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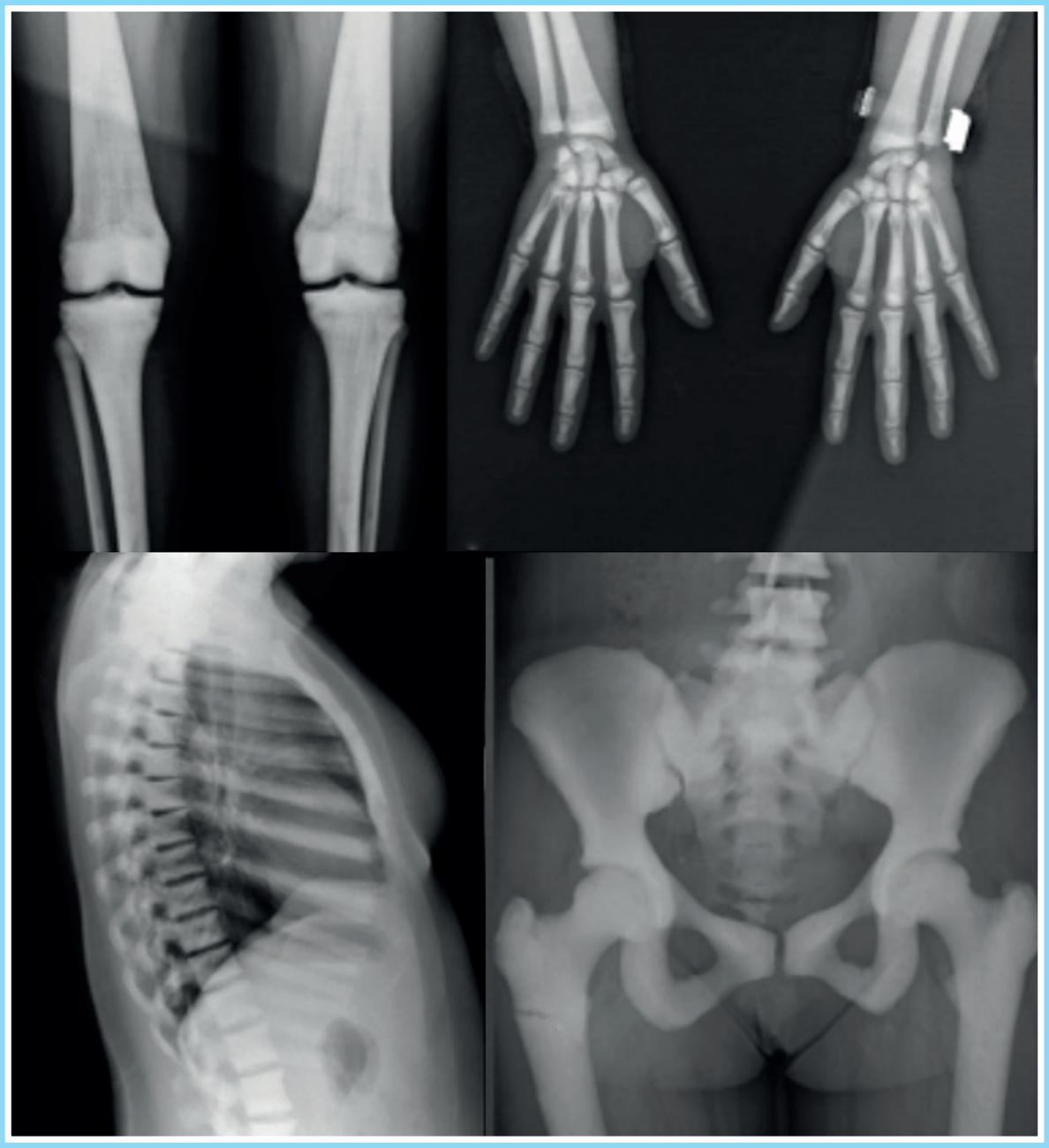

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Summary

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Cover image:

Plain radiographs corresponding to a patient with autosomal dominant osteopetrosis type II: anteroposterior radiograph of both knees, and both hands, lateral spine projection, and anteroposterior pelvic radiograph

I. Martín Martín, I. Peinado Ruiz, L. Estepa Díaz, R. Cáliz Cáliz. Osteopetrosis: characteristic radiological findings. Rev Osteoporos Metab Miner 2026;18(2):77-78. DOI: 10.20960/RevOsteoporosMetabMiner.00089

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Bone and liver diseases

Who thinks about osteoporosis when evaluating patients with chronic liver disease?

Well, I dare say that very few of the physicians who care for these patients do, and the same occurs in the context of research, since very few groups have rigorously analyzed its prevalence and pathogenic mechanisms. Therefore, it is important to disseminate information regarding the prevalence and incidence of osteoporosis in the major liver diseases, as well as to analyze the underlying mechanisms. The most extensively studied liver diseases have been cholestatic disorders, specifically primary biliary cholangitis (PBC) and end-stage liver disease. The prevalence of osteoporosis in PBC is around 35 % in the most significant studies, and depends on diagnostic criteria and the severity of liver damage, since its development is associated with age, postmenopausal status, duration of liver disease, and advanced histological stage (1). The prevalence of fractures is around 15 % in PBC and is even higher, approximately 22-36 %, in end-stage liver disease (2). These figures, however, correspond to studies published more than 10 years ago, and therefore may currently be lower due to improved control of cholestasis after the introduction of ursodeoxycholic acid and changes in the profile of patients awaiting liver transplantation. Nevertheless, this hypothesis has not been confirmed, since recent studies from the Swedish national registry and the Korean national health service, including between 4,000 and 5,000 patients with PBC, indicate a significantly higher incidence rate of fractures in patients with PBC vs controls (3,4). Similarly, analyses evaluating whether bone disease in patients awaiting liver transplantation had changed over a 20-year period showed that the prevalence of osteoporosis and fractures remained similar, although patients were older and had less severe liver damage in more recent years (5). However, other chronic liver diseases should not be overlooked, such as hemochromatosis, alcoholic liver disease, and nonalcoholic fatty liver disease. Both hemochromatosis and alcoholic liver disease are associated with an increased risk of osteoporosis and fractures; in hemochromatosis, it remains unclear whether osteoporosis is due to iron overload directly affecting bone tissue or occurs through complications such as liver cirrhosis or hypogonadism, while excessive alcohol consumption is an independent risk factor for osteoporosis and falls (6,7).

The mechanisms involved in osteoporosis associated with chronic liver disease are poorly understood, but most studies suggest that osteoporosis is mainly due to low bone formation. For this reason, it has been investigated whether sclerostin, which regulates osteoblastogenesis, plays a role in the low bone formation associated with chronic cholestasis. Serum sclerostin levels have been found to be elevated, and furthermore, sclerostin is expressed in the bile ducts of patients with PBC, especially in the early stages of the disease when inflammation/cholangitis is present (8). Additionally, and probably in more advanced stages of liver disease, substances retained during cholestasis, such as bilirubin and bile acids, may participate in reducing bone formation by decreasing proliferation, differentiation, and mineralization and by increasing apoptosis of osteoblastic cells, as observed in in vitro studies (9,10). Based on clinical and histological observations, it has been proposed that bone resorption increases as cholestasis worsens (11). Recently, in vitro studies have demonstrated that bilirubin may contribute to osteoporosis in advanced cholestatic liver disease by increasing osteoclast viability and decreasing apoptosis (2).

There are other factors involved in liver-bone interactions. Vitamin K, which is necessary for osteocalcin carboxylation, may be reduced in patients with severe cholestasis, representing an additional contributing factor. In this regard, undercarboxylated osteocalcin has been found to be elevated in a small group of patients with PBC, and its values decreased after administration of vitamin K1 (13). On the other hand, reduced IGF-1 levels have been described in liver cirrhosis (14), as well as a high prevalence of hypogonadism in hemochromatosis, chronic alcoholism, cirrhosis, and end-stage liver disease (15). Vitamin D deficiency, poor nutrition, and sarcopenia, which are frequent in most advanced liver diseases, complete the spectrum of bone disease in chronic liver diseases (15).

The treatment of osteoporosis associated with liver disease has been poorly investigated. In addition to adequate calcium intake and vitamin D supplementation to achieve serum 25OHD levels ≥ 30 ng/mL, different drugs used in postmenopausal osteoporosis have been tested, although studies are scarce and involve a limited number of patients. Most studies have been conducted in PBC and in patients before and after transplantation, with data focused on bone mineral density rather than fracture outcomes. Thus, hormone replacement therapy and raloxifene have been tested in very small series of patients with PBC, while more studies have been performed with oral bisphosphonates and zoledronate (16,17). Currently, 3 trials with denosumab have been published, again involving a limited number of patients (18,19), and one case report with romosozumab (20). All of these studies have shown stability or increases in bone mineral density, of varying magnitude, with few adverse effects.

The final takeaway is that patients with chronic liver disease may develop osteoporosis during the course of their illness due to a profound imbalance between bone formation and resorption caused by liver dysfunction, cholestasis, vitamin D deficiency, poor nutrition, and hypogonadism, with the magnitude depending on the type of liver disease and the severity of the condition.

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Original

MINDIN as a mediator of tumor-bone interaction: involvement of integrins in prostate cancer metastatic progression

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Abstract

Bone is a dynamic organ subjected to constant remodeling, a process that depends on the coordinated activity of osteoblasts, osteoclasts, and osteocytes. Bone is also a common site of metastasis for solid tumors, as occurs in prostate cancer, whose bone metastasis is associated with a poor prognosis. Furthermore, it has been proposed that, before the establishment of metastases, tumors induce changes in distant organs, promoting the formation of premetastatic niches that favor tumor implantation. MINDIN, an extracellular matrix protein, has been identified as a potential biomarker of prostate cancer and is highly expressed in patients with bone metastases. This protein can modify both tumor cells and the bone environment, and it has been suggested that it may promote tumor growth and metastatic dissemination. MINDIN may also act as a ligand for integrins, a family of transmembrane receptors that play a key role in the regulation of tumor cell adhesion, signaling, and migration. Based on these observations, we hypothesized that MINDIN may promote the formation of the bone premetastatic niche through the modification of specific integrin expression in tumor and bone cells. A murine tumor model was used to study the influence of MINDIN on integrin expression and its possible involvement in the formation of the bone premetastatic niche, together with in vitro experimental models employing osteoblastic (MC3T3-E1), osteocytic (MLO-Y4), and prostate tumor (TRAMP-C1) cell lines. In the experimental animal model previously developed by our group, in which a primary tumor was established in the absence of overt bone metastases, an increase in *integrin β6* gene expression was observed in both the prostates and tibias of tumor-bearing mice, an effect that was reversed by MINDIN silencing. These data were confirmed in our in vitro experimental model, where TRAMP-C1 cells stimulated with MINDIN showed increased *integrin β6* expression, whereas MINDIN silencing significantly reduced its expression. In osteoblastic cells (MC3T3-E1), MINDIN also induced overexpression of integrin β6. In contrast, in osteocytic cells (MLO-Y4), MINDIN did not induce β6 overexpression, but instead increased *integrin α2* expression. These results suggest possible cell-specificity in the interaction between MINDIN and integrins, where MINDIN may favor the creation of premetastatic niches in bone through the overexpression of *integrin β6*.

Keywords:
MINDIN. Integrins. Prostate cancer. Bone metastases.

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INTRODUCTION

Bone is a metabolically active organ that undergoes continuous remodeling throughout life. The bone remodeling process, in which bone resorption and formation are coupled, serves to adjust bone architecture to the mechanical demands placed on bone. In addition, bone is the principal storage organ for calcium, phosphorus, and magnesium. Plasma concentrations of these minerals depend on the balance between bone formation and resorption, regulated by hormones such as parathyroid hormone (PTH), calcitonin, and 1,25-dihydroxyvitamin D (1). This process is mediated by osteoblasts and osteoclasts, which carry out bone formation and resorption in a coordinated manner, respectively. Furthermore, a third type of bone cell, osteocytes, acts as a sensor of mechanical stimulation and is capable of communicating with osteoblasts and osteoclasts by modulating their activity (2,3).

Although not all solid tumors metastasize to bone, bone represents a common target organ in the metastatic dissemination of cancers such as prostate, breast, lung, kidney, and thyroid cancer, with the development of metastatic disease constituting a serious threat to the survival rate of patients who develop these types of tumors (4,5). Bone metastasis is a frequent complication in advanced stages of patients with prostate cancer, one of the tumors associated with the highest mortality and morbidity in developed countries (6). Tumors cause two different (although not mutually exclusive) types of skeletal lesions. The most common form, represented for example by breast cancer, is the osteolytic lesion, associated with altered bone remodeling caused by increased osteoclastic activity and the consequent osteolysis (7-10). On the other hand, there is a second type of lesion known as osteoblastic lesions, characteristic for example of prostate cancer, characterized by increased osteoblastic activity, increased osteoid, and an increased mineralization rate (11,12). Nevertheless, the existence of a resorptive component mediated by osteoclasts as a prior step for the establishment of osteoblastic lesions is currently recognized (11,12).

Recent studies have described prometastatic changes in organs where metastases subsequently develop (13). Such changes induce the formation of premetastatic niches that favor the implantation of tumor cells in specific target organs (13). The cellular complexity of bone, together with its continuous regulation of metabolism and bone remodeling, raises the possibility that the formation of the bone premetastatic niche is the consequence of a complex network of combined or sequential modifications in bone cell functions (14). Preventing the different stages required for tumor cells to leave the primary tumor, migrate, and establish themselves within the bone microenvironment is one of the main strategies to prevent the dissemination of bone metastases (14). The invasion of primary tumor cells into skeletal niches is associated with the activation of bone cells that release growth factors and cytokines, which in turn promote tumor growth within metastases (4). As a result, the so-called "vicious cycle" of bone metastases is generated, altering bone physiology and disrupting bone remodeling (4).

MINDIN, also known as spondin-2, is a secreted extracellular matrix protein belonging to the class of molecules containing thrombospondin type 1 repeats. MINDIN has recently been suggested to act as a specific diagnostic biomarker for prostate cancer (15,16), with higher concentrations of this protein being found in the serum of patients with prostate cancer and bone metastases (17). Furthermore, MINDIN has been shown to be highly expressed in other tumors, such as lung cancer (18), liver cancer (19,20), colorectal cancer (CRC) (21), gastric cancer (22), clear cell renal carcinoma (23), ovarian cancer (24), breast cancer (25), pancreatic cancer (26), and Barrett adenocarcinoma (27). Although MINDIN overexpression is significantly associated with the progression of colorectal, prostate, and ovarian tumors, and is used as a predictor of poor survival prognosis, the role of MINDIN in other tumors remains controversial due to the lack of studies and available information (19,20,22,24-32). Of note, MINDIN is capable of inducing proliferation, migration, and the acquisition of bone-like molecular characteristics in prostate tumor cells, a process known as osteomimicry (33,34). Furthermore, MINDIN is capable of modifying the expression of osteoblastic and osteoclastic markers within the bone environment, with increased gene expression of tartrate-resistant acid phosphatase (TRAP) and osterix, as well as increased TRAP protein expression. These changes have suggested that MINDIN may favor tumor progression and the formation of bone metastases (33,34).

Integrins are heterodimeric transmembrane receptors composed of alpha and beta subunits that function as anchoring and cellular signaling molecules (35). There are 18 alpha subunits and 8 beta subunits, giving rise to at least 24 known distinct integrin heterodimers with different functions and ligand-binding activities. Integrins act as anchoring molecules by mediating adhesion between the cellular cytoskeleton and the extracellular matrix (ECM). They also function as bidirectional signaling molecules by mediating signaling from the outside to the inside of the cell and vice versa, thereby controlling a series of vital cellular functions such as adhesion, polarity, differentiation, migration, and cell division (36-38). In pathological states, defective integrin signaling may alter these functions and lead to abnormal cell division, migration, and adhesion, which are characteristic features of cancer and metastasis (39-43). It has been observed that tumor processes are associated with dysregulation of integrin expression, in some cases showing overexpression *(β1, β3, β6)* and in others decreased expression *(α2, α3, α4, α5, α7)* (39-44) (Table I). Former studies have demon-

Table I. Summary of integrin subunits involved in tumor development and metastasis

Integrin α/β subunit	Dysregulated expression	References
α2	Decreased expression in prostate adenocarcinoma and increased expression in bone metastases	47-50
α3, α4, α5, α7	Decreased expression in prostate adenocarcinomas	47,48
α6	Overexpressed in bone metastases	48,51,52
β1	Increased expression in prostate adenocarcinoma	53,54
β3	Expressed in prostate adenocarcinoma and metastatic lesions; absent in normal cells	53,54
β6	Expressed in prostate adenocarcinoma and bone metastatic lesions; absent in normal cells	55,56

strated that the MINDIN protein can interact with integrins in neutrophils and that this MINDIN-integrin interaction is essential for the recruitment of inflammatory cells in certain in vivo models (45,46). However, it remains unknown whether MINDIN can alter the integrin expression profile in tumor and bone cells to promote tumor progression and bone metastasis processes (45,46).

Based on all these observations, we hypothesized that MINDIN may modify the integrin expression profile in bone and prostate tumor cells to potentially favor the development of bone metastases. To this end, in the present study we investigated the effects of the protumoral protein MINDIN on the gene expression profile in bone cells (osteoblasts and osteocytes) and prostate tumor cells of those integrins that are key in the development of bone metastases.

MATERIALS AND METHODS

ANIMAL MODEL

Three-month-old male C57BL/6 mice (Charles River, Wilmington, MA, United States) were used and maintained in cages under standard conditions: room temperature of 20 ± 0.5 °C, relative humidity of 55 ± 5 %, and a 12 h/12 h light/dark cycle. Animals remained unrestricted in movement and received a standard pellet diet (Teklad Global 18 % Protein Rodent Diet, Envigo, Madison, WI) and tap water *ad libitum.*

Surgical intervention was performed under aseptic conditions with mice anesthetized by isoflurane inhalation and administration of xylazine (10 mg/kg) and ketamine (25 mg/kg). Through a lower midline abdominal incision, 50 µL of vehicle (PBS), 5 × 10^5 TRAMP-C1 cells transfected with scrambled siRNAs, or 5 × 10^5 TRAMP-C1 cells transfected with three siRNAs directed against MINDIN (s97640, s97638, s87252; Life Technologies, Paisley, United Kingdom) were injected into the right posterior prostatic lobe. An injection was considered technically correct when the bleb was localized within the prostatic lobe. After the intervention, the abdominal cavity, muscle, and skin were closed with surgical staples.

One month later, primary prostate tumors and tibias were extracted and stored in Trizol (Thermo Scientific) for subsequent RNA extraction and analysis by quantitative real-time PCR. All procedures were approved by the Institutional Animal Care and Use Committee of Universidad San Pablo CEU (Madrid, Spain).

CELL CULTURES

For the experiments of the present study, the following cell lines were cultured at 37 °C with 5 % CO_2 and 95 % humidity in media supplemented with 1 % streptomycin/penicillin: mouse mesenchymal preosteoblastic cells (MC3T3-E1 subclone 4, ATTC, CRL-2593). These cells were cultured in alpha MEM medium without ascorbic acid ("minimum essential medium", Thermofisher, A1049001) supplemented with 10 % FBS (fetal bovine serum) and 1 % L-glutamine. Murine osteocytic MLO-Y4 cells (generously provided by Lynda Bonewald) were cultured in alpha MEM medium (Thermofisher, 22571038) supplemented with 2.5 % FBS and 2.5 % calf serum (CS; Thermofisher, 16010159). We used the murine prostate adenocarcinoma cell line TRAMP-C1 (ATCC® CRL-2730 TM), cultured in DMEM medium ("Dulbecco's Modified Eagle Medium", Thermofisher, 11965092) supplemented with 10 % FBS and 1 % L-glutamine.

Cells were seeded in 6-well culture plates and, after 24 hours, stimulated with MINDIN (5 ng/mL) for 6 or 24 hours. Following stimulation, each sample was collected in Trizol for subsequent total RNA extraction and quantitative real-time PCR analysis.

CELL SILENCING

TRAMP-C1 cells were silenced with a mixture of three siRNAs (20 nM each) directed against different coding sequences of murine *MINDIN* (s97640; s97638; s87252) (Life Technologies, Paisley, United Kingdom) using Lipofectamine RNAiMax (Life Technologies) and following the manufacturer's instructions. A scrambled

silencer not directed against any specific genomic sequence (control siRNA-A, Santa Cruz Biotechnology, Dallas, TX) was used as a negative control to verify the specificity of the MINDIN siRNA-dependent changes in the different evaluated parameters. The efficacy of MINDIN silencing was evaluated by real-time PCR: at 24 hours it was 0.05 ± 0.0060 vs 1 ± 0.03; $p < 0.0001$; at 48 hours, 0.15 ± 0.1 vs 1 ± 0.07; $p < 0.001$; and finally, after 15 days, 0.4 ± 0.13 vs 1 ± 0.01; $p < 0.001$.

REAL-TIME POLYMERASE CHAIN REACTION (PCR)

Total RNA was isolated using a standard procedure (Trizol, Life Technologies), and 2 µg of RNA from each sample were reverse-transcribed using a high-capacity cDNA reverse transcription kit ("High Capacity RNA to cDNA Kit", Thermofisher, 4387406) following the manufacturer's instructions.

Real-time PCR expression analysis was performed based on fluorescence emission from the fluorochrome SYBR Green premix ex Taq (Takara, Otsu, Japan), using an ABI PRISM 7500 system (Applied Biosystems). The number of mRNA copies was calculated for each sample using the threshold cycle (Ct) value. The *18S* or *β-ACTIN* genes (used as constitutive expression controls) were amplified in parallel as control genes, allowing the calculation of the number of amplification cycles required to reach an arbitrary Ct intensity. Relative gene expression was defined as relative expression compared with the control, calculated as $2^{-\Delta\Delta Ct}$, where ΔΔCt = ΔCt treatment – ΔCt control and ΔCt = Ct target gene – Ct control gene *(18S/β-ACTIN).* Gene expression from animal samples was represented as individual data points by calculating $2^{-\Delta Ct}$, as previously described (57). The specificity of each amplicon was confirmed by the presence of a single peak in the dissociation curve for each qPCR reaction.

STATISTICAL ANALYSIS

Data are expressed as means ± standard error. Data distribution was analyzed and, since they did not fit a normal distribution, differences between experimental groups were evaluated using non-parametric analysis of variance (Kruskal-Wallis). Determination of possible differences between experimental groups was performed using Dunn's or Mann-Whitney tests. GraphPad Prism software was used for statistical analyses and outlier detection. Outlier exclusion followed standard criteria based on standard deviation according to software analysis. A value of $p < 0.05$ was considered statistically significant.

RESULTS

MINDIN EXPRESSION IN PRIMARY PROSTATE TUMORS MODIFIES INTEGRIN GENE EXPRESSION IN THE PROSTATES AND TIBIAS OF MICE

In the present study, we characterized the gene expression patterns of integrins that could favor the metastasis of prostate tumor cells within the bone microenvironment. We used a C57BL/6 mouse model that recapitulates the progression of prostate cancer from hyperplasia to prostatic intraepithelial neoplasm, potentially metastasizing to lymph nodes, lungs, and bone. In this previously developed model, 1 month after orthotopic injection of TRAMP-C1 prostate cancer cells, overexpression of the tumor marker *PSCA* (1966 ± 834 vs 1 ± 0.4; $p = 0.024$) and *MINDIN* (714.25 ± 348.32 vs 1 ± 0.3; $p < 0.0001$) (34) was observed in the tumor group compared with the control group, indicating establishment of the primary tumor at this time point. Prostate tumors generated by TRAMP-C1 cells silenced for MINDIN showed a drastic decrease in *MINDIN* expression (28.57 ± 16.6 vs 714.25 ± 348.32; $p = 0.0012$) without affecting expression of the tumor burden marker PSCA (1136.3 ± 315.3 vs 1966 ± 834; $p < 0.43$), compared with the tumor group expressing *MINDIN* (33,34). In this model, since no changes in *PSCA* or *MINDIN* expression were observed in the tibias, we can conclude that tumor metastasis to bone had not occurred at the studied time point (33).

However, we had previously observed changes in the bones of this model, reflected by an increase in gene expression of the bone resorption marker TRAP and modification of bone microarchitecture; these changes were reversed when *MINDIN* was silenced in the primary tumor (33).

Since changes to integrin levels have been described during tumor progression, suggesting that they may be molecules through which MINDIN exerts its actions, we performed an integrin profiling study. In this model, among the different key integrins involved in bone metastases that were studied (Figs. 1A-E and 2A-E), establishment of prostate tumors with *MINDIN* overexpression induced only an increase in *integrin β6* gene expression in primary prostate tumors (Fig. 1E) and mouse tibias (Fig. 2E). The observed changes were reversed when MINDIN was silenced in the primary prostate tumor (Figs. 1E and 2E). These results indicate that MINDIN is capable of modulating *integrin β6* expression both in the primary tumor and in bone, where the tumor does not yet appear to be established. This could suggest that modification of the integrin profile may be considered a change that promotes formation of the bone premetastatic niche.

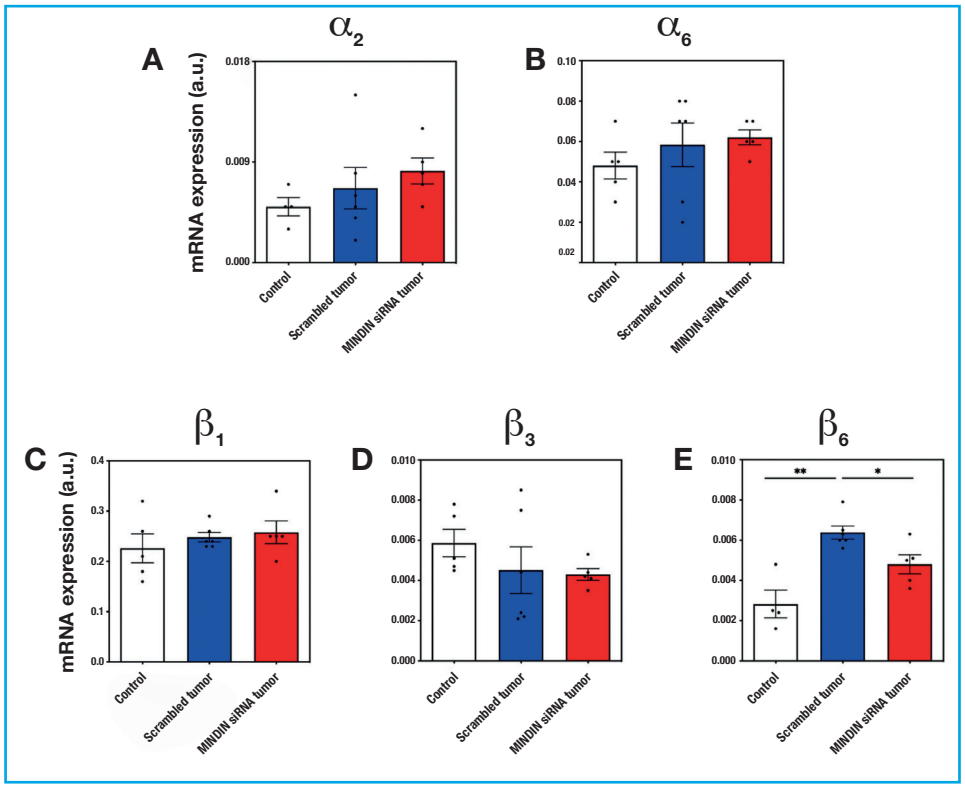

Figure 1. MINDIN induces integrin *β6* overexpression in prostate tumors in an in vivo mouse model. mRNA expression of integrins (A) *α2*, (B) *α6*, (C) *β1*, (D) *β3*, and (E) *β6* in mouse prostates evaluated by real-time PCR. Data are presented as mean ± SEM (n = 4-6 per group). $p < 0.05$; $^{**}p < 0.01$. The samples used were obtained from a previously developed and published animal model (33,34). The *18S* gene was used as the endogenous control.

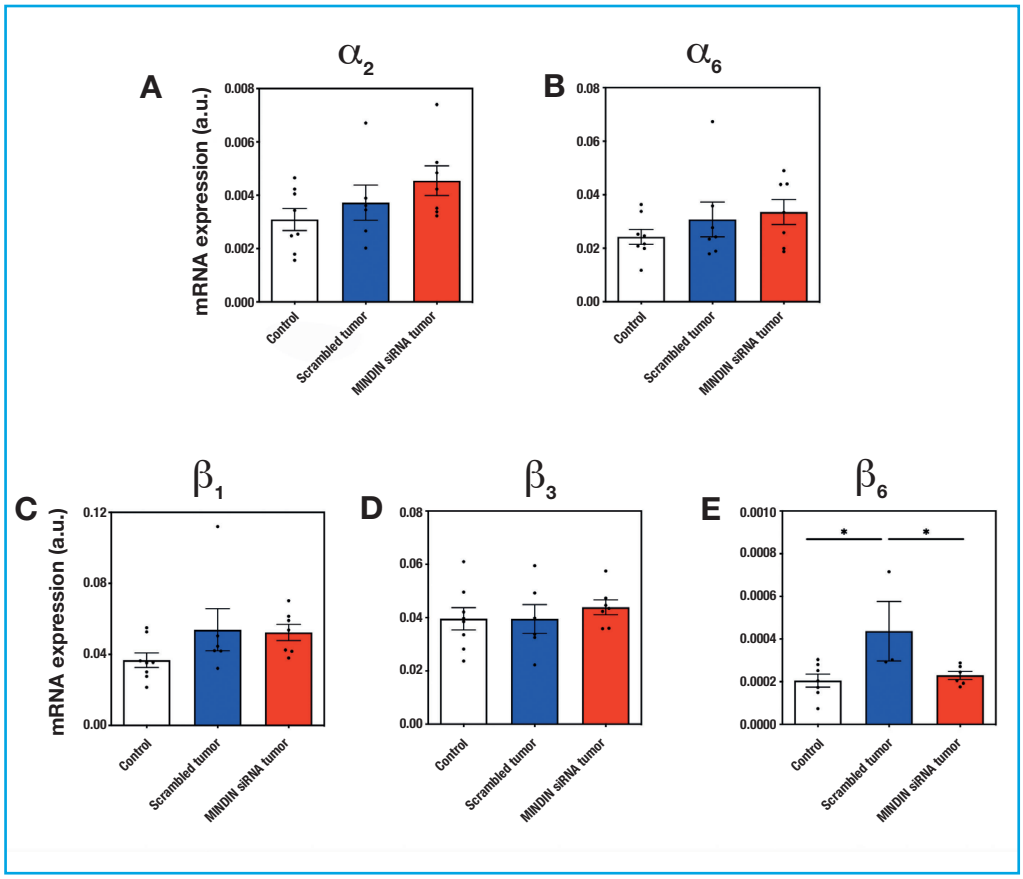

Figure 2. MINDIN induces *integrin β6* overexpression in tibias of mice with prostate tumors. mRNA expression of integrins (A) *α2*, (B) *α6*, (C) *β1*, (D) *β3*, and (E) *β6* in mouse tibias evaluated by real-time PCR. Data are presented as mean ± SEM (n = 3-7 per group). $p < 0.05$; $^{**}p < 0.01$. The samples used were obtained from a previously developed and published animal model (33,34). The *18S* gene was used as the endogenous control (33,34).

MINDIN INDUCES MODIFICATIONS IN INTEGRIN GENE EXPRESSION IN PROSTATE TUMOR CELLS

MINDIN has been reported to increase adhesion of different cell types to the extracellular matrix, including neutrophils, macrophages, lymphocytes, and prostate tumor cells (46,58). Therefore, since integrins are key transmembrane proteins involved in cell adhesion to the matrix, and considering the observed modification in *integrin β6* gene expression in the primary tumor, we investigated whether MINDIN secretion by prostate tumors could modify integrin expression to increase adhesion of prostate adenocarcinoma cells to bone surfaces. To this end, we used TRAMP-C1 prostate tumor cells stimulated with MINDIN. An increase in *integrin β6* expression was observed following stimulation with MINDIN for 6 or 24 hours (Figs. 3A-B). Furthermore, MINDIN silencing for 24 hours in TRAMP-C1 tumor cells caused a significant decrease *in integrin β6* expression (Fig. 3C). Although an increase in *integrin α2* expression levels was observed after 24 hours of stimulation with MINDIN, this increase did not reach statistical significance ($p = 0.117$).

MINDIN INDUCES CHANGES TO INTEGRIN GENE EXPRESSION IN OSTEOBLASTIC AND OSTEOCYTIC CELLS

To determine whether MINDIN could also modify the integrin expression profile in bone cells, we analyzed the effects of MINDIN in different bone cell types, including MC3T3-E1 osteoblastic cells and MLO-Y4 osteocytic cells. In MC3T3 osteoblastic cells, an increase *in integrin β6* gene expression was observed after both 6 and 24 hours of stimulation with MINDIN (Figs. 4A-B). However, in MLO-Y4 cells, no increase in *integrin β6* expression was observed ($p = 0.1$) after 6 hours of stimulation with MINDIN; instead, an increase in *integrin α2* expression was detected. These data suggest that, depending on the target cell type, MINDIN may modify the expression of different integrins (Figs. 4B-C).

Taken together, the data from the present study indicate that MINDIN can modify the transcriptional profile of several integrins associated with tumor progression and the establishment of metastases in both bone and tumor cells.

DISCUSSION

Bone metastases are painful, difficult to cure, and associated with a poor survival prognosis. Primary tumors create premetastatic niches favorable to tumor cells in secondary organs that subsequently lead to the development of metastases (59). Furthermore, during the initial phase of premetastatic niche formation, primary tumor cells produce various soluble factors to trigger the formation of an immature premetastatic niche (59). Therefore, identifying the factors that regulate tumor-bone interactions and increasing our

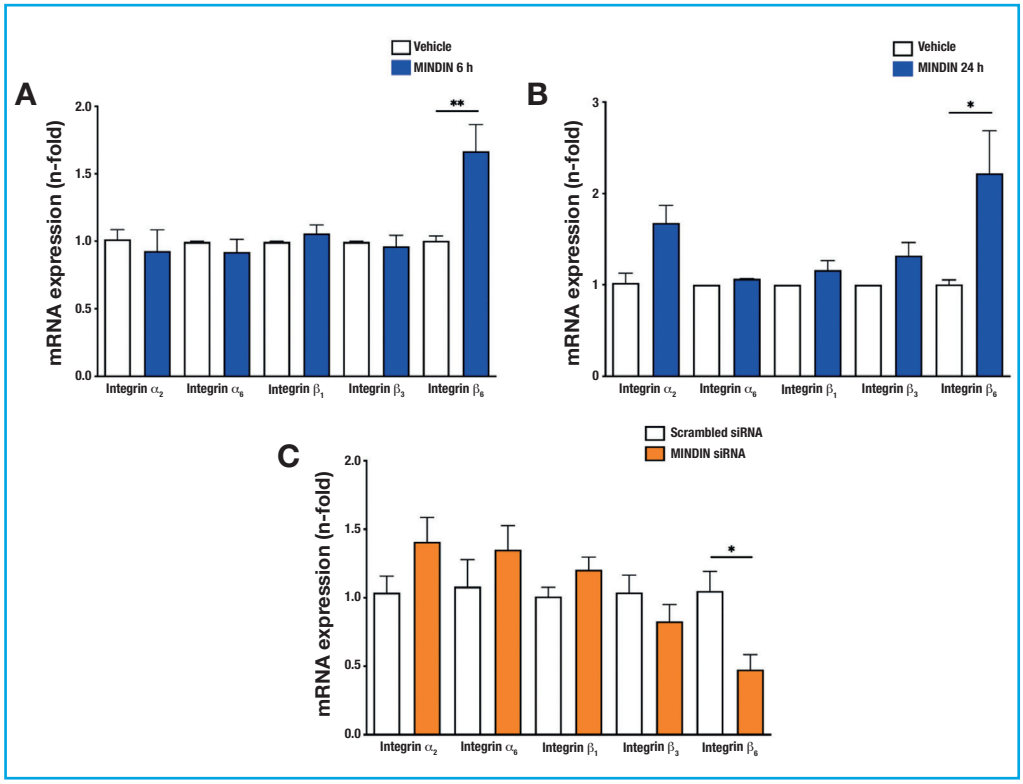

Figure 3. MINDIN induces *integrin β6* overexpression in prostate tumor cells. mRNA expression of integrins *α2*, *α6*, *β1*, *β3*, and *β6* in TRAMP-C1 prostate tumor cells stimulated with MINDIN for (A) 6 h, (B) 24 h, or (C) with MINDIN silenced, evaluated by real-time PCR. Data are expressed as mean ± SEM of 3 independent experiments performed in triplicate. $p < 0.05$ vs control; $^{**}p < 0.01$ vs control. The *β-ACTIN* gene was used as the endogenous control.

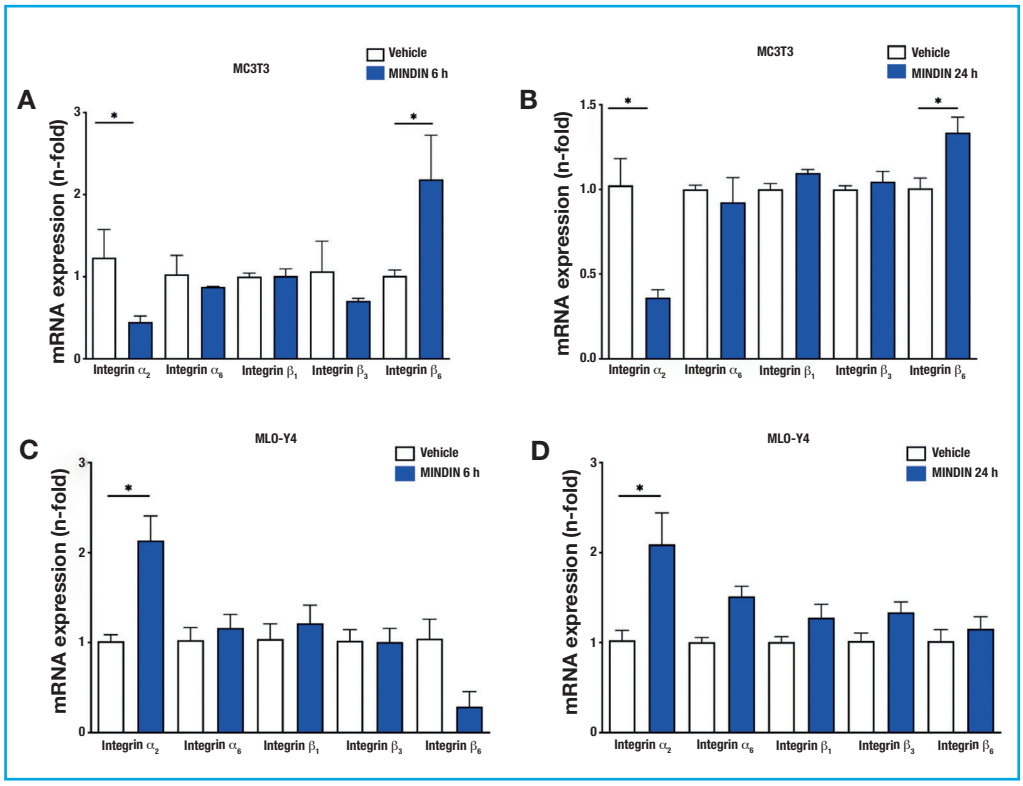

Figure 4. MINDIN induces *integrin β6* overexpression in osteoblasts and integrin *α2* overexpression in osteocytes. mRNA expression of integrins *α2*, *α6*, *β1*, *β3*, and *β6* in MC3T3 osteoblasts stimulated with MINDIN for (A) 6 h or (B) 24 h, and osteocytes stimulated with MINDIN for (C) 6 h or (D) 24 h, evaluated by real-time PCR. Data are presented as mean ± SEM of three independent experiments performed in triplicate. $p < 0.05$. The *β-ACTIN* gene was used as the endogenous control.

knowledge of the mechanisms controlling them are key to developing new therapeutic approaches. In the present study, we identified MINDIN, an extracellular matrix protein secreted by prostate tumors, as a factor capable of inducing and modulating integrin gene expression in both the primary tumor and bone. These changes in integrin expression could increase tumor cell adhesion to bone and favor the development of bone metastases, making them potential therapeutic targets against prostate cancer progression.

Dysregulation of *MINDIN* gene expression has been documented in several human tumors, such as prostate cancer (15,16), gastric cancer (60), and ovarian cancer (61). *MINDIN* expression has been described as being higher in samples from patients with more aggressive prostate cancer and poorer prognosis, and even higher in those with bone metastases (16,17). Evaluation of MINDIN by immunostaining in tumors with different Gleason scores revealed that MINDIN levels were highest in individuals with prostate cancer and bone metastases, followed by individuals without bone metastases, hyperplasia, and controls (16,17). MINDIN has also been shown to be a ligand for integrins, with MINDIN-integrin interactions being critical for the recruitment of neutrophils, macrophages, and T lymphocytes in inflammatory *in vivo* models (46,58). Furthermore, MINDIN has been observed to regulate Rho GTPase expression after interacting with $\alpha 4\beta 1$ and $\alpha 5\beta 1$ integrins, contributing to tumor development in colon carcinoma and hepatocellular carcinoma (58).

In male C57BL/6 mice from a previously published animal model (33,34), we observed that tumors induced by TRAMP-C1 prostate tumor cells caused changes in integrin gene expression, producing an increase in MINDIN-dependent *integrin β6* expression in primary prostate tumors. Although no changes in *MINDIN* or *PSCA* expression were observed in bone, indicating the absence of detectable bone metastases, an increase in *integrin β6* expression was observed in the tibias of tumor-bearing mice compared with controls. These changes could be related to modifications in *TRAP* gene expression and bone microarchitecture that had been previously observed (33). These results suggest that changes in integrin expression could be associated with the formation of bone premetastatic niches. However, further studies are required to confirm whether this increase is dependent on MINDIN.

Former data from our group revealed that MINDIN could be a key factor promoting increased adhesion of prostate adenocarcinoma cells to bone surfaces (34). Furthermore, MINDIN has been shown to induce activation of the ERK kinase pathway (34) in prostate adenocarcinoma cells. Increased adhesion generally requires increased expression of matrix-binding proteins such as integrins (62). It has also been documented that the MINDIN protein is capable of interacting with different integrins in immune and tumor cells (60,63). Therefore, it is possible that MINDIN binds to specific integrins in prostate cancer cells to enhance their adhesion to the bone extracellular matrix. In this

regard, a relationship between integrins and the Fak and Src kinases in cell adhesion has been observed (64). Former data show the suppressive action of Fak and Src kinase inhibitors on the ability of bone cells to promote adhesion of TRAMP-C1 cells, suggesting an interaction between these kinases and integrins in the adhesion of prostate cancer cells to bone (33). However, it remains unknown which integrins may be involved in the interaction between tumor cells and MINDIN to promote cell adhesion (33). In the present study, we demonstrated that stimulation of prostate tumor cells with MINDIN induces an increase in *integrin β6* expression, an increase that is reversed when *MINDIN* is silenced in tumor cells, indicating a possible dependence of *β6* expression on *MINDIN*. It should be emphasized that several studies have shown that integrin αvβ6 actively participates in TGF-β activation (56,65), extracellular matrix remodeling through regulation of *MMP2* expression (56), and facilitation of endothelial adhesion (66), which would favor colonization of the bone environment. However, our data do not necessarily imply that MINDIN binds directly to integrin β6.

Progression to invasive and metastatic carcinoma involves profound changes to the epithelial structure and function, as well as bone alterations to accommodate metastatic development. Integrins are essential mediators of tumorigenesis, adhesion, and migration. In particular, high *β6* expression in primary colon carcinomas, squamous cell carcinomas, pancreatic tumors, and breast tumors has been shown to act as a prognostic marker of aggressive disease and increased mortality in these patients (67-70). It has been observed that the *integrin β6* expression pattern is highly heterogeneous in primary prostate tumors; its expression is increased in cases of metastatic bone prostate cancer, whereas its expression is undetectable in neuroendocrine prostate tumors (55,56,71,72).

On the other hand, binding of MINDIN to integrins on osteoblasts and osteocytes could trigger signaling in these bone cells to facilitate adhesion of prostate tumor cells. The effects of MINDIN on bone are not restricted solely to modification of integrin expression, but also include actions of this protein on osteoblast proliferation, differentiation, and stimulation of the β-catenin signaling pathway, as well as modulation of different genes involved in bone formation and remodeling (for example, *Runx2, osteocalcin, osterix, OPG, and RANK-L*) (33). In the present study, we observed that stimulation with MINDIN induced an increase in *integrin β6* expression in osteoblasts and *integrin α2* expression in osteocytes, as well as a decrease in α2 expression in osteoblasts, suggesting that, depending on the cell type, MINDIN may modify the expression of different integrins. Although not widely described, integrin α2β1 is a principal receptor for type I collagen, the predominant component of bone, and it has been demonstrated that tumor cells with high *α2β1* expression exhibit greater affinity for bone tissue due to their ability to adhere to collagen (73).

However, in the animal model, only modifications in *integrin β6* were observed, despite osteocytes being the predominant cell type in bone. These changes observed in mice with prostate tumors induced by direct implantation of TRAMP-C1 cells into the prostatic lobe could be due to osteoblasts being more active cells (particularly in terms of collagen production and bone formation) and possessing a cellular machinery different from that of osteocytes, which may influence their ability to respond differently to proteins transported through the serum, such as MINDIN. In addition, because an early premetastatic model was used (1 month of nonmetastatic primary tumor formation), it is likely that the concentrations of MINDIN that may have entered the matrix interior, where osteocytes are located, were still too low to observe modifications in the integrins expressed by osteocytes.

A more detailed understanding of the molecular changes involved in the formation of the bone premetastatic niche, as well as the secretome factors that induce it, could provide new therapeutic targets or intervention protocols, thereby improving prognosis. The present data show that MINDIN expression in primary prostate tumors promotes changes in integrin expression associated with modifications of the bone microenvironment and gene expression in prostate tumor cells. Our data may suggest that MINDIN favors tumor progression and the creation of premetastatic niches in bone through overexpression of *integrin β6* in tumor and osteoblastic cells. However, further studies (including immunohistochemical analyses) are required to elucidate the changes occurring in the different cell types, as well as studies aimed at clarifying how MINDIN may influence *integrin β6* overexpression and promote tumor growth.

Although our study provides new insights into the role of MINDIN in integrin regulation and the possible formation of bone premetastatic niches in prostate cancer, it presents several limitations. First, the experimental models used, both *in vitro* (osteoblastic, osteocytic, and tumor cell lines) and *in vivo* (murine model), may not fully reproduce the complexity of the tumor and bone microenvironment in humans. Furthermore, the murine model employed did not develop established bone metastases, which limits direct evaluation of metastatic progression under real clinical conditions. Another aspect to consider is that the sample size in the animal experiments was limited. It should be emphasized that current data point toward a direct effect of MINDIN on integrin β6 expression, although an individualized correlation study would be a valuable approach for future investigations. Finally, although differential modulation of integrins by MINDIN was demonstrated in different cell types, additional studies

are required to confirm the molecular mechanisms involved and their relevance in human contexts. Future investigations using more representative models and larger sample sizes will be necessary to validate and expand these findings.

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Original

Association of early sports participation with bone mineral density and estimated fracture risk in older women

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Abstract

Background: achieving high peak bone mass during youth is a key protective factor against fractures and osteoporosis later in life. Early sports participation has been associated with long-term benefits for bone health.

Objective: to examine the association between early sports participation, bone mineral density (BMD), and 10-year fracture risk in older women.

Methods: this cross-sectional retrospective study included 52 older women (≥ 60 years; mean age, 70.90 ± 7.17 years), all sedentary or insufficiently active. Participants were divided into 2 groups: without (G0; $n = 29$) and with (G1; $n = 23$) a history of sports participation during childhood and/or adolescence. G1 was further subdivided into G1a (childhood), G1b (adolescence), and G1c (both periods). BMD was assessed by dual-energy x-ray absorptiometry (DXA) at the total body, lumbar spine, forearm, and femoral neck. Fracture risk was estimated using FRAX. Parametric and nonparametric tests were applied; $p < .05$.

Results: women with early sports participation presented significantly higher BMD at the total body; $p = 0.002$, lumbar spine; $p = 0.043$, and femoral neck; $p = 0.001$, as well as a lower estimated risk of major fractures; $p = 0.009$, and femoral neck fractures; $p = 0.034$. The strongest effects were observed among participants who practiced sports during adolescence or during both developmental periods.

Conclusions: early sports participation is associated with higher BMD and lower fracture risk in inactive older women. Encouraging organized physical activity from early life may represent an effective preventive strategy for maintaining bone health throughout aging.

Keywords:
Bone mineral density. Osteoporosis. Fracture. Exercise. Aging.

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INTRODUCTION

Peak bone mass, corresponding to the maximum accumulation of bone mineral density (BMD) achieved primarily during childhood and adolescence, represents a crucial stage of human development and determines bone strength throughout life. Evidence indicates that higher BMD levels attained during youth are strongly associated with reduced fracture risk and delayed onset of conditions such as osteoporosis in later decades (1). This association is partly explained by the high sensitivity of the skeleton to mechanical stimuli during prepuberty and early puberty. During this period, impact activities such as jumping and running not only promote greater structural gains in bone mass but also increase bone formation markers and reduce bone resorption markers (2).

School-based interventions involving impact exercises performed over several months have demonstrated significant increases in BMD, especially in vulnerable regions such as the lumbar spine and femur (3). In addition to increasing BMD, exercise stimuli promote modifications in bone geometry and cortical thickening. An 8-year longitudinal study indicated that vigorous physical activity during childhood is associated with continuous gains in cortical thickness maintained into adulthood (4).

Participation in moderate- to vigorous-intensity exercise during adolescence has also been associated with greater hip bone strength in adulthood, even among individuals who are currently inactive (5). The combination of physical activity and an adequate diet, including balanced calcium, protein, and vitamin D intake, enhances bone development. However, further studies are needed to investigate the isolated effects of each factor (6). In this context, the present study is noteworthy for specifically examining the association between physical activity during adolescence and bone health in adulthood, highlighting the originality of the investigation.

Considering the importance of BMD, skeletal sensitivity during growth, and the long-term effects of early physical activity, further understanding of this association is needed. Therefore, the primary objective of the present study was to investigate the association between sports participation during childhood and adolescence and BMD in older women. As a secondary objective, the study aimed to estimate the 10-year fracture risk according to sports participation during childhood and adolescence. This study seeks to contribute to the understanding of how early physical experiences may influence bone health decades later, assisting healthcare professionals in developing preventive strategies from childhood focused on promoting bone health and reducing the risk of osteoporotic fractures in older age.

METHODS

STUDY TYPE AND SAMPLE SELECTION

We conducted this cross-sectional, retrospective, quantitative study (7) at a university in Recife, Pernambuco, Brazil. The protocol was approved by the Research Ethics Committee (HUOC/UPE; opinion No. 6.855.902), and data collection was performed between January and June 2025 in accordance with the Declaration of Helsinki.

Eligible participants were postmenopausal women aged ≥ 60 years who were sedentary or insufficiently active (categories A and B) (8), were not receiving pharmacological treatment or vitamin supplementation for osteoporosis and had no diagnosis of dementia or cognitive impairment. Women classified as active or very active according to established criteria (8), those with difficulties understanding the interview, or those with physical limitations preventing BMD assessment were excluded.

Participants were recruited through a public invitation disseminated on social media. Interested individuals underwent an initial prescreening to verify eligibility according to the inclusion and exclusion criteria, followed by scheduling of the assessments.

Sample size was calculated according to World Health Organization guidelines for cross-sectional studies (9), adopting a 95 % confidence level, an absolute margin of error of 11 %, and an expected prevalence of 20 % for organized sports participation during childhood or adolescence, based on previous Brazilian data (10). The minimum estimated sample size was 50 participants. As the sample size calculation was based on exposure prevalence, analyses involving BMD outcomes should be interpreted as exploratory.

STUDY DESIGN

After the initial selection and prescreening, 67 older women were recruited and had their evaluations scheduled at a university laboratory. On the day of evaluation, participants underwent a sociodemographic interview that included assessment of current and past health conditions, as well as administration of the International Physical Activity Questionnaire (IPAQ) (8), both aimed at identifying participant characteristics, health status, and verifying compliance with the previously established inclusion and exclusion criteria.

Of the 67 older women recruited, 15 were excluded because they were classified as active or very active, resulting in a final sample of 52 participants. The exclusion of these individuals aimed to ensure the study

focus on women with low or insufficient levels of physical activity. This measure was intended to avoid bias resulting from physiological adaptations associated with regular and intense sports practice, which could influence the evaluated parameters, such as BMD.

Participants who met the eligibility criteria completed an interview regarding early sports participation during childhood and adolescence, as proposed by Fernandes and Zanesco (11). Based on their responses, participants were allocated into the following groups:

- G0: group without sports participation during childhood or adolescence (n = 29).
- G1: group with sports participation during childhood and/or adolescence (n = 23):
  - G1a: group with sports participation only during childhood (n = 5).
  - G1b: group with sports participation only during adolescence (n = 9).
  - G1c: group with sports participation during both childhood and adolescence (n = 9).

Finally, participants underwent BMD assessment using dual-energy x-ray absorptiometry (DXA) at the following sites: whole body, lumbar spine (L1-L4), forearm, and femoral neck (12). Each evaluation session was conducted by the same examiner to ensure procedural standardization. BMD assessment was performed by a trained examiner blinded to participants' group allocation.

INSTRUMENTS

Current physical activity level was assessed using the short version of the IPAQ, validated for the Brazilian population (8). The instrument evaluates the frequency and duration of physical activities performed during the previous 7 days and classifies individuals as sedentary, insufficiently active, active, or very active.

Sports participation during childhood (7-10 years) and adolescence (11-17 years) was retrospectively assessed using 2 dichotomous (yes/no) questions regarding engagement in organized and supervised sports activities outside school for at least 1 year (11). This instrument was selected because of its ease of understanding and high reproducibility (κ = 1.00; p = 0.001) and has been used in epidemiological studies (10,12,13). To facilitate recall, examples of common sports and school-stage temporal references were provided during the interview.

BMD was assessed by DXA using a Hologic device (Discovery CI/WI; software QDR4500W, version 11.2). Total body BMD and regional BMD at the lumbar spine (L1-L4), femoral neck, and forearm were evaluated according to standardized positioning protocols (14). All DXA scans were performed by a single trained examiner blinded to group allocation.

Fracture risk was estimated using FRAX adapted for the Brazilian population (15), incorporating femoral neck BMD and clinical risk factors, including age, sex, history of fractures, family history of fractures, corticosteroid use, smoking, alcohol consumption, rheumatoid arthritis, and secondary osteoporosis. The model estimated the 10-year probability of major osteoporotic and hip fractures (16).

STATISTICAL ANALYSIS

Numerical variables were described as mean ± SD. Data normality was assessed using the Shapiro-Wilk test, and homogeneity of variances between groups was evaluated using Levene test, both considered prerequisites for the application of parametric tests. Data entry was performed blindly, without prior knowledge of the group to which each participant belonged, and was subsequently verified by a second investigator to ensure data accuracy.

For comparisons between groups G0 (no sports participation during childhood and/or adolescence) and G1 (sports participation during this period), the independent-samples Student t test was applied to variables with normal distribution and homogeneity of variances. For variables that did not meet these assumptions ($p < 0.05$ in the Shapiro-Wilk or Levene tests), the Mann-Whitney U test was used. In both cases, a significance level of $p < 0.05$ was adopted. Effect size was calculated according to Cohen d formula and interpreted as follows: negligible ($\geq$ -0.15 and < 0.15), small ($\geq$ 0.15 and < 0.40), medium ($\geq$ 0.40 and < 0.75), large ($\geq$ 0.75 and < 1.10), and very large ($\geq$ 1.10 and < 1.45).

For comparisons among groups G0 (no sports participation), G1a (participation only during childhood), G1b (participation only during adolescence), and G1c (participation during both childhood and adolescence), 1-way ANOVA was used for variables that met assumptions of normality and homogeneity of variance. When these assumptions were not met, the nonparametric Kruskal-Wallis test was applied as an alternative. All statistical analyses were performed using IBM SPSS Statistics software, version 28.0.

RESULTS

The sample consisted of 52 older women, with a mean age of 70.9 ± 7.2 years. Participants were allocated into groups according to early sports participation. Detailed anthropometric and clinical characteristics of the total sample and each group are presented in table I.

Table I. Sample characterization (n = 52)

Variables	Mean ± SD	95 %CI	p
Age (years)	70.90 ± 7.17	68.90-72.90	0.333
Body mass (kg)	66.50 ± 10.6	63.50-69.50	0.771
Height (cm)	155.00 ± 7.10	153.00-157.00	0.986
BMI (kg/m^2)	27.70 ± 3.40	26.60-28.80	0.482
Bone tissue (kg)	2.00 ± 0.75	1.79-2.21	0.001*
BMD FB (kg/cm^2)	0.958 ± 0.52	0.932-0.984	0.733
BMD LS (kg/cm^2)	0.897 ± 0.59	0.854-0.940	0.001*
BMD forearm (kg/cm^2)	0.419 ± 0.11	0.387-0.450	0.001*
BMD FN (kg/cm^2)	0.711 ± 0.11	0.681-0.741	0.210
Major fractures (%)	4.05 ± 2.66	3.31-4.79	0.001*
Hip fractures (%)	1.18 ± 1.44	0.77-1.58	0.001*

*p < *0.05. BMI: body mass index; BMD: bone mineral density; FB: full body; LS: lumbar spine; FN: femoral neck.*

When comparing bone parameters between groups G0 and G1, group G1 showed significantly higher values. Total bone tissue was 21.31 % greater in G1 (p = 0.038), with a medium effect size (ES = 0.53); total body BMD was 8.11 % higher (p = 0.002), with a large effect size (ES = 0.89); lumbar spine BMD showed a 10.10 % increase (p = 0.043), with a medium effect size (ES = 0.57); and femoral neck BMD was 15.14 % higher (p = 0.001), with a large effect size (ES = 1.06). Furthermore, the estimated 10-year fracture risk was lower in group G1: major osteoporotic fractures showed a 38.90 % lower probability compared with G0 (p = 0.009), with a large effect size (ES = 0.75); and hip fractures were 47.79 % less likely (p = 0.034), with a medium effect size (ES = 0.50) (Table II).

When comparing bone parameters between group G0 and the subgroups that practiced sports during childhood and/or adolescence (G1a, G1b, and G1c), significant differences were observed in total body BMD (p = 0.047), with group G1b showing the highest values (1.013 ± 0.09 kg/cm^2), corresponding to values 17.93 % higher than those of G0. Regarding femoral neck BMD (p = 0.018), the best results were found in group G1c (0.794 ± 0.14 kg/cm^2), with values 19.04 % higher than those of G0.

Table II. Differences between the group without sports participation during childhood or adolescence (G0) and the group with sports participation during childhood and/or adolescence (G1) in bone tissue, bone mineral density (full body, lumbar spine, forearm, and femoral neck), and fracture risk (major osteoporotic fractures and hip fractures)

Variable	G0 (n = 29) Mean ± SD	G1 (n = 23) Mean ± SD	Δ (%)	Confidence interval		p (ES)
				Lower	Upper	
Bone tissue (kg)	1.83 ± 0.66	2.22 ± 0.82	0.39 (21.31)	-0.80	2.35	0.038*(0.53)
BMD FB (kg/cm^2)	0.925 ± 0.08	1.000 ± 0.08	0.075 (8.11)	-0.120	0.280	0.002*(0.89)
BMD LS (kg/cm^2)	0.859 ± 0.03	0.945 ± 0.10	0.086 (10.01)	-0.171	0.002	0.043*(0.57)
BMD forearm (kg/cm^2)	0.409 ± 0.07	0.431 ± 0.14	0.022 (5.38)	-0.085	0.041	0.486 (0.19)
BMD FN (kg/cm^2)	0.667 ± 0.08	0.768 ± 0.11	0.101 (15.14)	-0.154	0.047	0.001*(1.06)
Major fractures (%)	4.90 ± 2.80	2.99 ± 1.99	1.91 (38.90)	0.498	3.31	0.009*(0.75)
Hip fractures (%)	1.49 ± 1.60	0.778 ± 1.12	0.712 (47.79)	-0.076	1.51	0.034*(0.50)

*p < *0.05. G0: group without sports participation during childhood or adolescence; G1: group with sports participation during childhood and/or adolescence; ES: effect size; BMD: bone mineral density; FB: full body; LS: lumbar spine; FN: femoral neck.*

Regarding the 10-year fracture risk estimates, the lowest values were observed in group G1b. For major osteoporotic fractures (p = 0.022), this group had a risk of 2.53 ± 1.02 %, representing a 48.73 % lower risk compared with G0. For hip fractures (p = 0.019), the risk in G1b was 0.36 ± 0.25 %, corresponding to a 74.10 % lower risk of hip fracture compared with G0 (Table III).

DISCUSSION

The results of this study show that older women with a history of sports participation during childhood and/or adolescence have better bone health parameters and a lower estimated fracture risk compared with those who did not practice sports during these periods (p < 0.05). The group that reported early sports participation (G1) showed significantly higher BMD values at the total body, lumbar spine, and femoral neck compared with the group without this history (G0), in addition to a lower estimated risk of major osteoporotic fractures and hip fractures over the subsequent 10 years. These findings indicate that early exposure to structured physical activity may contribute to long-term skeletal benefits, even in the absence of regular physical activity during older age.

The association between sports participation during youth and better bone health in later life reinforces the role of physical activity during growth as a key determinant of achieving higher peak bone mass (1,2), which may exert a protective effect even in the presence of low levels of physical activity during senescence. Previous studies suggest that combined interventions involving adequate diet and physical activity during childhood and adolescence promote BMD accumulation, although evidence regarding the long-term persistence of these effects remains limited (6). Although the present study focused on childhood and adolescence, it is important to emphasize that regular physical exercise during older age—particularly resistance training—may also positively influence BMD in postmenopausal women. High-intensity training (≥ 70 % 1RM), performed 3 times per week for at least 40 minutes per session, appears to be optimal (17).

These findings reinforce the importance of understanding the impact of different life stages on bone health and suggest that the benefits of early physical activity may persist over time, even in the absence of continuous stimuli throughout adulthood.

The magnitude of the differences observed between the groups analyzed in this study reinforces the clinical relevance of the findings. Femoral neck BMD, a skeletal site highly vulnerable to osteoporotic fractures, was 15.14 % higher in group G1, with a large effect size (ES = 1.06). Furthermore, the 47.79 % difference in FRAX-estimated hip fracture risk suggests a potential protective effect of early physical activity, even among sedentary or insufficiently active older women. Reduction in fracture risk is a particularly relevant finding, considering that falls—a common precursor to fractures—represent the leading cause of death from unintentional injuries among adults aged ≥ 65 years (18). In this context, a recent systematic review demonstrated that physical exercise interventions are associated with reduced falls across several high-quality trials and provide significant benefits for multiple health outcomes (19).

Thus, the findings presented here may have important implications for public health policies aimed at pre-

Table III. Differences among the group without sports participation during childhood or adolescence (G0), the group with sports participation only during childhood (G1a), the group with sports participation only during adolescence (G1b), and the group with sports participation during both childhood and adolescence (G1c) in bone tissue, bone mineral density (full body, lumbar spine, forearm, and femoral neck), and fracture risk (major osteoporotic fractures and hip fractures)

Variable	G0 (n = 29) Mean ± SD	G1a (n = 5) Mean ± SD	G1b (n = 9) Mean ± SD	G1c (n = 9) Mean ± SD	F (df1, df2)	p
Bone tissue (kg)	1.83 ± 0.66	2.23 ± 1.00	2.28 ± 0.90	2.17 ± 0.72	0.974 (3, 12.1)	0.437
BMD FB (kg/cm^2)	0.925 ± 0.08	0.972 ± 0.10	1.013 ± 0.09	1.005 ± 0.07	3.49 (3, 12.9)	0.047*
BMD LS (kg/cm^2)	0.859 ± 0.03	0.993 ± 0.05	0.942 ± 0.03	0.923 ± 0.08	1.70 (3, 14.7)	0.211
BMD forearm (kg/cm^2)	0.409 ± 0.07	0.440 ± 0.05	0.412 ± 0.16	0.445 ± 0.17	0.406 (3, 12.7)	0.0752
BMD FN (kg/cm^2)	0.667 ± 0.08	0.728 ± 0.06	0.763 ± 0.07	0.794 ± 0.14	4.71 (3, 13.8)	0.018*
Major fractures (%)	4.90 ± 2.80	3.70 ± 1.59	2.53 ± 1.02	3.02 ± 2.79	4.42 (3, 14.1)	0.022*
Hip fractures (%)	1.49 ± 1.60	1.42 ± 1.53	0.36 ± 0.25	0.83 ± 1.33	4.80 (3, 12.7)	0.019*

*$p < 0.05$. *G0: group without sports participation during childhood or adolescence; G1a: group with sports participation only during childhood; G1b: group with sports participation only during adolescence; G1c: group with sports participation during both childhood and adolescence; BMD: bone mineral density; FB: full body; LS: lumbar spine; FN: femoral neck.*

venting falls and fractures by highlighting the importance of building a robust skeletal foundation during youth.

Subgroup analysis revealed that the benefits varied according to the developmental period during which sports participation occurred. Group G1c, which practiced sports during both childhood and adolescence, showed the highest femoral neck BMD values, whereas group G1b, with sports participation exclusively during adolescence, exhibited the lowest fracture risks, especially for major osteoporotic fractures. These findings suggest that adolescence may represent a critical period for consolidating bone adaptations induced by mechanical stimuli, consistent with the literature indicating puberty as the stage of greatest velocity of bone mass acquisition, influenced by hormonal and biological maturation changes (3). Considering that most Brazilian adolescents currently do not participate in sports activities and fail to meet minimum physical activity recommendations (20), it is necessary to develop and implement strategies that facilitate physical exercise across multiple settings, including schools and leisure-time activities.

Prioritizing physical activity during adolescence should therefore be considered a long-term investment in musculoskeletal health, with the potential to reduce the burden of osteometabolic diseases in the aging population.

Despite its cross-sectional and retrospective design, the present study used a validated self-report instrument to assess sports participation during childhood and adolescence, which has been widely applied in adult populations and has demonstrated high reproducibility in previous studies (11,12). In addition, the exclusion of participants who were currently active or very active helped isolate the potential long-term effects of early sports participation, thereby reducing confounding related to current physical activity levels.

Nevertheless, some limitations should be acknowledged. Owing to the cross-sectional and retrospective design, causal relationships cannot be established, and the findings should be interpreted as associations. The assessment of early sports participation relied on a dichotomous self-report measure (yes/no) encompassing broad developmental periods, which limited exposure precision because information regarding intensity, duration, type of sport, and mechanical loading characteristics was not collected and may have been subject to recall bias despite the high reproducibility of the instrument.

The sample size was relatively small, and subgroup analyses resulted in small numbers of participants, which may have reduced statistical power and contributed to instability in some estimates. In addition, recruitment through social media may have introduced selection bias, with possible overrepresentation of individuals with greater digital literacy and healthier profiles, thereby limiting the generalizability of the findings. Important factors potentially influencing BMD throughout life—such as nutritional intake (eg, calcium and vitamin D), sun exposure, medication use, hormonal status, reproductive history, and menstrual characteristics—were not controlled for and should be addressed in future investigations.

Despite these limitations, the findings support the hypothesis that regular sports participation during growth is associated with more favorable bone health indicators during older age, reinforcing the relevance of early-life physical activity as a potential contributor to long-term skeletal integrity.

CONCLUSIONS

This study aimed to investigate whether participation in organized sports during childhood and adolescence is associated with bone health and fracture risk in older women. The findings indicate that women who reported early engagement in sports activities presented higher BMD values at the total body, lumbar spine, and femoral neck, as well as a lower estimated 10-year risk of major osteoporotic and hip fractures compared with those without such history. These findings suggest a positive association between early exposure to mechanical loading and skeletal parameters later in life.

Although the cross-sectional and retrospective design does not permit causal inference and potential recall bias should be considered, the findings suggest that participation in organized sports during youth may be associated with more favorable bone health outcomes in later life. Promoting structured physical activity during childhood and adolescence may therefore represent an important strategy within broader public health approaches aimed at supporting skeletal health throughout the lifespan. Future longitudinal and controlled studies including larger and more diverse populations are necessary to confirm these associations and to better elucidate the long-term relationship between early sports participation and bone health.

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Original

Transition of patients with pediatric-onset metabolic bone diseases: variability in follow-up and proposal for transition bone densitometry

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Abstract

Objectives: to evaluate variability in the transition of patients with metabolic bone diseases (primary pediatric osteoporosis, secondary osteoporosis, and low bone mass) from pediatric units to adult specialties, and to analyze the use of bone densitometry and the existence of structured protocols.

Material and methods: a 17-question survey was designed and addressed to professionals from the different specialties involved in the follow-up of patients with metabolic bone diseases. It was distributed through the following scientific societies: Sociedad Española de Reumatología Pediátrica, Sociedad Española de Reumatología, Sociedad Española de Endocrinología Pediátrica, Sociedad Española de Endocrinología y Nutrición, Sociedad Española de Investigación Ósea y del Metabolismo Mineral, and Asociación Española de Pediatría. A total of 147 responses were collected and a descriptive analysis was performed.

Results: during pediatric age, follow-up was mainly conducted by Rheumatology (51 %) and Endocrinology (44 %). After transfer, Adult Rheumatology predominated (primary osteoporosis 47.6 %, secondary osteoporosis 44.5 %). Regarding loss to follow-up, a total of 45 % of respondents were unaware whether patients remained under follow-up, 17 % estimated a loss of 20-40 %, and 15 % estimated a loss of 0-20 %. Only 10 % had a formalized protocol. A total of 22 % performed bone densitometry during transition, and 45 % used the same device throughout all stages. During pediatric age, explored sites included lumbar spine (55 %), hip (27.4 %), and whole body (15 %). Hip assessment decreased to 19 % during transition, despite its potential usefulness as a reference for adulthood.

Conclusions: there is substantial heterogeneity in follow-up and use of bone densitometry during transition. We propose the concept of "transition bone densitometry" as an applicable strategy to improve continuity of care in these patients.

Keywords:
Bone density.
Transitional care.
Adolescents.
Follow-up studies.
Osteoporosis.

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INTRODUCTION

Adolescence is an important period in an individual's development, which, according to the World Health Organization (WHO), ranges from 10 to 19 years of age (1). Adolescence is a variable phase of life during which personal identity is formed (2) and constitutes a particularly delicate period for patients with chronic diseases. This is because characteristics inherent to this phase, such as vulnerability and questioning of authority, coexist with others associated with chronic illness, such as the gradual acquisition of responsibility for one's own health. In addition, during adolescence there is a greater risk of the emergence of risk behaviors (dietary habits, toxic habits, accidents, etc.), which must be identified to ensure appropriate prevention or treatment. Furthermore, it has been described that during adolescence and the transfer to adult units there is a greater risk of loss to follow-up and fragmentation of medical visits than during other periods of life (1).

Transition in chronic diseases is a planned process that goes beyond the mere administrative transfer from pediatric care to adult specialties. The main objective of this process is to ensure continuity of care, promote the patient's progressive autonomy, and preserve well-being during this stage of life (3). In recent years, this process has gained increasing recognition, with the implementation of different transition models adapted to health care systems and to the characteristics of each chronic disease (3). In the field of pediatric rheumatology, specific transition strategies have been developed mainly for juvenile idiopathic arthritis (JIA), with the creation of recommendations that include coordination between pediatric and adult teams and patient preparation tools for disease self-management (4,5). These initiatives have demonstrated their usefulness in reducing loss to follow-up, improving therapeutic adherence, and facilitating comprehensive care during adulthood in pediatric-onset chronic diseases (4-6).

Despite these advances, transition in the management of patients with pediatric-onset metabolic bone diseases (MBDs), such as primary pediatric osteoporosis, secondary osteoporosis, and low bone mass, remains an underdeveloped area both in clinical practice and in the related literature, despite the need to prevent complications and fractures. Recently, the TEAM project (Transition to Adulthood in Patients with Metabolic Bone Diseases), which brought together eight scientific societies, presented recommendations in 2023 for transition in metabolic bone diseases and a practical algorithm for their implementation, highlighting the need for coordination among multidisciplinary teams, individualized plans, and active patient involvement in the process (7). However, this document does not detail how clinical follow-up during transition should be performed, including key aspects such as bone densitometry (DXA), which constitutes an unmet area of need.

Interpretation of DXA in the pediatric population presents relevant differences vs routine adult practice, both in image acquisition and in the regions of interest evaluated. According to the recommendations of the International Society for Clinical Densitometry (ISCD) (8), in children and adolescents the preferred sites are the lumbar spine and total body less head, the latter being especially useful for global assessment of bone mass and body composition and not routinely used in adult densitometric practice. Similarly, hip measurement is not systematically recommended in the pediatric population because of technical and interpretative limitations. These methodological particularities condition the comparability of densitometric studies throughout growth and become especially relevant during the transition period to adulthood, when diagnostic criteria and evaluated regions of interest change.

Therefore, we aimed to determine the current situation of transition in patients with MBDs in Spain through a survey addressed to professionals involved in the management of these pediatric diseases and in adolescents and young adults, distributed through the main national scientific societies. The endpoints, results, and conclusions are presented in this study.

ENDPOINTS

PRIMARY ENDPOINT

To evaluate variability in the transition process of patients with metabolic bone diseases (primary pediatric osteoporosis, secondary osteoporosis, and low bone mass) from pediatric units to adult care.

SECONDARY ENDPOINTS

- To identify the specialties involved in the transition process and follow-up of these diseases during pediatric and adult age.
- To describe current practices in bone densitometry during pediatric age and the transition stage.
- To evaluate the existence of structured protocols related to transition in MBDs.
- To develop a conceptual proposal for transition bone densitometry based on the findings obtained.

MATERIAL AND METHODS

We conducted a cross-sectional, descriptive study based on a national survey addressed to professionals involved in the management of MBDs in pediatric, adolescent, and young adult patients.

For the purposes of this study, transition was considered the period between the end of follow-up in the

pediatric clinic and consolidation of follow-up in specialized adult care. This process involves the progressive preparation of adolescents with chronic diseases for integration into the adult health care system and should not be limited solely to the moment of administrative transfer, but rather constitutes a planned and gradual process that develops throughout adolescence and young adulthood (9). In most health care systems, transfer of care usually occurs between 14 and 18 years of age.

The survey was specifically designed for this study by the Metabolic Bone Diseases Working Group of the Sociedad Española de Reumatología Pediátrica (SERPE), following a review of the available literature on transition in chronic diseases and recent national recommendations. The content was agreed upon by the investigators before dissemination. The questionnaire included 17 closed-ended questions (multiple-choice and dichotomous) related to clinical follow-up, the use of bone densitometry, the specialties involved in the transition process, and the existence of structured protocols. An open-ended question was also included to identify the center and city of origin of the respondent. However, due to heterogeneity in completion and lack of standardization in responses, it was not possible to perform a reliable analysis of the characteristics of participant centers.

The complete survey is included as supplementary material (https://www.revistadeosteoporosisymetabolismomineral.com/files/525/ADMA1-00117-02.pdf). It was distributed during the 1st and 2nd quarters of 2024 through the main national scientific societies related to the management of MBDs*:* Sociedad Española de Reumatología Pediátrica (SERPE), Sociedad Española de Reumatología (SER), Sociedad Española de Endocrinología Pediátrica *(SEEP),* Sociedad Española de Endocrinología y Nutrición (SEEN), Sociedad Española de Investigación Ósea y del Metabolismo Mineral (SEIOMM), and Asociación Española de Pediatría (AEP*). Responses were collected anonymously* using an electronic form. A descriptive analysis of the obtained data was performed, expressing categorical variables as absolute frequencies and percentages. It was not possible to estimate the overall response rate because the exact number of professionals who received the invitation through the different scientific societies was unavailable.

RESULTS

A total of 147 valid responses were obtained.

Regarding the specialty of the respondents, 40 % corresponded to General Pediatrics, 21 % to Pediatric Endocrinology, 19 % to Pediatric Rheumatology, and 7.5 % to Adult Rheumatology. Regarding the dissemination channel, 58 % of respondents became aware of the survey through the Asociación Española de Pediatría (AEP), 25 % through the Sociedad Española de Reumatología Pediátrica (SERPE), 13 % through Sociedad Española de Endocrinología Pediátrica (SEEP), and 3 % through Sociedad Española de Reumatología (SER), with no responses directly originating from Sociedad Española de Investigación Ósea y del Metabolismo Mineral (SEIOMM). The distribution across scientific societies also reflected differences in the effectiveness of the dissemination channels used.

Regarding follow-up during pediatric age, patients with primary pediatric osteoporosis (PO) were mainly managed by Pediatric Rheumatology (51.4 %) and Pediatric Endocrinology (45.1 %), whereas 6.3 % of respondents indicated that no structured follow-up existed. A similar pattern was observed in the follow-up of secondary pediatric osteoporosis (resulting from osteopenia-inducing diseases or medications). In patients with low bone mass (LBM) without fractures, management was more variable, with greater participation of general pediatrics (13 %) and up to 11 % of professionals reporting that they did not know whether follow-up was performed or who was responsible for it.

The results regarding respondents' answers concerning follow-up by specialties during pediatric and adult age are shown in table I.

After transfer to adulthood, follow-up of these patients was predominantly carried out by Adult Rheumatology, especially in cases of primary osteoporosis (47.6 %) and secondary osteoporosis (44.5 %). However, it was striking that approximately 45 % of respondents were unaware whether the patient continued to receive medical care after discharge from pediatric consultation, highlighting a substantial loss of traceability.

In line with this, when analyzing respondents' perceptions regarding loss to follow-up during transition, 17 % estimated that between 20 % and 40 % of patients were lost to follow-up, while 15 % considered the loss to be below 20 %. Nevertheless, more than half of respondents (55 %) did not know or did not answer this question, reflecting the lack of formalized pathways and emphasizing the difficulty in estimating the true loss to follow-up in this population.

Regarding the existence of a standardized protocol for transition in patients with MBDs, only 10 % of centers had a specific protocol for primary pediatric osteoporosis, a percentage that decreased to 5.5 % in cases of low bone mass without fractures (Fig. 1).

With respect to the use of DXA in the pediatric population, substantial heterogeneity was observed in the explored regions of interest.

Table I. Follow-up of metabolic bone disease by specialty

	Primary PO, *n* (%)	Secondary PO, *n* (%)	LBM, *n* (%)
Follow-up by specialty during pediatric age			
Pediatric Rheumatology	74 (51.4)	69 (47.6)	61 (41.8)
Pediatric Endocrinology	65 (45.1)	64 (44.1)	66 (45.2)
General Pediatrics	14 (9.7)	13 (9)	19 (13)
Rheumatology	12 (8.3)	12 (8.3)	10 (6.8)
Nephrology	8 (5.6)	12 (8.3)	8 (5.5)
Others	10 (7)	15(10.3)	14(11.6)
None	9 (6.3)	10 (6.9)	16 (11)
Follow-up by specialty during adulthood			
Rheumatology	70 (47.6)	65 (44.5)	44 (30.1)
Endocrinology	17 (11.6)	13 (8.9)	15 (10.3)
Internal Medicine	8 (5.4)	11 (7.5)	8 (5.5)
Family Medicine	9 (6.1)	7 (4.8)	20 (13.7)
Others	0	2 (1.4)	0

PO: Pediatric osteoporosis; LBM: low bone mass.

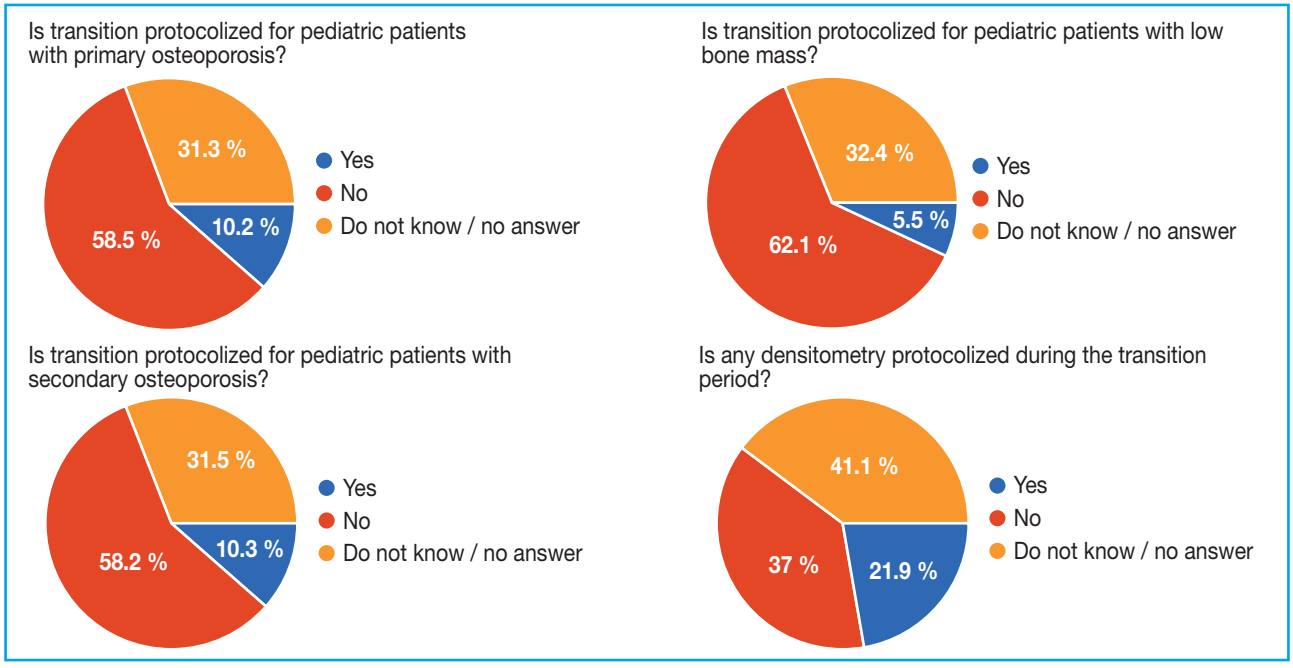

Figure 1. Protocolization of MBD transition.

A total of 55 % of respondents reported requesting lumbar spine DXA during childhood, whereas only 15 % performed whole-body DXA, and as many as 27.4 % requested hip DXA, decreasing to 19 % during the transition period.

On the other hand, although structured transition was protocolized in only a minority of centers, 22 % of professionals reported performing DXA during the transition to adult care. Regarding technical continuity, 45 %

of respondents indicated that the same densitometer was used during pediatric age, the transition period, and adulthood, facilitating longitudinal interpretation.

DISCUSSION

Transition in pediatric-onset chronic diseases constitutes a particularly sensitive stage during which continuity of clinical follow-up must be guaranteed. In the case of MBDs, our results demonstrate marked interhospital variability in the specialties involved both during pediatric age and after transfer to adult specialties. This heterogeneity becomes more pronounced during the transition period, where a lack of structured protocols was observed, in line with recent publications from national groups and despite the recommendations already proposed (7). This situation is also accompanied by a significant loss of patient traceability after discharge from pediatric care.

Although transition in pediatric chronic diseases has been widely addressed in the international literature, evidence specifically focused on metabolic bone diseases is limited. Recent reviews highlight the absence of standardized models and the need for specific recommendations for pediatric-onset bone diseases, as well as the heterogeneity in transition organization (10). In specific disorders such as osteogenesis imperfecta, difficulties in continuity of care and variability in the implementation of transition programs have also been described (11,12). Taken together, these data suggest that the challenges observed in our study are not exclusive to our setting and reinforce the need for structured strategies to improve continuity of care in these diseases.

One of the most relevant findings of our study is the limited systematization in the use of DXA during transition. Despite being a key tool for the diagnosis and monitoring of MBDs, only 22 % of respondents reported using it routinely in this context.

Particularly striking was the disparity in the regions of interest explored during pediatric age, as well as the inclusion of sites not recommended in this population, such as the hip (8,13), whose use was twice as frequent as whole-body assessment, despite the latter being recommended. This finding raises the hypothesis that, in some centers, adult protocols may be being applied without adaptation to pediatric age.

Of note, the percentage of respondents requesting hip DXA decreased from 27 % during pediatric age to 19 % during transition, despite the potential usefulness of this site in prospective densitometric evaluation in adulthood, allowing the availability of an initial reference point.

On the other hand, only 45 % of centers reported using the same densitometer throughout all stages of care, limiting longitudinal comparability of results and their evolutionary interpretation.

These data reinforce the need to emphasize a shared strategy among the different medical specialties involved in the transition process.

In this context, our working group proposes the concept of "transition DXA" (Table II), understood as a planned evaluation acting as a technical and clinical bridge between pediatric and adult stages, ensuring diagnostic continuity and facilitating medium- and long-term therapeutic decision-making. Transition DXA should form part of a structured transition protocol. However, in the absence of such a protocol, it could still be applied in adolescents with MBDs in whom persistence into adulthood is suspected. Transition DXA would integrate the regions of interest specific to pediatric and adult age, using pediatric software for interpretation, and would include the following regions: spine, total body less head, and hip. Similarly, densitometric studies should be performed on the same device to allow valid comparison over time.

The age period during which this transition DXA should be applied has not yet been established, but from a pathophysiological perspective, it could extend until peak bone mass acquisition, which usually occurs around 20 years of age (14,15). The official positions of the International Society for Clinical Densitometry (ISCD) (8) recognize the particularities of densitometric evaluation in the adolescent population, as well as the complexity of changing diagnostic criteria upon reaching adulthood, but do not define a specific strategy for the transition period. In this context, our proposal for "transition DXA" is aligned with these official positions, integrating pediatric and adult regions in a structured manner and aiming to fill a practical gap in the longitudinal densitometric follow-up of these patients.

Table II. Transition bone densitometry*

Up to puberty (12-16 years)	Pediatric DXA	– Lumbar spine – Total body less head ~(consider necessary adjustments for height)
From 12-16 to 20 years	Transition DXA	– Lumbar spine – Total body less head – Proximal femur
From 20 years onward	Adult DXA	– Lumbar spine – Proximal femur

**It is advisable that the examination always be performed using the same densitometer.*

We believe that this proposal may constitute a first step toward protocolization of densitometric follow-up in patients with MBDs, contributing to improved continuity of care and quality of management throughout the life cycle. This proposal should be evaluated in prospective studies assessing its feasibility and clinical applicability.

This study has limitations inherent to its survey-based design. There is a possible selection bias, since the professionals who responded may have had a greater interest in transition in MBDs, which may limit the generalizability of the results. In addition, the responses reflect individual perceptions rather than audited clinical data and therefore may not accurately represent real-world practice. The questionnaire was specifically designed for this study and was not subjected to a formal external validation process, which could influence the reproducibility of the results. Nevertheless, its content was agreed upon by a group of experts in MBDs after review of the available literature.

Although the survey was disseminated through several scientific societies, homogeneous representation of all healthcare settings and the entire national territory cannot be ensured. Similarly, we could not estimate the overall response rate, since the survey was distributed through different scientific societies without access to the exact number of professionals who received the invitation. Finally, although an open-ended question was included to identify the hospital or workplace, variability in completion prevented homogeneous categorization of the level of care (tertiary referral center, regional hospital, etc.) or comparative analysis between centers. Future studies should incorporate structured variables that allow analysis of these organizational differences.

CONCLUSIONS

The results of this study demonstrate considerable heterogeneity in the transition process of patients with pediatric-onset MBDs, both in the organization of follow-up and in the use of diagnostic tools such as DXA. This variability, together with the lack of structured protocols and limited traceability after discharge from pediatric care, underscores the need to improve coordination among specialties.

Our results also highlight marked variability in densitometric practice during pediatric age and the transition period, including the use of regions of interest not recommended in the pediatric population and the absence of technical continuity in a relevant number of centers. These findings suggest heterogeneous application of existing recommendations and reinforce the need for shared strategies that facilitate longitudinal interpretation of bone mass from pediatric age through adulthood.

In response to this need, we propose the concept of "transition DXA" as a key tool to ensure diagnostic continuity between pediatric and adult stages. In addition to its clinical value, transition DXA could represent a simple and feasible first step to implement in centers that do not yet have a formal transition protocol, thereby promoting more homogeneous and higher-quality care in this vulnerable population.

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Brief Communication

Can the BES TEST help in assessing the risk of fragility fractures in patients with normal or osteopenic DEXA T-score?

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Abstract

Introduction: osteoporosis is characterized by reduced bone mass and deterioration of bone microarchitecture, leading to bone fragility. Dual-energy x-ray absorptiometry (DEXA), which measures bone mineral density (BMD), is considered the gold standard for the diagnosis of osteoporosis and osteopenia. However, BMD is only one among several fracture risk factors. Consequently, DEXA should be associated with fracture risk assessment tools (FRAX, DeFRA, or FRA-HS). Therefore, the development of additional tests capable of measuring trabecular structure properties (quality), rather than only bone density, is needed.

Methods: the Bone Elastic Structure Test (BES TEST) is a CE-marked, registered software medical device that measures bone elastic response to loads. It is based on an internationally patented method using high-definition digital radiographs of the proximal epiphysis of 3 fingers to investigate the biomechanical functionality of the trabecular structure in terms of its contribution to bone strength.

Results: the main objective of the study is to assess the reliability and performance of the BES TEST in patients with fragility fractures and normal or osteopenic DEXA T-scores. Previous data suggest that the BES TEST could be a useful complementary tool for completing fracture risk assessment. Therefore, we conducted a randomized controlled trial in which 50 female patients, divided into 2 groups (25 nonfractured and 25 recently fractured), underwent baseline lumbar spine, femoral, and femoral neck DEXA, together with a baseline BES TEST. Both examinations were repeated after 18 to 24 months, with the addition of spine morphometry.

Conclusions: preliminary data confirm the reliability and reproducibility of both DEXA T-score and BSI T-score. The expected result is confirmation that BSI, associated with DEXA and FRAX-score or DeFRA-score, can help assess the risk of fragility fractures.

Keywords:
BES TEST.
Osteoporosis.
Osteopenia.
Fragility fractures.
DEXA.

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INTRODUCTION

Osteoporosis (OP) is a condition characterized by reduced bone mass and deterioration of bone microarchitecture. This process leads to bone fragility and a consequent high probability of fractures. Dual-energy x-ray absorptiometry (DEXA), which measures bone mineral density (BMD), is considered the gold standard for the diagnosis of osteoporosis and osteopenia. DEXA expresses results in terms of T-score, a statistical value indicating the number of standard deviations below the average value of young White adults. BMD is commonly measured at the lumbar spine, left femur, and left femoral neck. Wrist BMD can also be measured when both femurs are unavailable (eg, because of bilateral prostheses), whereas DEXA total-body scanning is currently limited to the study of sarcopenia through the measurement of muscle mass and body fat mass (1,2).

According to WHO criteria, 4 different degrees of bone mineral density can be defined: a) normal, when the BMD value is within 1 standard deviation of the T-score reference range (T-score ≥ –1); b) osteopenia, when the BMD value is between 1 and 2.5 standard deviations below the young adult mean; c) osteoporosis, when the BMD value is 2.5 standard deviations below the young adult mean; and d) severe osteoporosis (or established osteoporosis), when the BMD value is 2.5 standard deviations below the young adult mean and 1 or more fragility fractures have occurred. Fragility fractures are the consequence of low-energy trauma, defined according to WHO criteria as a fall from standing height (1,3,4). The correct densitometric diagnosis is based on the lowest T-score found in the spine, femur, or femoral neck (5,6). However, it is clear that BMD is only 1 among several fracture risk factors. For this reason, DEXA should be associated with fracture risk assessment tools.

FRAX (Fracture Risk Assessment Tool, developed in 2008 by the Centre for Metabolic Bone Diseases of the University of Sheffield; the Italian version was revised in 2013) (7), DeFRA (software developed by the Italian Society for Osteoporosis, Mineral Metabolism and Bone Diseases in 2012 and recently revised), and FRAHS (developed by the Italian Society for General Practitioners in 2017) (8,9) are currently used alongside DEXA to estimate the risk of major fractures. These algorithms are designed to predict the 10-year fracture risk based on the most common and severe risk factors for fragility fractures: spine and femoral BMD, smoking, daily alcohol intake (> 3 units), previous fragility fractures, corticosteroid use (daily dose), arthritis or chronic inflammatory connective tissue diseases, age at menopause, low body weight (BMI < 18.5), and family history of major osteoporotic fractures. Clearly, DEXA can evaluate only BMD, but not bone quality; therefore, alterations in bone microarchitecture are probably responsible for the high number of fragility fractures occurring in patients with osteopenia (10,11).

Given that DEXA alone cannot be considered a reliable predictive tool for preventing fragility fractures, the development of additional tests capable of measuring trabecular structure properties (quality), rather than only bone density, is needed.

TBS (Trabecular Bone Score) is a textural index of bone microarchitecture derived from DEXA images. It analyzes gray-level variations in the lumbar spine, providing information regarding trabecular bone microarchitecture (12).

However, TBS remains under discussion because of several limitations. It can only be applied to lumbar spine DEXA images, which are themselves limited by the frequent presence of osteophytes and aortic calcifications (13,14).

Conversely, the Bone Elastic Structure Test (BES TEST) measures the elastic response of bone to loading. Based on a direct discrete numerical approach (15), the BES TEST analyzes low-dose (< 0.005 mSv) planar radiographic projections of the proximal epiphysis of the first phalanx of the hand to perform a noninvasive biomechanical evaluation of trabecular bone microarchitecture through engineering simulations of load application, thereby quantifying pathological alterations in bone microarchitecture (16). BES TEST is a CE-marked, registered software medical device based on an internationally patented method. The BES TEST software processes radiographic images by transforming them into numerical structural models that are used to simulate compression loading through the Cell Method approach, which is highly effective in terms of robustness, computation time, memory requirements, and accuracy of results (17,18).

The simulation outcomes are combined into an index, the Bone Structure Index (BSI), which reflects the ability of trabecular bone structure to absorb loads (19). The BES TEST outputs are independent of bone mineral density as measured by DEXA (19).

Because of the clear visualization of trabecular structure on standard radiographs, the proximal phalanges of the second, third, and fourth fingers are used as the most suitable regions for evaluation (19). The outcomes obtained with the BES TEST are expressed as BSI T-scores. As with DEXA, these data are derived by comparing the patient's BSI with the average BSI of young White women aged 22 to 45 years and calculating the difference in number of SDs (20).

Previous studies suggest that the BSI T-score may predict fracture risk over the subsequent 3 years and that a value below –1.4 could represent the cutoff point (19). Moreover, no correlation has been observed between BSI and DEXA T-score (20).

For these reasons, we performed a randomized controlled trial to assess the reliability of the BES TEST in patients with fragility fractures and normal or osteopenic DEXA T-scores.

PARTICIPANTS, STUDY DESIGN, MATERIAL, AND METHODS

We conducted a clinical trial approved by the Ethics Committee of Istituti Clinici Scientifici Maugeri - IRCCS, Pavia (EC2387; February 4, 2020). The study was conducted in full compliance with the principles outlined the Declaration of Helsinki.

Fifty consecutive female outpatients were enrolled and divided into 2 groups. Group A consisted of 25 female patients with no history of recent fragility fractures, whereas group B consisted of 25 female patients with a recent fragility fracture. Sample size calculation was also approved by the same ethics committee on the basis of previous data.

All patients underwent baseline lumbar spine, femoral, and femoral neck DEXA (Hologic QDR 4500 densitometer) together with a baseline BES TEST. Both examinations were repeated after 18 to 24 months, with the addition of spine morphometry.

BES TEST performance characteristics were as follows: intraoperator CV, 0.06; 95 %CI, ± 8 BSI; interoperator CV, 0.11; 95 %CI, ±10.8 BSI (21), in line with current OP diagnostic standards.

Inclusion criteria were female sex, age between 40 and 75 years, and lumbar and femoral DEXA T-scores > –2.5 SD.

Exclusion criteria included treatment with glucocorticoids or antiosteoporotic drugs.

The trial began in 2020 but was interrupted because of the COVID-19 pandemic, resulting in the dropout of 3 patients. At present, 44 patients have completed the study and follow-up.

STATISTICAL ANALYSIS

Statistical comparisons between groups were performed using the unpaired Student *t* test. Statistical significance was established at $p < 0.05$.

Currently, we are collecting data and analyzing the final patient cohort. Furthermore, all patients are being contacted to update their clinical status. We are particularly interested in determining whether: a) they experienced a new fragility fracture during the year following the second BES TEST and DEXA evaluation; b) they are currently receiving treatment for osteoporosis; c) new fracture risk factors have been identified; d) they have experienced falls; and e) they have undergone a new DEXA scan, morphometric evaluation, or other relevant blood or urine tests.

RESULTS

Preliminary data showed that, at baseline and after 18 to 24 months, no significant differences were observed in DEXA T-scores between the fractured (F) and nonfractured (NF) groups; $p = 0.09$. In contrast, the BSI T-score differed significantly between the F and NF groups; $p = 0.0001$.

No significant differences were observed in femoral neck DEXA T-scores at baseline vs 18- to 24-month follow-up. Similarly, no significant differences were found in femoral DEXA T-scores or spine DEXA T-scores between baseline and follow-up evaluations.

In group B (fractured patients), no significant differences were observed in BSI T-score between baseline and 18- to 24-month follow-up. Likewise, no significant differences were detected in femoral neck DEXA T-scores, femoral DEXA T-scores, or spine DEXA T-scores between baseline and follow-up assessments (Fig. 1).

DISCUSSION

The BES TEST does not measure bone quantity but rather evaluates the elastic structural integrity and biomechanical competence of bone, thereby providing insight into functional bone strength. By focusing on trabecular bone elasticity, bone alterations can be monitored within weeks. BES TEST complements den-

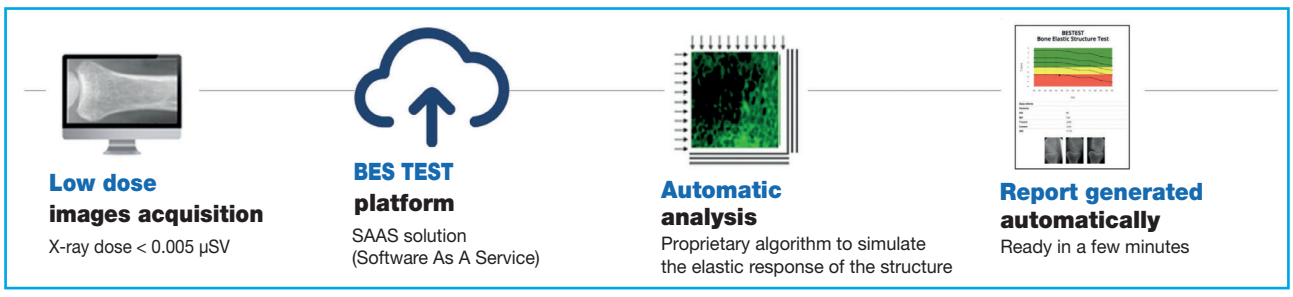

Figure 1. BES TEST workflow.

sitometry as a low-dose monitoring tool for bone follow-up (20) in rheumatology (22), oncology (23), nephrology (24), and rare diseases (25).

Evidence from a small study applying the test to both the hand and foot demonstrated similar trends across anatomical sites, indicating sensitivity to systemic physiological changes and supporting the concept that skeletal fragility reflects a generalized condition rather than a site-specific phenomenon ("La valutazione della Bone Elastic Structure (BESTEST™) in segmenti scheletrici sottoposti a diverso carico," oral presentation at the SIOMMMS National Conference 2022; abstract unpublished).

This concept is further supported by studies showing strong correlations between hand bone measurements and fracture-relevant skeletal sites. Bone mineral density assessed at peripheral hand bones correlates with femoral neck DEXA values, and quantitative ultrasound measurements at the finger phalanges can effectively assess fracture risk and detect age-related bone changes, with diagnostic sensitivity comparable to lumbar densitometry (26-28).

In a previous study (20), 351 consecutive White women were enrolled in a population study and contacted after a 3-year follow-up period to evaluate the incidence of fragility fractures; 166 of 351 responded to follow-up contact.

A total of 91 out of 351 patients experienced fractures, whereas 75 remained fracture-free. Fractured patients were slightly older than nonfractured patients; p = 0.00485. Body mass index (BMI) was similar between groups; p = 0.1243, and no correlation was found between BMI and BSI T-score in either group.

The mean BSI T-score was –1.5 (range, –3.4 to 0.8) in fractured patients and –0.4 (range, –3.2 to 2.4) in nonfractured patients. These findings suggested that the BSI T-score may predict fracture risk during the following 3 years and that patients with values below –1.4 should undergo careful evaluation (20).

In 2020, we published the first pilot study comparing BES TEST with DEXA (22). We enrolled 9 patients younger than 74 years with recent fragility fractures despite normal or osteopenic femoral neck T-scores on DEXA evaluation (group A). Conversely, group B consisted of volunteer female patients who had undergone DEXA and BES TEST evaluations in 2015 during the Trieste NEXT 2015 event (Ethics Committee of the University of Trieste approval No. 66, November 11, 2015).

All patients in both groups were analyzed according to the previously described BES TEST method, and results were expressed as mean ± SD. Comparisons between groups were performed using the unpaired Student t test. Statistical significance was established at $p < 0.05$.

In that second study (22), we confirmed that no correlation exists between BSI and DEXA T-score. Moreover, fractured patients showed significantly lower BSI T-scores compared with femoral neck DEXA T-scores. In contrast, nonfractured patients had normal BSI T-scores despite osteopenic femoral neck DEXA T-scores (22). Indeed, fracture risk in these patients may be more accurately assessed by combining DEXA with FRAX or DeFRA algorithms (28).

CONCLUSIONS

Current clinical recommendations suggest treatment of nonfractured osteoporotic patients only when FRAX or DeFRA indicate a substantial fracture risk, in order to avoid overtreatment and the consequent increase in adverse effects (29,30).

Because a high proportion of fragility fractures occur in patients with normal or osteopenic DEXA T-scores, this phenomenon is probably related to the limited ability of DEXA to distinguish and quantify bone quality. This limitation is especially evident in patients with secondary osteoporosis, such as those receiving chronic glucocorticoid therapy. Preliminary data from our trial confirm the reliability and reproducibility of both DEXA T-score and BSI T-score measurements. The expected outcome is confirmation that BSI, when combined with DEXA and FRAX or DeFRA scores, may improve the assessment of fragility fracture risk.

BES TEST should be further investigated to determine its ability to detect rapid skeletal changes during pharmacologic treatment, particularly in chronic inflammatory diseases such as rheumatoid arthritis. In these conditions, inflammation combined with glucocorticoid therapy may rapidly induce bone fragility that DEXA alone is unable to identify. Therefore, BES TEST could represent a valuable complementary tool, potentially helping to tailor pharmacologic therapy and, when necessary, rehabilitation programs aimed at reducing fall risk.

LIMITATIONS

The small sample size represents a clear limitation of this study. Additional trials involving larger patient populations are required.

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Image in Osteology

Osteopetrosis: characteristic radiological findings

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We present images corresponding to a 40-year-old woman evaluated in the Rheumatology Department with a diagnosis of osteopetrosis. She had a family history involving first-degree relatives (mother and two daughters). The diagnosis was established during adolescence, and since then she has suffered multiple bone fractures (metatarsals, ribs, left shoulder, and right femur).

Laboratory studies showed bone metabolism parameters within the normal range, including calcium 9.4 mg/dL (normal value: 9.0-10.5 mg/dL), phosphorus 3.6 mg/dL (2.7-4.5 mg/dL), alkaline phosphatase 82 U/L (40-120 U/L), 25-hydroxyvitamin D 42 ng/mL (30-100 ng/mL), and PTH 38 pg/mL (15-65 pg/mL). Complete blood count revealed anemia (Hb 9 g/dL; normal value: 12-16 g/dL). Genetic testing revealed a mutation in the CLCN7:C.857G > A p(Arg286Gln) gene.

Plain radiographs showed characteristic findings of osteopetrosis, such as diffuse increased bone density with loss of differentiation between cortical bone and bone marrow, trabecular thickening, and generalized sclerosis (Figs. 1 and 2).

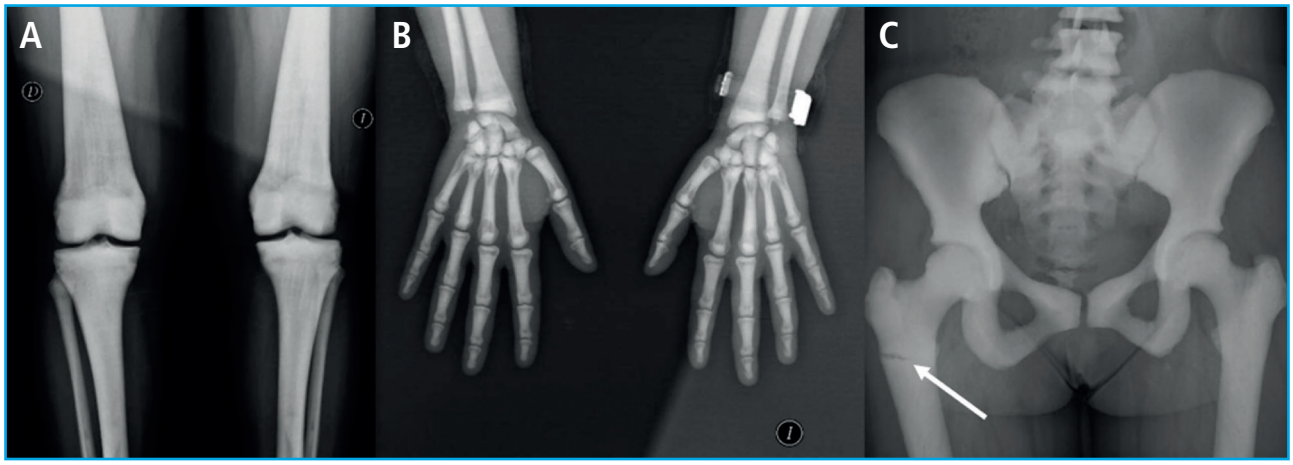

Figure 1. Plain radiographs corresponding to a patient with autosomal dominant osteopetrosis type II. A. Anteroposterior radiograph of both knees: diffuse increase in bone density, with loss of differentiation between cortical bone and marrow. B. Anteroposterior radiograph of both hands: trabecular thickening and "bone within bone" sign. C. Anteroposterior pelvic radiograph: diffuse bone sclerosis involving the iliac bones, sacrum, and femoral metaphyses, with loss of cortical-medullary differentiation, characteristic of osteopetrosis. In the right proximal femur, an incomplete transverse fracture is identified, visible as a faint radiolucent line (arrow).

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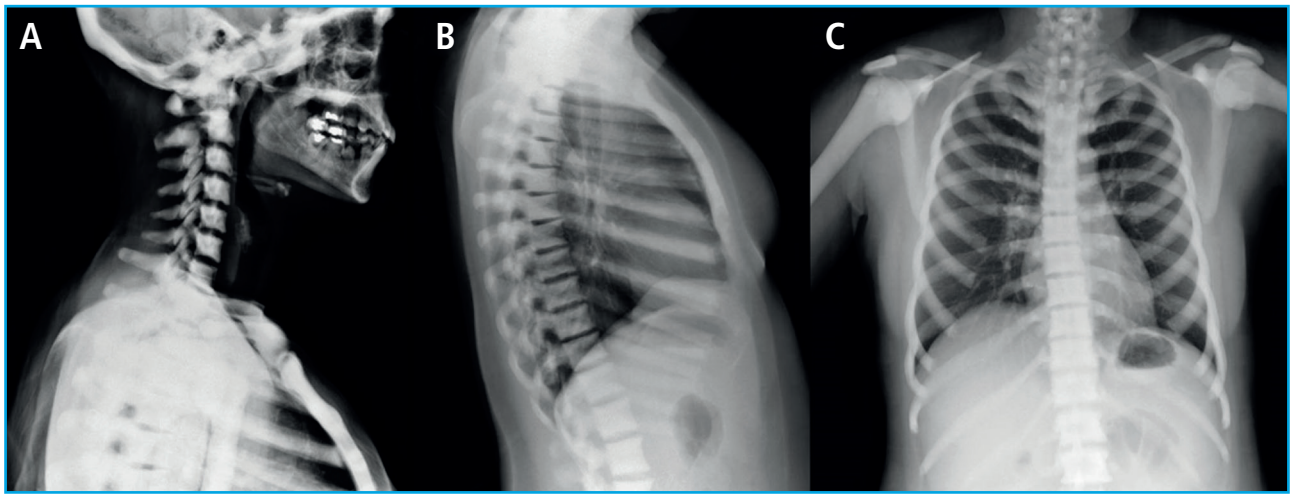

Figure 2. Plain radiographs corresponding to a patient with autosomal dominant osteopetrosis type II. A. Lateral cervical spine projection: note the thickening of the skull base. B. Lateral spine projection: sclerosis of the vertebral endplates is observed, giving the characteristic appearance of "sandwich" vertebrae. C. Anteroposterior projection of the thoracic spine and ribs: generalized bone sclerosis.

The case presented here is consistent with autosomal dominant osteopetrosis type II (also known as Albers-Schönberg disease). An evaluation of the anemia syndrome was performed, including complete blood count, reticulocyte count, peripheral blood smear, ferritin, serum iron and iron-binding capacity, vitamin B12, and folic acid, which revealed neither nutritional deficiencies nor evidence of hemolysis. Abdominal ultrasound demonstrated mild-to-moderate splenomegaly (14 cm), probably related to extramedullary hematopoiesis, a phenomenon described in osteopetrosis due to bone marrow involvement. However, no findings compatible with true hypersplenism were identified —including the absence of thrombocytopenia and a reticulocyte count within the low-normal range—, and therefore this possibility was considered only as an unconfirmed clinical hypothesis.

Osteopetrosis, or "marble bone disease," encompasses a group of rare genetic disorders, with an estimated incidence of 1 case per 250,000 births in the autosomal recessive forms and 1 per 20,000 in the dominant forms. It is characterized by an abnormal increase in bone density due to a defect in osteoclast differentiation or function, which alters bone remodeling and results in dense but fragile bones, with predisposition to fractures and bone marrow compromise.

Mutations in at least 10 implicated genes have been identified, accounting for approximately 70 % of cases. Inheritance may be autosomal recessive —more severe, with neonatal onset, presenting with fractures, hypocalcemia, compressive neuropathies, and bone marrow failure—, dominant —milder and diagnosed during childhood or adolescence—, or X-linked, generally associated with complex multisystem syndromes.

Diagnosis is based on clinical presentation and characteristic radiological findings, such as the "bone within bone" sign and "sandwich" vertebrae and may be confirmed by genetic testing. Identification of the implicated gene allows refinement of prognosis, guidance of treatment, and assessment of the risk of familial recurrence.

Although treatment is usually symptomatic, in severe forms hematopoietic stem cell transplantation may correct the osteoclastic defect and improve survival. Severe untreated infantile forms have a limited life expectancy, whereas adult-onset forms usually follow a benign course.

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