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# Reduced Bone Density in Androgen-Deficient Women with Acquired Immune Deficiency Syndrome Wasting

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**Women with acquired immune deficiency syndrome wasting are at an increased risk of osteopenia because of low weight, changes in body composition, and hormonal alterations. Although women comprise an increasing proportion of human immunodeficiency virus-infected patients, prior studies have not investigated bone loss in this expanding population of patients. In this study we investigated bone density, bone turnover, and hormonal parameters in 28 women with acquired immune deficiency syndrome wasting and relative androgen deficiency (defined as free testosterone ≤3.0 pg/ml, weight ≤90% ideal body weight, weight loss ≥10% from preillness maximum weight, or weight <100% ideal body weight with weight loss ≥5% from preillness maximum weight). Total body (1.04 ± 0.08 *vs.* 1.10 ± 0.07 g/cm$^2$, human immunodeficiency virus-infected *vs.* control respectively; $P < 0.01$), anteroposterior lumbar spine (0.94 ± 0.12 *vs.* 1.03 ± 0.09 g/cm$^2$; $P = 0.005$), lateral lumbar spine (0.71 ± 0.14 *vs.* 0.79 ± 0.09 g/cm$^2$; $P = 0.02$), and hip (Ward's triangle; 0.68 ± 0.14 *vs.* 0.76 ± 0.12 g/cm$^2$; $P = 0.05$) bone density were reduced in the human immunodeficiency virus-infected compared with control subjects. Serum N-telopeptide, a measure of bone resorption, was increased in human immunodeficiency virus-infected patients, compared with control subjects (14.6 ± 5.8 *vs.* 11.3 ± 3.8 nmol/liter bone collagen equivalents, human immunodeficiency virus-infected *vs.* control respectively; $P = 0.03$). Although body mass index was similar between the groups, muscle mass was significantly reduced in the human immunodeficiency virus-infected *vs.* control subjects (16 ± 4 *vs.* 21 ± 4 kg, human immunodeficiency virus-infected *vs.* control, respectively; $P < 0.0001$). In univariate regression analysis, muscle mass ($r = 0.53$; $P = 0.004$) and estrogen ($r = 0.51$; $P = 0.008$), but not free testosterone ($r = -0.05$, $P = 0.81$), were strongly associated with lumbar spine bone density in the human immunodeficiency virus-infected patients. The association between muscle mass and bone density remained significant, controlling for body mass index, hormonal status, and age ($P = 0.048$) in multivariate regression analysis.**

**These data indicate that both hormonal and body composition factors contribute to reduced bone density in women with acquired immune deficiency syndrome wasting. Anabolic strategies to increase muscle mass may be useful to increase bone density among osteopenic women with acquired immune deficiency syndrome wasting. (*J Clin Endocrinol Metab* 86: 3533–3539, 2001)**

ANDROGENS ARE A critical determinant of bone density in men. In hypogonadal non-HIV-infected men, bone density is reduced in association with serum testosterone concentrations and increases in response to testosterone replacement (1). Similarly, prior studies in non-HIV-infected women have demonstrated important effects of muscle mass and androgens on bone density (2–5). We have previously shown that androgen deficiency is highly prevalent among women with acquired immune deficiency syndrome (AIDS) wasting (6). Furthermore, we have shown significant loss of lean body and muscle mass in the large subpopulation of androgen-deficient women with AIDS wasting (6).

Women comprise one third of the new cases of HIV infection and an ever-increasing proportion of AIDS cases (7). Although hormonal parameters and body composition may be significantly affected among HIV-infected women (6), little is known regarding the effects of these factors on bone in this expanding population of patients. We hypothesized that bone density would be reduced in androgen-deficient women with AIDS wasting, and therefore investigated bone density, bone turnover, body composition, and hormonal factors in this group of patients compared with age- and body mass index (BMI)-matched healthy control subjects. Given the risk for significant morbidity related to bone loss in this population, our rationale in performing this study was to determine the risk factors for osteopenia in this population.

Abbreviations: AIDS, Acquired immune deficiency syndrome; AP, anterioposterior; BCE, bone collagen equivalents; BMI, body mass index; CV, coefficient of variation; DXA, dual x-ray absorptiometry; HIV, human immunodeficiency virus; LMP, last menstrual period; NRTI, nucleoside reverse transcriptase inhibitor; NTX, N-telopeptides of type I collagen; PI, protease inhibitor.

## Subjects and Methods

### *Subjects*

Twenty-eight ambulatory, HIV-infected women were recruited for a study of physiological testosterone administration from the multidisciplinary HIV clinic at Massachusetts General Hospital and via community and primary care provider advertisements from September 1998 to October 2000. Study subjects were selected based on a relatively low free testosterone level (≤3 pg/ml, the median of the normal range; Endocrine Sciences, Inc., Calabasas Hills, CA). Of the 49 subjects screened, 92% exhibited free testosterone level of 3 pg/ml or less, and 57% of these were enrolled in the study. HIV status was verified by ELISA and Western blot testing in all subjects. Further inclusion criteria included age between 18–45 yr; weight 90% or less of ideal body weight, loss of 10% or more of preillness maximum weight, or weight less than 100% of ideal body weight with weight loss 5% or more of the preillness maximum weight; and current use of acceptable birth control (intrauterine device or barrier method). Exclusion criteria included use of androgen replacement, oral/injected contraceptives, or anabolic agents within the past 3 months, positive pregnancy test, breast feeding, new opportunistic infection within 4 wk of the study, initiation of new antiretroviral therapy within 6 wk of the study, use of glucocorticoids, clinically significant liver

disease and/or serum glutamic-oxaloacetic transaminase level more than 5 times normal, clinically significant renal disease and/or serum creatinine above 2 mg/dl, hemoglobin less than 8 g/dl, and active substance or alcohol abuse. In this study we report baseline bone density and turnover in the study subjects before anabolic intervention compared with those in a simultaneously recruited group of age- and BMI-matched healthy subjects (see below). Regularly menstruating subjects were studied in the early follicular phase.

### *Control subjects*

Twenty-one healthy, eumenorrheic control subjects were simultaneously recruited and matched for age and BMI with the HIV-infected subjects. Subjects were studied within 1 wk of initiation of their menstrual cycle in the early follicular phase. Control subjects were not receiving any medications and had no illnesses known to affect bone.

### *Body composition and bone density assessment*

Fat and lean body mass were determined by dual x-ray absorptiometry (DXA) using a Hologic-4500 densitometer (Hologic, Inc., Waltham, MA). Total body, lumbar spine [anteroposterior (AP) and lateral], and hip (total hip, trochanter, femoral neck, and Ward's triangle) bone density were measured by DXA. The DXA technique has a precision error of 3.0% for fat and 1.5% for lean body mass. The *in vivo* precision for the measurement of bone density using the DXA technique is 0.5–1.5% at the AP lumbar spine (8). The SD of the lumbar spine bone density is 0.01 $g/cm^2$ (9).

Muscle mass was calculated from a 24-h urine creatinine measurement obtained as an in-patient on a meat-free, protein-replaced diet, which was initiated 3 d before the in-patient protocol. A constant of 18 kg muscle/g urinary creatinine was used to calculate muscle mass from the urinary creatinine (10).

### *Laboratory methods*

Blood sampling was performed after an overnight 12-h fast. Serum estradiol was measured by RIA kit (Diagnostics Systems Laboratories, Inc., Webster, TX) with an intraassay coefficient of variation (CV) of 3.2–5.3%. Serum total and free testosterone levels and SHBG were measured by Endocrine Sciences, Inc. (Calabasas Hills, CA). The free testosterone concentration was determined as the product of the percent free testosterone measured by equilibrium dialysis and the total testosterone concentration. The intraassay CV of free testosterone is 6.9%, and the intraassay CV for total testosterone is less than 8.1%. The intraassay CVs were developed using pooled sera covering the range of the assay. The normal range for total testosterone is 10–55 ng/dl, and that for free testosterone is 1.1–6.3 pg/ml in adult females. The sensitivity of the total testosterone assay is 3 ng/dl, and the sensitivity of the percent free testosterone assay is 0.1%. SHBG was measured by immunoradiometric assay (sensitivity, 0.2 nmol/liter; intraassay CV, <4%). LH was measured by RIA with a sensitivity of 0.1 mIU/ml and an intraassay CV of 2.6% (Nichols Institute Diagnostics, San Juan Capistrano, CA). FSH was measured by a radioisotopic kit with a sensitivity of 0.2 mIU/ml and an intraassay CV of 1.6–2.3% (Nichols Institute Diagnostics).

Serum osteocalcin was measured using a two-site immunoradiometric assay (Nichols Institute Diagnostics). The lower detection limit of the assay was 0.5 ng/ml, and the intraassay CV was 3.2–5.2%. Serum N-telopeptides of type I collagen (NTX) was measured using a competitive ELISA with an intraassay CV of 4.6% (Ostex International, Inc., Seattle, WA).

CD4 counts were measured by flow cytometry (FACS scan analyzer, Becton Dickinson and Co., San Jose, CA). Viral loads were measured using an assay with a lower limit of detection of 400 copies.

### *Statistical analysis*

HIV status was coded as a dichotomous variable. Bone density measurements, serum bone markers, muscle mass measurements, sex hormone levels, viral load and CD4 counts, and bone markers were treated as continuous variables. The *t* test was used to compare continuous variables among the different groups. A logistic regression model was used to analyze the relationship between bone density and muscle mass while controlling for age, body mass index, and hormone levels (JMP Statistical Discovery Software, SAS Institute, Inc., Cary, NC). Results are the mean ± SD unless otherwise indicated. Given our sample size, we were able to detect a difference in muscle mass of 5 kg between our two groups with a power of more than 0.95 ($\alpha = 0.05$). We were able to detect a difference in lumbar spine bone density of 0.09 $g/cm^2$ between our two groups with a power of more than 0.85 ($\alpha = 0.05$).

## Results

### *Demographic data*

Women with AIDS wasting and HIV-negative healthy female controls were similar according to age and body mass index (Table 1). Minority status was not different between the two groups. Immunological parameters among the HIV-infected patients are shown in Table 2. Viral load was undetectable in 10 of 28 patients. Seventy-five percent of the HIV-infected subjects were receiving antiretroviral therapy at the time of the study.

### *Hormonal status*

Five of the 28 women with AIDS wasting were amenorrheic compared with none of the HIV-negative female healthy controls ($P < 0.01$). Five additional HIV-infected patients had a history of hysterectomy, but none had oophorectomy. Estrogen levels were similar between the 2 study groups ($P = 0.82$). In addition, LH and FSH levels were not different between the 2 groups (Table 1). SHBG levels were higher in HIV-infected patients compared with control subjects (147 ± 65 *vs.* 113 ± 44 nmol/liter, HIV-infected patients *vs.* controls, respectively; $P = 0.04$). The free testosterone concentration was reduced in the HIV-infected patients compared with control subjects (1.9 ± 1.0 *vs.* 2.4 ± 0.8 pg/ml, HIV-infected *vs.* controls, respectively; $P = 0.05$). Adjustment of the estradiol level for SHBG (free estradiol index) (11) did not show a difference between the groups (0.88 ± 0.66 *vs.* 1.11 ± 0.95, HIV-infected patients *vs.* controls, respectively; $P = 0.33$). In contrast, the free androgen index (11), total testosterone/SHBG, was significantly reduced in the HIV-infected patients compared with the control group (0.006 ± 0.004 *vs.* 0.008 ± 0.002; $P = 0.01$).

Timing of hormonal sampling was similar in the two study groups. Eumenorrheic HIV-infected women were studied, on the average, 5.9 d after their last menstrual period (LMP) compared with the eumenorrheic healthy control subjects, who were studied 4.2 d after their LMP ($P > 0.05$). No relationship was seen between time since LMP and estradiol levels ($r = 0.14$; $P = 0.66$), as sampled within the early follicular phase in the HIV-infected group.

The free testosterone concentration was not significantly reduced in a subanalysis limited to eumenorrheic HIV-infected patients (1.9 ± 1.0 *vs.* 2.4 ± 0.8 pg/ml, eumenorrheic HIV-infected and healthy controls, respectively; $P = 0.11$). Estradiol (31.7 ± 25.8 *vs.* 29.6 ± 22.3 pg/ml, eumenorrheic HIV-infected and healthy controls, respectively; $P = 0.81$), LH (6 ± 3 *vs.* 6 ± 4 IU/liter; $P = 0.84$), and FSH (10 ± 10 *vs.* 11 ± 3 IU/liter; $P = 0.93$) were similar between the groups in subanalyses comparing eumenorrheic HIV-infected patients to control subjects. Similar SHBG levels were found between eumenorrheic HIV-infected women and controls (137 ± 73 *vs.* 113 ± 44 nmol/liter;

**TABLE 1.** Comparison of demographic, bone density, body composition, bone marker, and hormonal data between women with AIDS wasting and healthy HIV-negative female controls

| Variable | HIV-infected women with wasting (n = 28) | HIV-negative female controls (n = 21) | *P* value |
|---|---|---|---|
| Demographic data | | | |
| Age (yr) | 37 ± 4 | 36 ± 4 | 0.19 |
| Body mass index (kg/m$^2$) | 21.5 ± 3.5 | 22.5 ± 2.0 | 0.30 |
| Bone density data | | | |
| AP lumbar spine (g/cm$^2$) | 0.94 ± 0.12 | 1.03 ± 0.09 | 0.005 |
| AP lumbar spine *t*-score | −1.20 ± 1.03 | −0.16 ± 0.95 | <0.001 |
| Lateral lumbar spine (g/cm$^2$) | 0.71 ± 0.14 | 0.79 ± 0.09 | 0.02 |
| Lateral lumbar spine *t*-score | −1.39 ± 1.66 | −0.49 ± 1.13 | 0.04 |
| Femoral neck (g/cm$^2$) | 0.79 ± 0.12 | 0.83 ± 0.09 | 0.24 |
| Femoral neck *t*-score | −0.72 ± 1.04 | −0.20 ± 0.81 | 0.07 |
| Trochanter (g/cm$^2$) | 0.65 ± 0.11 | 0.70 ± 0.09 | 0.07 |
| Trochanter *t*-score | −0.67 ± 0.97 | −0.04 ± 0.90 | 0.02 |
| Total hip (g/cm$^2$) | 0.88 ± 0.13 | 0.94 ± 0.10 | 0.07 |
| Total hip *t*-score | −0.69 ± 0.96 | −0.05 ± 0.83 | 0.02 |
| Ward's triangle (g/cm$^2$) | 0.68 ± 0.14 | 0.76 ± 0.12 | 0.05 |
| Ward's triangle *t*-score | −0.66 ± 1.10 | 0.16 ± 1.03 | 0.01 |
| Total body (g/cm$^2$) | 1.04 ± 0.08 | 1.10 ± 0.07 | <0.01 |
| Total body *t*-score | −0.75 ± 0.96 | −0.05 ± 0.81 | <0.01 |
| Body composition data | | | |
| Muscle mass (kg) | 16.0 ± 4.0 | 21.0 ± 4.0 | <0.0001 |
| % Fat mass, DXA | 25 ± 6 | 28 ± 6 | 0.10 |
| Total body fat mass, DXA (kg) | 15.0 ± 5.5 | 17.5 ± 5.0 | 0.11 |
| Total lean mass, DXA (kg) | 41.0 ± 6.5 | 42.0 ± 4.0 | 0.69 |
| Bone turnover data | | | |
| Osteocalcin (μg/liter) | 25.1 ± 20.3 | 24.6 ± 11.3 | 0.92 |
| N-Telopeptides (nmol/liter BCE) | 14.6 ± 5.8 | 11.3 ± 3.8 | 0.03 |
| Hormonal data | | | |
| Estradiol (pg/ml) | 31.2 ± 24.5 | 29.6 ± 22.3 | 0.82 |
| Free testosterone (pg/ml) | 1.9 ± 1.0 | 2.4 ± 0.8 | 0.05 |
| SHBG (nmol/liter) | 147 ± 65 | 113 ± 44 | 0.04 |
| LH (IU/liter) | 9 ± 9 | 6 ± 4 | 0.18 |
| FSH (IU/liter) | 16 ± 25 | 11 ± 3 | 0.30 |

**TABLE 2.** Medication and disease status data in HIV-infected study subjects

| Variable | |
|---|---|
| Viral load (copies/ml) | 24,800 ± 70,900 |
| Viral load <400 copies (%) | 37 |
| CD4 count (cells/mm$^3$) | 335 ± 192 |
| Current antiretroviral therapy (%) | 75 |
| Current protease inhibitor use (%) | 29 |
| Current nucleoside reverse transcriptase inhibitor use (%) | 75 |
| Current nonnucleoside reverse transcriptase inhibitor use (%) | 32 |

n = 28, women with AIDS wasting.

$P = 0.23$). SHBG levels did not correlate with lumbar spine bone density (r = −0.15; $P = 0.45$) in HIV-infected patients.

Of the five amenorrheic subjects, two had elevated LH and FSH levels indicative of gonadal failure. Similarly, of the five hysterectomy patients, two had elevated LH and FSH levels in the postmenopausal range. Estradiol and free testosterone levels in amenorrheic HIV-infected women with or without hysterectomy were not different compared with levels in eumenorrheic women with AIDS wasting [estradiol: amenorrheic with hysterectomy *vs.* eumenorrheic HIV-infected subjects, 33.2 ± 27.7 *vs.* 31.7 ± 25.8 pg/ml ($P = 0.91$); amenorrheic without hysterectomy *vs.* eumenorrheic HIV-infected subjects, 22.7 ± 18.5 *vs.* 31.7 ± 25.8 pg/ml ($P = 0.49$); free testosterone: amenorrheic with hysterectomy *vs.* eumenorrheic HIV-infected subjects, 1.8 ± 1.0 *vs.* 1.9 ± 1.0 pg/ml ($P = 0.93$); amenorrheic without hysterectomy *vs.* eumenorrheic HIV-infected subjects, 2.0 ± 1.4 *vs.* 1.9 ± 1.0 pg/ml ($P = 0.90$)]. SHBG levels in amenorrheic HIV-infected women also were not different compared with levels in eumenorrheic women with AIDS wasting [amenorrheic with hysterectomy *vs.* eumenorrheic HIV-infected subjects, 146 ± 41 *vs.* 137 ± 73 nmol/liter ($P = 0.79$); amenorrheic without hysterectomy *vs.* eumenorrheic HIV-infected subjects, 192 ± 68 *vs.* 137 ± 73 nmol/liter ($P = 0.16$)].

*Bone density*

AP and lateral lumbar spine density [0.94 ± 0.12 *vs.* 1.03 ± 0.09 g/cm$^2$ ($P = 0.005$) and 0.71 ± 0.14 *vs.* 0.79 ± 0.09 g/cm$^2$ ($P = 0.02$), respectively] were significantly reduced in HIV-infected patients compared with control subjects, respectively. The corresponding *t* scores for the AP and lateral lumbar spine densities were also decreased in the HIV-infected patients compared with control subjects [−1.20 ± 1.03 *vs.* −0.16 ± 0.95 ($P = <0.001$) and −1.39 ± 1.66 *vs.* −0.49 ± 1.13 ($P = 0.04$), HIV-infected *vs.* controls, respectively]. Bone density was reduced for the greater trochanter [0.65 ± 0.11 *vs.* 0.70 ± 0.09 g/cm$^2$, HIV-infected *vs.* controls, respectively ($P = 0.07$); *t* scores: −0.67 ± 0.97 *vs.* −0.04 ± 0.90 ($P = 0.02$)], Ward's triangle [0.68 ± 0.14 *vs.* 0.76 ± 0.12 g/cm$^2$ ($P = 0.05$); *t* scores: −0.66 ± 1.10 *vs.* 0.16 ± 1.03 ($P = 0.01$)], and total hip [0.88 ± 0.13 *vs.* 0.94 ± 0.10 g/cm$^2$ ($P = 0.07$); *t* scores, −0.69 ± 0.96 *vs.* −0.05 ± 0.83 ($P = 0.02$)] [Table 1]. Total body bone density and corresponding *t* scores were

also significantly reduced in HIV-infected patients compared with control subjects (Table 1). In addition, using standard WHO criteria, a significantly greater percentage of HIV-infected patients compared with control subjects were osteopenic at the lumbar spine [50% (14 of 28) *vs.* 14.3% (3 of 21); $P = 0.04$, HIV-infected *vs.* control] and the hip [48.1% (13 of 27) *vs.* 9.5% (2 of 21); $P = 0.005$]. The percentages of patients with osteoporosis at the lumbar spine [3.6% (1 of 28) *vs.* 4.8% (1 of 21), HIV-infected *vs.* controls] were not different between the 2 groups. None of the patients had osteoporosis at the hip.

In a subanalysis limited to eumenorrheic HIV-infected women and HIV-negative controls, bone density was reduced at the lumbar spine compared with that in normal controls ($0.97 \pm 0.08$ *vs.* $1.03 \pm 0.09$ g/cm$^2$, HIV-infected *vs.* normal controls, respectively; $P = 0.049$). However, bone density was not significantly different between these two groups (eumenorrheic HIV-infected *vs.* healthy controls subjects) at the other measured sites (data not shown).

Viral load ($r = -0.05$; $P = 0.79$) and CD4 count ($r = 0.13$; $P = 0.52$) did not correlate with lumbar spine bone density. In addition, there was no difference in lumbar spine bone density according to CD4 count subgrouping ($0.93 \pm 0.12$ *vs.* $0.94 \pm 0.13$ g/cm$^2$, $<200$ *vs.* $\geq 200$, respectively; $P = 0.88$) or according to HIV RNA detectability ($0.91 \pm 0.16$ *vs.* $0.95 \pm 0.10$ g/cm$^2$, undetectable *vs.* detectable; $P = 0.43$).

Analysis according to current medication exposure status in the HIV-infected subjects [protease inhibitor (PI)-treated *vs.* non PI-treated; nucleoside reverse transcriptase inhibitor (NRTI)-treated *vs.* non NRTI-treated; non-NRTI-treated *vs.* non-NRTI-nontreated in the HIV-infected subjects] did not demonstrate any difference in lumbar spine (AP and lateral), total hip, or total body bone density between subgroups (data not shown). Bone density was reduced at the femoral neck ($0.76 \pm 0.12$ *vs.* $0.87 \pm 0.11$ g/cm$^2$, NRTI-treated *vs.* non-NRTI-treated, respectively; $P = 0.04$) and Ward's triangle hip region ($0.65 \pm 0.14$ *vs.* $0.77 \pm 0.10$ g/cm$^2$; $P = 0.05$) in patients exposed to NRTIs. Further analysis according to WHO criteria for osteopenia and osteoporosis did not reveal any differences according to NRTI therapy at either the femoral neck (NRTI-exposed *vs.* non NRTI-exposed, respectively, 40% *vs.* 28.6%, $P = 0.68$; 5% *vs.* 0%, $P = 1.0$, osteopenia and osteoporosis, respectively), or Ward's triangle (NRTI-exposed *vs.* non NRTI-exposed, respectively, 40% *vs.* 14.3%, $P = 0.36$; 5% *vs.* 0%, $P = 1.0$, osteopenia and osteoporosis, respectively). Subsequent analyses according to the length of medication therapy also did not reveal any association between duration of PI or reverse transcriptase inhibitor therapy and site-specific or total body bone density (data not shown).

### *Body composition*

DXA-measured total body fat and lean mass were not different between the BMI-matched study groups. In contrast, muscle mass, as determined by urinary creatinine excretion measurements, was significantly reduced in women with AIDS wasting ($16 \pm 4$ *vs.* $21 \pm 4$ kg, HIV-infected *vs.* controls, respectively; $P < 0.0001$) compared with HIV-negative controls (Table 1).

In a subanalysis looking only at women with AIDS wasting according to menstrual status, body composition measures were not different between groups. HIV-infected women who had undergone a hysterectomy had similar DXA-measured total body fat and lean mass compared with HIV-infected eumenorrheic women (total body fat, $17.3 \pm 7.4$ *vs.* $13.7 \pm 4.5$ kg, women with hysterectomy *vs.* eumenorrheic women, respectively, $P = 0.20$; total lean mass, $40.2 \pm 5.2$ *vs.* $39.2 \pm 5.8$ kg, $P = 0.73$). Similarly, these parameters were not different between amenorrheic HIV-infected women without hysterectomy and eumenorrheic HIV-infected women (total body fat, $14.4 \pm 5.2$ *vs.* $13.7 \pm 4.5$ kg, amenorrheic *vs.* eumenorrheic women, respectively, $P = 0.75$; total lean mass, $41.8 \pm 4.9$ *vs.* $39.2 \pm 5.8$ kg, $P = 0.38$). Muscle mass was also similar among these groups ($17.1 \pm 5.2$ *vs.* $16.7 \pm 3.7$ kg, amenorrheic without hysterectomy *vs.* eumenorrheic HIV-infected women, respectively, $P = 0.83$; $14.7 \pm 2.7$ *vs.* $16.7 \pm 3.7$ kg, amenorrheic with hysterectomy *vs.* eumenorrheic HIV-infected women respectively, $P = 0.28$).

### *Bone markers*

Serum NTX was significantly increased among HIV-infected patients compared with control subjects ($14.6 \pm 5.8$ *vs.* $11.3 \pm 3.8$ nmol/liter bone collagen equivalents (BCE), HIV-infected *vs.* controls, respectively; $P = 0.03$). In contrast, osteocalcin concentrations were not different between the study groups (Table 1). In a subanalysis limited to eumenorrheic patients, NTX was not different between the groups ($13.0 \pm 5.0$ *vs.* $11.3 \pm 3.8$ nmol/liter BCE, HIV-infected *vs.* controls, respectively; $P = 0.28$), whereas osteocalcin was reduced in the eumenorrheic HIV-infected patients ($16.3 \pm 10.9$ *vs.* $24.6 \pm 11.3$ $\mu$g/liter, HIV-infected *vs.* controls, respectively; $P = 0.04$).

### *Univariate and multivariate regression modeling*

In univariate regression analysis, muscle mass and estrogen were significantly associated with lumbar spine bone density (Table 3). Increasing age and low BMI tended to be associated with reduced lumbar bone density, but these associations did not reach statistical significance. No association was seen between testosterone and bone density.

In multivariate regression analysis among only HIV-infected patients, muscle mass remained a significant predictor of lumbar spine bone density, controlling for age, BMI, estrogen, and testosterone (Table 4). Lumbar spine bone density increased 0.012 g/cm$^2$ for each kilogram increase in muscle mass (Fig. 1).

## Discussion

Women with AIDS wasting are at significantly increased risk for bone loss secondary to a number of factors, including low weight, alterations in body composition, and irregular menstrual cycles. In this study we investigated bone density at multiple sites in a group of women with AIDS wasting and demonstrated significant bone loss in this group of patients compared with healthy age- and BMI-matched control subjects at the lumbar spine and hip. Our data suggest that changes in body composition and hormones are strong risk factors for osteopenia in this group.

**TABLE 3.** Univariate modeling with lumbar spine bone mineral density

| Variable | Correlation (r) | *P* value |
|---|---|---|
| Demographics | | |
| Age | −0.30 | 0.12 |
| Body mass index | 0.27 | 0.16 |
| Immune function | | |
| Viral load | −0.05 | 0.79 |
| CD4 count | 0.13 | 0.52 |
| Body composition | | |
| Total body fat mass | 0.28 | 0.15 |
| Total body lean mass | 0.30 | 0.13 |
| Muscle mass | 0.53 | 0.004 |
| Hormonal status | | |
| Free testosterone | −0.05 | 0.81 |
| Estradiol | 0.51 | 0.008 |
| SHBG | −0.15 | 0.45 |
| Bone markers | | |
| Serum NTX | −0.32 | 0.11 |
| Serum Osteocalcin | −0.40 | 0.04 |

n = 28, women with AIDS wasting.

**TABLE 4.** Multivariate modeling with lumbar spine bone mineral density

| Variable | Estimate | Confidence interval | SE | *P* value |
|---|---|---|---|---|
| Age | −0.006 | −0.02, 0.006 | 0.005 | 0.31 |
| Body mass index | 0.011 | −0.002, 0.024 | 0.006 | 0.08 |
| Estradiol | 0.001 | −0.001, 0.003 | 0.001 | 0.25 |
| Free testosterone | 0.0003 | −0.042, 0.042 | 0.020 | 0.99 |
| Muscle mass | 0.0122 | 0.0001, 0.0243 | 0.006 | 0.048 |

n = 28, women with AIDS wasting. Whole model, $P = 0.01$; whole model, $r^2 = 0.52$.

Potential factors contributing to reduced bone density in HIV-infected patients include reduced muscle mass, hormonal factors, and medication effects. Changes in body composition seen among women with AIDS wasting may contribute to osteopenia. In this study we demonstrate significant sarcopenia in HIV-infected patients compared with age- and BMI-matched control subjects, consistent with prior reports in the literature. Muscle mass, measured by urinary creatinine excretion, was significantly reduced in the HIV-infected patients compared with control subjects. Renal function was normal in all subjects. In contrast, lean body mass, as assessed by DXA, was not significantly different between the groups. Prior comparison of these two techniques by Proctor *et al.* has shown that urinary excretion methods of assessing total body muscle mass remains the more sensitive index of total body skeletal muscle mass (12). DXA does not specifically measure muscle mass and is best used to define fat and fat-free mass. The latter may be affected by changes in hydration status, which may explain the differences in the determination of muscle mass between the two techniques.

Body composition indexes have been found to be strong predictors of bone density in prior studies of women. Visser *et al.* reviewed the data from the Framingham Heart Study in women 72–93 yr old and found that both muscle mass and percent body fat were associated with total body BMD as measured by DXA (2). In adolescent girls with anorexia nervosa, bone mass measured by DXA was strongly correlated to lean tissue mass (3). In addition, weight and BMI have been found to be proportionate to bone density in premenopausal (13) and postmenopausal (14) women, respectively.

In our population of women with AIDS wasting, AP and lateral lumbar spine bone density were significantly correlated to muscle mass. In addition, the association between reduced lumbar spine bone density and reduced muscle mass in this population remained significant, controlling for other known predictors of bone density (age, BMI, estrogen, and free testosterone). In contrast, neither lean body mass nor fat mass was associated with lumbar spine bone density.

Androgen deficiency is a second potential factor contributing to reduced bone density in this population. The HIV-infected subjects in this study were selected based on a relatively reduced serum free testosterone concentration (defined as less than the median of the normal range for free testosterone by equilibrium dialysis methodology) independent of menstrual function, for subsequent participation in a study of androgen replacement. Thus, our results cannot be generalized to the entire population of women with AIDS wasting, but, rather, to the subset with this range of testosterone. Nonetheless, 92% of female patients with AIDS wasting screened for the study demonstrated free testosterone levels of 3 pg/ml or less (median of the normal range for the assay), and 49% demonstrated a free testosterone concentration below 1.1 pg/ml (lower limit of the normal range for the assay), suggesting a high prevalence of androgen deficiency in this population. These data are consistent with prior data from our group and others showing reduced androgen levels in women with AIDS wasting (6). Although androgen levels were reduced in the HIV-infected patients, the serum free testosterone level was not associated with lumbar spine or hip bone density. In contrast, muscle mass was highly predictive of bone density in multivariate modeling including age, BMI, estrogen, and free testosterone as variables.

Prior studies have not assessed the effect of testosterone on bone density in HIV-infected women. As our data now suggest significant bone loss in this population, future studies will be necessary to investigate treatment strategies for this group. Although the testosterone concentration was not shown to be a significant predictor of bone density, testosterone administration may have important indirect effects by increasing muscle mass in this sarcopenic population. Indeed, testosterone replacement in hypogonadal men has been shown to normalize previously reduced bone density (15, 16).

Menstrual status and estrogen exposure are factors known to affect bone density. Numerous studies in the literature demonstrate that postmenopausal and amenorrheic women are at increased risk for osteopenia and osteoporosis. Among our HIV-infected subjects with wasting, estrogen levels were not different from those in healthy control subjects measured in the early follicular phase. Although estradiol levels can fluctuate across the follicular phase, this variation was limited by the restriction of sampling to the early follicular phase of the menstrual cycle in both patient groups. Estrogen levels were significantly associated with lumbar spine bone mineral density in a univariate analysis ($P < 0.01$). However, estrogen did not remain a significant predictor of spinal bone density in a multivariate model controlling for free testosterone level, BMI, age, and muscle mass, in which muscle

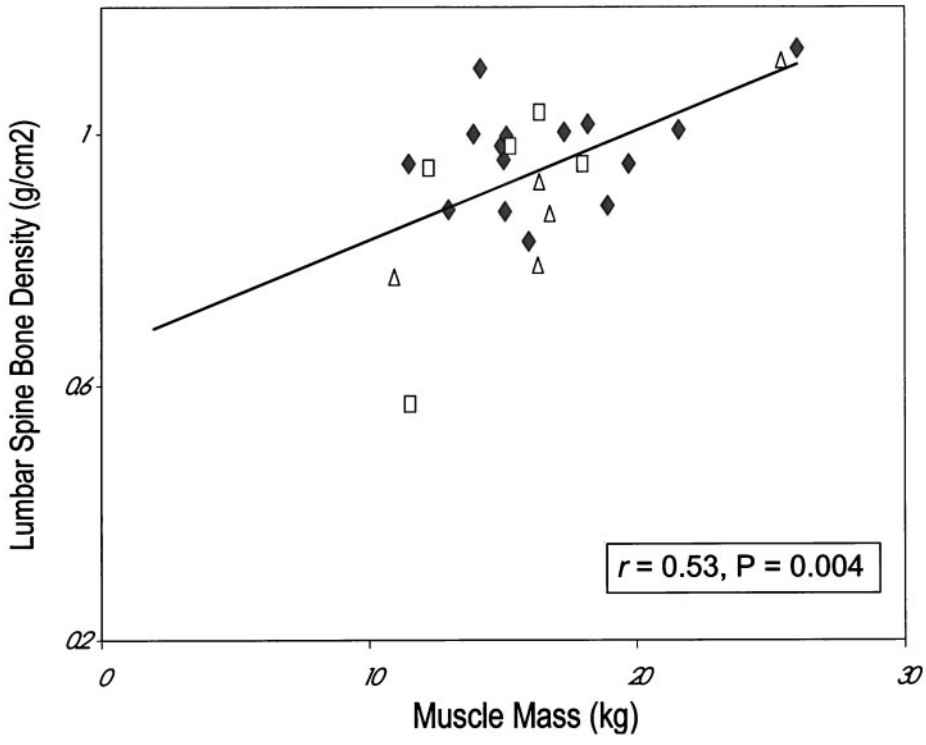


FIG. 1. Muscle mass *vs.* lumbar spine bone density among women with AIDS wasting. □, Amenorrheic status post hysterectomy; △, amenorrheic with uterus; ◆, eumenorrheic subjects.

mass was the only significant predictor of bone density. Furthermore, a subanalysis among HIV-infected women according to menstrual status did not show a difference in body composition. Ten of the 28 women with AIDS wasting did not have menses; half of these subjects had a history of hysterectomy, but none had oophorectomy. As amenorrhea itself is a risk factor for reduced bone density, we performed a subanalysis including only HIV-infected women with regular menses. In this subanalysis, lumbar spine bone density was reduced compared with control subjects. Among eumenorrheic HIV-infected subjects, LH and FSH levels did not exhibit any relationship with bone density.

SHBG has been negatively correlated to bone mineral density in postmenopausal women (17). This is presumably due to the concomitant reduction in free androgen and estradiol levels. In our population SHBG was significantly elevated compared with levels in normal controls. However, estradiol levels were not different between groups, and although free testosterone levels were lower in the AIDS wasting group, free testosterone was not a strong predictor of bone density in univariate or multivariate analyses. Moreover, in a subanalysis among eumenorrheic subjects, the difference in SHBG between groups did not persist. In analyses restricted to HIV-infected subjects, menstrual status did not appear to have an effect on the level of SHBG. In addition, SHBG did not correlate to lumbar spine bone mineral density.

Assessment of bone turnover in our population of women with AIDS wasting demonstrated increased NTX in the combined group of eumenorrheic and amenorrheic women, but no difference in osteocalcin levels. Serum NTX is a marker of bone resorption that correlates well to urine bone resorption marker (18) and predicts long-term changes in vertebral bone mineral density in elderly women receiving alendronate therapy (19). In contrast, osteocalcin is considered to reflect bone formation, but is also a marker of increased bone turnover. In this study increased osteocalcin more than NTX predicted reduced bone density, but osteocalcin was not significant as a predictor of bone density in multivariate modeling. The mechanism of the increased NTX in our HIV-infected population is unclear. One potential explanation relates to menstrual function. In a subanalysis limited to eumenorrheic patients, NTX was not different between groups, whereas osteocalcin was reduced in the HIV-infected women. Taken together, these data suggest that NTX and osteocalcin increase as expected in the amenorrheic patients and that the differences between HIV-infected and control subjects with respect to bone turnover are at least in part due to the abnormal menstrual function in HIV-infected patients.

The association between osteopenia and combined antiretroviral therapy has only been recently observed. Tebas *et al.* suggested that PIs may play a major role in the development of reduced bone density in HIV-infected men (20). The mechanism by which such drug therapy would result in decreased bone mineral density remains unclear. In contrast, we did not find any relationship between PI therapy and bone density. We did find an association between NRTI therapy and femoral neck and Ward's hip region bone densities; however, there was no difference in the proportion of patients meeting osteopenia or osteoporosis WHO criteria between the NRTI-exposed and non-NRTI-exposed groups at both of these sites. In addition, we did not find any relationship between the duration of any type of antiretroviral therapy and site-specific or total body bone density.

In conclusion, this is the first report of osteopenia among women with AIDS wasting. Our data demonstrate reduced lumbar spine, hip, and total body bone density and increased

bone resorption in women with AIDS wasting. Reduced muscle mass is the most significant and consistent predictor of reduced bone density in this population. Estrogen deficiency may also play a role in determining bone density. Additional longitudinal randomized controlled trials are necessary to investigate the utility of physiological androgen replacement as well as estrogen-androgen combination therapy to build muscle mass and reduce bone resorption in women with AIDS wasting.

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