Changes in Calciotropic Hormones and Biochemical Markers of Bone Metabolism in Patients With Human Immunodeficiency Virus Infection

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Data on the bone metabolism of human immunodeficiency virus (HIV)-infected patients are still extremely rare. To investigate the influence of HIV infection on the calciotropic hormones and markers of bone metabolism, we therefore performed a cross-sectional study on 100 patients (65 males and 35 females) with proven HIV infection. The following criteria were used for exclusion from the study: age less than 20/more than 50 years, confinement to bed, wasting symptoms, treatment with agents containing ketoconazole, renal or hepatic insufficiency, clinical or echographic signs of liver cirrhosis, endocrine diseases, or treatment with medications known to influence bone metabolism. Bone mineral content (BMC) was determined by single-photon absorptiometry on the left forearm. Reduced BMC was found among the male and female HIV-infected patients. Additional long-term use of heroin resulted in a severe loss of mineralization in the respective females. The markers of bone metabolism were determined in urine and serum samples. Significantly lower osteocalcin concentrations were found, indicating a reduced bone formation rate whose severity showed a significant correlation with the progressive loss of CD4 helper cells and was independent of low vitamin D_3 levels (1,25-dihydroxycholecalciferol) and alterations of protein metabolism. Increased urinary excretion of cross-links as an expression of enhanced bone resorption was likewise significantly correlated with the loss of CD4 helper cells and independent of the vitamin D concentration and protein metabolism. It is therefore concluded that the changes in bone metabolism are mainly due to mechanisms of the impaired immune defense of HIV-infected patients.

INFLAMMATION MEDIATORS are involved in the regulation of many metabolic and endocrine processes. The integration of mediators into the pathology of osteoporosis has been the subject of numerous experimental and clinical studies in recent years. In patients with acute rheumatoid arthritis, for instance, the increase of inflammatory activity is associated with increased urinary excretion of cross-links as an expression of enhanced bone resorption.[4]

Not only autoimmune diseases but also infectious diseases of various origins show features of increased inflammatory activity. For this reason, data on the bone metabolism of human immunodeficiency virus (HIV)-infected patients appear to be of well-founded interest, not least under the aspect of the constantly changing physiologic states during the course of disease. Alterations of bone metabolism have been observed in numerous studies of small groups of male HIV-infected patients; hypocalcemic phases,[27] hypercalcemic phases,[6,9,14,20] reduced serum osteocalcin levels,[13,21] and hypoparathyroidism[13,14] have been reported. However, in addition to the disease process itself, social factors seem to be of importance in the pathogenesis of possibly HIV-associated osteoporosis, for instance, a sufficient and balanced diet including vitamins and minerals or the abuse of drugs and alcohol.

Starting from these assumptions, we performed a larger cross-sectional study of HIV-infected patients in which we analyzed urine and serum samples for calciotropic hormones and markers of bone metabolism, with the aim to test the hypothesis that the rate of bone formation decreases with an increasing duration of the disease expressed as the total number of CD4 cells, and that the bone resorption rate increases and the markers of bone formation and resorption are independent of vitamin D supply and malnutrition. And finally, which influence on the markers of bone metabolism is exerted by the sex of the patient and by the long-term use of heroin?

SUBJECTS AND METHODS

Patients

One hundred patients (65 males and 35 females) with a confirmed serodiagnosis (including Western blot analysis) of HIV-1 infection participated in the study. Additionally, we recruited 30 heroin-dependent subjects (15 males and 15 females). They were examined as outpatients and subdivided into the following 6 groups: group I, male heroin-dependent patients (n = 24; age range, 25 to 43 years); group II, male patients not using heroin or other habit-forming drugs, including alcohol (n = 41; age range, 29 to 56 years); group III, male heroin-dependent patients without any serologic signs of HIV infection (n = 15; age range, 19 to 41 years); group V, female heroin-dependent patients (n = 20; age range, 26 to 41 years; 11 amenorrheic women); group VI, female patients meeting the criteria for group 2 (n = 15; age range, 27 to 48 years; 1 amenorrheic and 1 perimenopausal woman); and group VII, 15 female heroin-dependent patients (n = 15; age range, 18 to 40 years; 5 amenorrheic women). Secondary amenorrhea was defined as the first observation of the absence or the loss of menstruation. At the time of examination, none of the patients had concomitant opportunistic infections, acute or chronic hepatitis with increased transaminase activities, alterations of the liver parenchyma under sonomorphologic criteria, wasting symptoms, gastrointestinal disorders such as pancreatic insufficiency or malabsorption syndrome, or chronic diarrhea.

The patients did not use any medications known to influence the characteristics of bone metabolism or the endocrine system, but the necessary medication including antiretroviral therapy was continued. Patients received nucleosidanaloga like zidovudine (Retrovir; Glaxo Wellcome, Hamburg, Germany), zalcitabin (HIVID; Roche, Grenzach-Wyhlen, Germany), or didanosin (Videx; Bristol-Meyers Squibb, München, Germany). The recruitment of patients was finished at the

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end of 1995. Therefore, our patients received no agents like protease inhibitors for additional antiretroviral therapy because admission to this treatment in Germany was provided by the German government in 1996. Patients with a CD4 cell count less than 250 received a daily treatment with cotrimoxazol (trimethoprim 80 mg/sulfamethoxazol 400 mg; Bactrim; Roche) for the prevention of *Pneumocystis carinii* pneumonia. Five subjects had a mycobacterium infection formerly and received daily rifambutin 300 mg (Mycobutin; Pharmacia & Upjohn, Erlangen, Germany), isoniazid 5 mg/kg body weight (Isocid comp; Fatol, Schiffweler, Germany), and Ethambutol-HCl 20 mg/kg body weight (EMB-Fatol; Fatol). A soor mycosis resulting from infection with candida species was treated locally with amphotericin B–containing agents. Patients with cytomegaly-associated chorioretinitis were excluded from the study because it is well known that treatment with foscarnet (Foscavir; Astra, Wedel, Germany) or gancyclovir (Cymeven; Roche) affects the serum calcium level.

The respective clinical data are shown in Tables 1 and 3. Fasting blood samples were obtained by puncture of a cubital vein. The serum was frozen and stored at −30°C until analysis. Twenty-four-hour urine samples were collected after a gelatin-free diet and stored without additives at −30°C until analysis. An age-matched control group consisting of 40 healthy individuals (20 males, group 4; 20 females, group 8) was formed to compare the results. All subjects were kept busy all day long in departments of the German health service. None of the subjects performed extreme physical exercise. Their values for biochemical markers were in accordance with the normal range of the laboratory.

SUBJECTS AND METHODS

Lymphocyte subpopulations (CD4 cells) were determined using monoclonal antibodies and the FACscan autoanalyzer (both from Becton Dickinson, Mountain View, CA). Hydroxyproline was determined in 24-hour urine after a gelatin-free diet, using a kit from Organon Diagnostika (Espoo, Finland). Serum calcium, phosphate, creatinine, and albumin and urinary excretion of calcium, phosphate, and creatinine were measured by standardized laboratory methods. Plasma parathyroid hormone (PTH) (1-84) was determined by a radioimmunoassay (RIA) from the Nichols Institute (Wijchen, The Netherlands). The excretion of total pyridinolines was measured using a RIA kit from Biermann (Bad Nauheim, Germany). The reference value for this kit was set at values less than 50 nmol pyridinoline/nmol creatinine ratio. The serum osteocalcin level was measured using a commercial RIA kit from Incstar (Stillwater, MN). The interassay and intraassay coefficient of variation was 5% and 10%, respectively (reference value, 2.0 to 6.5 pg/mL). The calcitonin level was measured using a RIA kit from Biermann (normal, 5 to 15 pg/mL). The vitamin D_3 concentration (1,25-dihydroxycholecalciferol [$1,25(OH)_2D_3$]) in serum was determined using a RIA from Nichols Diagnostics (detection limit, 5.0 ng/mL), and 25 $(OH)D_3$ was also determined using a RIA from Nichols Diagnostics (normal, 8 to 80 ng/mL). Prior to the assay, the serum was chromatographed on a C_{18}OH column to remove the lipid components.

The measurement of bone mineral content (BMC) was performed using single-photon absorptiometry on the distal radius of the left forearm with a ^{125}I source (200 Ci, Osteometer DT; FA Christiansen, Ballerup, Denmark) and calculated as the mean of 6 scans. BMC was measured at the start of each investigation. The markers of the gonadal axis were measured by commercially available kits (FSH and LH RIA, Biermann; prolactin and total testosterone RIA, BYK Gulden, Konstanz, Germany).

Measurement of estradiol. Serum (1 mL) was extracted twice with diethylether. The extract was evaporated to dryness under a stream of nitrogen and dissolved in ethanol (1 mL). Duplicates of 0.4 mL were used for RIA and evaporated with nitrogen. Standard curves were prepared in duplicate over the range from 10 to 1,000 pg. To each tube (unknown and standard), about 7,000 cpm 2,4,6,7-^{3}H-estradiol-17β (Amersham Buchler, Karlsruhe, Germany) in 0.1 mL buffer, pH 7.0, and 0.1 mL antiserum (obtained from ICN, Heidelberg, Germany) were added. After incubation at 20°C for 20 hours, the tubes were kept at 4°C for 2 hours and then 0.2 mL charcoal suspension was added to each tube. They were shaken for 15 seconds and centrifuged at 1,500 × *g* for 5 minutes. The supernatants were decanted into counting vials containing 5 mL Rotiszint eco plus (Carl Roth, Karlsruhe, Germany). The within-assay coefficient of variation was 9.8% (n = 10). Cross-reactions were observed with dihydroequilin-17β (7.2%), 6β-OH-estradiol-17β (7.2%), 17β-dihydroequilinen (7.0%), 16-keto-estradiol-17β, estriol (1.3%), and estradiol-17α (0.8%).

Statistical Analysis

The mean ± SD in the different groups were compared by Student's *t* test. Differences at *P* less than .05 were considered statistically significant. Correlation analysis with a determination of Pearson's correlation coefficient was performed to examine the degree of (linear) relationship between the variable ''CD4 cell'' and another variable. The partial correlation coefficient accounts for the linear influence of a control variable and is a numeric measure for the linear correlation between 2 variables. Secondarily, the correlation was calculated after adjustment for a given variable—residual correlation. However, in all computations, the independent variable is the CD4 cell.[5]

RESULTS

The physical and clinical characteristics of the patients are shown in Tables 1 to 4. The body weight and body mass index were significantly lower in the 2 groups of male HIV-infected

Table 1. Characteristics (mean ± SD) of the Males Grouped by Heroin Abuse and HIV Infection

Characteristic	Group I (n = 24; HIV, heroin)	Group II (n = 41; HIV, no heroin)	Group III (n = 15; no HIV, heroin)	Control IV (males, n = 20)	
Weight (kg)	70.5 ± 4.2†	67.5 ± 6.3†	71.6 ± 7.2	78.5 ± 4.7	
Body mass index (kg/m²)	29.3 ± 3.4	27.9 ± 3.8	28.9 ± 4.6	34.5 ± 5.5	
Age (yr)	36.5 ± 4.5	40.2 ± 6.9	37.8 ± 3.6	35.4 ± 4.1	
Smoker (total)	5	28	10	5	
Unemployed (total)	4	14	8	0	
Cotrim (total)	7	21	0	0	
Tuberculostatica (total)	3	2	0	0	
Amphotericin B (total)	8	20	0	0	
CD_4 cells (total)	224.5 ± 51.5†‡	242.5 ± 32.5†‡	645.5 ± 104.5†	890 ± 69.1	610-970 (total)

*$P > .05$, HIV-afflicted patients of groups I (heroin addicts) *v* II (without heroin consumption).

†$P < .05$, HIV-afflicted patients (I and II) or male heroin addicts (III) *v* controls (IV).

‡$P < .05$, HIV-afflicted patients (I and II) *v* male heroin addicts without HIV infection.

Table 2. Characteristics (mean ± SD) of Males Grouped by Heroin Abuse and HIV Infection

Characteristic	Group I (n = 24; HIV, heroin)	Group II (n = 41; HIV, no heroin)	Group III (n = 15; no HIV, heroin)	Control IV (males, n = 20)	Range
Serum Ca^{2+} (mmol/L)	2.34 ± 0.24	2.2 ± 0.09†	2.27 ± 0.105†	2.6 ± 0.23	2.20-2.65 (mmol/L)
Serum D_3 (pg/mL) $1,25(OH)_2D$	30.25 ± 2.5†	30.29 ± 7.28†	34.8 ± 9.6†	67.45 ± 4.25	44-75 (pg/mL)
Serum D_3 (ng/mL) 25OHD	38.6 ± 17.4†	44.6 ± 14.2	39.1 ± 8.9†	69.5 ± 13.5	8-80 (ng/mL)
PTH (pg/mL)	26.57 ± 7.36†	27.97 ± 4.80†	31.55 ± 7.4	37.8 ± 4.8	10-65 (pg/mL)
Osteocalcin (ng/mL)	2.34 ± 0.46†	3.11 ± 0.35†	2.29 ± 0.49†	3.6 ± 0.39	2.0-6.5 (ng/mL)
Calcitonin (ng/L)	7.25 ± 0.51	9.01 ± 0.95	9.57 ± 0.95	8.53 ± 0.65	<10 (pg/mL)
Creatinine (mg/dL)	1.031 ± 0.162	0.985 ± 0.163	1.06 ± 0.107	0.87 ± 0.15	0.68-1.09 (mg/dL)
Albumin (g/L)	49.34 ± 5.6	42.15 ± 5.6	43.61 ± 7.2	46.5 ± 3.7	35.8-50.6 (g/L)
Cross-links (nmol Pyd/mmol Crea)	64.34 ± 20.45†	69.82 ± 9.85†‡	44.8 ± 9.6	31.5 ± 6.2	<50 (nmol Pyd/mmol/Crea)
Urinary Ca^{2+} (mmol/24 h)	2.78 ± 0.35†	3.25 ± 0.52†	2.68 ± 0.95	1.07 ± 0.31	<6.2 (mmol/L)
CD_4 cells (total)	224.5 ± 51.5†‡	242.5 ± 32.5†‡	645.5 ± 104.5†	890 ± 69.1	610-970 (total)
BMC (%)	87.62 ± 3.5†‡	87.97 ± 3.7†‡	98.45 ± 3.9	101.7 ± 2.8	
Testosterone (300-1,000 ng/dL)	445.6 ± 65.3*	667.8 ± 58.4†‡	481.5 ± 79.5	534.4 ± 45.8	(300-1,000 ng/dL)

*$P > .05$, HIV-afflicted patients of group I (heroin addicts) *v* II (without heroin consumption).
†$P < .05$, HIV-afflicted patients (I and II) or male heroin addicts (III) *v* controls (IV).
‡$P < .05$, HIV-afflicted patients (I and II) *v* male heroin addicts without HIV infection (III).

patients in comparison to the control group. Table 1 shows the investigated demographics as the mean ± SD of the respective groups.

In comparison to the control group and the group of noninfected male heroin addicts (group III), male HIV-infected patients showed a significant reduction of BMD in the left forearm ($P < .05$) irrespective of whether they were drug-dependent. There were no significant differences between controls and male heroin addicts of the third group. Serum PTH levels showed a similar decrease in the 3 male groups in comparison to the control group, but this decrease was only statistically nonsignificant in groups I and II.

When pooling the male HIV-infected patients of groups I and II (n = 65), serum PTH levels decreased with the progressive loss of CD4 helper cells (Table 5). The male patients in groups I and II had lower serum osteocalcin concentrations than the respective control group, with statistical significance.

Due to a poor analysis of the PTH measurement, groups I and II were pooled to substantiate a correlation between the loss of CD4 helper cells and the reduced osteocalcin levels, but no linear correlation and no statistical difference were observed.

The excretion of cross-links in the 24-hour urine samples of the various groups is shown in Tables 2 and 4. Both male groups had increased urinary cross-link excretion, which was significant in comparison to the control group ($P < .05$). The cross-link excretion pattern of the pooled male groups I and II showed a significant linear correlation with the CD4 helper cell count.

The mean vitamin $1,25(OH)_2D_3$ concentration was different between both group I and the controls (group IV), but patients with a reduced CD4 helper cell count also had reduced $1,25(OH)_2D_3$ levels (Table 6). This linear correlation was statistically significant in group I (drug-dependent males), while no correlation could be established in the other groups, possibly due to the small number of subjects. To underline the influence of the low-normal $1,25(OH)_2D_3$ concentration on the bone metabolism of the patients, the correlations between cross-link excretion/osteocalcin levels and the CD4 helper cell count were computed after adjustment of the interfering variable,

Table 3. Characteristics (mean ± SD) of the Females Grouped by Heroin Abuse and HIV Infection

Parameter	Group V (n = 20; HIV, heroin)	Group VI (n = 15; HIV, no heroin)	Group VII (n = 15; no HIV, heroin)	Control (females, n = 20)	Range
Weight (kg)	50.3 ± 3.7	52.5 ± 4.7	55.8 ± 12.1	54.2 ± 5.2	
Body mass index (kg/m^2)	27.56 ± 3.7	27.45 ± 3.05	27.7 ± 3.9	26.2 ± 2.2	
Age (yr)	39.4 ± 8.3	32.7 ± 7.8	34.7 ± 5.2	33.2 ± 2.7	
Smoker (total)	4	11	8	6	
Unemployed (total)	5	4	7	0	
Cotrim (total)	7	8	0	0	
Tuberculostatica (total)	0	1	0	0	
CD_4 cells (total)	317.5 ± 32.9†‡	271.5 ± 47.1†‡	603.5 ± 95.5†	815.5 ± 47.1	610-970 (total)
Prolactin (mU/L)	36.4 ± 16.92	39.7 ± 21.9	41.71 ± 18.2	29.7 ± 6.3	(20-50 mIU/L)
FSH (mU/mL)	11.7 ± 6.8	7.80 ± 7.2	14.9 ± 6.3	9.2 ± 6.1	(2.2-19.6)
LH (mU/mL)	9.06 ± 0.6	16.95 ± 0.8	18.6 ± 3.7	14.8 ± 1.1	(2.5-99.5 mIU/mL)
Serum estradiol (pg/mL)	79.2 ± 43.7	83.7 ± 31.5	62.4 ± 26.8	93.7 ± 27.5	(30-300 pg/mL)
Amenorrhea	11	1	5	0	

*$P > .05$, HIV-afflicted patients of group V (heroin addicts) *v* VI (without heroin consumption).
†$P < .05$, HIV-afflicted patients (V and VI) or female heroin addicts (VII) *v* controls (VII).
‡$P < .05$, HIV-afflicted patients (V and VI) *v* female heroin addicts without HIV infection (VII).

Table 4. Characteristics (mean ± SD) of the Females Grouped by Heroin Abuse and HIV Infection

Parameter	Group V (n = 20; HIV, heroin)	Group VI (n = 15; HIV, no heroin)	Group VII (n = 15; no HIV, heroin)	Control (females, n = 20)	Range
Serum Ca^{2+} (mmol/L)	2.63 ± 0.07*‡	2.2 ± 0.07*	2.31 ± 0.075	2.40 ± 0.17	2.20-2.65 (mmol/L)
Serum D_3 (pg/mL) $1,25(OH)_2D$	22.49 ± 7.81†	22.33 ± 3.4†	26.72 ± 7.39†	55.3 ± 7.2	44-75 (pg/mL)
Serum D_3 (ng/mL) 25OHD	34.9 ± 18.4	40.45 ± 18.1	40.3 ± 17.5	62.7 ± 12.8	8-80 (ng/mL)
PTH (pg/mL)	43.05 ± 4.75*	22.3 ± 7.2*†	34.65 ± 5.37	36.45 ± 4.3	10-65 (pg/mL)
Osteocalcin (ng/mL)	3.05 ± 0.49*	2.25 ± 0.478*†	2.39 ± 0.74†	3.2 ± 0.48	2.0-6.5 (ng/mL)
Calcitonin (ng/L)	10.8 ± 2.75	7.97 ± 2.05	10.25 ± 1.15	9.54 ± 1.6	<10 (pg/mL)
Creatinine (mg/dL)	0.86 ± 0.07	0.92 ± 0.03	1.07 ± 0.17	0.89 ± 0.09	0.68-1.09 (mg/dL)
Albumin (g/L)	42.5 ± 5.1	40.26 ± 5.4	39.35 ± 6.2	44.7 ± 7.1	35.8-50.6 (g/L)
Cross-links (nmol Pyd/mmol Crea)	35.41 ± 9.50*†	59.6 ± 11.5*†‡	33.5 ± 14.8	19.7 ± 6.8	<50 (nmol Pyd/mmol/Crea)
Urinary Ca^{2+} (mmol/24 h)	3.09 ± 0.28†‡	2.36 ± 0.31†	2.72 ± 0.34†	1.72 ± 0.35	<6.2 (mmol/L)
BMC (%)	103.2 ± 3.57*	85.67 ± 1.97*†‡	99.2 ± 2.07	100.3 ± 1.8	

*$P > .05$, HIV-afflicted patients of group V (heroin addicts) *v* VI (without heroin consumption).
†$P < .05$, HIV-afflicted patients (V and VI) or female heroin addicts (VII) *v* controls (VII).
‡$P < .05$, HIV-afflicted patients (V and VI) *v* female heroin addicts without HIV infection (VII).

$1,25(OH)_2D_3$, using the method of quadratic correlation of residues as described in the statistical analysis. Significant correlations between the osteocalcin level and the number of CD4 helper cells were found in the 2 male groups, suggesting that the bone formation rate decreases with progression of HIV disease and does not depend on the degree of vitamin D_3 supply. Therefore, we estimated the serum level of $25(OH)D_3$. There was a trend for lower serum levels of $25(OH)D_3$ with the progressive loss of CD4 cells in patients from groups I and II. Moreover, there was no correlation between serum $25(OH)D_3$ and the total CD4 cell count.

No such correlation could be statistically established for the excretion of cross-links in the various groups. Table 7 shows the correlations in the pooled group of patients ($N = 100$) irrespective of sex and drug consumption. It is again apparent that the correlation between a low bone formation rate and the loss of CD4 helper cells also exists without the influence of the interfering variable $1,25(OH)_2D_3$, and the increased urinary excretion of cross-links in patients with a reduced CD4 helper cell count is statistically significant and not dependent on the vitamin D level.

To investigate the influence of malnutrition on the markers of bone metabolism, we used the same mathematical method as for vitamin D to determine the correlations between osteocalcin levels/cross-link excretion and serum albumin. In the pooled patient group ($N = 100$), we found a correlation between osteocalcin, cross-link excretion, and CD4 helper cell count, without the influence of serum albumin as an interfering variable. The demographics of the female patients corresponded in the main to those of the respective control group. In the 3 female groups, similar PTH levels were found in the control group and in groups V and VII, whereas patients in group VI had significantly lower values. However, in the female patients, reduced bone density at the distal radius was only observed in group VI (drug-free). This reduction was statistically significant ($P < .05$) in comparison to the very similar bone densities in the female controls and the group of drug-dependent women from groups V and VII. Individual measurements in the group of female heroin-dependent patients revealed no reduction of bone density (Table 4).

A significant reduction of osteocalcin was found in the female patients of group VI. This reduction was considerably less pronounced in group V, which showed no significance in comparison to the control group but did show significance in comparison to group VI.

Increased urinary cross-link excretion was also observed in groups V and VI, but this excretion was much higher in the women of group VI versus the drug-dependent women of group 5 ($P < .05$).

DISCUSSION

Reduced BMC in the distal part of the left forearm was observed in HIV-infected male patients. Although we studied a considerably larger number of propositi, this observation is in agreement with that of Serrano et al,[21] who examined 22 patients, among them 9 women, by dual-photon absorptiometry and found no significant BMD reduction at the measuring points L2 to L4, neck, and intertrochanteric area of the femur and no correlation between BMD and the severity of the clinical picture. In the same study, however, they observed a marked decrease in bone turnover, reduced formation without changes in mineralization, and a decrease in osteoclast number in

Table 5. Correlations Between the Number of CD4 T-Lymphocytes and Markers of Bone Metabolism in 100 Patients Infected With HIV-1

Parameter	CD4 Group I (n = 24)	CD4 Group II (n = 41)	CD4 Group V (n = 20)	CD4 Group VI (n = 15)	CD4 Total (N = 100)
Osteocalcin (ng/mL)	.30	.53*	.17		−.03, .35*
PTH (pg/mL)	.31	.09	.27	−.10	.28*
$1,25(OH)_2D_3$ (pg/mL)	.40*	.15	.42	.13	.23*
$25(OH)D_3$ (ng/mL)	.29	.13	.30	.19	.11
Ca^{2+} (mmol/L)	−.02	−.23	.17	−.41	.14
Cross-links (mol Pyd/mmol Crea)	−.40*	−.40	−.77*	−.10	−.47*
BMC (%)	−.24	.02	.80*	−.24	.10

*$P < .05$.

Table 6. Partial Correlation of the Linear Regression Between the Number of CD4 T-Lymphocytes and Osteocalcin in 100 Patients With AIDS

Parameter	Osteocalcin (ng/mL)				
	Group I (n = 24)	Group II (n = 41)	Group V (n = 20)	Group VI (n = 15)	Total (N = 100)
CD4 (total) 1,25$(OH)_2D_3$ settled	.33*	.54*	−.001	−.08	.37*
CD4 (total) albumin settled	.24	.57*	.32	−.03	.34*

*$P < .05$.

transiliac bone biopsies from patients with a low CD4 helper cell count. The alteration of the histomorphometric parameters of formation and turnover were altered more in patients who presented with a greater disease severity according to the Centers for Disease Control classification and the number of CD4 helper cells.[21]

We likewise studied a marker of bone formation, namely osteocalcin, and found reduced levels in all groups, but this reduction was significant only in the group of drug-independent women. This finding has already been described in earlier reports.[8-10] A positive correlation between CD4 helper cells and osteocalcin as stated by Serrano et al[21] was only observed in our group of drug-dependent males and in the pooled male group. This correlation was independent of the existing low-normal or reduced vitamin D_3 levels and the reduced albumin values and is the expression of severe nutritional protein deficiency. The loss of CD4 helper cells is associated with reduced bone formation and offers itself as an important marker for the interpretation of the immune response. It has been shown that interleukin levels increase with the degree of the immune response.[16,18] These cytokine alterations are apparently so grave that acquired immune deficiency syndrome (AIDS) has been postulated to be a ''disease of the cytokines.''[17] Several studies have shown elevated tumor necrosis factor alpha (TNF-α) levels in the serum of HIV-infected subjects and AIDS patients. Normal TNF levels were found in asymptomatic patients and also in patients in the stage of lymphadenopathy, whereas all AIDS patients had elevated TNF-α levels.[17] Bertolini et al[3] have demonstrated that TNF causes bone resorption in fetal rat bone and stimulates the activity of osteoclasts in cultured bone. Our observation of increased urinary cross-link excretion as an expression of increased bone resorption in patients with advanced CD4 helper cell loss can be easily related to these experimental findings. Unfortunately, we had only incomplete data on the serum TNF-α levels of our propositi and were therefore unable to determine the statistical significance of these values. However, Serrano et al[21] reported reductions in osteoclast activity. Therefore, our finding of increased urinary excretion of cross-links as an expression of an elevated bone resorption rate does not conform with this result. Increased excretion of pyridinium cross-links has been demonstrated in osteoporosis due to inflammatory processes associated with osteoporosis and to corticosteroids.[2,25] It is important to consider the contribution of immobilization to the process of bone loss.[26] For this reason, we only recruited outpatients for this study.

Reduced or low-normal vitamin D_3 (1,25$(OH)_2D_3$) was found in all of our propositi. These concentrations were independent of drug dependence in the male and female groups. Haug et al[12] found reduced 1,25$(OH)_2D_3$ and slightly reduced 25$(OH)D_3$ levels in HIV-infected patients with symptomatic AIDS. In comparison to a group of asymptomatic HIV patients, this reduction was statistically significant. This finding is in line with our observation in the pooled group ($N = 100$) that lower vitamin 1,25D levels are associated with the progressive loss of CD4 helper cells. Serum levels of 1,25$(OH)_2D_3$ correlated even more closely with survival than the CD4 cell counts used to predict survival and progression to AIDS in HIV-infected patients.[8] Low vitamin 1,25D levels are responsible, in part, for an increased bone turnover rate.

Despite low vitamin D, the serum PTH values of HIV patients are in the low-normal range. This has been demonstrated by several groups[15,19] and was also observed in our 2 male groups. However, to our knowledge, no data on both the PTH values and markers of bone metabolism in HIV-infected females are available so far. Surprisingly, our female habitual drug users had PTH values in the upper-normal range, whereas Pedrazzoni et al[19] have reported reduced PTH values in chronic opioid abusers. In addition to this special feature, the females of our group V were characterized by a higher BMD and a distinctly milder bone turnover. This finding could not be explained from the clinical examinations or the supplementary analysis of markers of the thyroid axis, the gonadal axis, and protein metabolism, and it remains unclear. However, previous reports on calcium-regulatory hormones in opioid abusers were virtually limited to the finding of higher levels of serum calcitonin[23,25] and are in accordance with our results. The percentage of amenorrheic women is higher in the group of drug users than in group V. In women with systemic lupus erythematosus, which is associated with alterations of the CD4/CD8 lymphocyte system, amenorrhea is one of the most likely contributing factors in osteoporosis.[7] However, there is no evidence for a reduced BMC in our patients of group V. In addition to endocrine aspects, metabolic problems such as increased resting energy expenditure[10,11] and a reduced rate of protein synthesis[24] must be included in the discussion. Apart from the liver-dependent transfer protein transferrin (which was not determined in this study), serum albumin is suitable for

Table 7. Partial Correlation of the Linear Regression Between the Number of CD4 T-Lymphocytes and Cross-Links in 100 Patients With AIDS

Parameter	Cross-Links (mmol Pyd/mmol Crea)				
	Group I (n = 24)	Group II (n = 41)	Group V (n = 20)	Group VI (n = 15)	Total (N = 100)
CD4 (total) 1,25$(OH)_2D_3$ settled	−.28*	−.36*	−.85*	−.02	−.42*
CD4 (total) Albumin settled	−.33	−.14	−.66*	−.06	−.43*

*$P < .05$.

estimating the absolute visceral protein mass and is an indirect measure for liver function and liver mass, as well as substrate availability. However, knowing the close association between protein metabolism, osteocalcin, and other markers of bone metabolism, we were able to prove that the correlations between CD4 helper cell loss, osteocalcin, and urinary excretion of cross-links are statistically significant and independent of the respective albumin concentrations.

In summary, HIV-infected patients show alterations of the calciotropic hormones and bone metabolism in the sense of an increased bone resorption rate. Alterations of bone formation are also demonstrable, but these are less pronounced, statistically not significant, not present in all groups, and not dependent on disturbances of protein metabolism and the insufficient supply of vitamin D. The habitual long-term use of heroin does not seem to be of additional relevance for the bone metabolism of male HIV-positive patients. Bone turnover in female patients is diminished, and bone resorption and formation markers correlate with the progressive loss of CD4 helper cells. However, recent findings suggesting a resynchronization of bone formation and resorption during the highly active antiretroviral therapy in those patients, and support the idea of an interaction between cytokines and bone in the bone remodeling process.[1]

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