Effect of Acquired Immune Deficiency Syndrome Wasting on the Protein Metabolic Response to Acute Exercise

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Wasting is a major complication of human immunodeficiency virus (HIV) infection, which remains prevalent even in the era of highly-active antiretroviral therapy. We have previously shown that progressive resistance exercise can increase lean body mass (LBM) significantly in patients with wasting, and that exercise does not increase circulating HIV RNA concentrations. We examined the effect of 1 bout of moderately difficult exercise on whole body protein kinetics in 10 patients with HIV wasting and 12 patients with HIV infection without wasting. At baseline, there were no differences between the groups in whole body leucine flux, oxidation, or nonoxidative leucine disposal (NOLD, a measure of whole body protein synthesis). Six days after exercise, NOLD was significantly higher in the wasted patients compared with the nonwasted ones (82.2 ± 16.7 *v* 66.5 ± 15.2 μmol/kg LBM/h, $P < .03$). The change in NOLD between baseline and day 6 was significantly different between the 2 groups (+9.0 ± 9.2 *v* −3.3 ± 5.7 μmol/kg LBM/h, $P < .02$). These data indicate that the ability to respond to exercise with protein synthesis is maintained in HIV wasting.

WASTING IS A MAJOR cause of disability, morbidity, and mortality in human immunodeficiency virus (HIV) infection.[1-3] Although the prevalence of wasting has declined in the developed world with the advent of highly active antiretroviral therapy (HAART), it remains a serious problem in emerging countries.[4,5] Studies of acquired immune deficiency syndrome (AIDS) wasting have shed new light on the mechanisms underlying catabolic conditions. Macallan et al[6] found that whole-body leucine flux was increased by 25% in patients with advanced HIV disease compared with controls, while patients with less advanced disease had intermediate values. Recently, Yarasheski et al[7] found a lower whole body protein breakdown rate and a higher rate of appearance of glutamine in plasma of patients with AIDS wasting compared with HIV-positive, nonwasted patients or to healthy HIV-negative controls. In contrast, the leucine oxidation rate was highest in the HIV-infected, nonwasted patients, as was the fractional rate of mixed muscle protein synthesis.[7] The investigators interpreted these observations as indicating that with wasting there is failure to match muscle synthesis to the increased rate of breakdown that occurs with HIV infection and an increase in protein utilization by glutamine-dependent tissues, including immune cells and enterocytes.

Progressive resistance exercise training (PRT) is the only physiologic way to increase muscle mass and strength. To assess the safety and metabolic impact of exercise in this population, we have recently studied the response of a group of patients with HIV infection with and without wasting to a single 15-minute bout of stepping exercises of moderate difficulty.[8] Participants stepped up and down a 60-cm step at a cadence of 15 steps per minute, always performing an eccentric contraction with 1 leg and a concentric contraction with the other leg. The exercise caused a mild acute phase response, with an increase in circulating neutrophil counts, serum creatine kinase concentration, and 3-methylhistidine appearance in the urine. Nevertheless, there was no increase in plasma viral load, as measured by reverse transcriptase-polymerase chain reaction (RT-PCR). We concluded that weight lifting exercise is likely to be safe in patients with HIV infection, and that PRT should not be avoided in these patients because of concern about increased HIV viremia. We and others have demonstrated that PRT can reverse wasting in such patients.[9-11] We now report the results of 1 bout of exercise on whole-body leucine kinetics.

MATERIALS AND METHODS

Study Population

Subjects were eligible for this study if they were infected with HIV and were participants in an ongoing, longitudinal study of nutritional status during HIV infection (Nutrition for Healthy Living, Tufts University School of Medicine). HIV infection was documented by enzyme-linked immunosorbent assay (ELISA) in all subjects. Weight loss or AIDS (based on the revised 1993 Centers for Disease Control [CDC] criteria[12]) were not entry requirements. All subjects had normal renal function (serum creatinine <1.2 mg/dL), hepatic function (aspartate aminotransferase [AST] and alanine aminotransferase [ALT] < twice the upper limit of normal, total bilirubin and alkaline phosphatase within the normal range), and were able to give informed consent. All subjects were sedentary except for 2 who performed mild aerobic exercise 2 to 3 times per week. No subject was performing resistance training. Thirty-one HIV-infected volunteers expressed interest in the study and were given informed consent forms. Two subjects consented to the study, but did not have adequate venous access and were removed from the study before any metabolic determinations were performed. Five others agreed to enter the study, but were not compliant with the study protocol (n = 2) or decided not to participate after their initial agreement (n = 3). The other 24 patients participated in the study, 21 completed the study successfully, and data from these subjects are reported here. Three of the subjects had technical problems

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that prevented full kinetic data from being available: in 2 subjects with a history of intravenous drug use, venous access was inadequate at follow-up to allow completion of the protocol, and in 1 subject the pump failed at follow-up and plateau in tracer enrichment was not achieved. The study was approved by the Human Investigation Review Committee of Tufts University and New England Medical Center.

Wasting was determined by CDC criteria as either a body mass index (BMI) less than 20 kg/m^2 or unintentional loss of weight of 10% or more of baseline weight. Because all participants were also followed in the Nutrition for Healthy Living cohort study, measured weight was used to assess the latter parameter. Thus, wasting was often present in persons whose BMI was still within the "normal" range. To compare lean mass between patients with and without wasting and the seronegative controls, the "lean body mass index" (kg LBM/ht^2) was calculated as previously described.[13]

Assessment Protocol

Participants were admitted to the Tufts University School of Medicine General Clinical Research Center (GCRC) at New England Medical Center on day 1, 3 days before a single bout of acute exercise, which was performed on day 4. Subjects were discharged on day 5 and readmitted on the morning of day 9 for 4 additional days. Subjects were instructed in a meat-free diet by a registered dietitian and asked to begin this diet on day −3 and to continue it during the study. Participants kept dietary records for 3 days before the first admission and during the 4 days between the first and second admission to allow assessment of their usual dietary intake and compliance with the meat-free diet. Mean energy and protein intakes per kilogram body weight were 143 kJ/kg (34.0 kcal/kg) and 1.2 g protein/kg, respectively, and did not differ between groups. These intakes were not significantly different from outpatient 3-day food records collected by the subjects over the previous 6-month period as part of their participation in the main cohort study.

During the 2 admissions, all meals were provided by the GCRC staff. Body composition was assessed by dual-energy x-ray absorptiometry (DXA) using a Hologic QDR 2000 (Waltham, MA) with version 5.64A software. Grams of lean mass, fat mass, and bone mineral were measured by a single technician using a standardized protocol.

Protein Metabolism

Protein metabolic studies were performed on days 3 and 10, the first 1 day before exercise and the second 6 days after exercise.[14] The 6-day assessment time was chosen to (1) reduce the effect of any immediate change in energy balance that the exercise bout could cause so that the results would better reflect more profound effects of exercise on protein metabolism independently of energy; and (2) to examine the possible effect of exercise on viral replication, which was expected to peak 5 to 6 days after the exercise. On the day of each isotope study, a primed continuous infusion of L-[1-^{13}C] leucine (99 atom%, Cambridge Isotope Laboratories, Andover, MA) was administered for 4 hours to determine leucine flux, oxidation, and synthesis using the reciprocal pool approach.[15] All subjects were studied in the postabsorptive state. At approximately 7:00 AM, after an overnight fast, 1 catheter was inserted into an antecubital vein for tracer infusion, and a second catheter was inserted in retrograde fashion into a hand vein for blood sampling. Venous blood samples were "arterialized" by keeping this hand at 38°C throughout the infusion procedure. At approximately 8:00 AM, the isotope infusion was begun with the administration of priming doses of $NaH^{13}CO_3$ (0.2 $mg \cdot kg^{-1}$) (Cambridge Isotope Laboratories) and L-[1-^{13}C] leucine (7.6 $\mu mol \cdot kg^{-1}$) (Cambridge Isotope Laboratories). The L-[1-^{13}C] leucine was diluted in sterile saline and infused using a calibrated syringe pump (Harvard Apparatus, Natick, MA) for 4 hours at a rate of 0.347 $mL \cdot min^{-1}$ (0.127 $\mu mol \cdot kg^{-1} \cdot min^{-1}$).

Blood samples were obtained 60 minutes before the infusion and hourly for the first 2 hours, then every 30 minutes during the third hour and every 15 minutes for the last hour. Plasma (or serum for α-ketoisocaproic acid, or KIC) was separated immediately by centrifugation at 4°C and kept frozen at −70°C for subsequent analysis of isotopic enrichments. α-KIC was isolated and derivatized as previously reported.[14,16] The isotope enrichment of plasma ^{13}C-KIC was determined as its quinoxalinol-t-butyl-dimethyl-silyl (TMS) derivative by gas chromatography-electron impact mass spectrometry (Hewlett-Packard Model 5888P, Palo Alto, CA) with selected monitoring of ions at *m/z* 232 and 233 at the appropriate retention times for the derivative.[16] Measurement of L-[1-^{13}C] leucine concentration in the infusates was performed using the method of Moore and Stein,[17] adapted to a Perkin-Elmer Lambda 1 UV/VIS spectrophotometer (Coleman/Perkin-Elmer, Oak Brook, IL).

Expired air samples were collected for measurement of $^{13}CO_2$ enrichment using a disposable mylar balloon and transferred to a 15-mL evacuated collection tube (Exetainer; Labco, High Wycombe, Buckinghamshire, England). Carbon dioxide production rates were determined before the start of the infusion and at 150 and 225 minutes during the infusion using a ventilated hood system as previously described.[18] The ^{13}C enrichment of expired CO_2 was measured by isotope ratio mass spectrometry at *m/z* 45 and 44 ions (Hydra; Europa Scientific, Crewe, UK).[19] The ^{13}C enrichment values were used with the CO_2 production rates to calculate whole body leucine oxidation.

Plasma HIV and CD4 Assays

Plasma HIV RNA was measured in a research retrovirology laboratory using a Roche Amplicor Monitor reverse transcriptase polymerase chain reaction assay according to the manufacturer's instructions (Roche Molecular Systems, Somerville, NJ). Blood samples were collected in acid citrate dextrose (ACD) tubes before the exercise protocol and 2, 6, 24, and 168 hours (1 week) after the exercise session. Plasma was separated within 3 hours, frozen at −70°C, and thawed just before assaying. All samples from individual subjects were batched and assayed together. For the 3 subjects found to have less than 400 copies/mL, results were checked by repeating the measurements using the ultrasensitive Amplicor Monitor assay system with a detection limit of approximately 25 copies/mL. T-cell subsets (total CD4 and CD8 cells) were measured by the Clinical Immunology Laboratory at New England Medical Center using flow cytometry.

Exercise Protocol

All subjects completed the exercise protocol, consisting of 15 minutes of a 60-cm (vertical distance) step aerobic at a cadence of 1 step per second. Thus, each person completed 225 concentric-eccentric cycles in 15 minutes. Even fit participants found this exercise difficult. One subject required a 30-second rest period after 5 minutes and again after 10 minutes in order to complete the protocol.

Statistical Analysis

The major outcome of the study was change in leucine flux, oxidation, and nonoxidative leucine disposal (NOLD, a measure of protein synthesis) from baseline to postexercise. Leucine metabolism was calculated using the stochastic model of Matthews et al.[15] Under this model, Flux = Oxidation + Synthesis = Intake + Breakdown. In the fasting state, Intake = 0, so the rate of leucine release from protein breakdown equals the calculated flux. Leucine flux was calculated from the plasma enrichment of KIC to reflect the intracellular leucine pool[20] and to eliminate sampling site inconsistencies.[21] Leucine oxidation was calculated from $^{13}CO_2$ production, and the isotopic enrichment of KIC in plasma, corrected using a bicarbonate retention factor of 0.70.[22] The rate at which leucine was incorporated into protein synthesis was calculated as flux minus oxidation.

Data and residuals were examined graphically and statistically for

normality. Baseline variables were compared between wasted and nonwasted patients using *t* tests. Leucine kinetics (flux, oxidation, and NOLD) were compared at the follow-up time point between the 2 groups using analysis of variance (ANOVA) to adjust for lean body mass. Change in leucine kinetics in response to the exercise bout was assessed by analysis of covariance (ANCOVA) adjusting for baseline kinetic measured (eg, percent change in flux or oxidation), without the assumptions inherent in using ratios of physiologic variables, as previously pointed out by Toth et al.[23] Post hoc contrasts were analyzed using Tukey's honestly significant difference (HSD) test to assess the significance of differences between groups if the overall analysis was significant. Results were considered statistically significant if the result of a 2-tailed *P* value was $<.05$.

RESULTS

Subject Characteristics

Table 1 shows the demographic and laboratory characteristics of the study population. There were 18 men and 4 women infected with HIV in the study, of whom 7 were African-American, 14 were Caucasian, and 1 was Native-American. The patients' risk factors for HIV infection were injection drug use in 9, homosexual contact in 12, and unknown in 1. Their mean age was 38 years. Eighteen of the patients were taking zidovudine, either alone (at the beginning of the study) or in combination with 3TC, or were taking 3TC with or without d4T. Twelve of the patients (4 with wasting) were taking a protease inhibitor (indinavir in 8 subjects, saquinavir in 3, saquinavir + ritonavir in 1). Two patients were taking no antiretroviral therapy at all. No patients were taking glucocorticoids, anabolic steroids, or growth hormone. Patients were not admitted within 1 month of an acute infection or a change in their antiretroviral regimen.

Table 1. Baseline Demographic, Clinical, and Laboratory Features of the Study Participants

Parameter	HIV-Seropositive, Wasted	HIV-Seropositive, Nonwasted
Age, yr (range)	37.5 (31-50)	40.4 (29-55)
Sex M:F	10:0	8:4
BMI, kg/m^2		
Men	21.4 (2.3)*	25.9 (3.5)
Women	—	31.3 (3.0)†
Lean body mass, kg		
Men	57.7 (8.4)	58.6 (8.4)
Women	—	43.1 (3.7)
Lean mass index, kg/m^2		
Men	16.7 (1.4)	18.1 (2.2)
Women	—	16.7 (1.6)
% Body fat		
Men	14.5 (5.1)*	25.3 (7.6)
Women	—	39.6 (11.3)
Hemoglobin, g/L	13.7 (1.9)	13.9 (0.8)
Serum creatinine, mg/dL	0.86 (0.24)	0.87 (0.13)
AST, U/L	40.5 (24.5)	32.0 (30.3)
CK (baseline), U/L	120 (109)	89 (39)
Median CD4 count, #/mm^3 (range)	100 (38-352)†	365 (174-681)
HIV RNA, copies/mL	3.6×10^4 (7×10^4)	4.4×10^4 (7×10^4)

NOTE. Data are mean (SD) unless otherwise indicated.

Abbreviations: AST, aspartate aminotransferase; CK, creatine kinase.

*$P < .01$ *v* other group.

†$P < .05$ *v* other group.

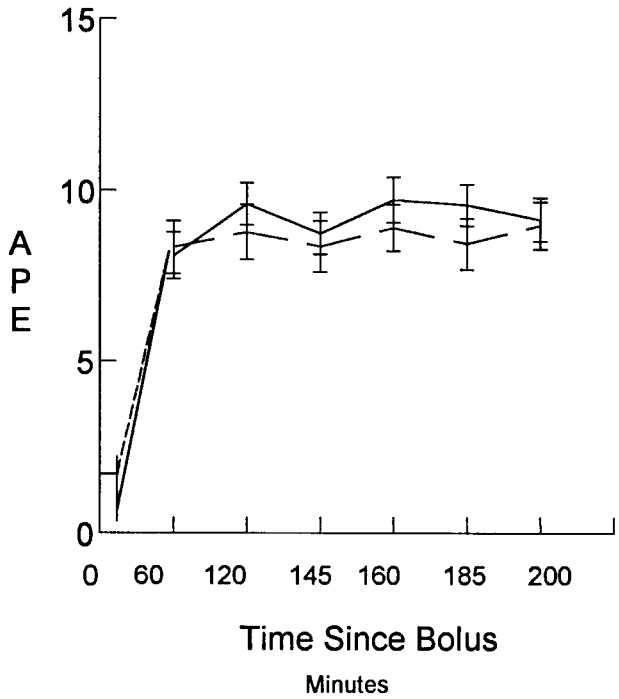

Fig 1. ^{13}C enrichments (means ± SE) of plasma α-KIC during infusion of ^{13}C-leucine at baseline (—) and after exercise (— — —); n = 22 subjects.

Baseline Protein and Energy Metabolism

A plateau in isotope enrichment was achieved at comparable levels by 2 hours in both baseline and follow-up infusions (Fig 1). There were no differences at baseline between the groups in leucine flux, oxidation, or NOLD after adjustment for lean body mass. However, the point estimate of leucine oxidation was 24% higher in the HIV-positive group compared with the wasted group ($P < .12$, Table 2). Resting energy expenditure was comparable in the 2 groups at baseline. No association was seen between protein parameters and medication use, HIV RNA levels, or CD4 counts.

Metabolic Response to Exercise

Six days after exercise, leucine flux did not differ significantly between patients with and without wasting (116.9 ± 17.2 *v* 104.8 ± 17.3 μmol/kg LBM/h, $P < .19$, Table 2). The trend toward higher leucine oxidation in the HIV-positive, nonwasted patients persisted after exercise ($P < .07$), but the change in oxidation did not differ between wasted and nonwasted patients ($P < .17$). In contrast, NOLD 6 days after the exercise bout was significantly higher in the wasted patients compared with the nonwasted ones (82.2 ± 16.7 *v* 66.5 ± 15.2 μmol/kg LBM/h, $P < .03$). The change in NOLD between baseline and day 6 was significantly different between the 2 groups (+9.0 ± 9.2 *v* −3.3 ± 5.7 μmol/kg LBM/h, $P < .02$). Resting metabolic rate was not different at the follow-up evaluation, confirming that the goal of measuring changes in pro-

Table 2. Leucine Kinetic Parameters (imol per kg of LBM per hour) in Subjects Before and After Exercise

μmol/kg/h	HIV-Positive, Wasted (n = 10)	HIV-Positive, Nonwasted (n = 12)	*P*
Leucine flux			
Pre	112.4 (28.5)	118.5 (28.8)	.86
Post	116.9 (17.2)	104.8 (17.3)	.19
Change	4.5 (8.8)	−13.7 (7.5)	.10
Leucine oxidation			
Pre	39.2 (12.2)	48.7 (21.2)	.12
Post	34.7 (9.1)	38.3 (7.1)	.07
Change	−4.5 (7.7)	−10.4 (7.6)	.17
Leucine NOLD			
Pre	73.2 (22.9)	69.8 (21.1)	.42
Post	82.2 (16.7)	66.5 (15.2)	.03
Change	9.0 (9.2)	−3.3 (5.7)	.02
REE			
Pre	1,924 (232)	1,919 (225)	.76
Post	1,911 (244)	1,967 (240)	.47
Change	−13 (119)	48 (210)	.23

NOTE. Data shown are mean (SD). *P* values compare the subject groups within each time point.

tein kinetics at a time when energy metabolism was not perturbed by the exercise bout had been met.

Because the wasted subjects were all men, and the nonwasted group included 4 women, we examined the response to exercise in men alone to exclude a possible confounding effect by sex. The results were substantially the same, with no significant difference in leucine flux change ($P < .11$) or oxidation ($P < .33$), and a significant difference in NOLD ($P < .03$). Similarly, we found no differences in the leucine kinetic parameter changes between patients taking a protease inhibitor and those not taking one.

DISCUSSION

Despite the marked improvement in survival of patients with HIV infection, wasting, poor physical conditioning, and exercise intolerance continue to be important problems in the developed world, as well as in emerging countries. For example, in the parent cohort from which we recruited study patients, 18% demonstrate evidence of wasting even with widespread use of highly active antiretroviral therapy (Gorbach SL, et al, unpublished observations). In the present study, we required 10% weight loss or a BMI less than 20 kg/m^2 for the definition of wasting, so that our wasted subjects had a relatively severe degree of weight loss. The patients who participated in this study varied widely in their body composition, severity of illness, and level of HIV viremia. The exercise regimen used here was therefore constrained by the most ill patients and was chosen to be difficult, but feasible, even in relatively frail subjects. This goal was met, as all subjects completed the exercise protocol, although 1 subject needed a rest period during the exercise bout (see Roubenoff et al[8] for more details). Thus, an acute phase response was induced by the exercise, as was a metabolic response at the whole body level.

At baseline, we found no difference in whole body protein breakdown, oxidation, or synthesis between patients with wasting and those without. This is in contrast with the observations of Macallan et al[6] earlier in the HIV epidemic, before effective treatment was available: these investigators found higher whole body leucine flux in patients with more advanced disease, although they did not separate their groups according to wasting. More recently, Yarasheski et al[7] found a lower whole body protein breakdown rate in patients with AIDS wasting compared with HIV-asymptomatic patients or seronegative controls. However, our results and those of the 2 previous studies are similar in terms of leucine oxidation: there was a trend toward higher leucine oxidation in the present study in the HIV-positive group compared with the wasted group (24% higher, $P < .12$, Table 2).

This is the first study to examine protein metabolism in response to exercise in patients with HIV infection. We found a higher level of whole-body protein synthesis in response to 1 bout of exercise in patients with wasting than in those without wasting. The increased protein synthetic response in the wasted patients, 6 days after exercise, suggests that despite their wasted state, they are still quite capable of responding to an anabolic stimulus. The ability to respond to exercise in the wasted state contrasts with the reduced responsiveness to growth hormone administration shown by McNurlan et al[24] and may indicate that PRT can be useful in situations in which pharmacologic anabolic treatment fails.

Even though this exercise intervention was milder than the eccentric protocols previously used in our laboratory,[25] it was forceful enough to cause significant increases in peripheral neutrophil counts, creatine kinase levels, and urinary 3-methylhistidine excretion.[8] We studied our subjects 6 days after their bout of exercise, a time that is probably past the peak metabolic response to this exercise protocol, which occurs at day 4 in healthy subjects, as measured by serum creatine kinase levels.[26] With a more strenuous protocol of acute eccentric exercise using a motor-driven cycle ergometer, Fielding et al[25] demonstrated that muscle protein breakdown was still significantly elevated at day 10 in healthy adults. However, the protocol used in this study was chosen because it is safer for ill patients and causes less muscle injury. We chose day 6 for our follow-up metabolic measures to allow any effect of exercise on viral replication to be fully expressed, because our primary concern at the time this study was designed (before the advent of highly active antiretroviral therapy) was that exercise could exacerbate HIV viremia and thus induce harmful effects. Because viral replication takes several days to become detectable in the blood, we designed our follow-up period accordingly. In the event, we saw no effect of exercise on HIV RNA levels, despite induction of an acute phase response.[8] Thus, the leucine kinetic response we observed is more likely to be driven by endogenous hormonal and cytokine responses to exercise than by an effect of HIV-mediated immune response per se.

The timing of our postexercise tracer infusion may have fortuitously served to highlight a difference in metabolic response to exercise between wasted and nonwasted subjects, if the stimulus of exercise was more prolonged in the wasted subjects. Although we did not measure leucine kinetics at an intervening time point between baseline and day 6 postexercise, it is likely that both groups had an increase in their protein turnover during days 2 to 3 after exercise, but that only in the

wasted subjects was the effect still detectable at day 6. Our exercise protocol involved lifting subjects' own body weight, and thus should have protected wasted subjects because of their antecedent weight loss. Because much of the weight loss in the wasted group was fat loss, they were able to perform the exercise without incident. Indeed, there was no difference in the degree of acute phase response caused by the exercise in the wasted patients compared with the other 2 groups or in their subjective response to the bout of exercise.[8] Thus, it is likely that the level of stimulation to the muscle was comparable in the study groups.

These results are complementary to those recently published by Yarasheski et al[7] who found that whole body NOLD was reduced in HIV-positive wasted subjects compared with both healthy controls and to HIV-positive nonwasted patients at rest. While we found no difference in NOLD at rest in our subjects, the increase in NOLD in response to exercise suggests that wasted patients retain the ability to respond to exercise in an anabolic manner. Yarasheski et al also found a significantly higher leucine oxidation rate in their HIV-infected nonwasted subjects, while we also found a trend toward higher leucine oxidation in the HIV-positive nonwasted patients ($P < .12$, Table 2). Unlike Yarasheski et al, we did not measure muscle protein synthesis and cannot directly address whether wasted subjects respond to exercise with increased muscle protein synthesis. Nevertheless, based on their persistent increase in NOLD 6 days after 1 bout of exercise, wasted subjects appear primed for synthesis if they are given a signal such as exercise. Taken together with the larger response to resistance training previously demonstrated in wasted patients,[9-11] these data suggest that exercise can exert an important beneficial effect on muscle mass and protein metabolism in AIDS wasting.

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