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# Factors associated with low bone mineral density in a Brazilian cohort of vertically HIV-infected adolescents

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SUMMARY

*Objective:* To assess the prevalence and factors associated with low bone mineral density (BMD) in HIV-infected adolescents.
*Methods:* This was a cross-sectional study of a Brazilian cohort of vertically HIV-infected adolescents. Body composition and lumbar spine (LS) and total body (TB) BMD were estimated by dual-energy X-ray absorptiometry (DXA). Low BMD was considered for a *Z*-score $\leq -2$ standard deviations. Pubertal development, anthropometric data, laboratory measurements, antiretroviral regimen, and time of immunological and virological recovery were evaluated as factors associated with a low BMD.
*Results:* Seventy-four adolescents aged $17.3 \pm 1.8$ years were studied. Low BMD was present in 32.4% of them. LS and TB BMD *Z*-scores were positively correlated with weight, body mass index (BMI), BMI *Z*-score, total body fat, and nutritional status. Patients on tenofovir had lower LS and TB BMD *Z*-scores. Time on tenofovir was indirectly correlated with LS and TB BMD *Z*-scores. No difference was found regarding levels of calcium, parathyroid hormone, or 25-hydroxyvitamin D according to BMD status.
*Conclusions:* Control of the HIV infection, especially before the initiation of puberty, might have a positive influence on bone gain. Body composition and nutritional status had a positive influence on BMD that was more evident in females, suggesting that nutritional intervention may have a positive impact on BMD.


## 1. Introduction

Universal access to antiretroviral therapy (ART) and especially to highly active antiretroviral therapy (HAART), which combines drugs with different modes of action, has resulted in a significant decline in mortality rates, allowing many vertically HIV-infected children to reach adolescence.[1] This increasing survival rate is also evident in Brazil, mostly since 1996 when the public health system started the distribution of HAART.[2]

As a consequence, long-term complications of the HIV infection and HAART side effects have been the focus of many studies. A decrease in height-for-age has been described in HIV-infected children, and this difference in linear growth is already significant at the age of 15 months.[3] HIV can infect bone cells and alter bone metabolism, and patients fail to achieve the expected gains in bone size, mass, and strength.[4] ART may also have direct and indirect effects on phosphate and vitamin D metabolism, which contributes to low bone mineral density (BMD) due to impaired mineralization.[5] HIV patients may also have hypovitaminosis D, even subclinical, which is now recognized as one of the most important influences on skeletal integrity.[6]

Due to the lack of studies addressing factors related to low BMD in HIV-infected adolescents in Brazil, the aim of this study was to assess the prevalence and factors associated with low BMD in a cohort of vertically HIV-infected adolescents, followed-up at the Clementino Fraga Filho University Hospital, at the Federal University of Rio de Janeiro (HUCFF-UFRJ).

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## 2. Methods

### 2.1. Study population

All vertically HIV-infected adolescents followed-up at the Infectious Diseases Service of the HUCFF-UFRJ were considered for this study. One patient was excluded due to neoplasia and two refused participation. The remaining 74 selected patients were not receiving bone-active drugs (glucocorticoids, anticonvulsants, bisphosphonates, calcium, and vitamin D supplements), had no hepatitis C virus co-infection or other detectable disease that could affect bones, and denied drug addiction or alcohol consumption; one was a current cigarette smoker. Pregnancy was excluded by β-human chorionic gonadotropin (HCG) testing. Some patients had been pregnant but were included: one patient at 6 months after abortion and four patients at 12 months after parturition. Also, opportunistic infections were controlled in two patients before inclusion (pulmonary pneumocystosis and cerebral toxoplasmosis). The study was performed during the period May 2008 to May 2011.

The diagnosis of perinatal HIV infection was retrospective, as most of the children were the index case in the family, while others were diagnosed by screening of a family index case. The mean age at first HIV test was 4.7 ± 3.6 years. In 80% of the cases, the infection was also confirmed in the mother. In the remaining 20%, composed of orphan children at the time of diagnosis, a history of HIV infection in the mother could be retrieved, with the exception of three cases. Over 95% were breastfed, therefore we consider that most of the HIV infections were acquired around peripartum or in the early postnatal period.

The study protocol was approved by the Ethics Committee of the HUCFF-UFRJ. Written informed consent was obtained from all participants and their parents/guardians.

### 2.2. ART history and virological and immunological evaluations

ART was categorized into the following: (1) none; (2) dual therapy (use of two nucleoside or nucleotide analogue reverse transcriptase inhibitors (NRTIs)); and (3) HAART consisting of NRTIs + non-nucleoside reverse transcriptase inhibitor (NNRTI) or protease inhibitor (PI). The ART used at the time of the study and previous exposure to dual therapy were considered. Time between birth and initiation of treatment, time on ART (defined as the time between the initiation of any antiretroviral and the time of the dual-energy X-ray absorptiometry (DXA) exam), time on tenofovir (TDF), time of undetectable viral load (viral load <50 copies/ml), time between birth and the first undetectable viral load, and CD4+ <200 cells/mm$^3$ were analyzed. Immunological stage was determined according to the US Centers for Disease Control and Prevention (CDC) classification system.[7]

### 2.3. Laboratory measurements

Blood was drawn after overnight fasting for routine exams (hemogram, glucose, calcium, phosphorus, albumin, creatinine, and liver enzymes). Calcium was corrected for albumin levels ($Ca_{corr} = Ca + 0.8 \times [4 - alb]$). Other exams were CD4+ cell counts measured by FACSCalibur (Becton-Dickinson, NJ, USA) and viral load by bDNA (Versant HIV-1 RNA 3.0 third generation, Chiron/Bayer/Siemens; the lower limit of detection was 50 copies/ml). Serum samples were collected with protection from light and stored at −80 °C until analysis of hormones: 25-hydroxyvitamin D (25(OH)D) by high-performance liquid chromatography (HPLC; Chromsystem, Germany; normal range 30–80 ng/ml) and intact parathyroid hormone (PTH) by chemiluminescence (Immulite 2000, Siemens, USA; normal values 12–65 pg/ml).

### 2.4. Assessment of BMD and body composition

DXA scans were performed on the lumbar spine (LS) and total body (TB) to assess BMD and body composition (percent body fat and lean mass), using Prodigy software v. 11.40, adequate for child and adolescent assessment. The BMD *Z*-score was considered the best measure to compare all patients because the population included male and female patients of different ages. Lower than expected BMD was defined as *Z*-scores at LS and/or TB equal to or lower than 2 standard deviations from the reference data (*Z*-score ≤−2 SD).[8]

Weight, height, and body mass index (BMI) were used as indicators. BMI-for-age was classified as follows: obesity (*Z*-score >2), overweight (*Z*-score ≤2 to >1), normal (*Z*-score ≤1 to ≥−2), thinness (*Z*-score <−2 to ≥−3), severe thinness (*Z*-score <−3), in accordance with the World Health Organization (WHO) 2007 reference.[9]

### 2.5. Assessment of pubertal development

Pubertal stage was self-assessed privately using a validated method with Tanner diagrams.[10] Subjects were given a standardized series of drawings showing the five developmental stages with explanatory text to assess their own pubertal development.

### 2.6. Dietary intake

The dietary assessment was based on a single 24-h recall conducted by a trained registered nutritionist, using measuring cups, spoons, and portion-size images to increase the accuracy of the recall. The household measurements were converted into grams and milliliters for quantitative analysis of energy and nutrient intake using the software Avanutri online (version online; 2010, Rio de Janeiro, Brazil). Only energy, macronutrients (carbohydrate, protein, and lipid), calcium, and vitamin D results are presented here. Calcium and vitamin D consumption were categorized according to the estimated average requirement (EAR).[11]

### 2.7. Statistical analysis

The Mann–Whitney test was employed to verify statistical differences in continuous variables and Fisher's exact test for categorical variables. Spearman's coefficients between continuous variables and BMD *Z*-scores at LS and TB were calculated. The level of significance adopted was $p < 0.05$. Analyses were performed using SPSS v. 17.0 (SPSS Inc., Chicago, IL, USA).

## 3. Results

### 3.1. Demographic data and clinical, virological, and immunological characteristics of the studied population

We studied 41 females and 33 males. Their mean age was 17.3 ± 1.8 years (range 13.4–21.2 years). Because the Brazilian population is highly mixed, we classified the race as white (36.5%) and non-white (63.5%). At the time of evaluation, six of the patients were off therapy, one was on two NRTIs, and 67 (90.5%) were on HAART: 14 (18.9%) on NRTIs + NNRTI and 53 (71.6%) on NRTIs + PI. Antiretroviral therapy history, analyzed in 70 patients (48 normal BMD and 22 low BMD), revealed that the majority, 53 patients (75.7%), had previous exposure to two NRTIs; 32 (45.7%) had been exposed to TDF and 29 were currently using TDF (for 2.9 ± 1.5 years). Patients were 6.3 ± 3.8 years old at initiation of ART and the duration of treatment was 11.1 ± 3.5 years. Virological and immunological evaluations analyzed in 71 patients (48 normal BMD and 23 low BMD) demonstrated that

viral load was undetectable in 34 patients (47.9%) during the last 3.8 ± 2.9 years, and CD4+ was <200 cells/mm$^3$ in 16 patients (22.5%).

### 3.2. Bone mineral density, use of antiretroviral therapy, and laboratory measurements

No patient made reference to fragility fractures, bone pain, or deformities. LS and/or TB BMD were low in 24 of the 74 patients (32.4%), 23 of whom were receiving ART. Mean BMD *Z*-scores at LS and TB were compared to the following variables: use of dual therapy, TDF, PI, and presence of undetectable viral load. Patients exposed to TDF showed a lower BMD *Z*-score at LS (−1.82 ± 1.12 vs. −1.28 ± 0.93, $p = 0.021$) and TB (−1.51 ± 1.09 vs. −0.88 ± 1.04, $p = 0.022$). Patients on PI tended to show a lower BMD *Z*-score at LS (−1.74 ± 1.09 vs. −1.12 ± 0.95, $p = 0.055$), but not at TB (−1.31 ± 1.12 vs. −0.88 ± 1.10, $p = 0.163$). When analyzing patients according to BMD status (normal or low BMD), the only significant differences were related to use and time on TDF and serum phosphorus, and these were the only significant differences for ART and laboratory measures (Table 1). Patients on TDF compared to those who had never used this drug had similar levels of serum phosphorus (4.23 ± 0.80 mg/dl vs. 4.17 ± 0.96 mg/dl, $p = 0.723$) and a lower BMD *Z*-score at LS (−1.85 ± 1.12 vs. −1.29 ± 0.93, $p = 0.019$) and TB (−1.53 ± 1.10 vs. −0.88 ± 1.05, $p = 0.019$). Time on TDF was inversely correlated to BMD *Z*-score at LS ($r = -0.446$; $p = 0.013$) and TB ($r = -0.383$; $p = 0.037$). In relation to anthropometry, patients on TDF showed a trend for a lower BMI *Z*-score (−0.76 ± 1.18 vs. −0.18 ± 0.92, $p = 0.093$). However, time on TDF did not show any correlation with the BMI *Z*-score. Although we found that low BMD was independently associated with BMI and TDF exposure, the model was unstable because of the small sample size.

Most patients with an undetectable viral load and a good immunological CDC classification had normal BMD *Z*-scores when compared to those with less control over the HIV infection, although this difference was not statistically significant. Other clinical parameters such as time from birth to initiation of treatment, time on ART, and time of undetectable viral load, as well as serum 25(OH)D and PTH were not associated with the BMD *Z*-scores or gender.

### 3.3. Bone mineral density, demographics, pubertal development, and anthropometric data

Body composition and nutritional status were positively associated with BMD *Z*-scores, especially in females (Table 2). A low BMD *Z*-score was identified in 11 of the 17 (64.7%) patients classified as 'thinness' or 'severe thinness', and only in 13 of 57 (22.8%) patients classified as 'normal' or 'overweight', although we did not observe differences in the percentages of thinness and severe thinness between the genders (data not shown). The pubertal development group tended to be associated with BMD status. All four patients with pubertal delay (Tanner stage 2) had an age range of 15.4–17.6 years and *Z*-scores between −2 and −3.8 SD. We also observed a statistically significant difference in the presence of Tanner stage 5 among females (22/35, 62.9%) and males (6/30, 20.0%) ($p = 0.001$). The one cigarette smoker had a normal BMD.

Anthropometric data and correlation with BMD are presented in Figure 1. The LS BMD *Z*-score tended to correlate with percent body fat ($r = 0.203$, $p = 0.083$). The TB BMD *Z*-score tended to correlate with percent body fat ($r = 0.224$, $p = 0.055$) and lean body mass ($r = 0.200$, $p = 0.087$), and inversely with time between birth and first undetectable viral load ($r = -0.339$, $p = 0.050$). The height *Z*-score was not correlated to BMD *Z*-scores.

Race did not influence the results of BMD in the group. Low BMD was found in 37% of white and 29.8% of non-white patients ($p = 0.609$). *Z*-scores of LS and TB for whites and non-whites were −1.63 ± 1.21 vs. −1.44 ± 0.98, $p = 0.271$ and −1.17 ± 1.26 vs. −1.14 ± 0.99, $p = 0.682$, respectively.

### 3.4. Bone mineral density and dietary intake

Dietary assessment data were not different between patients with normal and low BMD, even when divided by gender. Calcium

**Table 1**
Laboratory measurements, antiretroviral therapy history, and virological and immunological evaluations in relation to bone mineral density in a Brazilian cohort of vertically HIV-infected adolescents[a].

| Variables | Normal BMD ($n = 50$)[b] | Low BMD ($n = 24$)[b] | *p*-Value |
|---|---|---|---|
| Use of PI | 33 (66) | 20 (83.3) | 0.100 |
| Use of TDF | 18 (37.5) | 14 (63.6) | 0.037 |
| Time on TDF, years | 2.4 ± 1.2 | 3.6 ± 1.4 | 0.019 |
| Previous use of two NRTIs | 38 (79.2) | 15 (68.2) | 0.241 |
| Time between birth and initiation of treatment, years | 6.5 ± 3.6 | 6.0 ± 4.2 | 0.558 |
| Time between birth and the first undetectable viral load, years | 12.3 ± 4.3 | 14.9 ± 2.1 | 0.149 |
| Use of ART | 45 (90) | 23 (95.8) | 0.360 |
| Time on ART, years | 11.0 ± 3.6 | 11.1 ± 3.5 | 0.746 |
| CD4+ cell count, cells/mm$^3$ | 479.3 ± 318.3 | 438.0 ± 287.3 | 0.326 |
| CD4+ cells <200/mm$^3$ | 12 (25.0) | 4 (17.4) | 0.556 |
| Viral load, $\log_{10}$ copies/ml | 2.2 ± 2.0 | 1.8 ± 1.9 | 0.290 |
| Undetectable viral load | 22 (45.8) | 12 (52.2) | 0.466 |
| Time of undetectable viral load, years | 4.4 ± 3.3 | 2.9 ± 1.7 | 0.302 |
| CDC immunological stage | | | 0.089 |
| 1 | 3 (6.3) | 0 | |
| 2 | 12 (25.0) | 3 (13.0) | |
| 3 | 33 (68.7) | 20 (87.0) | |
| PTH, pg/ml | 37.3 ± 27.0 | 35.7 ± 15.8 | 0.716 |
| Phosphorus, mg/dl | 3.9 ± 0.8 | 4.6 ± 0.8 | 0.014 |
| $Ca_{corr}$, mg/dl | 9.2 ± 0.9 | 8.9 ± 0.8 | 0.223 |
| Albumin, g/dl | 5.8 ± 6.1 | 4.5 ± 0.4 | 0.359 |
| 25-Hydroxyvitamin D, ng/ml | 37.1 ± 10.3 | 40.2 ± 18.2 | 0.490 |

ART, antiretroviral therapy; BMD, bone mineral density; $Ca_{corr}$, calcium corrected for albumin levels ($Ca_{corr} = Ca + 0.8 \times [4 - alb]$); CDC, US Centers for Disease Control and Prevention; NRTIs, nucleoside or nucleotide analogue reverse transcriptase inhibitors; PI, protease inhibitor; PTH, parathyroid hormone; TDF, tenofovir.

[a] Results are presented as *n* (%) or mean ± standard deviation.

[b] Antiretroviral therapy history was analyzed in 70 patients (48 normal BMD and 22 low BMD); virological and immunological evaluations were analyzed in 71 patients (48 normal BMD and 23 low BMD).

**Table 2**
Demographic and anthropometric data in relation to bone mineral density status according to gender in a Brazilian cohort of vertically HIV-infected adolescents[a].

| Variables | Total ($n=74$) | | | Female ($n=41$) | | | Male ($n=33$) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Normal BMD ($n=50$) | Low BMD ($n=24$) | $p$-Value | Normal BMD ($n=28$) | Low BMD ($n=13$) | $p$-Value | Normal BMD ($n=22$) | Low BMD ($n=11$) | $p$-Value |
| Female:male ratio | 28:22 | 13:11 | 0.882 | | | | | | |
| Age, years | 17.4 ± 2.0 | 17.2 ± 1.3 | 0.673 | 17.4 ± 1.9 | 17.3 ± 1.3 | 0.833 | 17.4 ± 2.1 | 17.1 ± 1.3 | 0.789 |
| Weight, kg | 54.4 ± 9.5 | 47.6 ± 8.7 | 0.003 | 53.9 ± 8.5 | 45.4 ± 8.8 | 0.012 | 55.0 ± 10.9 | 50.2 ± 8.2 | 0.089 |
| Height, m | 1.59 ± 0.10 | 1.58 ± 0.07 | 0.738 | 1.55 ± 0.05 | 1.56 ± 0.06 | 0.790 | 1.65 ± 0.12 | 1.62 ± 0.07 | 0.078 |
| Height-for-age, $Z$-score | −1.03 ± 1.02 | −1.29 ± 0.96 | 0.183 | −0.97 ± 0.64 | −0.98 ± 1.00 | 0.846 | −1.11 ± 1.34 | −1.62 ± 0.07 | 0.138 |
| BMI, kg/m$^2$ | 21.4 ± 3.1 | 18.9 ± 2.9 | 0.004 | 22.4 ± 3.3 | 18.8 ± 3.3 | 0.007 | 20.1 ± 2.3 | 19.1 ± 2.4 | 0.252 |
| BMI-for-age, $Z$-score | −0.20 ± 0.82 | −0.91 ± 1.25 | 0.059 | 0.09 ± 0.8 | −1.07 ± 1.5 | 0.041 | −0.54 ± 0.7 | −0.73 ± 0.9 | 0.651 |
| Lean body mass, kg | 38.9 ± 8.3 | 34.6 ± 11.9 | 0.166 | 33.6 ± 3.0 | 27.6 ± 10.2 | 0.038 | 45.6 ± 8.0 | 42.9 ± 7.8 | 0.181 |
| Total body fat, % | 23.0 ± 11.9 | 18.6 ± 9.0 | 0.133 | 31.6 ± 8.2 | 25.5 ± 5.6 | 0.015 | 12.0 ± 4.4 | 10.4 ± 3.8 | 0.311 |
| Total body fat, kg | 12.6 ± 7.6 | 8.6 ± 4.7 | 0.038 | 17.2 ± 6.7 | 11.6 ± 4.3 | 0.014 | 6.8 ± 3.7 | 5.1 ± 1.9 | 0.143 |
| Nutritional status | | | <0.001 | | | 0.004 | | | 0.048 |
| Severe thinness | 0 | 8 (33.3) | | 0 | 5 (38.5) | | 0 | 3 (27.3) | |
| Thinness | 6 (12) | 3 (12.5) | | 2 (7.1) | 1 (7.7) | | 4 (18.2) | 2 (18.2) | |
| Normal | 35 (70) | 12 (50) | | 18 (64.3) | 6 (46.1) | | 17 (77.3) | 6 (54.5) | |
| Overweight | 9 (18) | 1 (4.2) | | 8 (28.6) | 1 (7.7) | | 1 (4.5) | 0 | |
| Obesity | 0 | 0 | | 0 | 0 | | 0 | 0 | |
| Pubertal development[b] | | | 0.087 | | | 0.376 | | | 0.166 |
| Tanner stage 1–2 | 1 (2.4) | 4 (17.4) | | 0 | 1 (8.3) | | 1 (5.3) | 3 (27.3) | |
| Tanner stage 3–4 | 20 (48.8) | 11 (47.8) | | 7 (31.8) | 4 (33.3) | | 13 (68.4) | 7 (63.6) | |
| Tanner stage 5 | 20 (48.8) | 8 (34.8) | | 15 (68.2) | 7 (58.4) | | 5 (26.3) | 1 (9.1) | |

BMD, bone mineral density; BMI, body mass index.
[a] Results are presented as $n$ (%) or mean ± standard deviation.
[b] Pubertal development was analyzed in 64 patients.

and vitamin D intake were inadequate in 72.9% and 91.5% of them, respectively, but were not associated with BMD status. Patients with an undetectable viral load consumed more energy (3248.1 ± 1298.8 kcal vs. 2736.4 ± 1460.1 kcal, $p = 0.037$), carbohydrate (448.3 ± 209.7 g vs. 336.9 ± 149.8 g, $p = 0.011$), and calcium (896.8 ± 581.6 mg vs. 719.5 ± 793.1 mg, $p = 0.041$).

## 4. Discussion

Despite the steady increase in studies evaluating bone loss in HIV patients, most have not included adolescents. To our knowledge, this is the first study evaluating factors related to low BMD in adolescents perinatally infected by HIV-1 in Latin America.

Adolescence is characterized by an accelerated bone turnover, but formation must exceed resorption to guarantee a progressive bone gain until peak bone mass is achieved. Any chronic disease during this period may compromise bone gain or even induce bone loss.[12] This may be true in HIV-infected adolescents exposed to malnutrition, opportunistic infections, and long-term antiviral therapy.

The current WHO definition of osteopenia and osteoporosis in adults cannot be applied to children and adolescents because they have not reached peak bone mass. For this reason, some authors prefer not to classify bone status in children or adolescents.[13] Our patients were classified as having low BMD according to the 2007 International Society for Clinical Densitometry Pediatric Official Positions.[8] We found a high prevalence of low BMD among vertically HIV-infected adolescents, as almost a third of the patients had a low BMD $Z$-score at LS and/or TB. In 28 normal female adolescents of similar age range, ethnicity, social conditions, and nutritional status we previously identified that only two had BMD $Z$-scores at or below −2 SD,[14] which is very different from the prevalence of low BMD in these HIV-infected patients. Different criteria are applied in studies with young seropositive patients, which make it difficult to compare these with our findings. Gafni et al.,[12] studying children 8–16 years of age, found that 40% had low BMD $Z$-scores (<−2 SD), however measurements included not only LS and TB but also the femoral neck. Ramos et al.,[15] studying 35 vertically HIV-infected children, found that 40% of the patients had osteopenia (BMD $Z$-score <−1 SD for LS). McComsey and Leonard,[16] studying 23 vertically infected children, found that 48% had osteoporosis (BMD $Z$-scores ≤−2 SD) and 26% met the criteria for osteopenia (BMD $Z$-scores <−1 and >−2 SD) at LS.

HIV infection of osteoblasts may be related to a negative balance of bone remodeling.[5,17] The BMD of naïve HIV-infected patients seems to be lower than that in healthy controls, but this difference disappears after adjustment for age, height, lean mass, and fat mass.[13] In our sample, only six patients were not using ART and only one had a low BMD, a proportion apparently lower than that seen in the treated group (23 out of 68). However, the small sample precludes any further analysis.

Bone loss is expected after initiation of ART in adult populations,[18] but there is a lack of longitudinal studies regarding the impact of ART on BMD in young populations. A comparison between HAART-treated and HAART-naïve children has shown that HAART was a risk factor for low BMD.[13] PIs have been related to increased bone turnover, accelerated bone loss, and a higher prevalence of reduced BMD.[12,18] Some studies comparing bone loss with PIs or NNRTIs have associated the use of PIs with a greater loss of BMD,[17–19] but other studies have failed to show such a difference.[20,21] Although in our study a comparison between PIs and NNRTIs was not possible because of the small number of patients using NNRTIs, patients on PIs tended towards lower LS BMD, but not at TB. This could be explained by the higher turnover rates in trabecular bone, mainly involved in the maintenance of mineral homeostasis which predominates in vertebrae, when compared to the cortical bone responsible for support functions which composes most of the human skeleton.[5]

TDF has been associated with renal and bone toxicity in children,[22] with decreased renal tubular phosphate reabsorption leading to hypophosphatemia and decreased bone mineralization,[23] but the impact on BMD is still controversial. In a study of a 48-week treatment in children and adolescents, TDF was associated with a >6% decline in BMD $Z$-score in a third of the subjects. Decreases in BMD correlated with young age, suggesting that these patients may be at greater risk of bone toxicity,[22]

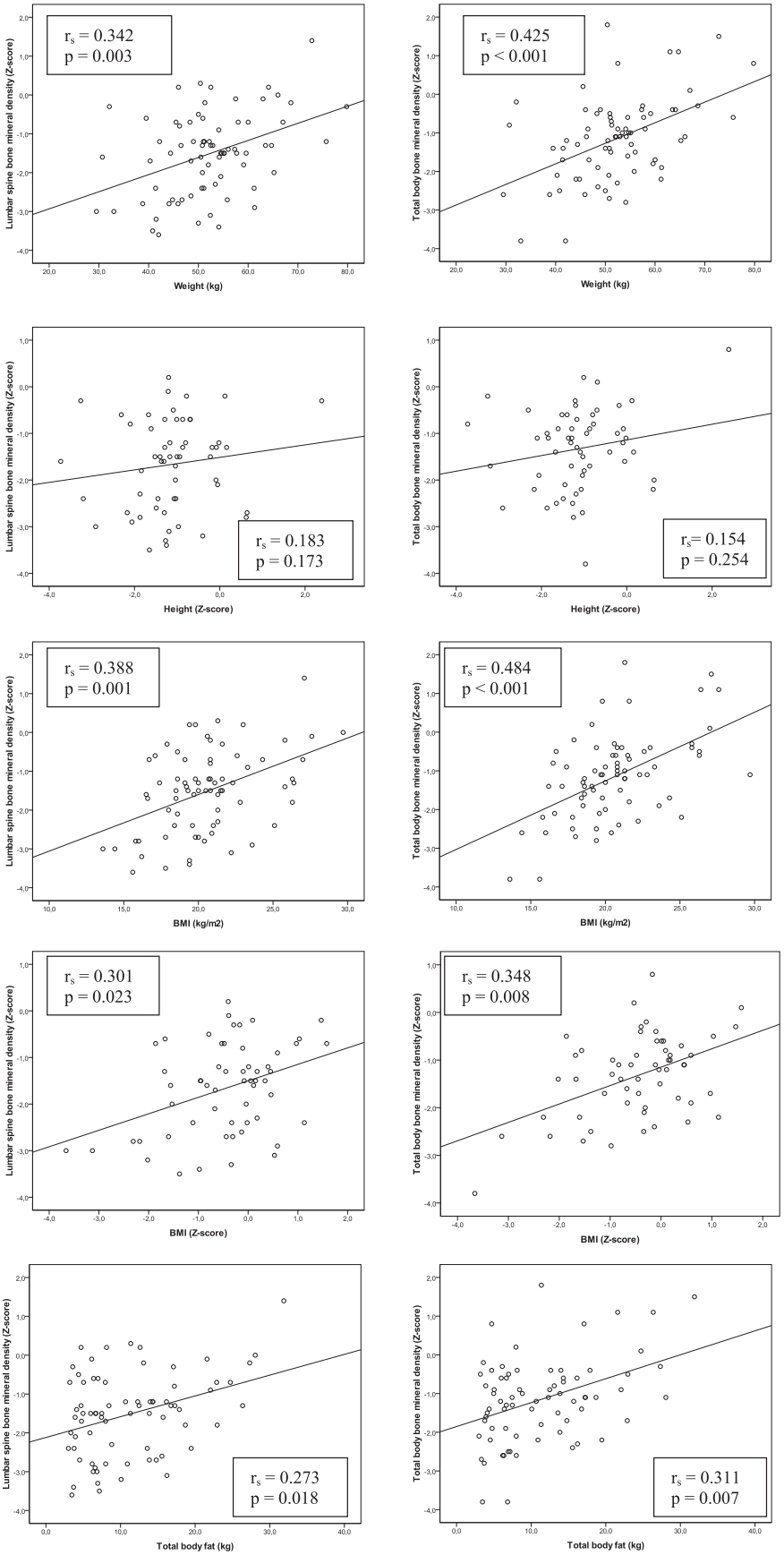


**Figure 1.** Scatter plot and correlation coefficients from parameters that influence bone mineral density in a Brazilian cohort of vertically HIV-infected adolescents.

although a full recovery can occur with TDF withdrawal.[24] Another study found low BMD in 40% of children treated with TDF.[12] Results of the present study reinforce the relationship between TDF and low bone mass, despite no significant alteration in phosphate metabolism. In fact, we found higher serum phosphorus among patients with low bone density, but the reason for this finding is unknown. We also found that time on TDF showed a trend to correlate with low BMD *Z*-scores. On the other hand, some studies have failed to show any correlation between TDF and low bone mass, even after long-term exposure to the drug.[25,26]

NRTIs can reduce BMD by elevating lactic acidemia, a mechanism related to calcium hydroxyapatite loss, especially in the trabecular bone, due to the lability of calcium storage.[27] Our study did not find differences in BMD *Z*-scores when comparing adolescents treated with two NRTIs before the introduction of HAART.

Several studies have directly highlighted HIV factors associated with low BMD, especially: duration of infection, HIV viral burden, and a more advanced HIV disease (reflected by a lower CD4+).[28] Our patients who achieved an undetectable viral load at an earlier age tended to have a better TB BMD *Z*-score, suggesting that control of the HIV infection, especially before the initiation of puberty, might have a positive influence on bone gain. Recent treatment guidelines for HIV-infected children suggest the initiation of HAART before 1 year of age, but early initiation of treatment can expose children to the negative influence HAART has on bones. Thus it will be interesting in the future to assess BMD in this group of children to evaluate the impact of earlier treatment on bone health.

Vitamin D and PTH are also related to bone health. Vitamin D deficiency has the potential to cause osteomalacia or accelerated bone loss due to secondary hyperparathyroidism.[29] In our sample these hormones did not seem to influence BMD.

Delayed sexual maturity has also been observed in perinatally infected children and adolescents.[30,31] During pubertal development a significant gain in bone mass is directly correlated to age, height, and weight through childhood, and differences between genders become evident. Both the starting age of the pubertal spurt and growth process are earlier in girls, but the duration of the growth spurt and the maximal peak of growth are greater in boys.[32] Approximately one-third of bone mass is accumulated between pubertal stages 2 and 5, especially in cancellous bone.[33] A study comparing HIV-infected adolescents and controls showed no difference in bone mass at Tanner stage 1–2 for either boys or girls, but a pronounced difference in later Tanner stages in boys. According to that study, infected boys and girls may acquire a similar amount of bone mass as those not infected, but at a later age.[31] In our study there was no control group. However, there was a trend to lower BMD *Z*-scores in early pubertal stages as compared to stage groups 3–4 and 5, suggesting that pubertal delay was an important factor for these HIV patients.

Many confounding factors are assumed to play a role in the pathogenesis of decreased bone mass in HIV/AIDS patients.[34] Studies evaluating specific body composition determinants and bone mineral show contradictory results. A 10-year longitudinal study found that the fat-free mass is the most important component of body composition that influences LS bone in young adulthood.[35] However, according to Reid,[36] between the determinants of BMD in humans, low weight appears to be the major component, and this relationship has also been identified in HIV patients.[4,37] Our results are in agreement with these findings, showing a positive influence of body composition on BMD. Weight, BMI, and body fat were associated with BMD, especially in females. A possible explanation for this finding could be the higher distribution of Tanner stage 5 among females when compared to males. These findings reinforce the importance of a healthy nutritional status in adolescents perinatally infected by HIV-1 to prevent low BMD.

Food consumption is another important factor for bone mineral accrual, especially because low calcium and vitamin D intake reduce BMD.[38] We detected important inadequacies in the intake of bone health-related nutrients in the adolescent HIV population, although calcium and vitamin D intake were similar to that described in other young Brazilian populations.[14,39] Overall nutritional status had a positive influence on bone and this might explain the trend for higher BMD in those patients with undetectable viral load, who also had a better diet in relation to energy, carbohydrate, and calcium. Dietary intake was determined by a single 24-h recall, so these values are only an estimate. Larger studies are clearly needed to better characterize the habitual intake of this population. Another limitation of our study is the lack of information regarding physical activity in the group studied.

In conclusion, we detected a high prevalence of low BMD among vertically HIV-infected adolescents. Among all possible risk factors demonstrated in the literature for bone loss in HIV patients, in our sample low BMD was associated with body composition, especially in females, and to the use of TDF. Besides that, control of the HIV infection, especially before the initiation of puberty, might have a positive influence on bone gain.

All HIV-infected adolescents in whom undernutrition is clinically observed should be screened for low BMD. This will lead to the identification of patients at great risk of skeletal fractures and guide treatment decisions. Attempts to improve nutritional status and an adequate intake of calcium and vitamin D may be beneficial to those with low BMD, which in turn correlates with a longer life expectancy and a better quality of life.

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