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ORIGINAL ARTICLE

Bone mass, body composition and vitamin D status of ARV-naïve, urban, black South African women with HIV infection, stratified by CD_4 count

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Abstract

Summary This is the first report examining vitamin D status and bone mass in African women with HIV infection using dual-energy X-ray absorptiometry (DXA) with an appropriate HIV-negative control group. Unlike previous publications, it demonstrates no difference in bone mineral density (BMD) or vitamin D status in HIV-positive patients, at different disease stages, vs. HIV-negative subjects.

Introduction Low bone mass and poor vitamin D status have been reported among HIV-positive patients; suggesting HIV or its treatment may increase the risk of osteoporosis, a particular concern for women in countries with high HIV prevalence such as South Africa. We describe bone mass and vitamin D status in urban premenopausal South African women, who were HIV positive but not on antiretroviral therapy (ARV).

Methods This study is a cross-sectional measurement of BMD and body composition by DXA and vitamin D status by serum 25-hydroxyvitamin D (25(OH)D) concentration. Subjects were recruited into three groups: HIV negative ($n=98$) and HIV positive with preserved CD_4 cell count (non-ARV; $n=74$) or low CD_4 cell counts prior to ARV initiation (pre-ARV; $n=75$).

Results The mean (standard deviation (SD)) age of women was 32.1 (7.2) years. Mean CD_4 (SD) counts ($\times 10^6$/l) were 412 (91) and 161 (69) in non-ARV and pre-ARV groups ($p<0.0001$). Pre-ARV women were significantly lighter and had lower mean BMI than the other two groups ($p<0.002$). The pre-ARV group also had significantly less fat and lean mass compared with non-ARV and HIV-negative subjects ($p \leq 0.05$). After full adjustment, there were no significant differences in BMD at any site ($p>0.05$) between the groups, nor was vitamin D status significantly different between groups ($p>0.05$); the mean (SD) cohort 25(OH)D being 60 (18) nmol/l.

Conclusion Contrary to previous studies, these HIV-positive women did not have lower BMD or 25(OH)D concentrations than HIV-negative controls, despite the pre-ARV group being lighter with lower BMI.

Keywords Body composition · Bone mineral density · Dual energy X-ray absorptiometry · HIV infection · Vitamin D

Introduction

HIV infection and the use of antiretroviral (ARV) medication have been associated with low bone mineral density (BMD) and poor vitamin D status. In a meta-analysis, the prevalence of low BMD in HIV-positive individuals was three times higher than in HIV-negative controls [1–3]. Similarly, studies have described high prevalence of low 25-hydroxyvitamin D

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(25(OH)D) concentrations in HIV-positive patients [4]. Some studies of the effects of HIV and/or its treatment on bone are limited by retrospective design, a preponderance of white, male subjects, and lack of HIV-negative controls [5] while others are prospective [6] and do include women [7, 8]. Other studies are limited by confounding by low body weight or other risk factors for low BMD, such as intravenous drug use (IDU), exposure to a large variety of ARV regimes and measurement of BMD and vitamin D status after varying duration of ARV exposure [6]. The few prospective studies focusing on women have also been limited by some of these aspects [6, 9], and as a result it is difficult to ascertain with certainty if HIV infection and/or its treatment or factors unrelated to HIV infection are contributing factors to the low bone mass and low vitamin D status described in the current literature. In contrast, there are data to suggest that after adjusting for body weight, BMD is normal or near normal, and that patients on ARV do not have increased rates of bone loss [10, 11]. As a result, there is not a definitive consensus on the contribution of HIV infection or ARV exposure on BMD in infected individuals.

In South Africa, estimates of HIV prevalence for 2010 are 10.5 % for the total population and 29.3 % for women attending antenatal clinics. The epidemic is described as “hyperendemic” because of the high prevalence and continuing drivers of transmission [12–14]. In South Africa, individuals generally become eligible for ARV treatment when their CD_4 count is less than a nationally specified threshold. By 2009, 56 % of those requiring ARV were able to receive them, with the government intending to increase ARV coverage to 80 % by 2011 [12].

Vitamin D has well-known associations with bone health via its role in calcium and phosphate homeostasis, and vitamin D status is considered an important modulator of immune function by some authors [14–16]. In South Africa, adults are largely dependent on the cutaneous synthesis of vitamin D to maintain vitamin D status, as only small amounts of vitamin D are obtained from the diet due to limited food fortification. In Johannesburg (26° S latitude), there is sufficient ultraviolet B (UVB) radiation in sunshine throughout the year for dermal synthesis of vitamin D [17]. Nevertheless, vitamin D deficiency has been described in tropical/subtropical countries despite the potential for adequate skin exposure to UVB-containing sunshine [18].

The aim of the study presented here was to describe BMD, body composition and vitamin D status in South African women with and without HIV infection, prior to a planned longitudinal study of this cohort to chart the changes in these outcomes over time. We hypothesised that HIV-positive women with low CD_4 counts, below the threshold that would make them eligible for ARV treatment, would have lower bone mass, less fat and muscle mass and inferior vitamin D status than HIV-positive women with preserved CD_4 counts and HIV-negative women in South Africa.

Methods

Subjects

Urban, black, premenopausal, South African women (n=247) were recruited from clinics in Soweto, Greater Johannesburg and enrolled into the study between February and July 2010. Subjects were recruited from a voluntary counselling and testing clinic and local health clinics. The aim was to recruit 95 HIV-negative and 73 (±10) in each of two HIV-positive groups (with or without low CD_4 counts). This sample size was based on calculations for the longitudinal study to detect a 2 % change in lumbar spine BMD, allowing for a between-individual coefficient of variation in BMD of 5 %, with 95 % confidence and 80 % power. For the study presented here, this sample size was sufficient for a comparison of three groups to allow the detection of mean differences between each pair of groups of around 0.4 standard deviation (SD) at 5 % significance and 80 % power. The study was approved by the University of the Witwatersrand Human Research Ethics Committee (HREC number: M101525) and the Gauteng Department of Health.

Eligible subjects were adult females (defined as aged greater than 18 years) and premenopausal (defined as regular menses). Other inclusion criteria included a documented negative HIV test within the last 12 weeks for HIV-negative women and a documented positive HIV test for all other women. Patient-retained clinic records were scrutinised whenever possible to confirm medical history, current CD_4 count, prior exposure to ARVs and concurrent medication use. Exclusion criteria included conditions associated with abnormal bone metabolism or current use of medication likely to affect bone or vitamin D status such as bisphosphonates. Pregnant and lactating women were excluded as were those with an acute medical condition. The group with the lowest CD_4 count were largely recruited after the other groups: May to June and February to April, respectively.

Study posters were displayed in the clinic and training sessions undertaken with clinic staff. Women who expressed an interest in the study underwent initial telephone screening, in their language, to ensure inclusion and exclusion criteria were met. Prior to enrolment, potential subjects completed a medical- and health-related questionnaire to assess past and current health status and medication use and to further assess compliance with inclusion and exclusion criteria. Women who remained eligible were enrolled in the full study after they had provided written consent.

The enrolled women consisted of HIV-negative (n=98) and HIV-positive (n=149) subjects. The HIV-positive women were recruited into two prespecified groups: those with relatively preserved CD_4 counts ($>350\times10^6$ cells/l), not requiring ARV therapy (non-ARV group; n=74) and those with low CD_4 counts (in the region of 200×10^6 cells/l) requiring ARV initiation (pre-ARV group; n=75) according to the current South Africa (SA) treatment guidelines [19]. HIV-negative status was confirmed using the Determine™ rapid HIV-antibody test (Alere San Diego, Inc., San Diego, CA, USA), while HIV-positive status was established using a second platform. HIV-positive women were either newly diagnosed or known to be HIV positive, but not on ARVs. All HIV-positive women provided an up-to-date (within 3 months) CD_4 count prior to enrolling into the study. All HIV-positive women received SA standard of care with respect to ARV provision and clinical follow up. Women requiring urgent ARV initiation were managed in such a way that there would be no delay in ARV initiation if they were to participate in the study.

Women attended the Developmental Pathways for Health Research Unit (DPHRU) facility at the Chris Hani Baragwanath Academic Hospital, after an overnight fast and underwent phlebotomy, anthropometry, and dual-energy X-ray absorptiometry (DXA) assessment of bone mass and body composition. After phlebotomy, subjects were given breakfast and each received ZAR 50.00 (≈US$6.25) for travel expenses.

Anthropometry

Height was measured to the nearest millimetre using a stadiometer (Holtain, Crosswell, UK). Weight was measured to the nearest 100 g using a digital scale (Tanita, TBF-410 MA Body Composition Analyzer, Tanita Corporation of America, Inc., Arlington Heights, IL, USA) with participants wearing light clothing and no shoes. BMI was calculated as the participant's weight in kilograms divided by the square of their height in metres (in kilogram per square metre). Underweight, normal, overweight, and obese were defined as BMI <18.5, 18.5–24.9, 25–29.9, ≥30.0 kg/m^2, respectively [20].

Bone absorptiometry and body composition measurements

DXA was performed using a Hologic QDR 4500A DXA (model: Discovery W (S/N 71201) software version 12.5:7 Hologic, Inc., Waltham, MA, USA) according to standard procedures. Scans were conducted using the automatic scan mode, i.e. 'array', 'fast array' or 'slow array', depending on the weight of the subjects. Subjects wore light clothing having removed metal objects, jewellery, etc. DXA was used to measure bone mineral content (BMC, in grams), bone area (BA, in square centimetre) and areal BMD (in grams per square centimetre), of whole body (WB), total hip (TH), femoral neck (FN) and lumbar spine (LS). The coefficients of variation (CV%) for repeated measurements of the manufacturer's phantom were 0.3, 0.4 and 0.2 % for BA, BMC and BMD, respectively. The CV for repeated measurement by the DXA operator of the LS and TH BMD were 0.7 and 1.0 %, respectively. DXA scans for WB were analysed using WB less head (WBLH) as many women wore wigs and hair weaves that could not be removed prior to scanning. This artificial hair was of similar density to soft tissue and therefore could cause measurement artefact. Total fat and lean body mass (in grams) were also measured by DXA.

Laboratory analysis

Blood was collected for 25(OH)D analysis, measured by chemi-luminescent immunoassay (Liason) kit (DiaSorin Inc., Stillwater, MN, USA). The blood samples were allowed to clot for a minimum of 20 min at room temperature, and the serum was aliquoted and stored at −20 C until analysed. All samples were run in duplicate. The inter-assay CV for low and higher 25(OH)D controls was 10 and 9 %, respectively, whereas the intra-assay CV was 8 and 6 %, respectively. The DPHRU laboratory participates in the International Vitamin D External Quality Assessment Scheme and holds the certificate of proficiency [21].

Statistical analysis

Data were analysed using DataDesk 6.3.1 (Data Description Inc, Ithaca, NY, USA) and summary statistics were documented as mean (SD) or median (interquartile range), depending on the distribution. Comparisons were made between the three groups of women using hierarchical linear models; ANOVA (or ANCOVA) and Scheffé post hoc tests were used to compare group means (standard error (SE)). To consider the possible influence of group differences in bone and body size, bone mineral data were adjusted for age, weight, height and bone area, and bone area was adjusted for age, weight and height, using ANCOVA [16]. Preliminary plots of the relationship between fat mass and lean mass in this sample population demonstrated non-linearity. Regression of fat mass on lean mass in the HIV-negative control group with data in natural logarithms gave a power exponent 2.05± 0.18 (SE) , indicating that fat mass-to-lean square mass best described the relationship in this population. The exponent was similar when the data from all three groups were included in the model; 2.07±0.14. Consequently, a fat mass-to-lean square mass term was used to describe differences in body composition between the groups, and logarithmic regression was used to adjust fat mass for lean mass in statistical models. BMD SD scores (SDS) were generated using HIV-negative subjects as the reference population (ref) against which the SDS for each individual HIV-positive woman (i) was derived as follows: $[(BMD_i - \text{mean } BMD_{ref})/SD_{ref}]$. A p value of ≤ 0.05 was considered to be statistically significant.

Table 1 Subject characteristics, anthropometric measurements and vitamin D status as measured by serum 25(OH)D

	Group 1 HIV-negative *n*=98	Group 2 HIV-positive, non-ARV *n*=74	Group 3 HIV-positive, pre-ARV *n*=75	Group effect ANOVA *p*
Age (years)	30.0 (8.1)	33.5 (6.1)[a]	33.4 (6.5)[a]	0.001
HIV status	Negative	Positive	Positive	
Current CD$_4$ count $\times 10^6$ cells/l	ND	412 (91)	161 (69)[b]	<0.001
Median (IQR)		420 (127;409)	175 (120;165)	
Min	NA	240	18	
Max	NA	604	275	
Gravidity median (IQR)	1 (0;2)	2 (2;3)[a]	2 (1;3)[a]	
Range	0–5	0–6	0–6	
Current hormonal contraceptive use (%)	34 (35.4)	26 (36.6)	25 (33.3)	0.9
Current smoking (%)	10.2	13.5	8	0.2
Height (cm)	157.6 (5.9)	159.4 (5.9)	159.2 (5.3)	0.06
Weight (kg)	69.7 (17.0)	72.0 (17.4)	62.3 (15.2)[c,d]	<0.001
BMI (kg/m^2) Median (IQR)	27.3 (23.1;31.7)	27.8 (23.3;32.3)	23.5 (20.5;27.0)[d,e]	<0.001
Overweight BMI >24.9 kg/m^2, <30 kg/m^2 (%)	35	28	28	
Obese BMI >30 kg/m^2 (%)	30	37	16	
Underweight BMI <18.5 kg/m^2 (%)	4	1	11	
WBLH Fat (kg)	26.1 (11.5)	26.1 (9.8)	19.7 (9.3)[b,e]	<0.0001
WBLH Lean (kg)	38.3 (60.8)	39.5 (62.4)	36.4 (48.1)[d]	0.005
Fat/lean^2 (kg/kg^2)*	17.32 (4.80)	15.92 (4.56)	14.58 (5.47)[a,f]	0.002
25(OH)D (nmol/l)	59.7 (16.5)	59.2 (16.5)	61.6 (22.3)	0.7
25(OH)D (nmol/l) >50 (%)	73.5	70.3	66.7	
25(OH)D (nmol/l) <50 (%)	26.5	29.7	33.3	
25(OH)D (nmol/l) <25 (%)	1.0	2.7	5.3	

All values are mean (SD) unless indicated. Letters are used to indicate significance of between-group differences as tested by ANOVA/Scheffé

25(OH)D 25 hydroxyvitamin D, *ARV* antiretroviral therapy, *cm* centimetres, *IQR* interquartile range, *kg* kilograms, *SD* standard deviation, *WBLH* whole body less head, *ND* not determined, *NA* not applicable

*Value multiplied by 1,000 to illustrate the relative differences in kilogram

[a] Significantly different from group 1, $p \leq 0.01$

[b] Significantly different from group 2, $p \leq 0.001$

[c] Significantly different from group 1, $p \leq 0.05$

[d] Significantly different from group 2, $p \leq 0.01$

[e] Significantly different from group 1, $p \leq 0.001$

[f] Significantly different from group 2, $p \leq 0.05$

Results

Subject characteristics

By design, the mean CD$_4$ count ($\times 10^6$ cells/l) in the pre-ARV group was significantly lower than that in the non-ARV group (412 (91) and 161 (69), respectively, $p<0.0001$). The mode of acquisition of HIV-infection was via heterosexual transmission in all subjects, only one subject reporting IDU in the past (Table 1).

Mean age (SD) was 32.1 (7.2) years with HIV-negative women being significantly but only slightly younger than both groups of HIV-positive women. The age ranges were similar in the three groups (18–49, 22–48 and 19–47 years in HIV-negative, non-ARV and pre-ARV women, respectively). Median (IQR) gravidity was 2 (1; 3) with both HIV-positive groups having a higher median gravidity compared to the HIV-negative group.

Anthropometry and body composition

HIV-negative women tended to be shorter than both groups with HIV-infection ($p=0.06$), while HIV positive, pre-ARV women were significantly lighter than the other two groups ($p<0.05$). Median (IQR) BMI of the study cohort was 26.1 (22.4; 31) kg/m^2 with BMI in pre-ARV women being significantly lower than in HIV-negative and non-ARV women. Combined overweight and obesity represented 65, 65 and

44 % of subjects in HIV-negative, non-ARV and pre-ARV women, respectively , while underweight was present in 4, 1 and 11 %, respectively (Table 1).

There were significant differences in fat mass between groups with pre-ARV women having significantly lower fat mass than non-ARV women ($p \leq 0.001$). Although lean mass was also lower in pre-ARV compared with non-ARV women ($p=0.005$) the pre-ARV group had lower fat mass-to-lean square mass ratio than the other two groups ($p=0.002$). When fully adjusting for lean mass using logarithmic regression, the pre-ARV group had significantly lower fat mass for their lean mass than the other two groups; such that for each unit of lean mass the pre-ARV group had a mean difference (SE) of 21 (5)% less fat than the controls, $p=0.0002$, and 16 (5)% less fat than the non-ARV group, $p=0.02$.

Bone measures

No significant differences in BMD at the TH, FN, LS and WBLH were found, and age and size adjustment did not reveal any differences between groups. When expressed as SD scores, there were no significant differences between pre-ARV and non-ARV groups in BMD for any site measured ($p>0.05$) and all the mean values were within a −0.5 SD of the HIV-negative reference group (Table 2). In addition, no significant differences were found in BMC values except at WBLH when fully adjusted for age, size and BA ($p=0.03$). Unadjusted BA was significantly greater in both groups of HIV-positive women than HIV-negative women at some sites but these differences disappeared after adjusting for age and size (see Electronic supplementary material (ESM) for BA and BMC data).

Vitamin D status

Mean (SD) 25(OH)D for the whole cohort was 60.1 (18.4) nmol/l and there were no significant differences between groups ($p>0.05$). 25(OH)D concentration <50 nmol/l in 29.6 % of individuals; with similar proportions in each of the groups in this category (26.5, 29.7 and 33.3 % in HIV-negative, non-ARV and pre-ARV, respectively). Very few subjects had a 25(OH)D concentration <25 nmol/l (1.0, 2.7 and 5.3 % in the three groups, respectively), despite the slightly greater number of pre-ARV subjects whose blood samples for 25(OH)D measurement were obtained during the winter months.

Discussion

The aim of this study was to determine whether South African HIV-positive women with preserved CD_4 counts differed from those with low CD_4 counts making them eligible for ARV and to compare each group with HIV-negative women. In this group of urban, South African women, pre-ARV women were significantly lighter than HIV-negative and non-ARV subjects and had lower fat mass than expected for their lean mass, raising the possibility that women with advancing HIV disease preferentially lose fat rather than lean mass. There were no significant differences between groups in BMC or BMD at any site before or after adjustment for age, BA, weight and height and the observed smaller BA in the HIV-negative women disappeared after adjustment for age, height and weight. There was no significant difference in vitamin D status between groups with the majority of subjects having a serum concentration >50 nmol/l.

The assessment of 'optimal' vitamin D status is problematic because varying cut offs are used to define sufficiency, insufficiency and deficiency [22]. A concentration below 25 nmol/l is generally recognised as indicating an increased risk of rickets and osteomalacia [23]. The 2010 Institute of Medicine report considered that a blood 25(OH)D concentration of 20 ng/mL (50 nmol/l) to be sufficient for good bone health in 'practically all individuals' [24]. However, it noted that evidence was lacking to make a similar statement regarding non-skeletal health. In the context of HIV infection and ARV use, the optimal vitamin D status remains undefined because there

Table 2 BMD of the three groups of South African women

	BMD (g/cm^2) Mean (SD)			Group effect[a] *p*
	Group 1 HIV-negative *n*=98	Group 2 HIV-positive, non-ARV *n*=74	Group 3 HIV-positive, pre-ARV *n*=75	
Total Hip	1.013 (0.131)	0.985 (0.124)	0.988 (0.125)	0.3
Femoral Neck	0.930 (0.114)	0.916 (0.125)	0.923 (0.131)	0.8
Lumbar Spine	1.018 (0.118)	1.021 (0.109)	1.006 (0.128)	0.7
WBLH	0.958 (0.079)	0.943 (0.071)	0.947 (0.080)	0.4

ARV antiretroviral therapy, *BMD* bone mineral density (in gram per square centimetre), *SD* standard deviation, *WBLH* whole body less head

[a] Group effect by ANOVA. There were no significant differences between pairs of groups by Scheffé post hoc tests

may be different requirements for maximal bone health and immune functioning compared with HIV-negative populations. However, in contrast to other reports [4, 25], in our study, there were no indications that HIV infection was associated with inferior vitamin D status because there were no significant differences in vitamin D status between the three groups, the distributions of 25(OH)D concentration were similar, and vitamin D status appeared to be generally adequate with very few women having a concentration <25 nmol/l.

Contrary to previous reports [9], we found no significant differences in BMD between either group of HIV-positive and HIV-negative women. Full adjustment for bone and body size did not alter these results. This lack of any differences is surprising as HIV-positive women with low CD_4 counts, requiring ARV initiation, were significantly lighter, with lower fat and lean mass, than the other women. However, it may reflect the selection criteria for this study because despite recruiting women with low CD_4 counts, of clinical concern, women with severe clinical disease received immediate ARV therapy and were thus excluded from the study. It may also be influenced by the fact that the subjects were not intravenous drug users and thus not exposed to the additional effect on BMD that this poses. Another limitation may be that the groups were different in terms of duration of hormonal contraception use, parity and total duration of lactation; however, at the time of the study, no women were pregnant or lactating. The findings are also limited by the fact that the sample of HIV-positive women was likely to be heterogeneous with respect to immune status and duration of infection. However, most other studies have also recruited HIV-positive subjects in a similar manner and this is unlikely to account for the different findings in our study.

The rates of combined overweight and obesity 65 % in HIV-negative and non-ARV subjects in this study were greater than the national average in South Africa of 51.5 % [26]; even women with advanced HIV-disease (pre-ARV group) had a combined overweight and obesity rate of 44 %. It is possible, therefore, that the typically high weight of South African women has a sparing effect on bone in those with HIV infection, even with CD_4 counts below the threshold for initiation of ARV intervention.

Historically, being overweight has been viewed as protective against osteoporotic fracture, although evidence is emerging that overweight and obesity may be a risk factor for leg fragility fractures in women [27]. In the study population of younger black women in South Africa, there were no significant differences in BMD SD score, expressed relative to the HIV-negative group, according to HIV status at any site. The effects of HIV and its treatment on fracture risk in South Africa are unknown.

The lack of difference between the groups which is at variance from previously reported studies may be the result of true lack of effect of HIV infection or reflect important differences in bone response to HIV between black Africans and Caucasians. The study design in which two distinct groups of HIV-positive women, based on South African eligibility criteria for ARV treatment plus the inclusion of a HIV-negative control group strengthens the finding that HIV infection with varying degree of immunosuppression does not appear to be driving alterations in BMD or vitamin D status in these young, urban women. The high rates of overweight may be masking more dramatic differences in BMD and vitamin D in those subjects with advanced clinical HIV disease not included in this study. Further work is required to address the effects of ARV exposure on bone and vitamin D status as well as the relative effect of 'traditional' osteoporosis risk factors in this population. The data from this study provide an insight into bone health, body composition and vitamin D status in African women living with HIV. They challenge our own hypotheses and previously reported differences in BMD and vitamin D status in HIV-positive subjects living in developed countries and highlight the importance of studying subjects prior to ARV exposure.

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Conflicts of interest There were no conflicts of interest.

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