Assessing bone mineral density in children and adolescents living with HIV and on treatment with tenofovir disoproxil fumarate: a systematic review

Avaliação da densidade mineral óssea em crianças e adolescentes que vivem com HIV e em tratamento com tenofovir disoproxil fumarato: uma revisão sistemática

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ABSTRACT

Objective: To investigate the impact of tenofovir disoproxil fumarate on bone mineral density and bone mineral content in children and adolescents infected with the human immunodeficiency virus.

Data source: The search procedure was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement. The search was carried out until April 2022 in Medical Literature Analysis and Retrieval System Online (Medline), Embase, Cochrane Central, Latin American and Caribbean Health Sciences Literature, Web of Science, Scopus, and MedRxiv. The combination of terms used was: (Children OR Youth OR Teenagers) AND HIV AND (Tenofovir OR "Antiretroviral therapy") AND ("Bone density" OR Osteoporosis OR Osteopenia). The protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO, CRD42022312851)

Data synthesis: The initial searches resulted in 1156 papers. After the exclusion of duplicate studies, three blinded reviewers analyzed the title and abstract of 563 papers, of which 57 remained to be read in full. Only nine papers met the eligibility criteria and were included in descriptive and risk-of-bias analyses. Regarding study design, four were cross-sectional, three were longitudinal before-after studies without a control group, and two were prospective cohorts. Among these nine papers, seven showed no significant association between tenofovir disoproxil fumarate use and reduced bone mass in young people. However, these papers did not have high methodological quality.

RESUMO

Objetivo: Investigar o impacto do tenofovir disoproxil fumarato sobre a densidade mineral óssea e o conteúdo mineral ósseo em crianças e adolescentes infectados pelo vírus da imunodeficiência humana.

Fontes de dados: O procedimento de busca foi executado de acordo com o Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement. A busca foi realizada até abril de 2022 nas seguintes bases de dados: Medical Literature Analysis and Retrieval System Online (Medline), Embase, Cochrane Central, Literatura Latino-Americana e do Caribe em Ciências da Saúde (Lilacs), Web of Science, Scopus, and MedRxiv. A combinação de termos utilizada foi: (Children OR Youth OR Teenagers) AND HIV AND (Tenofovir OR "Antiretroviral therapy") AND ("Bone density" OR Osteoporosis OR Osteopenia). O protocolo foi registrado na base International Prospective Register of Systematic Reviews — PROSPERO (CRD42022312851).

Síntese dos dados: As pesquisas iniciais resultaram em 1.156 artigos. Após a exclusão dos estudos duplicados, três revisores cegos analisaram título e resumo de 563 estudos, dos quais 57 permaneceram para leitura na íntegra. Somente nove artigos atenderam aos critérios de elegibilidade e foram incluídos para análises descritivas e de risco de viés. Com relação ao desenho dos estudos, quatro foram transversais, três foram estudos longitudinais antes-depois sem grupo controle e dois foram coortes prospectivas. Dos nove artigos, sete não mostraram associação significativa entre uso de TDF e redução de massa óssea em pessoas jovens. Entretanto, esses estudos não tiveram alta qualidade metodológica.

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Conclusions: Although most of the selected papers found no harmful effect of tenofovir disoproxil fumarate on bone mass, further primary research with higher methodological quality is needed so robust scientific evidences can be obtained.
Keywords: Child; Adolescent; HIV; Bone density; Tenofovir.

Conclusões: Embora a maioria dos estudos selecionados não tenha encontrado efeito prejudicial do TDF sobre massa óssea, novas pesquisas primárias com maior qualidade metodológica são necessárias para que sejam obtidas evidências científicas robustas.
Palavras-chave: Criança; Adolescente; HIV; Densidade óssea; Tenofovir.

INTRODUCTION

The human immunodeficiency virus (HIV) infection is an epidemic disease among children and adolescents, and around 2.8 million people infected are in this age group (0–19 years).[1] In 2020, an estimated 300,000 new children and adolescents acquired HIV worldwide.[1]

Tenofovir disoproxil fumarate (TDF) is one of the drugs used in antiretroviral therapy (ART). The Food and Drug Administration (FDA) approved its use in children and adolescents from two years of age. In Brazil, however, it is only part of the preferred therapy regimen for youth over 12 years of age.[2,3] For children from 3–12 years old, it is indicated as part of alternative therapy.[2,3]

In this age group, bone development is crucial, because more than 90% of the bone mass is obtained till the age of 20.[4] During infancy, bone mineral deposition occurs slowly.[5] From puberty and growth spurt, the rate of bone formation becomes fast until it reaches a peak, which will constitute a reserve of bone quantity and quality.[5-7] The peak of bone mineral density (BMD) occurs around age 25; however, it can vary according to the skeleton site.[5,8,9]

For every 10% increase in bone mass in adolescence, the risk of fractures is reduced by 50% and the development of osteoporosis is reduced by up to 13 years.[8] Therefore, people who come into adulthood with low bone mass will have a higher risk of bone fracture.[10] A lower peak of BMD is a problem, especially in girls, because pregnancy and lactation periods mobilize bone calcium to meet fetal growth needs and produce the mother's breast milk, which predisposes to a higher risk of osteoporosis.[11] Furthermore, women face a drop in estrogenic hormones after menopause causing higher rates of bone reabsorption.[9] Although in men the BMD loss starts later than in women, the gain in bone mass during their puberty is also a protective factor against osteoporosis — a disease with a high prevalence in both genders.[6]

Among HIV-infected individuals, the causes of low bone mass are multifactorial, involving smoking, physical inactivity, inadequate diet, the infection itself, chronic activation of the immune system, and adverse effects of antiretroviral therapy.[12] The virus promotes a proinflammatory condition that is not completely reversed by ART and causes increased reabsorption relative to bone formation.[12,13] Furthermore, in vitro studies showed that proteins present in HIV stimulate osteoclastic activity and suppress osteoblastic activity.[14-16]

The TDF is a tenofovir prodrug from the nucleoside reverse transcriptase inhibitor class, which inhibits HIV proliferation.[17] In murine models, it can decrease extracellular adenosine concentration, which increases osteoclast differentiation and causes osteopenia.[17] Furthermore, TDF is thought to be associated with bone mass loss due to its toxicity in the proximal renal tubule with consequent phosphate loss.[18] Clinical studies in adults have associated its use with reduced BMD.[19,20]

Because of TDF potential effect of downregulating bone turnover, it is hypothesized that there is an association between its use and reduced bone mass. However, in children and adolescents, the effect of TDF on bone mass and its clinical significance are still controversial.[14,18,21] In this context, a systematic review is necessary to analyze the scientific evidences available in the literature and to support future research on this topic. Therefore, the present paper aimed to answer the following research question: what is the impact of TDF on bone mineral content (BMC) and BMD in children and adolescents living with HIV?

METHOD

This paper is a systematic literature review, outlined according to the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).[22] Prior to the systematic review development, a protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO), record: CRD42022312851, available at: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022312851.

Longitudinal-observational studies, clinical trials, and cross-sectional studies that examined the effects of ART containing TDF on measures of bone mass in children and adolescents living with HIV were included in the eligibility criteria.

Adolescents are considered persons aged 10–19 years, as defined by the World Health Organization.[23] The bone mass measures considered for inclusion in the present study were BMD and BMC, both represented mainly in Z-score and measured by dual-energy X-ray absorptiometry (DXA). Exclusion criteria were: studies available only in the form of conference abstracts, review studies, case reports, studies that included children and adolescents with HIV and hepatitis B virus coinfection, patients with previous renal, bone, or endocrine diseases, use of vitamin D or non-antiretroviral drugs that affect bone metabolism. There were no exclusions regarding the language or period of the studies.

The searches for the studies to be reviewed were performed in the following online databases: Medline (in PubMed, 1966–2022), Embase (in ELSEVIER, 1947–2022), Cochrane Central (Central, until 2022), Lilacs (in Virtual Health Library – VHL, 1982–2022), Web of Science (in Clarivate, 1945–2018), and Scopus (in ELSEVIER, 1996–2018). To find gray literature, the MedRxiv database (up to 2022) was used. The last searches in the databases were performed on April 5, 2022.

The strategies for searches are available with the correspondent author and were elaborated with terms combined with Boolean operators, as follows: (Children OR Youth OR Teenagers) AND HIV AND (Tenofovir OR "Antiretroviral therapy") AND ("Bone density" OR Osteoporosis OR Osteopenia). These terms were adapted according to the availability of exact descriptors in each controlled vocabulary platform, with Emtree being used for Embase and Scopus, and Health Science Descriptors/Medical Subject Headings (DeCS/MESH) for the other databases.

A manual search was proceeded on the reference list of the included studies to look for potential papers that could be considered for inclusion in the analysis.

The searches and the entire study selection process were performed by three independent blinded reviewers. The initial triage was conducted in Rayyan – Intelligent Systematic Review Platform. First, the platform detected duplicate papers, which were checked by one of the reviewers to exclude studies that were indeed duplicates. Subsequently, the three reviewers performed initial triage by reading the title and abstract on the platform. Eligible papers were selected by reading the full text. In cases of disagreement, the final decision was reached by consensus after discussion among the three reviewers.

To check the reliability among the reviewers, the R program[24] was used to perform Fleiss' kappa test, which is an adaptation of Cohen's kappa used when there are three or more reviewers.[25,26] For this, each reviewer's decisions before the consensus meetings were considered. The level of reliability was interpreted according to the Kappa (k) values as follows: none (0–0.20); minimal (0.21–0.39); weak (0.40–0.59); moderate (0.60–0.79); strong (0.80–0.90); and near perfect (above 0.90).[26]

In the screening stage by title and abstract, Fleiss' Kappa test presented moderate reliability among the three reviewers (k=0.696; 95%CI 0.648–0.743; z=28.6; p<0.001), with 92% agreement between inter-raters. In full-text selection, Fleiss' Kappa test showed moderate reliability (k=0.748; 95%CI 0.577–0.918; z=8.59; p<0.001), and the percent agreement was 81.8%.

The data extracted from the included studies were organized in forms previously developed, contemplating the following items: paper identification (author and year), study design, study population (characteristics, country, sample size, age, gender of volunteers, follow-up time, and nutritional status), intervention or exposure group, control group, source of information on the use of TDF, bone mass status variables, and results. Data unavailable in the papers were requested from the authors by e-mail.

For papers with a longitudinal approach that provided bone mass variables only at baseline, cross-sectional data analysis was considered. The control group composed of individuals without HIV was not considered for data extraction. Research with no control group, but that followed the TDF group over time, regardless of whether it was intervention or exposure, was described as longitudinal before-after studies.

The evaluation of bias risk was conducted by three independent reviewers who had been previously trained, and disagreements were resolved by consensus after discussion.

For cross-sectional studies, the Quality Assessment Tool for Observational Cohort and Cross-sectional Studies Scale from the National Institutes of Health (NIH)[27] was used. For longitudinal before-after studies, the Quality Assessment Tool for Before-After (Pre-Post) Studies with no Control Group from the NIH[28] was used. For these two scales, the classification was made according to the percentage of "yes" answers. Thus, 70 to 90% of "yes" answers would classify the study as "good", 41 to 69% as "fair", and up to 40% as "poor".

For the longitudinal approach with the control group, the Newcastle-Ottawa Quality Assessment for Cohort Studies[29] scale was used. On this scale, classification was performed based on the total score of each study: low risk of bias (7–9 points); high risk of bias (4–6 points); and very high risk of bias (0–3 points).

A descriptive approach was used to highlight the results of the present paper. The data analysis was categorized by study approach, including:

1. Before-after longitudinal studies without a control group;
2. Longitudinal studies with a control group; and
3. Cross-sectional studies.

In addition, meta-analysis was considered as long as the studies met the following criteria:

1. Similar paper designs;
2. Use of similar units of bone mass measurements; and
3. Presence of similar intervention and control groups between studies.

RESULTS

Database searches identified 1156 papers. After the exclusion of duplicates, 563 references were screened by title and abstract analysis. Among these, 57 met the eligibility criteria, and their full texts were analyzed. Thus, nine studies were included in this review. Finally, a manual search was performed in the references of the included studies, but no article met the inclusion criteria (Figure 1).

Among the nine included studies, two were conducted in Italy,[30,31] one in the Netherlands,[32] one in Canada,[33] one in Thailand,[34] one in Brazil and Panama,[35] one in Thailand and Indonesia,[36] one in Zimbabwe,[13] and one did not inform the country of origin.[37] Regarding the paper approach, four were cross-sectional, three were longitudinal before-after studies without a control group, and two were prospective cohort studies (Tables 1 and 2).
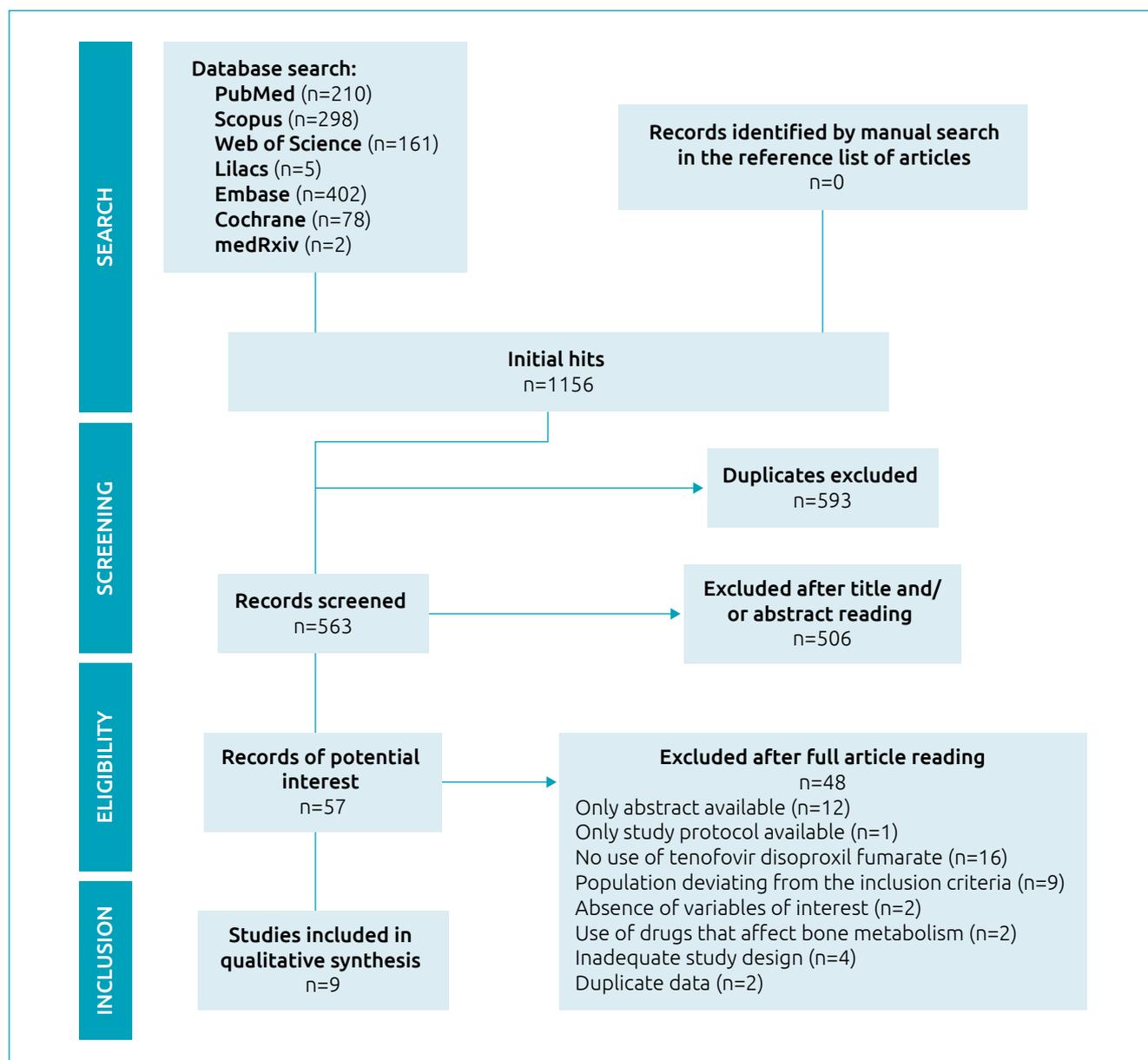

Figure 1. Study selection flowchart.

Table 1. Characteristics of the longitudinal studies included.

Author	1. Study population 2. Sample (n)	Objectives	1. Study design 2. Statistical analysis	Outcomes*
Hazra et al.[37]	1. HIV-infected children and adolescents who had progressive disease even after using 2 antiretroviral regimens. 2. n=18.	Provide preliminary TDF safety data.	1. Longitudinal before-after. 2. Wilcoxon Test and Spearman Correlation.	The median decrease in LS BMDz was statistically significant at week 24 (p=0.007) and at week 48 (p=0.02). However, reductions in BMDz at week 48 were not correlated with TDF pharmacokinetic parameters.
Negra et al.[35]	1. Adolescents infected with HIV-1, with previous ART failure and with a viral load greater than 1000 copies/mL. 2. n=81.	To evaluate the efficacy and safety of TDF in adolescents.	1. Longitudinal before-after. 2. Wilcoxon Test and Mann-Whitney Test.	Median LS BMD increased at weeks 144 (p<0.001), 192 (p<0.001), 240 (p<0.002) from baseline. The median reduction in LS BMDz adjusted for chronological age was -0.265 (p=0.001) at week 96. However, after 288 weeks there as an increase in LS BMDz.
Giacomet et al.[30]	1. Children and adolescents vertically infected with HIV with previous long-term ART without TDF. 2. n=26.	Evaluate the effect of TDF on bone mass in young people with HIV	1. Longitudinal before-after. 2. Linear mixed effects model.	BMD changes observed over the years in HIV-infected patients did not differ from those expected in healthy children and adolescents, indicating that the TDF-containing regimen did not impair skeletal mineralization. After 12 months, mean LS BMDz increased by 0.2 in the total sample.
Bunders et al.[32]	1. Children with HIV treated at Emma Children's Hospital between 2002 and 2010. Exposed to TDF (n=13) and controls (n=53). 2. n=66.	To determine the impact of ART on the BMD of HIV-infected children.	1. Longitudinal. 2. Univariate and multivariate linear regressions.	Despite the negative trend observed between femoral BMDz-score and TDF treatment, femoral (p=0.244) and lumbar spine (p=0.584) BMDz-scores were not significantly lower among children treated with TDF in univariate regression.
Aurpibul et al.[34]	1. HIV-infected children with viral suppression after regimen without TDF. Intervention (n=40) and controls (n=40). 2. n= 80.	Evaluate the efficacy, safety and pharmacokinetics of TDF.	1. Longitudinal with control group. 2. Analysis of variance for repeated measures.	Significant reduction in LS BMDz occurred in children receiving TDF in the first 24 weeks of treatment (p=0.011), however there were no significant changes after 96 weeks of follow-up. No correlation was seen between tenofovir and absolute change in LS BMD (p=0.086).

*The outcomes are presented according to the control for confounding variables if the study used group pairing or regression analysis. HIV: Human Immunodeficiency Virus; TDF: tenofovir disoproxil fumarate; BMDz: bone mineral density z score; ART: antiretroviral therapy; BMD: bone mineral density; LS: lumbar spine.

The selected papers included a total of 1040 individuals with HIV diagnoses.[13,30-37] Considering the six papers that had a control group,[13,31-34,36] 323 patients were in the TDF group while 613 were in the control group. The proportion of female participants varied from 38.7 to 61% and was unavailable in the study by Negra et al.[35] Of the five longitudinal research,[31,33-35,37] four reported clearly that the patients taking TDF during the follow-up period were not using the drug before the start of the research.[31,34,37] The follow-up time varied from one year to 7.1 years between the studies. Two of these five studies included only patients with prior ART failure.[35,37] All papers presented

the BMC or BMD variables in Z-score form, which is the most suitable for analyzing bone mass in children and adolescents (Tables 1 and 2).

Regarding the concomitant use of other antiretrovirals that may interfere with bone mass, there are five, of the nine studies, with unavailable data.[13,31-33,35] According to Sudjaritruk et al.,[36] 58.1% of the TDF group also used protease inhibitors (PI). In other studies, PI[37] and efavirenz[31,34] were used in combination with TDF in all patients.

The overall methodological quality of studies included in this review ranged from low to moderate. Scores on the

Table 2. Characteristics of the cross-sectional studies included.

Author	1. Study population 2. Sample (n)	Objectives	1. Study design 2. Statistical analysis	Outcomes*
Zuccotti et al.[31]	1. HIV-infected children and adolescents on long-term ART or naïve to ART. Exposed to TDF (n=28). 2. n=86.	To evaluate the role of different antiretroviral regimens on bone mass measurements.	1. Cross-sectional. 2. Multiple linear regression.	Only stavudine (p=0.001) and PI (p=0.03) significantly interfered in reduction of LS BMDz. There was no correlation between the use of TDF and changes in LS BMDz (p>0.05). The LS BMC values of patients using TDF did not differ from healthy or ART naïve subjects (p>0.05).
Macdonald et al.[33]	1. Children and adolescents vertically infected with HIV. Exposed to TDF (n=11). 2. n=31.	To investigate the association of bone mass measurements with the use of ART.	1. Cross-sectional. 2. Univariate regression analysis.	There was no significant association between use of TDF and changes in LS BMCz (p>0.05) and TB BMCz (p>0.05) despite the positive trend.
Sudjaritruk et al.[36]	1. Vertically HIV-infected adolescents with long-term viral suppression on ART. Exposed to TDF (n=136). 2. n=394.	Determine the effect of TDF on metabolism and bone mass.	1. Cross-sectional. 2. Mann-Whitney test and multiple linear regression.	There was no significant difference in median BMDz between TDF users and non-users (p=0.27). The use of TDF had no significant association with changes in LS BMDz (p=0.09). There was no significant relationship between BMDz and duration of TDF treatment (p=0.34).
Rukuni et al.[13]	1. Children and adolescents using ART for at least 2 years. Exposed to TDF (n=95). 2. n=279.	Identify the prevalence of low bone density and investigate associated risk factors.	1. Cross-sectional. 2. Chi-square, Fisher's exact test; Univariate and multiple linear regressions.	TDF use was significantly associated with lower TBLH BMCz (p=0.015) and LS BMADz (p=0.012) relative to non-TDF users. TDF was associated with decreased LS-BMADz in univariate (p<0.0001) but not multivariate analysis (p=0.25). Regarding TBLH-BMCz, there was also this association in both the univariate (p=0.037) and multivariate (p=0.046) analysis. TDF use for more than 4 years was correlated with more pronounced TBLH-BMCz deficits in multivariate analysis (p=0.046).

*The outcomes are presented according to the control for confounding variables if the study used group pairing or regression analysis. HIV: Human Immunodeficiency Virus; ART: antiretroviral therapy; TDF: tenofovir disoproxil fumarate; PI: protease inhibitors; LS: lumbar spine; BMDz: bone mineral density Z-score; BMC: bone mineral content; BMCz: bone mineral content z score; BMADz: bone mineral apparent density; TBLH: total body less-head.

Newcastle-Ottawa scale were 5 and 6, indicating a high risk of bias. Bunders et al.[32] scored 3 for participant selection, 1 for comparability, and 1 for outcome. Aurpibul et al.[34] scored 3, 2, and 1, respectively.

On the NIH scale for longitudinal before-after studies, two of three studies had fair quality while one had poor quality (Table 3). As for the NIH scale for cross-sectional studies, there were two with fair risk and another two with poor quality (Table 4).

The design of this systematic review included a meta-analysis of BMC and BMD. However, upon reviewing the papers, it was impossible to perform this statistical procedure for the following reasons:

1. Different study designs;
2. Use of different bone mass measurement units;
3. Use of different measurements of central tendency and dispersion to show the data; and
4. Studies with different control or intervention groups.

DISCUSSION

The focus of this systematic review was to identify the possible effect of TDF on bone mass in children and adolescents with HIV, in terms of BMDz (Z-score of BMD) or BMCz (Z-score of BMC) measured by DXA.

BMDz or BMCz measures of central tendency were negative at baseline in four of five longitudinal studies, indicating bone mass below that expected for the age group of subjects.[30,32,35,37] The four cross-sectional studies followed the same tendency.[13,31,33,36]

Table 3. Quality analysis before-after studies included in this review.

Author	Defined goal	Specified population	Participation rate of eligible persons equal to 100%	Representative sample	Adequate sample size	Well-defined intervention/exposure	Reliable measurement of outcome	Blinding of evaluators	Follow-up loss ≤20%	Appropriate statistical analysis	Dependent variable measured multiple times before and after the intervention	Statistical analysis for intervention at the group level	Quality rating
Hazra et al.[37]	Yes	Yes	Not reported	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Not applicable	Fair
Negra et al.[35]	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Not applicable	Fair
Giacomet et al.[30]	Yes	No	Not reported	Yes	No	Yes	Yes	Not reported	Not reported	Yes	Yes	Not applicable	Poor

Label: ● (Yes) ● (No) ● (Not reported) ● (Not applicable)

Table 4. Quality analysis of cross-sectional studies.

Author, year	Defined goal	Specified population	Participation rate of eligible persons >50%	Pre-specified and uniform eligibility criteria	Representative sample size	Exposure measured before the outcome	Adequate follow-up time	Well-defined intervention/exposure	Reassessment of intervention/exposure	Well-defined outcome	Evaluators blinding	Follow-up loss ≤20%	Statistical adjustment for confounding factors	Quality
Rukuni et al.[13]	Yes	Yes	Yes	Yes	Yes	No	No	Not reported	Not applicable	Yes	Not reported	Not applicable	Yes	Fair
Sudjaritruk et al.[36]	Yes	No	Not reported	Not reported	Not reported	No	No	Not reported	Not applicable	No	Not reported	Not applicable	Yes	Poor
Zuccotti et al.[31]	Yes	Yes	Not reported	No	No	No	No	No	Not applicable	Yes	Not reported	Not applicable	Yes	Poor
Macdonald et al.[33]	Yes	Yes	Yes	Yes	No	No	No	Yes	Not applicable	Yes	Not reported	Not applicable	No	Fair

Label: ● (Yes) ● (No) ● (Not reported) ● (Not applicable)

The three longitudinal before-after studies[30,35,37] had a total of 104 participants, who took TDF-containing regime after the previous ART and with statistical analysis suitable for before-after studies. The period between the suspension of the previous ART and the introduction of TDF was one week in Hazra et al.,[37] the other studies did not provide such data.[30,35] Two papers found no association between TDF and decreased bone mass.[30,35] In contrast, Hazra et al.[37] found median reductions in BMDz of -0.38 at week 24 (p=0.007) and -0.30 at week 48 (p=0.020) compared to a baseline median of -1.18. In this study, all volunteers' ART contained PI, which may have influenced the decrease in BMDz, as there are reports of an association between PI and bone loss.[38,39]

In the same direction, Negra et al.[35] observed, at week 96, a decrease in BMDz of the chronological age-adjusted spine (p=0.001), but at week 288, there was an increase without statistical significance, indicating stabilization of bone mass. Such results should be considered carefully since the loss at follow-up was 96% at the end of the study. However, the results of Negra et al.[35] and Hazra et al.[37] supported the information that in the first two years after initiation of various ART regimens, BMD is reduced, but stabilizes thereafter.[18]

Giacomet et al[30] found significant increases in BMD of the lumbar spine and total body after 52 weeks of therapy containing TDF (p<0.0001). In contrast to Hazra et al.,[37] in the Giacomet et al.[30] study, all participants used efavirenz in combination therapy – a drug related to higher bone mass when

compared to protease inhibitors[40] — which may have influenced the increase in BMD. However, the statistical analyses were performed only for absolute BMD values, which represents a shortcoming. The BMDz and BMCz are the most accurate measures for diagnosing low bone mass in children and adolescents.[4,41,42]

Among the longitudinal researches with the control group, one had TDF as an intervention in open-label design[34] and another as exposure.[32] Bunders et al.[32] demonstrated no significant association between TDF use and changes in BMDz of the lumbar spine (p=0.584) and femoral neck (p=0.244). These results are limited because of the sample size, composed of only 13 children exposed to ART. However, an association was found between longer exposure time to ART and increased BMDz of the lumbar spine (p=0.027) after a mean of 7.6 years of ART use, which corroborates the hypothesis of BMD stabilization after an initial TDF associated reduction.[18]

Aurpibul et al.[34] found a significant decrease in BMDz of the lumbar spine only at week 24 (p=0.011) in TDF users. After 96 weeks, BMDz rose again despite not reaching the baseline value after 96 weeks of follow-up. Among the 40 children using TDF, 11 showed a significantly greater absolute reduction in BMDz of the lumbar spine than the group without TDF (p=0.002). However, the prevalence of low BMDz was 9% at the end of the study, with no significant differences between groups (p=0.395), and there was also no correlation between TDF and absolute BMD change (p=0.859).

The cross-sectional papers covered 790 patients, of whom 270 were using TDF. Three of them were adjusted for confounding factors by multivariate linear regression.[13,31,36]

Zuccotti et al.[31] found no association of TDF use with decreased bone mass in multiple regression (p>0.050). Similarly, Macdonald et al.[33] observed no association of TDF with BMCz values (p>0.050).

In Sudjaritruk et al.[36] despite TDF users had lower median BMDz than nonusers, statistical significance was absent (p=0.270). In multivariate regression, no association occurred between TDF and BMDz (p=0.090). On the other vein, TDF was a predictor of an increase in bone resorption markers (p=0.040) and a decrease in bone formation markers (p=0.040). Resorption markers may indicate a high rate of bone loss and predict fracture risk independently of BMD values,[43] and may change early about the radiopaque structure observed on DXA.[44]

Rukuni et al.[13] showed a significant reduction in BMCz of total body less head (p=0.015) and apparent BMDz of the lumbar spine (p=0.012) in young people on TDF when compared to those on ART without TDF, in addition to associating pronounced bone deficits with longer time of exposure to TDF. These findings are consistent with Schtscherbyna et al.,[45]

in which time of TDF use was inversely correlated with BMDz of lumbar spine in a cross-sectional design.

The bias risk evaluation showed that the papers included in this review did not have a robust methodology. Most of them do not require sample size calculation and had a small sample size, affecting the representativeness of the population.[46] All the studies have a risk of measurement bias due to the lack of shielding of evaluators from exposure.[27,28,46]

In longitudinal before-after research, extensive loss to follow-up,[35] and lack of multiple measurements of dependent variables before the intervention[30,37] were significant methodological problems. Reliability of TDF exposure was doubtful in two studies: one had only 38.3% adherence,[35] while another did not assess adherence.[30]

In the cross-sectional research, the bias occurred mainly due to issues inherent to the methodological design itself that prevent inference of causality:[46] the impossibility of measuring exposure before the endpoint and follow-up time.

The evaluation of BMDz and BMCz in children and adolescents needs to consider physiological and behavioral variables that can influence these outcomes, such as menarche in girls, sexual maturation, growth spurt, diet, physical activity, solar exposure, nutritional status, ethnicity, and bone age.[4,5,6,8,42,47-51] Furthermore, when evaluating children and adolescents with HIV, it can be viewed that there are additional confounding variables related to infection: viral load, CD4+, and therapeutic factors.[52,53]

Only Bunders et al.[32] considered ethnicity as an independent variable in the regression analyses. This variable showed no effect on BMDz of the lumbar spine but there was a positive association between black and mixed black ethnicities and BMDz of the femoral neck.

The importance of assessing nutritional status occurs because both obesity and malnutrition are relevant factors for the reduction of BMD in children.[51] Only one of the nine studies did not present height and weight Z-scores.[32] In studies with available data,[13,30,31,33-37] the mean or median of these measurements at baseline were negative, indicating that young people with HIV were shorter and lighter than healthy people, but the mean and/or median were within the limits considered for eutrophic individuals. Only Rukuni et al.[13] reported the presence of 26% of the sample with malnutrition. Despite the importance of nutritional status in the assessment of BMD, the studies did not present a statistical analysis of the association between these variables, which may configure a fragility of the results.

No study considered nutritional status and bone age as confounding factors. Among the longitudinal before-after studies[30,35,37] that compose the present review, none analyzed other

variables with the potential to interfere with bone mass, which limits the power of their findings.

Among the longitudinal papers with the control group, one performed adjustments for HIV-related confounding factors such as percentage of CD4 cells, viral load, and duration of ART by multivariate regression,[32] while another adjusted for age, sex, and CD4 cell count by group matching.[34] In the study with the control group by Bunders et al.,[32] 23% of TDF users were already using the drug before the start of the study, which makes it impossible to conclude if TDF-related bone mass changes were absent at the beginning of the research.

Regarding the follow-up time, the longitudinal studies ranged from 48 weeks to 7.1 years and only two lasted more than two years,[32,35] representing a limitation in determining the real effect of chronic exposure to TDF on bone mass.

Of the cross-sectional research, only Macdonald et al.[33] did not control for confounding variables. Other papers[13,31,33] did not cover the following factors: viral load,[31,36] CD4 count,[31] duration of ART,[13,31] current ART regimen,[13,31] menarche,[13,31,36] growth spurt,[13,31,36] physical activity,[31] diet,[31] solar exposure,[13,31] ethnicity,[13,31,36] nutritional status,[13,31,36] and bone age.[13,31,36]

Zuccotti et al.,[31] removed sexual maturity from its final analyses since it was not statistically significant. However, Arabi et al.[54] noted that BMD of lumbar spine was 43% higher in post-pubertal boys than in prepubertal boys, and 66% higher in post-pubertal girls (p<0.001). Burns et al.[55] recommend considering Tanner stage in the evaluation of BMDz because pubertal delay influences bone mass and prevents the use of BMDz as a diagnostic criterion for osteoporosis.

The study by Sudjaritruk et al.[36] showed that there were no differences in solar exposure, dietary calcium ingestion, and physical activity level between groups, thus, multivariate analyses to control for these factors were not necessary. Factors like age, Tanner stage, use of other antiretroviral drugs, and duration of ART were excluded from regression analysis because they showed statistical collinearity with TDF administration, which precludes conclusions about the impact of these variables on bone mass.

Rukuni et al.[13] controlled for the largest number of confounding factors in multivariate regression. After such adjustments, only early stages of Tanner (p=0.029) and, in contradiction to Hazra et al.,[37] higher age (p=0.0012) were significant covariables for low BMDz of the lumbar spine. In Rukuni et al. study,[13] measures of the central tendency of bone mass were negative at all pubertal stages but did not reach low bone mass values. The other studies did not provide this data.

Furthermore, other antiretroviral drugs, such as efavirenz and PI, are known to have potential to affect BMD.[4,38,39,40] As combinations of antiretroviral agents used in ART vary across research and among participants themselves in the same research, the observed effects on bone mass may not be exclusively attributable to TDF.

Seven of the nine studies reviewed in the present paper found no significant association between TDF and reduced BMD, but these results should be interpreted cautiously because of methodological limitations and lack of evaluation of potential confounding factors, which prevent conclusions about the real effect of TDF on bone mass.

The evidence of an association between TDF and bone mass has limitations due to the methodological designs of the papers included in this review. Randomized clinical research was not found. There was only one clinical trial with control and intervention group. Most studies were cross-sectional or longitudinal observational. Some of the papers did not show sample size calculations to ensure that the sample was representative of the general population. The studies did not use the same confounding factors in the multivariate regression analyses. Besides, the number of confounding factors was limited in most studies. Therefore, it is recommended that further research be conducted, including a sample size calculation, blinding the evaluators to exposure or intervention, and considering confounding factors in experimental design and data analysis.

The academic evidence available until now is insufficient to evidence the effect of TDF on bone mass in children and adolescents living with HIV. Although most available data indicate no association between the variables analyzed by the studies included in this review, the extant literature presents low or moderate methodological quality. Therefore, we recommend that studies of high methodological quality be conducted to identify the real effect of antiretroviral therapy containing TDF on bone health in this population.

Conflict of interests
The authors declare there is no conflict of interests.

Authors' contribution
Study design: Gusmão MBF, Melo LC. *Data collection*: Gusmão MBF, Oliveira VV, Santos NMS. *Data analysis*: Gusmão MBF, Oliveira VV, Santos NMS. *Manuscript writing*: Gusmão MBF, Melo LC. *Manuscript revision*: Melo LC. *Study supervision*: Melo LC.

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