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Bone mass in children and adolescents infected with human immunodeficiency virus☆

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KEYWORDS
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Child;
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Life style

Abstract

Objective: To describe bone mineral density (BMD) and bone mineral content (BMC) in children and adolescents infected with the human immunodeficiency virus (HIV), and to compare them with data from the National Health and Nutrition Examination Survey IV (NHANES IV).

Method: The study included 48 children and adolescents (7 to 17 years old) infected with HIV through vertical transmission. BMC and BMD were measured by dual energy absorptiometry X-ray, by calculating z-scores based on data from NHANES IV. The information on clinical and laboratory parameters of infection by HIV was obtained from medical records. Physical activity, calcium intake, and skeletal maturation were also assessed. Descriptive and inferential statistical procedures were used, with levels of significance set at 5%.

Results: Seropositive patients presented lower values compared to data from NHANES IV in all z-scores of bone mass (mean = −0.52 to −1.22, SD = 0.91 and 0.84, respectively). Based on the subtotal z-BMD, there was a prevalence of 16.7% of children and adolescents with low bone mass for age. Individuals using protease inhibitors presented a lower total z-BMD when compared to the group that did not use (−1.31 *vs.* −0.79, p = 0.02). There were no bone mass differences in relation to physical activity and calcium intake.

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Conclusions: In the present sample children and adolescents living with HIV have low bone mass for age, and the use of protease inhibitors appears to be related to such decreases.

PALAVRAS-CHAVE
Densidade óssea;
Criança;
Adolescente;
Vírus da imunodeficiência humana;
Síndrome da imunodeficiência adquirida;
Estilo de vida

Massa óssea em crianças e adolescentes que vivem com vírus da imunodeficiência humana

Resumo
Objetivo: Descrever a densidade mineral óssea (DMO) e conteúdo mineral ósseo (CMO) de crianças e adolescentes que vivem com o vírus da imunodeficiência humana e comparar com os dados do *National Health and Nutrition Examination Survey* IV (NHANES IV).
Método: Participaram do estudo 48 crianças e adolescentes (sete a 17 anos de idade) com infecção pelo vírus da imunodeficiência humana adquirida por transmissão vertical. A DMO e o CMO foram mensurados pela absorciometria por dupla emissão de raios-X, calculando-se escores-z com base nos dados do NHANES IV. Nos prontuários médicos foram obtidas as informações dos parâmetros clínicos e laboratoriais da infecção pelo vírus da imunodeficiência humana. Foram ainda avaliadas a atividade física, a ingestão de cálcio e a maturação esquelética. Utilizaram-se procedimentos da estatística descritiva e inferencial, estabelecendo níveis de significância de 5%.
Resultados: Os pacientes soropositivos demonstraram valores inferiores comparados aos dados do NHANES IV em todos os escores-z da massa óssea (média = −0,52 a −1,22, dp = 0,91 e 0,84, respectivamente). Com base no z-$DMO_{subtotal}$, há uma prevalência de 16,7% de crianças e adolescentes com massa óssea reduzida para a idade. Indivíduos que utilizaram inibidores de protease apresentaram um z-DMO_{total} inferior, comparado ao grupo que não utilizou (−1,31 vs. −0,79; p = 0,02). Não foram encontradas diferenças na massa óssea em relação ao nível de atividade física e ingestão de cálcio.
Conclusões: Na presente amostra, crianças e adolescentes que vivem com o vírus da imunodeficiência humana possuem baixa massa óssea para idade, e o uso de inibidores de protease parece estar relacionado a tais reduções.

Introduction

After the advent of antiretroviral therapy (ART), a reduction in morbidity and mortality due to human immunodeficiency virus (HIV) infection was observed. However, side effects of ART have been reported, such as lipodystrophy, metabolic disorders, and bone mass alterations.[1] In young individuals infected with HIV through vertical transmission, prolonged drug exposure may potentiate these effects, and significant reductions in bone mineral density (BMD) and bone mineral content (BMC) have been reported.[2-7] Despite the controversies, the factors that contribute to these decreases are calcium intake deficit, ART that includes protease inhibitors (PI), specific antiretrovirals (stavudine-d4T, tenofovir [TDF], and ritonavir [RTV]), advanced stages of infection, and lipodystrophy.[3,5,8,9] Although the benefits of regular physical activity in maintaining bone health is known, few studies have considered this variable in relation to BMD/BMC.[3,6,7]

Peak bone mineral accrual occurs mainly during puberty;[10] thus, concerns about decreases in bone mass in children and adolescents living with HIV is increased, as they could develop early complications

This study aimed to describe the BMD and BMC of children and adolescents infected with HIV through vertical transmission, and to compare these values with those of healthy individuals, taking into account age, gender, and ethnicity. BMD was also investigated as a function of calcium intake, level of physical activity, and the inclusion of PI in ART.

Methods

Ethical aspects

The research followed the ethical guidelines outlined in the Declaration of Helsinki and was approved by the Research Ethics Committee of the Hospital Infantil Joana de Gusmão (HIJG) (No. 077/2009). Parents or legal guardians signed the informed consent.

Subjects

The study sample consisted of 48 children and adolescents with HIV infection acquired by vertical transmission, of both genders, aged between 7 and 17 years, living in the city of Florianópolis, capital of the state of Santa Catarina, Brazil. Individuals were selected in a non-probabilistic way from the Specialized Care Service of the Hospital Dia - HIJG.

The following inclusion criteria were adopted: undergoing treatment at HIJG; availability of clinical and laboratory information in the medical record; absence of other diseases or medications that could alter body composition, considering that the main study investigated the alterations of three components of body composition.

Study variables, instruments and standardizations

Socio-demographic and anthropometric data

During an interview, information was collected on age, gender, and ethnicity of children and adolescents infected with HIV, as well as on the socioeconomic and educational level of parents or guardians. Height and body mass were measured by standardized procedures,[11] using a Tonelli wall stadiometer (120A – Criciúma, Brazil) with an accuracy of 0.1 cm, and a Tanita digital scale (BF683W – Arlington Heights, USA) with an accuracy of 0.1 kg. The measurements were performed twice by the same examiner, with technical errors of measurement of 0.19 cm and 0.51 kg for height and body mass, respectively. Nutritional status was assessed by the z-score, based on the parameters of height/age and body mass index (BMI)/age.[12]

Bone mass

Bone mass was measured through dual emission X-ray absorptiometry (DXA) on a Hologic device (Discovery WI Fan-Bean – Bedford, USA); the attenuation of X-rays was computed using a pediatric software. This method is safe, as the devices emit radiation from 4.2 to 5.2 μSv, equivalent to one day of sunshine. It has high concurrent validity, reproducibility, and technical accuracy when measuring bone mass.[13] In this study, the internal quality control included daily calibrations, according to the manufacturer. The CV of total BMD during the study period was 1%. During the assessments, the subjects used appropriate clothing, without shoes, earrings, and/or rings.

Based on the DXA results, the total and subtotal BMD and BMC variables were considered (except the head). The subtotal is a good measure of accuracy and reproducibility, as the total BMD/BMC can dilute the alterations in bone mass.[14] For logistical reasons, the specific parameters of the spinal column, femoral head, and 33% radius were not evaluated. Z-scores were calculated from the LMS values published by Kelly et al.,[15] using the supplement LMS Growth for MS Excel, developed by Pan & Cole. Brazilian data in the literature do not allow the determination of the z-score by age, gender, and ethnicity.[16]

Human immunodeficiency virus-infection clinical data

Medical records provided clinical (type/time of ART, immunological and clinical symptoms classifications) and laboratory data (HIV RNA viral load, and TCD4+ and TCD8+ lymphocytes) of the HIV infection. Participants were classified into the categories of immunosuppression and clinical symptoms of HIV evolution according to the propositions of the Centers for Disease Control and Prevention (CDC).[17] HIV RNA viral load was determined by the branched DNA method (b-DNA), and TCD4+ and TCD8+ lymphocytes were determined by flow cytometry.

Control and lifestyle variables

Bone age was determined through the radiographic evaluation of the left hand and fist, estimated by the Greulich-Pyle method,[18] performed by an HIJG radiologist with extensive experience with the method.

The level of physical activity was measured objectively by a motion sensor (pedometer; [Digi-Walker SW200 – YamaxCorp, Tokyo, Japan]), used for five days, two of them during the weekend. The use of the pedometer was standardized on the right side of the waist above the iliac crest, and patients started to use it in the morning, removing it only for activities in the water or bath/shower. At the end of the day, the subjects and/or guardians wrote down the number of recorded steps. This method has a high correlation with accelerometry (r = 0.86) and a moderate one with indirect measurements of energy expenditure (r = 0.64).

Individuals were included with data for at least four days. Five subjects did not meet this criterion and thus a gender-specific median value of steps was used. In the absence of physical activity level classification for the infected population, the proposed cutoff of 13,000 and 11,000 steps/day for healthy boys and girls, respectively, was used. For teenagers, the cutoff was 10,000 steps/day.[19] These values, on average, correspond to 60 minutes of moderate-vigorous physical activity, which classifies youth as either active or insufficiently active.[19]

Calcium intake was assessed using a simplified version of the food frequency questionnaire,[20] which shows moderate validity in estimating calcium (r = 0.70), adjusted for intra and interpersonal variability.[20] The questionnaire was administered to the subjects or guardians (for those younger than 13), during an interview with a nutrition student. The standardization of portion sizes was established in accordance with the suggestion of Pinheiro et al.[21] To determine the nutritional value of foods, the Brazilian Table of Food Composition and the USDA National Nutrient Database for Standard Reference were used.[22,23]

Bone diameter was used to control differences in BMD related to body and bone size. It has been suggested that DXA is incapable of detecting bone edges in subjects that are still growing.[24] Thus, femur diameter measurements followed standardizations[11] and were obtained using a digital caliper (Digimess – São Paulo, Brazil) with accuracy of 0.01 mm. The technical error of measurement was found to be 0.55 mm.

Statistical analysis

A Gaussian distribution was established by the Shapiro-Wilk's test, in addition to the analysis of graphic representation. Asymmetric data were normalized using $\log_{10}$ transformation. Descriptive statistics procedures were used in the presentation of data (measurements of

central tendency, dispersion, and relative and absolute frequencies). In inferential statistics, Student's *t*-test, the chi-squared test, and analysis of covariance (ANCOVA) were performed, adjusting for bone age, body mass, diameter of the femur, physical activity, calcium intake, duration of ART, and use of PI in ART. The Statistical Package for Social Sciences (SPSS) was used for statistical analyses and the significance level was set at 5% ($\alpha \leq 0.05$ or 95% CI).

Results

Most children and adolescents infected with HIV were under the guardianship of the biological family (64.6%), 20.8% lived with an adoptive family, and a small number (14.6%) lived under the tutelage of the state. The guardians had a degree of educational level up to incomplete elementary school in 22.9% of the cases, and only 6.3% reported complete college/university education. A *per capita* monthly income of up to two Brazilian minimum wages was reported by 47.9% of the guardians, while 37.5% reported an income higher than two Brazilian minimum wages.

Table 1 shows demographic and lifestyle data. In the studied group, there was a similar distribution between genders and ethnicity. The mean negative difference between bone and chronological age suggests delay in skeletal maturation, with no statistical difference between the genders. Five teenagers presented delayed skeletal maturation, using a cutoff of ≤ −2 standard deviations specific for age and gender.[18] Negative means of the z-score of height for age and of BMI for age were also found, with no statistical difference between the genders.

Regarding lifestyle, inadequate levels of physical activity were observed in 68.8% (n = 33), according to the pedometry criterion. Regarding calcium intake, 29 (60.4%) of the young individuals infected with HIV had inadequate calcium levels, when compared to the dietary reference intakes (DRIs).[25]

In general, the good immunological and virological status of individuals infected with HIV is evidenced (Table 2), due to the absence of immunosuppression or moderate immunosuppression (≈ 75% of the group) and due to undetectable HIV RNA viral load (58.3%, n = 28). Over half of individuals younger than 13 years of age (58.4%) were in the early stages of the disease (N - asymptomatic and A - mild symptoms), and 54.2% of adolescents (> 13 years) presented the symptoms of acquired immunodeficiency syndrome (AIDS). In general, ART was followed by triple therapy, and approximately 10% were using some medication compatible with viral resistance.

Regarding bone mass measurements (Table 3), higher values were observed in adolescents when compared to children (adjusted for gender and BMI, $p < 0.001$) and in males when compared to females (adjusted for age and BMI z-score, $p < 0.05$), reflecting bone mineral increase processes and sexual dimorphism, respectively. Lower values of total BMD z-score of for age (total BMD-z) and subtotal BMD z-score for age (subtotal BMD-z) were found in females when compared to males ($p < 0.05$), a difference that did not remain significant when the subtotal BMD z-score was analyzed for height (subtotal BMD-z/ height). The z-scores of BMC and BMD showed, in general, negative values for all parameters analyzed.

Considering subtotal BMC-z score, four subjects (males) had scores ≤ -2 SD, indicating low bone mineral content for age. When analyzing the subtotal BMD z-score, the prevalence increased to eight cases (16.7%). These z-scores

Table 1 Sociodemographic, clinical, and lifestyle parameters of children and adolescents infected with HIV, Florianópolis-SC, Brazil, 2011.

Variables	Female (n = 24)	Male (n = 24)	Total (n = 48)
Demographic data			
Age, mean (SD)[a]	12.5 (3.1)	12.9 (2.4)	12.7 (2.7)
Ethnicity, n (%)			
White	14 (58.3)	13 (54.2)	27 (53.3)
Black/Mixed-race	10 (41.7)	11 (45.8)	21 (43.8)
Bone age, mean (SD)[a]	12.4 (2.9)	12.3 (3.7)	12.4 (3.3)
Difference BA-CA, mean (SD)[a]	−0.12 (1.20)	−0.42 (1.11)	−0.27 (1.1)
Anthropometric and life style data			
Weight, mean (SD)[a]	39.2 (10.7)	41.3 (14.3)	40.3 (12.5)
Height, mean (SD)[a]	144.5 (13.7)	150.9 (16.3)	147.7 (15.2)
z-BMI/A, mean (SD)[b]	−0.03 (0.77)	−0.50 (0.95)	−0.27 (0.88)
z-H/A, mean (SD)[b]	−0.71 (0.84)	−0.55 (1.02)	−0.63 (0.93)
PA (steps/day), mean (SD)[a,c]	9179 (4539)	11797 (5922)	10488 (5385)
Calcium (mg/day), mean (SD)[a]	1010.4 (406.3)	1102.1 (353.6)	1057.3 (378.1)

BA-CA, bone age minus chronological age; PA, physical activity; SD, standard deviation; z-BMI/A, body mass index z-score for age; z-H/A, height z-score for age.
[a]Student's *t*-test for independent samples.
[b]Analysis of covariance adjusted by bone age (mean of 12.4 years).
[c]Standardized by log-transformation (log_{10}). No significant differences were found between genders in the analyzed variables ($p > 0.05$).

Table 2 Clinical, immunological and virological parameters in children and adolescents infected with human immunodeficiency virus, Florianópolis-SC, Brazil, 2011.

Variables	Female (n = 24)	Male (n = 24)	Total (n = 48)
Infection parameters[a]			
TCD4⁺ lymphocytes (cells/μL)	787 (544-961)	731 (528-1,137)	747 (531-1,088)
TCD4⁺ lymphocytes (%)	31.8 (22.1-37.3)	30.5 (26.4-35.3)	31.5 (23.3-35.6)
TCD8⁺ lymphocytes (cells/μL)	1,093 (810-1,249)	1,082 (715-1,463)	1,089 (746-1,430)
HIV RNA viral load (copies/mL)	49 (49-1,640)	49 (49-491)	49 (49-1,023)
HIV RNA viral load (log_{10})	1.69 (1.69-2.69)	1.69 (1.69-2.69)	1.69 (1.69-3.00)
CDC symptoms[b]			
< 13 years, n (%)			
A	8 (61.5)	2 (18.2)	10 (41.7)
B	–	5 (45.4)	5 (20.8)
C	3 (23.1)	2 (18.2)	5 (20.8)
> 13 years, n (%)			
1	2 (18.2)	1 (7.7)	3 (12.5)
2	2 (18.2)	6 (46.2)	8 (33.3)
3	7 (63.6)	6 (46.2)	13 (54.2)
CDC immunosuppression,[b] **n (%)**			
1	3 (12.5)	6 (25.0)	9 (18.8)
2	15 (62.5)	13 (54.2)	28 (58.3)
3	6 (25.0)	5 (20.8)	11 (22.9)
HIV RNA viral load, n (%)			
Undetectable	13 (54.2)	15 (62.5)	28 (58.3)
50-10,000 copies/mL	10 (41.7)	8 (33.3)	18 (37.5)
> 10,000 copies/mL	1 (4.2)	1 (4.2)	2 (4.2)
Antiretroviral therapy,[c] **n (%)**			
2 NRTI + 1 NNRTI	10 (41.7)	8 (33.3)	18 (37.5)
2 NRTI + 1 PI	11 (45.8)	11 (45.8)	22 (45.8)
Another regimen[d]	2 (8.3)	4 (16.7)	6 (12.5)

CDC, Centers for Disease Control and Prevention; HVI, human immunodeficiency virus; NNRTI, non-nucleoside reverse-transcriptase inhibitors; NRTI, nucleoside reverse-transcriptase inhibitors; PI, protease inhibitors.
[a]Median and interquartile values.
[b]CDC (2008).
[c]Two individuals do not receive antiretroviral therapy.
[d]n = 6, of which: 2 NRTI (n = 2), 1 NNRTI + 1 PI (n = 1), 1 NRTI + 1 NNRTI + 1 PI (n = 1), 2 NRTI + 1 NNRTI + 1 PI (n = 1) and 1 NRTI + 2 PI + 1 integrase inhibitor (n = 1).

were calculated based on specific values for age, gender, and ethnicity.[15]

There was no difference in the proportions of subjects with subtotal BMD z-score ≤ -2 SD between genders or ages. Although the z-scores between ≤ −2 and ≤ −1 SD did not define low bone mass for age, 27 children and adolescents (56.2%) had low values of subtotal BMC-z-score, whereas lower values of subtotal BMD-z-score were observed in 21 subjects (43.7%).

Lower total z-BMD was observed in the group that used PI in ART, regardless of body mass, femur diameter, bone age, physical activity, calcium intake, or ART duration (Table 4).

There were no significant differences in total z-BMD score of children and adolescents infected with HIV, compared by level of physical activity or daily calcium intake ($p > 0.05$). There were no significant differences in total z-BMD between stages N and A *versus* B and C, stages 1 and 2 *versus* 3, undetectable viral load versus viral load > 50 copies/mL, and absence/moderate immunosuppression *versus* severe immunosuppression ($p > 0.05$). All these analyses were adjusted for body mass, femur diameter, bone age, physical activity, daily calcium intake, time of ART, and use of PI in ART.

Discussion

The main finding of this study was the low z-score of bone mass in children and adolescents infected with HIV, when compared to healthy subjects from NHANES IV. The prevalence of low subtotal BMD for age was estimated at 43.7%, considering z-scores < −1 SD.

These data are innovative in the national literature, and reproduce research findings from other countries using absolute values,[2,4] z-score,[3,5,7] or quantitative

Table 3 Bone mass parameters in children and adolescents infected with human immunodeficiency virus, Florianópolis-SC, Brazil, 2011.

Bone mass	Infected with HIV (n = 48)								
	Children (n = 11)	Adolescents (n = 37)	F[a]	p	Male (n = 24)	Female (n = 24)	F[b]	p	Total (n = 48)
	Mean (standard deviation)								
BMC[c] (g)									
Subtotal[d]	605.7 (118.6)	1,148.3 (366.7)	37.782	< 0.001	1,080.3 (462.1)	967.7 (324.1)	2.902	0.096	1,024.0 (398.9)
Total	882.3 (142.7)	1,512.5 (419.8)	32.563	< 0.001	1,429.2 (519.3)	1,306.9 (392.0)	3.026	0.089	1,368.0 (459.3)
Subtotal/age) (z-score)	−0.87 (0.85)	−1.03 (0.78)	2.518	0.120	−1.05 (0.83)	−0.93 (0.76)	0.209	0.650	−0.99 (0.79)
Subtotal/height (z-score)	-0.91 (1.09)	−0.44 (0.85)	0.442	0.510	−0.53 (1.08)	−0.51 (0.70)	0.590	0.447	−0.52 (0.91)
BMD ($g \cdot cm^{-2}$)									
Subtotal[b]	0.602 (0.07)	0.817 (0.11)	28.761	< 0.001	0.791 (0.15)	0.744 (0.12)	7.096	0.011	0.768 (0.14)
Total	0.729 (0.06)	0.932 (0.11)	21.811	< 0.001	0.905 (0.14)	0.867 (0.13)	4.200	0.046	0.886 (0.13)
Subtotal/age (z-score)	−1.33 (0.92)	−1.19 (0.83)	0.754	0.390	−1.05 (0.79)	−1.40 (0.88)	7.591	0.008	−1.22 (0.84)
Total/age (z-score)	−1.38 (1.27)	−1.01 (0.80)	0.049	0.825	−0.89 (0.93)	−1.30 (0.89)	6.438	0.015	−1.10 (0.92)
Subtotal/height[b] (z score)	−0.66 (1.24)	−0.56 (0.93)	0.021	0.886	−0.76 (1.15)	−0.38 (0.70)	0.333	0.567	−0.58 (0.97)

BMC, bone mineral content; BMD, bone mineral density; BMI, body mass index; HIV, human immunodeficiency virus.
[a]Analysis of covariance in children and adolescents adjusted for sex and BMI.
[b]Analysis of covariance between genders adjusted for age, and BMI z-score.
[c]Normalized by log transformation (log_{10}), except for z-score.
[d]Subtotal = Total except the head.

Table 4 Analysis of covariance of the total BMD z-score in HIV-infected children and adolescents using protease inhibitor antiretrovirals in the treatment, Florianópolis, Florianópolis-SC, Brazil, 2011.

Total BMD Z-score	Infected by HIV			
	HAART without PI (n = 19)	HAART with PI (n = 26)	F*	p
Crude mean (SE)	−0.84 (0.19)	−1.26 (0.17)	2.717	0.106
Confidence interval	−1.23; −0.44	−1.61; −0.92		
Adjusted mean (SE)	−0.79 (0.16)	−1.31 (0.15)	5.433	0.025
Confidence interval	−1.11; −0.46	−1.60; −1.01		

BMD, bone mineral density; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; PI, protease inhibitor; SE, standard error.
*Analysis of covariance adjusted by body mass, femur diameter (mean = 85.4 mm), bone age, physical activity, calcium ingestion, and time of HAART (mean = 8.5 years). Total BMD z-score: z-score of total bone mineral density for age.

ultrasound measurements.[6] However, the results found are in disagreement with two studies. The first found low bone mass only through computed tomography assessment, inferring the inability of DXA to assess bone mass in subjects with small bones.[24] For this reason, femur diameter was used in the present study to adjust the values for bone size. The second study found no decreases in bone mass in a small number of antiretroviral treatment-naïve children who were infected through horizontal transmission,[26] probably due to the fact that their health status, growth, and maturation were little affected by the short time of exposure to HIV, in addition to the absence of antiretroviral therapy, which would promote greater bone integrity.

HIV infection and the host's response are involved in alterations in bone mass. In animal models, bone remodeling was induced by high activity of C-terminal collagen telopeptide and an increase in the number of osteoclasts, given the high activity of the receptor activator of NF-kB ligand.[27] *In vitro* analyses have demonstrated that HIV-specific proteins interfere in the differentiation of mesenchymal stem cells into osteoblasts; they also reduce the secretion of regulatory molecules and transcription factors activity of osteoblasts.[28] Additionally, HIV infection reduces the production of IGF-1.[6,7] HIV increases the production of interleukin-6 and stimulates the formation and activity of osteoclasts through the autocrine/paracrine factor, reducing bone mass.[6,7]

Nearly half of the children and adolescents in the sample had bone mass < -1 SD when compared to data from NHANES IV. However, this prevalence should be viewed with some caution. Kocks et al.[29] demonstrated that several databases used to build the z-scores can be highly correlated, but the values may differ significantly, varying in the proportion of subjects with low bone mass for age (15.4% to 27.9%).[29] Regarding the data from NHANES IV,[15] as they derive from a representative sample of healthy young individuals and are shown by age, gender, and ethnicity, they allowed for the calculation of specific z-scores. This strategy enabled a further discriminated analysis of bone health in children and adolescents infected with HIV.

In the present study, the mean total BMD z-score was significantly lower in the group that used PI in ART, when compared to those without PI, regardless of body mass, femur diameter, bone age, level of physical activity, calcium intake, and time of ART (Table 4). The deleterious effect of PI on bone mass has been demonstrated by the alteration of gene expression, which induces local inflammatory activity and the consequent reduction in the activity of osteoblasts exposed to ritonavir.[30] Exposure to lopinavir (LPV/r) produces a decrease in gene expression and activity of the alkaline phosphatase enzyme of osteoblasts, as well as a decrease in calcium deposits and an increase in osteoclast activity.[31]

In this study, 25 of the 26 subjects in the group that included PI in ART received low-dose ritonavir in addition to LPV/r. This specific PI did not demonstrate significant reductions in BMC in one study;[9] however, the results of the same study[9] showed reductions in BMC of the lumbar spine of those who received full-dose ritonavir. Furthermore, patients who received PI in this sample also received d4T and TDF (3/26 and 5/26, respectively), indicated as modulators in bone mass decrease.[8,9] These antiretrovirals were present only in ART with PI, so it can be assumed that the reductions found in the group that uses PI with ART may have been influenced by the three associated antiretrovirals (LPV/r, d4T, and TDF).

There were no significant differences in bone mass of the subjects regarding the physical activity recommendations, when controlled for other covariates. The positive association between physical activity and bone mass in healthy subjects is demonstrated by performing high-intensity activities, or with high gravitational or muscular load, promoting osteogenic stimulus.[32] In this context, some possible limitations that may explain the findings are that the specific sites in bone mass were not assessed, the measured physical activity represented only the period of data collection, the pedometry cut-off is nonspecific in the HIV group, and the size of the analyzed group may have reduced the power of the statistical analyses.

Meeting the recommendations for daily calcium intake showed no significant influence on bone mass. O'Brien et al.[5] found reductions in bone mass in a group of girls infected with HIV, which were attributed to inadequate calcium intake (20% to 50% below the recommendations) and to a multifactorial process. Calcium intake is usually determined through the use of the 24-hour food recall.

In the present study, this variable was estimated using a semiquantitative food frequency questionnaire, which despite the moderate validity (r = 0.70),[20] may have underestimated values.

There were no differences in bone mass due to the evolution of clinical symptoms of HIV infection, confirming some findings in the literature.[4,5] However, other studies[3,6] found that individuals in advanced stages present reductions in BMD and impaired bone quality and metabolism. In the present study, it was necessary to divide the group into different age groups (children [< 13 years] and adolescents [> 13 years])[17] to meet the infection classification system adopted by the CDC. There was no difference in bone mass in relation to immunosuppression, confirming the findings of Mora et al.[4] The status of HIV RNA viral load (undetectable *versus* > 50 copies/mL) also did not affect BMD. Rapid immunological and virological alterations can occur due to success or failure of the pharmacological interventions, whereas alterations in bone mass are slower and require approximately six months to be detected;[14] this may partly explain the findings.

The present study has other limitations that should be considered when interpreting the data: the cross-sectional design does not allow causality inference; the evaluated subjects represented, on average, individuals with good clinical and demographic characteristics, which restricts, in part, the external validity of the findings. However, the decrease in bone mass remains clear and the findings are quite distressing, as lower values tend to remain reduced with increasing age and, moreover, the highest bone mineral increase occurs in childhood and adolescence[10] and interruptions to this process may determine increased risk of osteopenia and early osteoporosis. Prolonged use of ART may occur in HIV infection, potentiating the reduction in bone mass.

This study reinforces the low bone mass for age in children and adolescents infected with HIV, regardless of the bone parameter used, when compared to data from NHANES IV. The use of PI in ART is related to reductions in bone mass, regardless of confounding factors. No differences were observed in bone mass in relation to physical activity and daily calcium intake; however, a small percentage of subjects who met both recommendations was observed in the group. Interdisciplinary interventions are necessary to promote physical activity and adequate nutrient intake in order to minimize the impact of HIV infection and ART, thus contributing to bone health.

Conflicts of interest

The authors have no conflicts of interest to declare.

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