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# Vitamin D Deficiency among HIV Type 1-Infected Individuals in the Netherlands: Effects of Antiretroviral Therapy

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## Abstract

Vitamin D regulates bone metabolism but has also immunoregulatory properties. In HIV-infected patients bone disorders are increasingly observed. Furthermore, low 1,25(OH)<sub>2</sub>D<sub>3</sub> levels have been associated with low CD4<sup>+</sup> counts, immunological hyperactivity, and AIDS progression rates. Few studies have examined the vitamin D status in HIV-infected patients. This study will specifically focus on the effects of antiretroviral agents on vitamin D status. Furthermore, the effect of vitamin D status on CD4 cell recovery after initiation of HAART will be evaluated. Among 252 included patients the prevalence of vitamin D deficiency (<35 nmol/liter from April to September and <25 nmol/liter from October to March) was 29%. Female sex, younger age, dark skin, and NNRTI treatment were significant risk factors in univariate analysis, although in multivariate analyses skin pigmentation remained the only independent risk factor. Median 25(OH)D<sub>3</sub> levels were significantly lower in white NNRTI-treated patients [54.5(27.9–73.8) nmol/liter] compared to white PI-treated patients [77.3(46.6–100.0) nmol/liter, *p* = 0.007], while among nonwhites no difference was observed. Both PI- and NNRTI-treated patients had significantly higher blood PTH levels than patients without treatment. Moreover, NNRTI treatment puts patients at risk of elevated PTH levels (>6.5 pmol/liter). Linear regression analysis showed that vitamin D status did not affect CD4 cell recovery after initiation of HAART. In conclusion, 29% of the HIV-1-infected patients had vitamin D deficiency, with skin color as an independent risk factor. NNRTI treatment may add more risk for vitamin D deficiency. Both PI- and NNRTI-treated patients showed higher PTH levels and might therefore be at risk of bone problems. Evaluation of 25(OH)D<sub>3</sub> and PTH levels, especially in NNRTI-treated and dark skinned HIV-1-infected patients, is necessary to detect and treat vitamin D deficiency early.

## Introduction

VITAMIN D IS REQUIRED for bone health and calcium homeostasis and has important immunoregulatory properties. In HIV-infected patients bone disorders, like osteopenia and osteoporosis, are increasingly observed.<sup>1,2</sup> Furthermore, low 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> [1,25(OH)<sub>2</sub>D<sub>3</sub>] levels and polymorphisms in vitamin D receptor have been associated with low CD4<sup>+</sup> counts, immunological hyperactivity, and AIDS progression rates.<sup>3–6</sup> However, only a few studies have examined the vitamin D status in HIV-infected patients (as recently reviewed<sup>7</sup>).

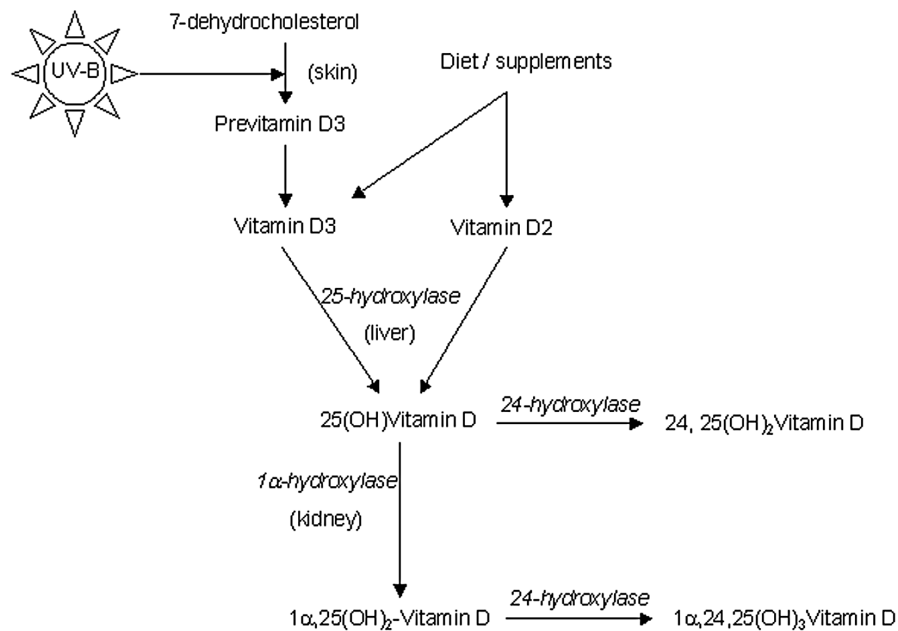
Plasma concentrations of 25-hydroxyvitamin D<sub>3</sub> [25(OH)D<sub>3</sub>] are the best indicator of vitamin D status.<sup>8</sup> Vit-

amin D is obtained from the diet and produced in the skin under the influence of sunlight. All reactions in vitamin D metabolism are catalyzed by enzymes from the cytochrome P450 (CYP450) enzyme system (Fig. 1). Previtamin D<sub>3</sub> is 25-hydroxylated in the liver to 25(OH)D<sub>3</sub> and then 1 $\alpha$ -hydroxylated in the kidney to 1,25(OH)<sub>2</sub>D<sub>3</sub>, the active metabolite.<sup>9</sup> Inactivation of vitamin D metabolites occurs mainly by renal 24-hydroxylation, which is stimulated by 1,25(OH)<sub>2</sub>D<sub>3</sub> and high calcium.

Vitamin D deficiency can be caused by several factors. The synthesis of previtamin D<sub>3</sub> in the skin decreases with age and skin pigmentation.<sup>8,10</sup> Also low sunlight exposure, obesity, and insufficient vitamin D intake are known risk factors for vitamin D deficiency.<sup>11,12</sup> In HIV-infected patients,

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**FIG. 1.** Vitamin D metabolism. 7-Dehydrocholesterol is, under influence of sunlight, in the skin converted to previtamin D<sub>3</sub>, which is transported to the liver by vitamin D-binding protein, where it is 25-hydroxylated (CYP27A1) to 25(OH)D<sub>3</sub>. 25(OH)D<sub>3</sub> is then 1 $\alpha$ -hydroxylated (CYP27B1) in the kidney to 1,25(OH)<sub>2</sub>D<sub>3</sub>, the active metabolite.<sup>9</sup> Inactivation of vitamin D metabolites occurs mainly by renal 24-hydroxylation, which is stimulated by 1,25(OH)<sub>2</sub>D<sub>3</sub> and high calcium.

HIV itself might lead to low 1,25(OH)<sub>2</sub>D<sub>3</sub> levels by inhibition of renal 1 $\alpha$ -hydroxylase, possibly induced by an inhibitory effect of tumor necrosis factor (TNF)- $\alpha$ .<sup>5</sup> Alternatively, 1,25(OH)<sub>2</sub>D<sub>3</sub> might be low due to utilization for maturation and proliferation of T lymphocytes during HIV infection.<sup>7</sup> In addition, highly active antiretroviral therapy (HAART) might interact with vitamin D metabolism: protease inhibitors (PIs) and nonnucleoside reverse transcriptase inhibitors (NNRTIs) can, respectively, inhibit or induce CYP450 enzymes. *In vitro*, PIs showed a strong inhibition of 1 $\alpha$ - and 25-hydroxylase activities, while showing mild inhibition of 24-hydroxylase,<sup>13</sup> which may *in vivo* result in low 1,25(OH)<sub>2</sub>D<sub>3</sub> levels. Also other drugs such as antiepileptic agents and tuberculostatics can lead to reduced vitamin D levels and subsequent low bone mineral density by either inducing or inhibiting CYP450.<sup>14–16</sup>

In this study, we examined the prevalence and causes of vitamin D deficiency among HIV-1-infected patients in the Netherlands. We examined, in particular, whether and which antiretroviral agents interfere with vitamin D metabolism. Additionally, the effect of vitamin D status on CD4<sup>+</sup> T cell recovery after initiation of HAART has been investigated.

## Materials and Methods

### Study subjects

From January 2006 to August 2006, a cross-sectional survey on vitamin D deficiency was done among 254 adult HIV1-infected patients visiting the Radboud University Nijmegen Medical Centre, The Netherlands. We excluded subjects with pregnancy, renal disorders (serum creatinine >220  $\mu$ mol/liter), and hepatic disorders (ASAT >200 or ALAT

>225 IU/liter). Two hundred and fifty-two patients (99.2%) could be included.

The study received approval by the local ethics committee and all subjects provided written informed consent.

### Study design

During their routine visit the vitamin D status of the patients was screened. Blood samples were collected for measurement of 25(OH)D<sub>3</sub>, intact parathyroid hormone (PTH), serum calcium, phosphate, creatinine, albumin, alkaline phosphatase, ASAT and ALAT, CD4 and CD8 cell counts, and viral load (VL). Patients' age, sex, weight, length, skin color/ethnicity, duration and stage of HIV infection [according to 1993 Centers for Disease Control (CDC) criteria<sup>17</sup>], duration and kind of antiretroviral therapy, and medications were collected from patients' medical history or the database of HIV monitoring foundation. BMI was calculated as weight in kg/length<sup>2</sup> (in meters) for each subject.

### Laboratory measurements

25(OH)D<sub>3</sub> was measured by high-pressure liquid chromatography (HPLC) with UV detection, after prior extraction on small SepPak columns. Tritiated 25(OH)D<sub>3</sub>, collected from the HPLC system during passage of the UV peak, was used to correct for procedural losses.

Serum intact PTH in the first 33 patients was measured using the Advantage PTH-N kit (Nichols, San Juan Capistrano, CA) and in the last 219 patients using the ELSA-PTH kit (CIS Bio International, Gif-sur-Yvette, France). The CIS BIO assay was recalibrated on the Advantage assay to give identical measurement results.

Serum calcium, phosphate, creatinine, albumin, alkaline phosphatase, ASAT and ALAT, cholesterol, triglycerides, and glucose were determined by automated standard laboratory techniques. Corrected calcium was calculated as follows:  $(\text{Calcium}) - [0.025 \times (\text{albumin})] + 1$ .

CD4 and CD8 cell counts were determined by standard flow cytometry (FACS Count System, Becton Dickinson). Viral load was measured with the Cobas Amplicor HIV-1 monitor test, with a minimal detection threshold before January 2000 of <400 HIV-1 RNA copies/ml and after January 2000 of <40 HIV-1 RNA copies/ml.

### Questionnaire

For further evaluation of risk factors associated with vitamin D deficiency an invalidated questionnaire on dietary vitamin D intake, intake of vitamin D supplements, and average sunlight exposure (hours/day) was taken by face-to-face interview. Two hundred and one patients (79.8%) participated in this questionnaire. Data on dietary questions were processed in a special developed spreadsheet (Microsoft Excel 2000) in order to calculate the daily vitamin D intake. The mean dietary vitamin D intake per patient was calculated by using the Dutch Food table 2004.<sup>18</sup> The recommended daily intake (RDI) of vitamin D is >2.5  $\mu\text{g}$  for people aged 18–50 years, >7.5  $\mu\text{g}$  for people 51–70 years, and >12.5  $\mu\text{g}$  for people >70 years.<sup>18</sup>

### Definition of vitamin D deficiency and elevated PTH levels

Vitamin D deficiency was defined as circulating plasma 25(OH)D<sub>3</sub> levels <35 nmol/liter in April–September or <25 nmol/liter in October–March. These levels are corrected for season, because sunlight radiation differs between summer and winter and can thereby directly influence the levels of vitamin D.

Elevated PTH levels were defined as serum intact PTH >6.5 pmol/liter.

### Effects of vitamin D deficiency on CD4 cell recovery

For this part only patients receiving HAART for >24 weeks were included. Patients were excluded when pregnant during initiation of HAART. As HIV-1 can induce a decline in CD4 cells only patients with undetectable HIV-1 viral loads, measured 24 weeks after the initiation of HAART, were included. Also patients that had fewer than three CD4 measurements available for regression analysis were excluded. One hundred and five patients could be included. CD4 cell counts and VL of included patients were retrospectively collected from the database of the HIV Monitoring Foundation starting 8 weeks after initiation of HAART for as long as the viral load remained undetectable. The first 8 weeks of CD4 cell recovery were excluded, because then CD4 cells increased rapidly due to a release of memory CD4 cells trapped in the lymphoid tissue.<sup>19</sup>

### Statistical analysis

Continuous variables are expressed as median (Q1–Q3) and categorical variables as number of cases (percentage). Continuous variables were compared by the Mann–Whitney *U* test or Kruskal–Wallis test when appropriate. Frequencies were compared by the  $\chi^2$  test or Fishers' exact test when ap-

propriate. Correlation between 25(OH)D<sub>3</sub> levels and PTH and CD4 cell count and low calcium and elevated PTH was tested using Spearman correlation.

Logistic regression analysis was used to identify significant risk factors for vitamin D deficiency. The following variables were entered: age, gender, BMI, skin color (white, Mediterranean, Asian, or black), type of HAART (PI based, NNRTI based, PI + NNRTI based, triple NRTI based, or no HAART), vitamin D intake, and sunlight exposure.

For CD4 cell analysis, a linear regression line of the CD4 cell response to HAART was calculated for each individual patient. The obtained slopes of each line represented the second phase CD4 cell recovery rate of each patient. Because CD4 cell recovery will probably stop around normal physiological levels, with a lower limit of 500, the CD4 cell recovery rates for each patient were calculated until a recovery of 500 and 600 cells was reached. Within these two groups, median CD4 cell recovery rates were compared between the vitamin D-deficient group and patients with normal vitamin D levels using the Mann–Whitney *U* test. Also, baseline CD4 cell values can influence the CD4 cell recovery rate; therefore the patients were stratified according to their baseline CD4 cell count in 0–200 and >200 CD4 cells and the former comparison was reapplied.

All analyses were performed using SPSS statistical software package release 14.0.2 (SPSS Software, Chicago, IL). A *p*-value  $\leq 0.05$  was considered statistically significant.

## Results

### Subject sample and characteristics

Overall, vitamin D status was determined for 254 patients. Two patients were excluded because of pregnancy and HIV-2 coinfection, respectively. Subsequently 252 patients could be included. The population characteristics are summarized in Table 1.

### Prevalence and risk factors of vitamin D deficiency

Data for patients according to presence or absence of vitamin D deficiency are shown in Table 1. The prevalence of vitamin D deficiency was 29.0% (73/252). Females had a significantly higher prevalence of vitamin D deficiency (58.1%) compared to males (24.7%) (*p* = 0.010). The median (Q1–Q3) 25(OH)D<sub>3</sub> levels were significantly lower in females, 32.25 (22.55–51.58) nmol/liter, compared to males, 56 (30–78.5) nmol/liter (*p* < 0.001). Postmenopausal females are at risk of developing vitamin D deficiency and therefore we compared the age of female patients with or without vitamin D deficiency. The median age of females with vitamin D deficiency was 36.5 (31.5–41.25) years and of females without vitamin D deficiency was 35.5 (29.5–43.75) years; this difference was not statistically significant.

The prevalence of vitamin D deficiency was 19% in white, 33% in Mediterranean, 44% in Asian, and 62% in black patients. Black patients were at a significantly higher risk of developing vitamin D deficiency compared to the other groups (OR 6.22, 95% 3.20–12.08). Median (Q1–Q3) serum levels of 25(OH)D<sub>3</sub> in white, Mediterranean, Asian, and black patients were 59.50 (36.20–82.75), 57.00 (22.75–64.00), 32.00 (21.50–57.50), and 27.10 (16.75–36.95) nmol/liter, respectively; the differences were statistically significant between white and

TABLE 1. PATIENT CHARACTERISTICS OF HIV-1 SEROPOSITIVE PATIENTS: OVERALL AND WITH AND WITHOUT VITAMIN D DEFICIENCY<sup>a</sup>

Variables	Overall (N = 252)	Vitamin D deficient (N = 73, 29%)	Normal vitamin D (N = 179, 71%)	p value <sup>b</sup>
<b>Clinical data</b>				
Male sex <sup>c</sup>	190 (75.4)	47 (64.4)	143 (79.9)	<0.001
Female sex <sup>c</sup>	62 (24.6)	26 (35.6)	36 (20.1)	<0.001
Age (years) <sup>d</sup>	41.00 (35–50)	38.0 (32.0–46.5)	43.0 (37.0–52.0)	0.005
BMI (kg/m <sup>2</sup> ) <sup>c</sup>	23.57 (21.6–25.7)	23.39 (21.51–25.60)	23.73 (21.64–26.02)	0.740
CD4 cell count (cells/ml) <sup>d</sup>	420 (290–600)	420 (310–560)	420 (280–625)	0.931
CD8 cell count (cells/ml) <sup>d</sup>	780 (580–1150)	670 (515–950)	860 (590–1205)	0.009
CD4/CD8 ratio <sup>d</sup>	0.54 (0.30–0.82)	0.62 (0.38–0.90)	0.51 (0.28–0.76)	0.055
VL (copies/ml) <sup>d</sup>	40 (40–400)	40 (40–500)	40 (40–400)	0.790
		285 (136–518)	275 (116–516)	0.781
<b>HIV stage (1993 CDC criteria)</b>				
A <sup>c</sup>	126 (50)	39 (53.4)	87 (48.6)	0.786
B <sup>c</sup>	52 (20.6)	14 (19.2)	38 (21.2)	<0.001
C <sup>c</sup>	74 (29.4)	20 (27.4)	54 (30.2)	<0.001
<b>Immune stage (1993 CDC criteria)</b>				
CD4 > 500 cells/mm <sup>3c</sup>	95 (37.7)	30 (41.1)	65 (36.5)	0.682
CD4 200–499 cells/mm <sup>3c</sup>	131 (52.0)	37 (50.7)	93 (52.2)	<0.001
CD4 < 200 cells/mm <sup>3c</sup>	26 (10.3)	6 (8.2)	20 (11.2)	<0.001
<b>Skin color</b>				
White <sup>c</sup>	184 (73)	35 (47.9)	149 (83.2)	<0.001
Mediterranean <sup>c</sup>	9 (3.6)	3 (4.1)	6 (3.4)	0.721
Asian <sup>c</sup>	9 (3.6)	4 (5.5)	5 (2.8)	0.287
Black <sup>c</sup>	50 (19.8)	31 (42.5)	19 (10.6)	<0.001
<b>Antiretroviral treatment</b>				
No treatment <sup>c</sup>	53 (21)	13 (17.8)	40 (22.3)	0.497
PI-based regimen <sup>c</sup>	61 (24.2)	14 (19.2)	47 (26.3)	0.260
NNRTI-based regimen <sup>c</sup>	104 (41.3)	38 (52.1)	66 (36.9)	0.034
PI- and NNRTI-based regimen <sup>c</sup>	11 (4.4)	2 (2.7)	9 (5.0)	0.518
Triple NRTI-based regimen <sup>c</sup>	23 (9.1)	6 (8.2)	17 (9.5)	1.000
HAART duration (weeks) <sup>d</sup>	238 (108–363)	264 (134–380)	213 (102–355)	0.236
<b>Metabolic parameters</b>				
25-hydroxyvitamin D (nmol/liter) <sup>d</sup>	49.00 (27.45–74.83)	20.4 (16–26.15)	62.00 (46.8–84.5)	<0.001
Parathyroid hormone (pmol/liter) <sup>d</sup>	4.1 (2.8–5.3)	5.1 (3.89–7.61)	3.57 (2.60–4.79)	<0.001
Calcium (mmol/liter) <sup>d</sup>	2.34 (2.27–2.39)	2.30 (2.23–2.36)	2.36 (2.30–2.41)	<0.001
Corrected calcium (mmol/liter) <sup>d</sup>	2.32 (2.26–2.38)	2.29 (2.25–2.36)	2.33 (2.27–2.40)	0.022
Phosphate (mmol/liter) <sup>d</sup>	0.93 (0.82–1.08)	0.95 (0.81–1.08)	0.92 (0.83–1.09)	0.760
Albumin (g/liter) <sup>d</sup>	41 (38–43)	40 (38–42)	41.5 (39–44)	0.017

<sup>a</sup>BMI, body mass index; PI, protease inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; HAART, highly active antiretroviral therapy.

<sup>b</sup>p-value of comparison of prevalence or values between vitamin D-deficient patients and patients with normal vitamin D with Chi-square test or Mann-Whitney *U* test, respectively.

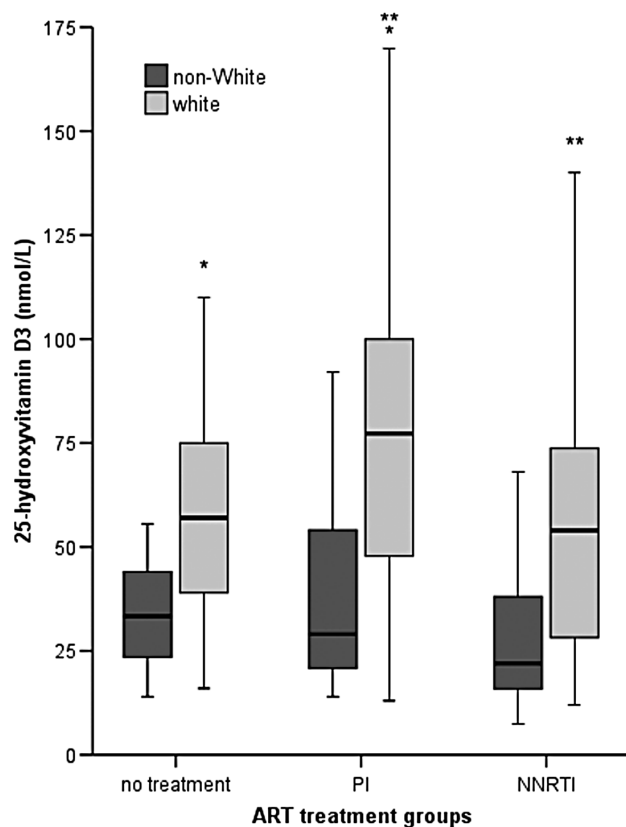
<sup>c</sup>Univariate analysis: data presented as *N*, %.

<sup>d</sup>Univariate analysis: data presented as median (Q1–Q3).

black ( $p < 0.001$ ) and between Mediterranean and black patients ( $p = 0.026$ ). Nonwhite patients were significantly younger [median(Q1–Q3): 34 (30–39.75) years] than whites [45(39–53) years], which may explain the significantly younger age of the vitamin D-deficient patients.

The prevalence of vitamin D deficiency was 24.5% in patients without treatment and 30% in patients receiving HAART: 23.0% in patients who received PI-based HAART and 36.5% in patients on NNRTI-based HAART. Prevalence tended to be higher among NNRTI-treated patients compared to PI-treated patients ( $p = 0.070$ ). Usage of NNRTIs

was of significant influence on vitamin D deficiency (OR 1.86, 95% CI 1.07–3.22). Median (IQR) 25(OH)D<sub>3</sub> levels did not differ between patients with and without treatment [48.00 (27.00–76.00) vs. 50.3 (34.6–72.2), respectively,  $p = 0.750$ ]. Median (IQR) 25(OH)D<sub>3</sub> levels were significantly lower for NNRTI-treated patients [42.5 (22.25–67.80) nmol/liter] compared to PI-treated patients [60.00 (29.00–89.20) nmol/liter,  $p = 0.017$ ]. In Fig. 2 plasma 25(OH)D<sub>3</sub> levels according to the therapy groups stratified for white and nonwhite skin color are shown. Within the white population the median 25(OH)D<sub>3</sub> levels of PI-treated patients [77.30 (46.85–100.00)



**FIG. 2.** Plasma 25-hydroxyvitamin D<sub>3</sub> levels for white and nonwhite patients in the different therapy groups. Box plots show the median (horizontal line in the center of each box), the 25th and 75th percentiles (bottom and top of each box), and the 10th and 90th percentiles (bottom and top of error bars). Plasma 25(OH)D<sub>3</sub> levels differed significantly only within the white group for PI-treated patients compared to NNRTI-treated patients (\* $p = 0.007$ ) and patients that received no treatment (\*\* $p = 0.049$ ).

nmol/liter] were significantly higher compared to NNRTI-treated patients [54.50 (27.90–73.78) nmol/liter;  $p = 0.007$ ] or no treatment groups [57.00 (68.00–78.35) nmol/liter;  $p = 0.049$ ]. Interestingly, no significant differences in 25(OH)D<sub>3</sub> levels were found among nonwhite patients treated with PI, NNRTI, or without treatment [29.00 (20.40–57), 22.00 (14.70–38.40), and 33.3 (23.25–44.53) nmol/liter, respectively].

In addition to female gender, younger age, lower CD8 cell count, black skin color, and NNRTI treatment, higher PTH, lower calcium, and lower albumin levels were also significantly associated with vitamin D deficiency in univariate analysis (Table 1).

Results of the questionnaire are shown in Table 2. There was no significant difference in either dietary vitamin D intake or sunlight exposure between the two groups. Overall, 25.9% of the patients (52/201) used nonprescribed vitamin D supplements, for example, multivitamins. Usage of a vitamin D supplement was not significantly different between vitamin D-deficient and -nondeficient patients ( $p = 0.532$ ). When the total intake of vitamin D from food and supplements is considered, 55 patients (27.3%) consumed less than the RDI. When patients consuming vitamin D supplements were excluded, 54 patients (36.2%) consumed less than the RDI. Again, no significant difference was found in percentage of subjects consuming less than the RDI between patients with and without vitamin D deficiency [34.1% ( $n = 14$ ) and 37.0% ( $n = 40$ ), respectively;  $p = 0.743$ ].

Multiple logistic regression analysis was used to identify significant risk factors for vitamin D deficiency in HIV-1-infected persons. Because the food questionnaire was done in only 201 patients, the multivariate analysis could be conducted only for that population. Only skin color remained a significant risk factor for vitamin D deficiency (AOR = 5.4, 95% CI 2.3–12.2,  $p < 0.001$ ). After stratification of the population into white and nonwhite groups no independent risk factor for vitamin D deficiency remained.

**TABLE 2.** QUESTIONNAIRE RESULTS OF HIV-1-INFECTED PATIENTS STRATIFIED FOR VITAMIN D DEFICIENCY<sup>a</sup>

	Vitamin D deficient (N = 53, 26%)	Normal vitamin D (N = 148, 74%)	p value <sup>b</sup>
Supplement			
Use of Vit D supplement <sup>c</sup>	12 (22.6)	40 (27.0)	0.532
Vit D supplement intake ( $\mu\text{g}/\text{day}$ ) <sup>d</sup>	5.00 (5.00–5.00)	5.00 (5.00–5.00)	0.311
Diet			
Dietary Vit D intake ( $\mu\text{g}/\text{day}$ ) <sup>d</sup>	4.3 (2.7–5.5)	4.5 (3.1–6.3)	0.143
Total Vit D intake ( $\mu\text{g}/\text{day}$ ) <sup>d</sup>	4.64 (2.83–7.30)	5.57 (3.7–7.98)	0.131
Total Vit D intake < RDI <sup>c</sup>	14 (26.4)	41 (27.7)	0.857
Total Vit D intake > RDI <sup>c</sup>	39 (73.6)	107 (72.3)	
Sunlight			
Sunlight exposure (min/day) <sup>d</sup>	60 (30–150)	90 (60–180)	0.168

<sup>a</sup>Vit D, vitamin D; RDI, recommended daily intake.

<sup>b</sup> $p$ -value of comparison of prevalence or values with Chi-square test or Mann-Whitney  $U$  test, respectively.

<sup>c</sup>N, %.

<sup>d</sup>Median (Q1–Q3).

### Prevalence of elevated PTH levels and effect of HAART

For two patients (with normal vitamin D) the PTH levels were missing. Overall, 42 patients (16.8%) had elevated PTH levels. Among the patients with vitamin D deficiency, 27 patients (37%) had elevated PTH levels. No patients (0/13, 0.0%) without treatment, five patients (5/14, 35.7%) receiving PI-based HAART, and 19 patients (19/38, 50%) on NNRTI-based HAART developed elevated PTH levels in addition to vitamin D deficiency. Patients treated with NNRTIs had a significantly higher risk of developing elevated PTH levels in addition to vitamin D deficiency (OR 3.93, 95% CI 1.65–9.37).

Among the patients with normal vitamin D levels, 15 (15/177, 8.5%) patients developed elevated PTH levels; one patient (1/39, 2.6%) had no treatment, four patients (4/47, 8.5%) received PI-based HAART, and nine patients (9/65, 13.8%) received NNRTI-based HAART. There was no significant difference in prevalence of elevated PTH levels among these treatment groups ( $p = 0.212$ ). In Fig. 3, the PTH levels of patients without and with PI or NNRTI treatment according to 25(OH)D<sub>3</sub> levels are shown. The upper left quadrant, with low vitamin D<sub>3</sub> and high PTH blood levels, shows an overrepresentation of NNRTI- and PI-treated patients.

There was a significant inverse correlation between serum PTH and 25(OH)D<sub>3</sub> levels ( $r = -0.394$ ,  $p < 0.001$ ). PTH levels of patients on PI treatment [4.27 (3.26–5.46) pmol/liter] or NNRTI treatment [4.61 (3.30–6.89) pmol/liter] were significantly higher than patients without treatment [2.98 (2.15–3.68) pmol/liter;  $p < 0.001$  and  $< 0.001$ , respectively]. The PTH levels of patients on NNRTIs were nonsignificantly higher than that of patients on PI treatment ( $p = 0.098$ ).

There is a significant relationship between low calcium levels and elevated PTH ( $r = 0.153$ ,  $p = 0.031$ ).

### Effects of vitamin D deficiency on CD4 cell recovery

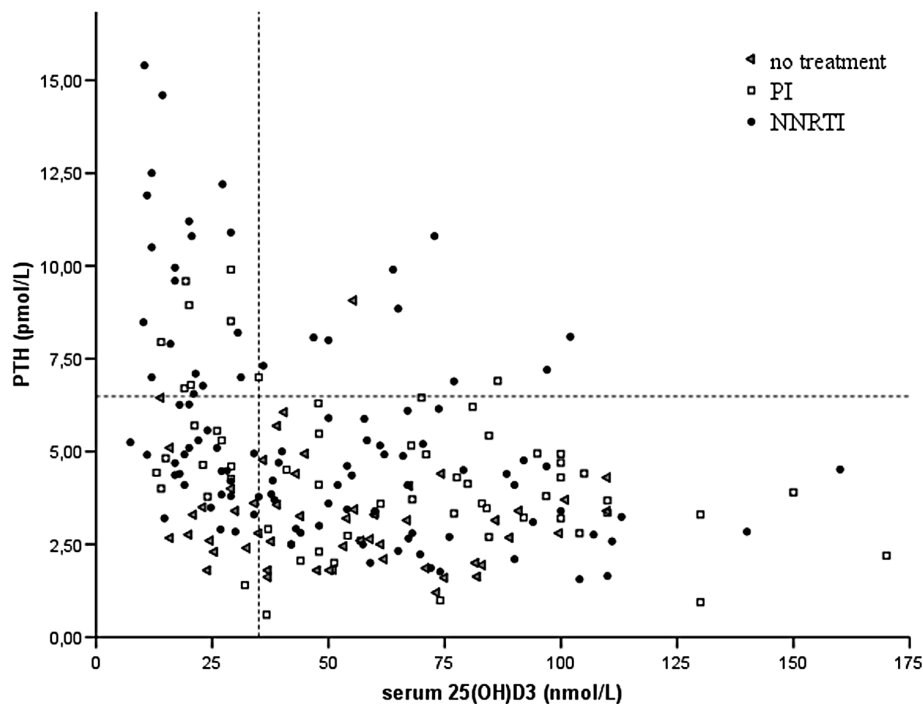
One hundred and five patients could be included in regression analysis with CD4 cell counts up to 500 cells, 31 patients (29.5%) had vitamin D deficiency, and 74 patients (70.5%) had normal vitamin D levels. There was no significant difference in median CD4 cell recovery rates between patients with and without vitamin D deficiency [median (IQR) 1.17 (0.69–2.91) and 1.10 (0.29–2.25), respectively;  $p = 0.177$ ]. Also with a cutoff of 600 CD4 cells/ml no statistical differences in CD4 cell recovery rates were found.

In addition, when patients were stratified according to their baseline CD4 cell count, no statistically significant differences in CD4 cell recovery rate were found (data not shown). Overall, there was no significant correlation between CD4 cell counts and 25(OH)D<sub>3</sub> levels ( $r = 0.059$ ,  $p = 0.351$ ).

### Discussion

We found a prevalence of 29% vitamin D deficiency among HIV1-infected patients. In univariate analysis, female sex, dark skin color, younger age, and NNRTI treatment were found to be significant contributors. Skin color remained the only independent risk factor for vitamin D deficiency in multivariate analysis.

The 25(OH)D<sub>3</sub> levels were significantly lower among NNRTI-treated patients as compared to PI-treated patients. When the group was stratified into whites and nonwhites, the 25(OH)D<sub>3</sub> levels remained significantly lower for white



**FIG. 3.** PTH levels of patients with NNRTI-based, PI-based, or no antiretroviral treatment according to serum 25(OH)D<sub>3</sub> levels (nmol/liter). Reference lines show low vitamin D levels (<35 nmol/liter) and elevated PTH levels (>6.5 pmol/liter). In the upper left corner patients with elevated PTH levels and vitamin D deficiency can be found.

NNRTI-treated patients compared to white PI-treated patients, while among the nonwhites no significant differences were found. These results suggest that NNRTI treatment may have increased the risk for vitamin D deficiency. Interestingly, NNRTI- as well as PI-treated patients had higher levels of PTH compared to patients without treatment. Moreover, NNRTI treatment puts patients at risk for elevated PTH levels. No effect of vitamin D status on CD4 recovery rate after initiation of HAART was found.

Vitamin D deficiency among HIV-infected patients has previously been reported in only two studies.<sup>20,21</sup> Kuehn *et al.* found a prevalence of 50% vitamin D deficiency (<50 nmol/liter) among 21 HIV-infected patients with advanced AIDS and hypocalcemia.<sup>21</sup> Coodly *et al.* found 17% vitamin D deficiency [average 25(OH)D-2SD] among adult HIV-infected patients.<sup>20</sup> These results cannot easily be compared with our study as the study populations are small, the patients also had hypocalcaemia, and different definitions of 25(OH)D deficiency were used. Whether the incidence of vitamin D deficiency in our study population is high can, however, not be firmly concluded, as we lack an HIV-negative control group. Also in the literature there are no other reports that compared vitamin D status between HIV-positive and HIV-negative controls. One study by Teichmann *et al.* reported that 25(OH)D levels were significantly lower in HIV-positive compared to HIV-negative individuals.<sup>6</sup>

In univariate analysis female sex, pigmented skin, younger age, and NNRTI treatment were significant contributors to the prevalence of vitamin D deficiency in our patient population. In the study by Teichmann *et al.*, lower 25(OH)D<sub>3</sub> levels were found among females compared to males; however no information on the prevalence of vitamin D deficiency was given.<sup>6</sup> In the literature the prevalence of vitamin D deficiency among adult HIV-negative females in Europe ranged from 26% to 80%, with causes being menopausal or veiling traditions.<sup>22,23</sup> In our study, the median age of females was younger than 50 years and showed no differences between patients with and without vitamin D deficiency. None of the females in the present study wore a veil.

Skin pigmentation is a known contributor to the development of vitamin D deficiency.<sup>8</sup> A study by Grootjans-Geerts reported high levels of vitamin D deficiency among non-western immigrants in the Netherlands.<sup>24</sup> The younger age of black patients in this study contributed to the significantly younger age of the vitamin D-deficient group. As a consequence, in multivariate analysis only skin pigmentation remained a significant risk factor for vitamin D deficiency.

Although HAART was no significant contributor for vitamin D deficiency in multivariate analysis, our results suggest that antiretroviral agents may affect 25(OH)D<sub>3</sub> levels. At first sight, we found no significant differences in 25(OH)D<sub>3</sub> levels between patients with and without antiretroviral therapy. In contrast, Ramayo *et al.* found significantly lower 25(OH)D<sub>3</sub> levels among naive compared to HAART-treated HIV-infected patients. This was most likely caused by undernourishment associated with HIV disease progression, given the fact that lower albumin levels were found among naive patients.<sup>25</sup> We specifically then looked at the effects of PIs and NNRTIs on 25(OH)D levels, after correction for skin color, and found among whites significantly lower 25(OH)D<sub>3</sub> levels in patients treated with NNRTIs compared to PI-treated patients. Furthermore, NNRTI-

treated patients had significantly higher PTH levels compared to patients without treatment. The low vitamin D levels may have been caused by increased catabolism of 25(OH)D<sub>3</sub> or 1,25(OH)<sub>2</sub>D<sub>3</sub> through induction of CYP450 by NNRTIs.

On the other hand, we found that white PI-treated persons had significantly higher 25(OH)D<sub>3</sub> levels compared to white NNRTI-treated patients and white patients without treatment. *In vitro*, PIs have been found to inhibit 1 $\alpha$ -hydroxylation of 25(OH)D<sub>3</sub> to 1,25(OH)<sub>2</sub>D<sub>3</sub>.<sup>13</sup> This may explain the elevated PTH levels that were found in our study. In addition, Madeddu *et al.* reported significantly lower 1,25(OH)<sub>2</sub>D<sub>3</sub> levels within all HIV-infected patients compared to controls, with the lowest levels found among PI-treated patients.<sup>26</sup>

Within the nonwhite population no significant differences in 25(OH)D<sub>3</sub> levels were found, which emphasizes the great contribution of skin pigmentation in vitamin D deficiency.

Low 1,25(OH)<sub>2</sub>D<sub>3</sub> levels have been associated with low CD4<sup>+</sup> counts.<sup>3-6</sup> However, in the present study and in that of Teichmann *et al.*, no associations between 25(OH)D<sub>3</sub> levels and CD4 cells were found.<sup>6</sup> Also no differences in CD4 recovery rates after initiation of HAART between patients with and without vitamin deficiency were found.

This is the first study evaluating the prevalence of vitamin D deficiency among a large cohort of adult HIV-1-infected patients. One drawback of the present study is the lack of an HIV-negative control group, which limits firm conclusions on incidence levels in HIV-infected patients. Nevertheless, this is the first study evaluating the effect of antiretroviral therapy on vitamin D status. In the present study it was shown that NNRTI-treated patients may be at risk for vitamin D deficiency. Both PI- and NNRTI-treated patients showed higher PTH levels and might therefore be at risk for increased bone mass loss.

An adequate vitamin D status is necessary for the maintenance of good bone mineral density. Therefore the vitamin D status of HIV-infected patients, especially those having dark skin color or receiving NNRTI- or PI-containing HAART, should be evaluated by measuring 25(OH)D<sub>3</sub> and PTH levels. By doing so vitamin D deficiency could be detected early and treated.

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