh-free diet containing defined, adequate amounts of protein and calories. Flesh-free meals were employed to reduce the effects of dietary creatine on 24-h urinary creatine excretion measurements used to estimate whole body muscle mass (37). Before admission, a research dietician surveyed each participant’s typical eating habits and designed the 2-day meal plan to provide 1.5 g eating habits and designed the 2-day meal plan to provide 1.5 g

### Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>High HIV RNA</th>
<th>Low HIV RNA</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>39 ± 4</td>
<td>40 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Height, cm</td>
<td>176 ± 3</td>
<td>176 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>79.5 ± 5.5</td>
<td>82.6 ± 6.0</td>
<td>0.23</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.9 ± 2.1</td>
<td>26.8 ± 2.3</td>
<td>0.23</td>
</tr>
<tr>
<td>FFM, kg</td>
<td>58.2 ± 3.2</td>
<td>59.4 ± 3.2</td>
<td>0.33</td>
</tr>
<tr>
<td>Legs FFM, kg</td>
<td>17.5 ± 1.2</td>
<td>18.4 ± 1.1</td>
<td>0.32</td>
</tr>
<tr>
<td>Arms FFM, kg</td>
<td>6.7 ± 0.6</td>
<td>6.9 ± 0.6</td>
<td>0.42</td>
</tr>
<tr>
<td>Fat, kg</td>
<td>21.3 ± 3.8</td>
<td>23.2 ± 4.3</td>
<td>0.28</td>
</tr>
<tr>
<td>TBW, kg</td>
<td>40.6 ± 3.8</td>
<td>42.3 ± 3.0</td>
<td>0.29</td>
</tr>
<tr>
<td>Muscle mass, kg</td>
<td>25 ± 2.5</td>
<td>26 ± 2.2</td>
<td>0.67</td>
</tr>
<tr>
<td>RMR, kcal/day</td>
<td>2,000 ± 105</td>
<td>2,069 ± 132</td>
<td>0.53</td>
</tr>
<tr>
<td>CD4, cells/µl</td>
<td>145 ± 38</td>
<td>217 ± 52</td>
<td>0.009</td>
</tr>
<tr>
<td>Plasma HIV RNA, copies/ml</td>
<td>167,236 ± 41,409</td>
<td>2,607 ± 2,236</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Values are means ± SE. High HIV RNA, baseline measurement of human immunodeficiency virus (HIV) RNA; Low HIV RNA, measurement 4.2 ± 1.8 mo after medication change. BMI, body mass index; FFM, fat-free mass; TBW, total body water; RMR, resting metabolic rate; NS, not significant.

### Table 2. Anti-HIV medication changes used to reduce plasma HIV RNA

<table>
<thead>
<tr>
<th>Subject</th>
<th>High HIV RNA</th>
<th>Low HIV RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>D4T, 3TC, ABC, RTV</td>
<td>3TC, ABC, NVP</td>
</tr>
<tr>
<td>2</td>
<td>AZT, D4T</td>
<td>DDI, 3TC, NFV</td>
</tr>
<tr>
<td>3</td>
<td>DD1, ABC, EFV, APV, RTV</td>
<td>DDI, TDF, LPV, RTV</td>
</tr>
<tr>
<td>4</td>
<td>D4T, 3TC, NFV</td>
<td>D4T, 3TC, ABC, RTV</td>
</tr>
<tr>
<td>5</td>
<td>EFV, APV</td>
<td>3TC, ABC, LPV, RTV</td>
</tr>
<tr>
<td>6</td>
<td>AZT, 3TC, IDV, RTV</td>
<td>D4T, ABC, LPV, RTV</td>
</tr>
<tr>
<td>7</td>
<td>AZT, 3TC</td>
<td>D4T, ABC, IDV, RTV</td>
</tr>
<tr>
<td>8</td>
<td>Naive</td>
<td>AZT, 3TC, EFV, NFV</td>
</tr>
<tr>
<td>9</td>
<td>D4T, HU, IDV, APV</td>
<td>D4T, DDI, IDV, RTV</td>
</tr>
<tr>
<td>10</td>
<td>AZT, 3TC, NFV</td>
<td>AZT, 3TC, EFV</td>
</tr>
<tr>
<td>11</td>
<td>Naive</td>
<td>AZT, 3TC, NVP</td>
</tr>
</tbody>
</table>

NRTI, nucleoside reverse transcriptase inhibitors: AZT, zidovudine; 3TC, lamivudine; D4T, stavudine; DDI, didanosine; ABC, abacavir; DDC, zalcitabine. NNRTI, nonnucleoside reverse transcriptase inhibitors: EFV, efavirenz; NVP, nevirapine. NRTI, nucleoside reverse transcriptase inhibitors: TDF, tenofovir. RTN, ribonucleotide reductase inhibitor: HU, hydroxyurea. PI, protease inhibitors: IDV, indinavir; RTV, ritonavir; NFV, nelfinavir; APV, amprenavir; LPV, lopinavir.

The subjects were instructed to eat all food provided and no other food/drink. Any small amount not consumed was weighed, and the daily intake record was recorded accordingly.

### Body composition. After an overnight fast, whole body and regional fat and fat-free mass (FFM) were measured using a Hologic QDR-2000 enhanced-array whole body dual-energy X-ray absorptiometer (Waltham, MA). Whole body images were obtained at baseline and after plasma HIV RNA was reduced. Both images for each subject were processed by the same technologist using Hologic software (v. 5.64A).

Estimates of total body muscle mass were derived by averaging the 24-h urine creatinine excretion values and by assuming that 1 g of urinary creatinine excreted per day is equivalent to 20 kg muscle mass (37). Urine creatinine concentration was measured using a stable isotope dilution assay and gas chromatography-mass spectrometry (Agilent 6890N Gas Chromatograph and Agilent 5973N Mass Selective Detector (GC-MS), Palo Alto, CA) as described (41).

Total body water (TBW; intra- and extracellular) was measured by the dilution of orally administered D₂O (0.25 g/kg; Cambridge Isotope Laboratories) into body fluids, as described previously (30, 43). Serum samples (2 ml) were obtained before and 3 and 4 h after administration of D₂O. Two hundred microliters of an internal standard were added ([2H₉]-tert-butanol), and D₂O enrichment was measured using ¹H magnetic resonance spectroscopy. TBW was calculated from the average of the 3- and 4-h samples (30).

Whole body glutamine and leucine rates of appearance and muscle protein synthesis rate. After the evening meal on day 2 (1800), whole body and skeletal muscle amino acid kinetics were determined during a 13-h overnight (1900–0800) intravenous infusion of [2-¹³C]glutamine (6.8 μmol·kg⁻¹·h⁻¹, −99 atom%; Cambridge Isotope Laboratories, Andover, MA) (9, 44). A primed (7.58 μmol/kg) constant intravenous infusion (7.58 μmol·kg⁻¹·h⁻¹) of [1-¹³C]leucine (−90 at%), Cambridge Isotope Laboratories) was used to measure the rates of whole body proteolysis, nonoxidative leucine disposal, and leucine oxidation, using the reciprocal pool model (15, 21, 32), and to measure the in vivo fractional rate of incorporation of [1-¹³C]leucine into skeletal muscle proteins (2, 40, 42, 44). Amino acid kinetics were measured after an overnight fast and expressed per kilogram of FFM.

In blood samples obtained before and at 30-min intervals during the last 2.5 h of the amino acid tracer infusions, plasma glutamine was isolated using a modification of a described anion-exchange chromatographic method (8). [U-¹³C₆]glutamine (Cambridge Isotope Laboratories) was added to the plasma before isolation and used as an
internal standard for the quantification of plasma glutamine concentra-
tion. Plasma glutamine was converted to the N-heptafluorobutyryl 
propyl ester derivative and analyzed using GC-MS in negative ion 
chemical ionization mode (7). 15N enrichment was determined by 
selected ion monitoring of ion intensity at mass-to-charge ratios (m/z) 
of 407 and 408, and glutamine concentration was determined by 
monitoring ions at m/z 407 and 412 at the appropriate retention time. 
Plasma Ra Gln was calculated as described (8, 9).

For quantitation of plasma leucine concentration, [U-13C6]leucine 
(Cambridge Isotope Laboratories) was added to the plasma before 
isolation and used as an internal standard. Plasma leucine was con-
verted to the N-heptafluorobutyryl propyl ester derivative and ana-
alyzed using GC-MS in negative ion chemical ionization mode. 
Leucine concentration was determined by monitoring ions at m/z 349 and 
355 at the appropriate retention time.

In the same blood samples, plasma α-ketoglutaric acid (KIC) 
was isolated and derivatized, and 13C enrichment was measured 
(selected ion monitoring of m/z 232 and 233) using GC-electron 
ionization-MS (32, 44). The plasma [α-13C]KIC enrichment value was 
used to calculate the rate of whole body protein breakdown (23, 40, 44).

Exhaled breath samples were collected into 20-mI evacuated tubes 
(Becton-Dickinson Vacutainer; Franklin Lakes, NJ) before and at 
the end of the amino acid tracer infusion. Breath samples were analyzed for 
13CO2 enrichment (m/z 45 and 44 ions) using a gas isotope ratio-
MS (Finnigan Delta+ XL-IRMS, Bremen, Germany). The 13CO2 
enrichment values were used in conjunction with 15-min measure-
ments of CO2 production (ml/min) made at the same time points using 
a Delta-Trap indirect calorimeter (Sensormedics, Yorba Linda, CA) to 
determine the rate of whole body leucine oxidation (23, 44). 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 (23, 40, 44).

Indirect calorimetry was also used to measure resting metabolic rate 
(RMR).

The fractional rate of mixed muscle protein synthesis was mea-
sured as the in vivo rate of incorporation of [13C]leucine into proteins 
isoalted from two vastus lateralis muscle samples obtained ~12 h 
after the end of the tracer infusion study. One muscle 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. Tissue free amino acids were 
exttracted from 10–20 mg of muscle by homogenization in 10% 
trichloroacetic acid (TCA). The N-heptafluorobutyryl propyl esters 
were isolated and used as an internal standard. Plasma leucine was con-
verted to the N-heptafluorobutyryl propyl ester derivative and ana-
alyzed using GC-MS in negative ion chemical ionization mode. 
Leucine concentration was determined by monitoring ions at m/z 349 and 
355 at the appropriate retention time.

The fractional rate of mixed muscle protein synthesis was mea-
sured as the in vivo rate of incorporation of [13C]leucine into proteins 
isolated from two vastus lateralis muscle samples obtained ~12 h 
after the end of the tracer infusion study. One muscle 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. Tissue free amino acids were 
exttracted from 10–20 mg of muscle by homogenization in 10% 
trichloroacetic acid (TCA). The N-heptafluorobutyryl propyl esters 
were isolated and used as an internal standard. Plasma leucine was con-
verted to the N-heptafluorobutyryl propyl ester derivative and ana-
alyzed using GC-MS in negative ion chemical ionization mode. 
Leucine concentration was determined by monitoring ions at m/z 349 and 
355 at the appropriate retention time.

The fractional rate of mixed muscle protein synthesis was mea-
sured as the in vivo rate of incorporation of [13C]leucine into proteins 
isolated from two vastus lateralis muscle samples obtained ~12 h 
after the end of the tracer infusion study. One muscle 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. Tissue free amino acids were 
exttracted from 10–20 mg of muscle by homogenization in 10% 
trichloroacetic acid (TCA). The N-heptafluorobutyryl propyl esters 
were isolated and used as an internal standard. Plasma leucine was con-
verted to the N-heptafluorobutyryl propyl ester derivative and ana-
alyzed using GC-MS in negative ion chemical ionization mode. 
Leucine concentration was determined by monitoring ions at m/z 349 and 
355 at the appropriate retention time.
after plasma HIV RNA was reduced (Table 3). It is important to note that the statistical power of these observations was low (16–44%) because these were not primary outcome measures. As a result, it is possible that alterations in circulating levels of IGF-I, testosterone, insulin, cortisol, or thyroid hormone contributed to changes in muscle protein synthesis rate and plasma R\textsubscript{Gln} after plasma HIV RNA was reduced.

Whole body and muscle amino acid kinetics. Plasma leucine and glutamine concentrations and the rates of protein breakdown, nonoxidative leucine disposal (i.e., protein synthesis), and leucine oxidation were not changed after plasma HIV RNA was reduced (Table 4; see Fig. 2). Whole body protein synthesis and proteolysis rates were similar to those reported previously for HIV-positive asymptomatic and AIDS-wasting subjects, and these were significantly greater than in seronegative subjects (44).

Table 3. Serum endocrine parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>High HIV RNA</th>
<th>Low HIV RNA</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone, ng/dl</td>
<td>604±135</td>
<td>722±178</td>
<td>0.28</td>
</tr>
<tr>
<td>IGF-I, ng/ml</td>
<td>204±18</td>
<td>235±22</td>
<td>0.08</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>11.4±3.3</td>
<td>14.2±3.4</td>
<td>0.30</td>
</tr>
<tr>
<td>C-peptide, ng/ml</td>
<td>1.9±0.3</td>
<td>1.4±0.3</td>
<td>0.06</td>
</tr>
<tr>
<td>Glucagon, pg/ml</td>
<td>113±19</td>
<td>97±13</td>
<td>0.08</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>8.9±3.6</td>
<td>11.6±3.6</td>
<td>0.08</td>
</tr>
<tr>
<td>Cortisol, µg/dl</td>
<td>12±1</td>
<td>14±2</td>
<td>0.57</td>
</tr>
<tr>
<td>TSH, µU/ml</td>
<td>1.6±0.3</td>
<td>2.1±0.3</td>
<td>0.15</td>
</tr>
<tr>
<td>T\textsubscript{d}, µg/dl</td>
<td>6.7±1.1</td>
<td>5.7±1.1</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Values are means ± SE. IGF-I, insulin-like growth factor I; TSH, thyroid stimulating hormone; T\textsubscript{d}, thyroxine.

Whole body and muscle amino acid kinetics. Plasma leucine and glutamine concentrations and the rates of protein breakdown, nonoxidative leucine disposal (i.e., protein synthesis), and leucine oxidation were not changed after plasma HIV RNA was reduced. When plasma HIV RNA was reduced, mixed muscle protein synthesis rate was significantly increased when plasma HIV RNA was reduced (Fig. 1). These synthesis rates were calculated using muscle tissue free [\textsuperscript{13}C]leucine as the precursor pool enrichment. The findings were exactly the same: muscle protein synthesis rate was significantly increased when plasma HIV RNA was reduced (High HIV RNA 0.044±0.005%/h vs. Low HIV RNA 0.055±0.006%/h, P=0.01). As expected, when [\textsuperscript{13}C]KIC was used as the precursor pool enrichment, the findings were exactly the same: muscle protein synthesis rate was significantly increased when plasma HIV RNA was reduced (High HIV RNA 0.044±0.005%/h vs. Low HIV RNA 0.055±0.006%/h, P=0.01). When [\textsuperscript{13}C]KIC was used as the precursor pool enrichment, the findings were exactly the same: muscle protein synthesis rate was significantly increased when plasma HIV RNA was reduced (High HIV RNA 0.044±0.005%/h vs. Low HIV RNA 0.055±0.006%/h, P=0.01).

Whole body and muscle amino acid kinetics. Plasma leucine and glutamine concentrations and the rates of protein breakdown, nonoxidative leucine disposal (i.e., protein synthesis), and leucine oxidation were not changed after plasma HIV RNA was reduced. When plasma HIV RNA was reduced, mixed muscle protein synthesis rate was significantly increased when plasma HIV RNA was reduced (Fig. 1). These synthesis rates were calculated using muscle tissue free [\textsuperscript{13}C]leucine as the precursor pool enrichment. The findings were exactly the same: muscle protein synthesis rate was significantly increased when plasma HIV RNA was reduced (High HIV RNA 0.044±0.005%/h vs. Low HIV RNA 0.055±0.006%/h, P=0.01). As expected, when [\textsuperscript{13}C]KIC was used as the precursor pool enrichment, the findings were exactly the same: muscle protein synthesis rate was significantly increased when plasma HIV RNA was reduced (High HIV RNA 0.044±0.005%/h vs. Low HIV RNA 0.055±0.006%/h, P=0.01). When [\textsuperscript{13}C]KIC was used as the precursor pool enrichment, the findings were exactly the same: muscle protein synthesis rate was significantly increased when plasma HIV RNA was reduced (High HIV RNA 0.044±0.005%/h vs. Low HIV RNA 0.055±0.006%/h, P=0.01). When [\textsuperscript{13}C]KIC was used as the precursor pool enrichment, the findings were exactly the same: muscle protein synthesis rate was significantly increased when plasma HIV RNA was reduced (High HIV RNA 0.044±0.005%/h vs. Low HIV RNA 0.055±0.006%/h, P=0.01).

Fig. 1. Mixed muscle protein synthesis rate was increased when plasma human immunodeficiency virus (HIV) RNA was reduced (*P=0.01). Symbols represent individual subjects. Muscle protein synthesis expressed as a fraction of whole body protein synthesis (Table 4) was 12±2% when HIV RNA was high and 15±2% when HIV RNA was lower (P=0.01), but still lower than 24–25% in seronegative control subjects (44).
proteolysis, but a smaller portion of the accelerated whole body protein turnover rate was utilized to synthesize muscle protein (12%). We suggest that this represents dysregulated muscle amino acid metabolism, partly because in HIV-seronegative subjects muscle protein accounted for 24–25% of the whole body protein synthesis rate (44).

HAART substantially reduced plasma HIV RNA, and muscle protein synthetic rate (%/h) increased to a normal value, but it was still only 15% of the whole body protein synthetic rate. HAART improved muscle amino acid kinetics but did not correct them. Muscle mass and muscle mass-to-FFM ratio were not significantly increased after plasma HIV RNA was reduced, despite the fact that muscle protein synthesis rate was increased and muscle proteolytic activity was reduced. This indicates that, even when plasma HIV RNA was reduced to therapeutically acceptable levels, the muscle protein synthesis rate relative to whole body protein synthesis was still lower than normal. This failure to completely restore muscle protein synthesis to normal may explain why muscle and body cell mass are not restored to normal in patients effectively treated with HAART (22, 26, 38). We suggest that some factor associated with a high HIV RNA adversely affects muscle amino acid metabolism and that lowering circulating HIV RNA to near-undetectable levels does not completely eliminate the suppressive effects of this factor on muscle protein accretion. For example, one candidate factor associated with HIV infection that possesses the potential to affect muscle amino acid metabolism is the HIV-1 accessory protein Vpr (virus protein-R) (18, 31).

Reducing plasma HIV RNA reduced muscle chymotrypsin-like catalytic activity. This implies that proteolytic activity for at least one enzyme in the 20S proteasome core was reduced. The mechanism for this inhibition may be the anti-HIV medications used to reduce plasma HIV RNA. In several cell types (T cell lymphocytes and lymphoma, hepatoma), protease inhibitors [indinavir (IDV), ritonavir (RTV), saquinavir (SQV)] and reverse transcriptase inhibitors alone [lamivudine (3TC), zidovudine (azidothymidine; AZT)], but especially in combination, inhibit chymotrypsin-like and trypsin-like catalytic activities, but not PGPH activity, in isolated proteasomes (1, 19, 28). The relevance of these in vitro observations has been questioned on the basis of the supraphysiological drug concentrations used (50–100 µM) (17). In each of the 11 subjects studied here, these medications (3TC, AZT, IDV, RTV, SQV) constituted at least one component of the anti-HIV regimen used to lower HIV RNA (Table 2). Our in vivo findings suggest that physiological concentrations of anti-HIV medications may inhibit muscle proteasome chymotrypsin-like catalytic activity. We suspect, and evidence supports the notion, that HIV infection activates the ubiquitin-proteasome proteolytic pathway (13, 20) and anti-HIV medications may reduce or restore this activation to lower or more normal levels. Effective HAART appeared to lower the rate of muscle proteolysis but suboptimally increased the rate of muscle protein synthesis relative to whole body protein synthesis, so muscle mass was not dramatically increased.

Plasma Ra Gln declined when plasma HIV RNA was reduced, and this indicates that muscle proteolysis or de novo synthesis of glutamine was reduced. About 65% of plasma Ra Gln derives from de novo synthesis in skeletal muscle, and ~35% of plasma Ra Gln derives from direct release of glutamine from muscle proteolysis (27). The gastrointestinal tract (enterocytes), kidneys, liver, and immune cells (T-lymphocytes, macrophages) all utilize glutamine as a source of energy. In other cachectic conditions (cancer, trauma, surgery, catabolic hormone excess), plasma Ra Gln and muscle glutamine synthetase expression are elevated (14). Our findings support the notion that, in the cachectic state characterized by high plasma HIV RNA, leucine incorporation into skeletal muscle proteins is reduced, perhaps because intramuscular leucine transamination for the synthesis of glutamine is activated, and less leucine is available for muscle protein synthetic pathways (14). The undesirable result of activated synthesis of glutamine (and alanine) from leucine (and other branched-chain amino acids) in severe illness is muscle protein wasting (14). In high plasma HIV RNA-induced cachexia, presumably there exists an increased utilization of nitrogen or glutamine in enterocytes, T-lymphocytes, macrophages, kidneys, or liver.

We have not identified the cell or tissue where glutamine uptake is increased in HIV infection, but T-lymphocytes are actively destroyed by HIV infection and turn over rapidly in HIV-infected people. It is interesting to note that, in two small randomized, double-blind, placebo-controlled studies, glutamine-containing nutritional supplements increased body

![Fig. 2. Plasma glutamine (Gln) rate of appearance was reduced when plasma HIV RNA was reduced (*P = 0.04). FFM, fat-free mass. Symbols represent individual subjects.](image-url)

![Fig. 3. Muscle chymotrypsin-like activity was reduced when plasma HIV RNA was reduced (*P = 0.03), whereas the catalytic activity of other enzymes present in the 20S proteasome core [trypsin-like, peptidyl glutamyl peptide hydrolase (PGPH)] was not affected.](image-url)
weight, body cell mass, lean body mass, and intracellular water content in AIDS-wasting patients (6, 34). In another study, glutamine supplementation reduced the severity of nelfinavir-associated diarrhea in HIV-infected people (16). Taken together, these findings support the notion that glutamine is a conditionally essential amino acid in HIV-infected people with high plasma HIV RNA. A reduction in circulating levels of HIV RNA reduced the rate of muscle glutamine export, suggesting that either 1) the signal that activated muscle glutamine efflux was removed, or 2) the requirements for glutamine by other cells/tissues in the body were reduced.

It is reassuring that the amino acid kinetic measurements agreed with the static measurement of muscle mass and that the reduction in plasma HIV RNA correlated with the change in muscle mass. The changes in muscle protein synthesis and proteolysis rates predicted an increase in muscle mass (1.3–1.4 kg) that only minimally exceeded the indirect estimate for the increase in muscle mass (1 kg). The small difference may be accounted for because the increase in muscle protein synthesis and the reduction in catalytic activity are not instantaneous and simultaneous events that occur after switching/starting medications that reduce HIV RNA. Also, not all muscle chymotrypsin-like catalytic activity in the proteasome is devoted to myofibrillar protein breakdown, and other proteolytic pathways exist in muscle. On the basis of the average reduction in plasma HIV RNA, the average increase in muscle protein synthesis rate, the amount of time between the baseline and low HIV RNA measurements, and the assumption that muscle is 19% protein, we estimate that, for each 10,000 copies/ml reduction in plasma HIV RNA, ~3 g of additional muscle protein are synthesized per day.

It is possible that these findings can be attributed to a favorable effect of lowering plasma HIV RNA on circulating levels of testosterone, IGF-I, insulin, C-peptide, glucagon, leptin, cortisol, or thyroid hormone levels (anabolic or catabolic hormones). Although these serum hormone levels were not changed after HIV RNA was reduced, the sample size was limited, and we have not eliminated the possibility that several other endocrine/autocrine/paracrine factors or cytokines that were not measured changed in a manner that can explain these findings.

There are some limitations. The medication regimens used to lower plasma HIV RNA in these subjects were very different. Each HIV-infected subject was treated with very complex and variable medication regimens at baseline, and no standard, single medication regimen is safe and efficacious for lowering plasma HIV RNA in every subject. Many factors that were not under our control determined what regimen was used to lower HIV RNA. As a result, we cannot attribute the improvements in muscle amino acid metabolism to a single medication or drug class. Despite the variability in medication regimens, the changes in muscle amino acid metabolism were consistent, with one exception; subjects receiving ABC tended to have the smallest increments or a slight decrement in muscle protein synthesis rate (Fig. 1). The reasons for this are unknown. In human muscle cells, in vitro cytotoxicity and mitochondrial toxicity of ABC is less than for other nucleoside reverse transcriptase inhibitors (e.g., zalcitabine and stavudine) and nucleotide reverse transcriptase inhibitors (tenofovir) (4, 5). We conclude that lowering plasma HIV RNA is either directly or indirectly associated with improved muscle amino acid metabolism.

Despite the reduction in plasma HIV RNA to very low levels in this study, this does not mean that the HIV virus and its potential effects were completely eradicated from these subjects. Even with undetectable HIV RNA in the circulation, HIV replication continues in tissue sanctuaries for HIV (lymph nodes, macrophages, dendritic cells). Our working hypothesis is that this active, low-level HIV replication or a viral protein produced during HIV replication persists in the low plasma HIV RNA condition, and this explains the observation that the reduction in plasma HIV RNA correlated with the change in muscle mass. The changes in muscle mass were increased when the plasma HIV RNA level was high. Muscle amino acid metabolism was improved but not completely normalized when plasma HIV RNA was reduced, so muscle mass was only marginally affected. These findings support the existence of biological interactions between factors associated with chronic viral infection and muscle amino acid metabolism. The HIV-related factor that regulates muscle amino acid metabolism is under investigation. Glutamine may be conditionally essential in HIV-infected people with high plasma HIV RNA. Identifying anti-HIV medication regimens that effectively reduce plasma HIV RNA without adversely affecting amino acid, glucose, and lipid metabolism is a clinically important challenge.

ACKNOWLEDGMENTS

We thank the subjects for time and altruism. Sherry Claxton, RD, Mary Hoffmann, Amanda Becker, Jennifer Chen, Erin Laciny, and Jill Schulte provided technical assistance.

GRANTS

Support, through National Institutes of Health grants, was provided by the nurses and staff at the AIDS Clinical Trials Unit (AI-25903), the General Clinical Research Center (RR-00036), and the Clinical Nutrition Research Unit (DK-56341). K. E. Yarasheski was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-49393, DK-54163, and DK-59531 and by the Mass Spectrometry Research Center (RR-00954).

REFERENCES


