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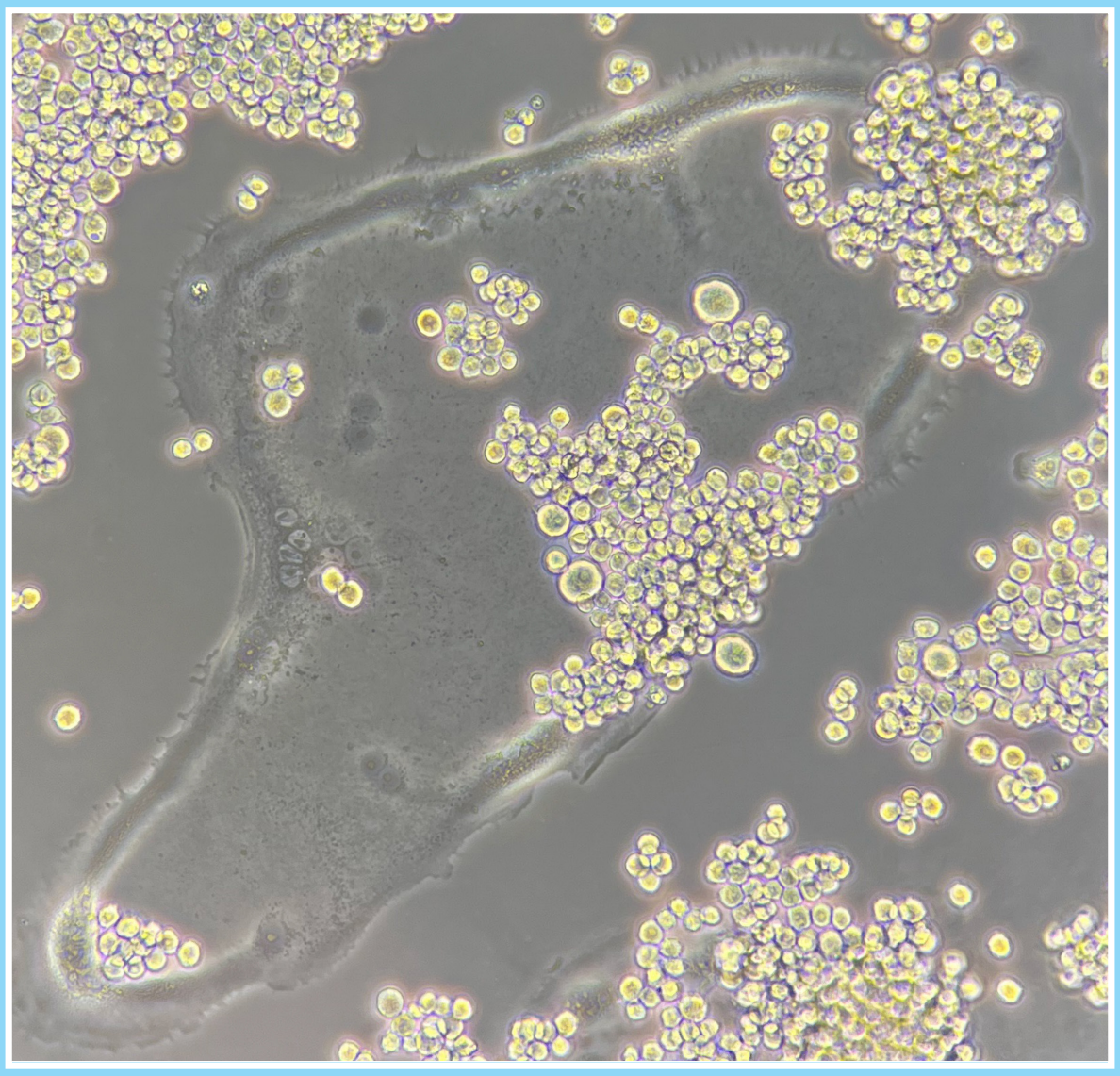

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Osteoclast differentiated from the murine RAW 264.7 cell line

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Original

Immunomodulatory role of MINDIN in bone microenvironment under inflammatory conditions

Luis Álvarez-Carrión[1,3], Martina Romeo[1], Irene Gutiérrez-Rojas[1,2], Gonzalo García de Casasola-Rodríguez[1], Juan Antonio Ardura Rodríguez[2,3], Verónica Alonso Rodríguez[1,3]

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Abstract

Introduction: bone is a dynamic organ undergoing constant remodeling, a process involving bone cells (osteoclasts and osteoblasts) and immune system cells (such as macrophages and lymphocytes). This interaction between the two cell types is studied by the field of osteoimmunology, which focuses on how immune factors influence bone formation and resorption, especially in inflammatory contexts such as osteoporosis or osteomyelitis.

Aim: MINDIN, an extracellular matrix protein with immunomodulatory functions, has previously been described as a mediator in the recruitment and activation of macrophages, although its role is poorly understood. Therefore, the present study aimed to clarify the role of MINDIN as an immunomodulator in bone and its actions on bone remodeling in an inflammatory context.

Material and methods: in *ex vivo* mouse ulna model under inflammatory conditions induced by LPS was used to analyze the role of MINDIN in macrophage polarization and bone remodeling.

Results: stimulation with MINDIN promoted a proinflammatory profile characterized by overexpression of F4/80 and iNOS, without altering CD206 levels. In addition, MINDIN increased the expression of osteoclastic markers such as RANKL and TRAP, as well as modifying the expression of AXL, a marker of osteomorphs, depending on the inflammatory context. Correlation analysis between proinflammatory and bone remodeling genes revealed significant associations suggesting a close interaction between immune activation and bone resorption.

Conclusion: taken together, these results identify MINDIN as a potential mediator at the interface between inflammation and bone remodeling, with potential relevance in inflammatory osteolytic diseases such as osteomyelitis, rheumatoid arthritis, and bone metastases.

Keywords:
MINDIN.
Macrophages.
Polarization.
Osteoimmunology.

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INTRODUCTION

The skeletal and immune systems share numerous molecules, including cytokines, transcription factors, receptors, and hormones. Under physiological conditions, interactions between bone cells (such as osteoclasts and osteoblasts) and immune cells are essential for bone development, repair, regeneration, and homeostasis (1). Despite its apparently static nature, bone is a highly dynamic organ that undergoes continuous remodeling throughout life, involving both bone cells (2) and various immune cells. During this process, old and/or damaged bone is replaced through the coordinated activity of osteoclasts, which resorb bone, and osteoblasts, which produce and deposit new bone matrix (3-5). Bone remodeling maintains the adult skeleton throughout life, and an estimated 10 % of the skeleton is remodeled each year (6).

Immune cells and bone cells have been shown to interact, a form of cellular communication referred to as osteoimmunity. This interdisciplinary field examines how interactions between osteoclasts or osteoblasts and various immune cells maintain the balance between bone resorption and formation, with particular emphasis on molecular signaling in inflammatory settings such as osteoporosis, cancer, and periodontal disease. In this regard, proinflammatory cytokines such as IL-6, TNF-α, IL-1β, and IL-11 released by activated T lymphocytes have been shown to induce osteoclast formation and promote bone resorption (1,7-11).

Osteoclasts are large, motile, multinucleated cells responsible for bone resorption. They develop from myeloid progenitors or bone marrow osteomacs through several stages of differentiation (12-16). Osteoclast fission processes have recently been described that give rise to small daughter cells termed osteomorphs. These osteomorphs retain the ability to fuse again and form functional osteoclasts and are therefore considered a quiescent reservoir for osteoclast activation. Unlike osteoclasts, which adhere to the bone surface, osteomorphs are present in bone marrow and the bloodstream, suggesting a dynamic role in regulating bone turnover and skeletal plasticity (17).

Macrophages are phagocytic immune cells derived from monocytes that infiltrate virtually all tissues (18). They have a fundamental role in maintaining tissue homeostasis and in extracellular matrix turnover, tissue repair, and angiogenesis (18-20). Macrophages are notably heterogeneous because they can adapt their phenotype and functions to their local environment, generating populations with distinct properties (18), a process known as polarization. In a simplified model, macrophages occupy 2 extremes of phenotypic polarization according to their functions and the stimuli to which they respond. These extremes define the M1/M2 paradigm: M1 macrophages exert proinflammatory and antitumor functions and express markers such as iNOS and TNF-α, whereas M2 macrophages exert anti-inflammatory and protumor functions and are characterized by markers such as CD206 and IL-10.

With aging and in certain diseases, bone resorption and formation become imbalanced, leading to bone loss and increased fracture risk (21). In particular, osteomyelitis caused by *Staphylococcus aureus* infection is associated with extensive bone destruction believed to be mediated primarily by bone-resorbing osteoclasts (22,23). Culture-model studies suggest that factors produced by *S. aureus* and/or elevated inflammatory cytokine levels may induce osteoclast formation (24-27).

MINDIN (also known as spondin-2) is a highly conserved extracellular matrix protein containing F-spondin domains 1 and 2 (FS1 and FS2) at the N-terminus and type 1 thrombospondin repeats (TSRs) at the C-terminus (28,29). Previous studies have shown that MINDIN can initiate innate immune responses by recruiting macrophages and neutrophils to sites of inflammation and can bridge innate and adaptive immunity (30). Mice deficient in MINDIN expression have also been reported to have impaired clearance of influenza virus and bacterial infections. This is because MINDIN is a pattern-recognition molecule that can bind directly to bacterial LPS and influenza virus particles to initiate innate immune responses; it also functions as an opsonin in macrophage-mediated bacterial phagocytosis (30-32). Nevertheless, the mechanisms by which MINDIN promotes phagocytosis remain poorly understood.

The aim of this study was to clarify the role of MINDIN as an immunomodulator in bone and its effects on bone remodeling in an inflammatory setting.

MATERIALS AND METHODS

EX VIVO EXPERIMENTAL MODEL

An *ex vivo* ulna culture model was established using 2- to 4-month-old female C57BL/6J mice over five days. The ulnas were harvested, the skin and muscle were removed, and the epiphyses were excised. Bones were cultured in six-well plates containing α-MEM (Thermo Fisher Scientific®, Rockford, IL, United States) supplemented with 2.5 % fetal calf serum, 2.5 % fetal bovine serum, 1 % penicillin (100 units/mL), and streptomycin (100 µg/mL), in a humidified incubator at 37 °C with 5 % CO_2. After the first 24 hours of culture, explants were treated with or without bacterial lipopolysaccharide (LPS, 100 ng/mL; Sigma-Aldrich®, Darmstadt, Germany) to induce an inflammatory environment. After a further 24 hours, explants were either stimulated or not stimulated with MINDIN (5 ng/mL, the lowest con-

centration observed in patient serum; R&D Systems®, Minneapolis, MN, United States) during the last three days of culture. Bones were stored in TRIzol™ for RNA extraction and subsequent real-time PCR analysis. The samples used are presented in table I.

Table I

Bone sample group	Number of samples
Control	8
MINDIN-stimulated	5
LPS-stimulated	5
LPS + MINDIN-stimulated	6

REAL TIME QUANTITATIVE PCR (RT-qPCR)

Total RNA was isolated using a standard procedure (TRIzol™, Life Technologies), and 2 µg of RNA was reverse-transcribed using the High-Capacity RNA-to-cDNA Kit (Thermo Fisher, 4387406) according to the manufacturer's instructions.

Gene expression was assessed by real-time PCR based on fluorescence emitted by SYBR Green Premix Ex Taq (Takara, Otsu, Japan), using the ABI PRISM 7500 system (Applied Biosystems). Messenger RNA copy numbers were calculated for each sample using the cycle threshold (Ct). The 18S or β-actin genes, used as housekeeping controls, were amplified in parallel, allowing calculation of the number of amplification cycles required to reach an arbitrary Ct threshold. Relative gene expression versus control was calculated as $2^{-\Delta\Delta Ct}$, where ΔΔCt = ΔCt(treatment) – ΔCt(control) and ΔCt = Ct(target gene) – Ct(internal control gene, 18S). Gene expression in animal samples was displayed as individual data points calculated using $2^{-\Delta Ct}$, as previously described (33). Amplicon specificity was confirmed by a single peak in the dissociation curve for each qPCR reaction. The thermal cycling program is shown in table II.

Gene expression was analyzed for the macrophage marker F4/80; markers of polarization toward the M1 (iNOS) or M2 (CD206) phenotype; and bone remodeling markers.

Table II

Cycles	Temperature	Time
1	95 °C	10 minutes
45	95 °C	15 seconds
	60 °C	15 seconds
	72 °C	15 seconds
1	95 °C	0 seconds
	65 °C	15 seconds
	95 °C	0 seconds
1	40 °C	30 seconds

(OPG, RANKL, TRAP), as well as osteomorph markers (Axl) (Table III).

STATISTICAL ANALYSIS

Data are expressed as means ± standard error. Data distributions were examined and, because they did not follow a normal distribution, differences between experimental groups were assessed using nonparametric analysis of variance (Kruskal–Wallis test). Differences between individual experimental groups were assessed using Dunn's or Mann–Whitney tests. Correlations were analyzed using Spearman's nonparametric correlation test, yielding a correlation coefficient (*r*) and associated *p* value. Statistical analyses were performed using GraphPad Prism (version 10.1.1). *p* values < 0.05 were considered statistically significant.

RESULTS

MINDIN INDUCES A PROINFLAMMATORY AND BONE-RESORPTIVE PROFILE

The role of MINDIN in macrophage recruitment and polarization was investigated in an ex vivo model of LPS-induced inflammation in mouse ulnas.

Independent stimulation with either MINDIN or LPS increased expression of the macrophage marker

Table III

Gene	Forward primer (5'--- > 3')	Reverse primer (5'--- > 3')
RNA18S	ATGCTCTTAGCTGAGGTGCCCG	ATTCCTAGCTGCGGTATCCAGG
OPG	CAGAGCGAAACACAGTTTG	CACACAGGGTGACATCTATTC
INOS	CCTGCTTTGTGCGAAGTGTC	CCCTTTGTGCTGGGAGTCAT
CD206	CCACAGCATTGAGGAGTTTG	ACAGCTCATCATTTGGCTCA
RANK-L	TGTACTTTCGAGCGCAGATG	AGGCTTGTTTCATCCTCCTG
TRAP	CACGAGAGTCCTGCTTGTC	AGTTGGTGTGGGCATACTTC
Axl	GGAAAGAGGTGAACTGGTAGTC	CCATGACGTCTCGTAGTTTCTC

F4/80 (Fig. 1A) and the M1 polarization marker iNOS (Fig. 1B), without changes in the M2 macrophage marker CD206 (Fig. 1C). Nevertheless, sequential stimulation with LPS followed by MINDIN reduced expression of F4/80 (Fig. 1A) and the M1 marker iNOS (Fig. 1B).

Furthermore, markers of bone remodeling (OPG and RANKL), active and differentiated osteoclasts (TRAP), and osteomorphs (AXL) were also investigated under these conditions. As observed in the polarization analysis, independent stimulation with LPS increased RANKL expression compared with untreated controls, whereas stimulation with MINDIN alone under basal conditions did not significantly change RANKL, TRAP, or OPG expression (Fig. 2A–C). Under inflammatory stimulation (LPS), subsequent MINDIN exposure significantly reduced RANKL expression compared with LPS alone, but not TRAP expression. Conversely, stimulation with MINDIN alone decreased expression of the osteomorph marker AXL. Sequential treatment with LPS and MINDIN did not reduce AXL expression (Fig. 2D).

Afterwards, to investigate the molecular mechanisms involved in bone inflammation, correlations between mRNA expression of inflammatory and bone remodeling markers were analyzed. This analysis sought to clarify how sustained inflammation may interfere with physiological bone formation and resorption and the role of MINDIN as a mediator of the innate immune response. Positive correlations were observed between RANKL and TRAP (Fig. 3A); F4/80 and TRAP, F4/80 and RANKL, F4/80 and iNOS, and F4/80 and AXL (Fig. 3B–E); and OPG and AXL (Fig. 3F). These findings suggest a close interaction between the immune (macrophage) and skeletal (osteoclast) systems.

DISCUSSION

Aging and certain bone diseases, such as osteomyelitis, alter remodeling and increase bone loss. This is partly attributable to the recruitment of osteoclast precursors that promote bone resorption. This study identified MINDIN, an extracellular matrix protein, as an immunomodulatory factor capable of inducing macrophage recruitment and polarization in bone, potentially accelerating bone resorption.

Over the last few years, research has explored the immune system as a key regulator of bone homeostasis and repair after fracture or tissue damage. In this context, the ability of macrophages—essential immune cells—to polarize toward proinflammatory or anti-inflammatory phenotypes decisively influences intercellular interactions and the activation of mechanisms essential for bone regeneration, such as new blood vessel formation (angiogenesis) and bone tissue formation (osteogenesis) (20-22,34). The findings of this study reinforce the concept that MINDIN is not merely a structural extracellular matrix protein but also an active immunological and osteoimmunological modulator. Its ability to induce polarization toward the M1 phenotype and regulate key genes involved in bone resorption suggests that MINDIN may amplify local inflammatory responses and activate osteolytic pathways under pathological conditions.

Of note, the translational relevance of MINDIN lies in its dual function as an extracellular matrix protein and an immunomodulatory signaling molecule. These findings show that MINDIN not only serves as a structural component but actively promotes a proinflammatory macrophage phenotype (M1) characterized by

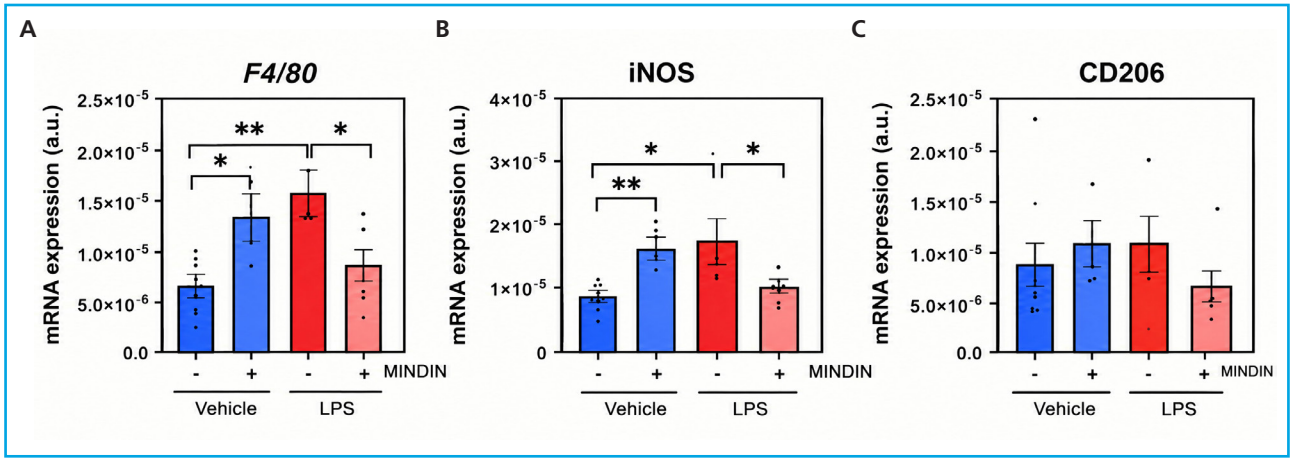

Figure 1. MINDIN induces inflammation in ex vivo mouse bones. mRNA expression of (A) F4/80, (B) iNOS, and (C) CD206 in mouse ulnas measured by real-time PCR. After 24 hours in culture, ulnas were stimulated or not stimulated with bacterial LPS and subsequently with or without MINDIN during the final three days. Data are presented as mean ± SEM (n = 7–10 per group). *$p < 0.05$; **$p < 0.01$.

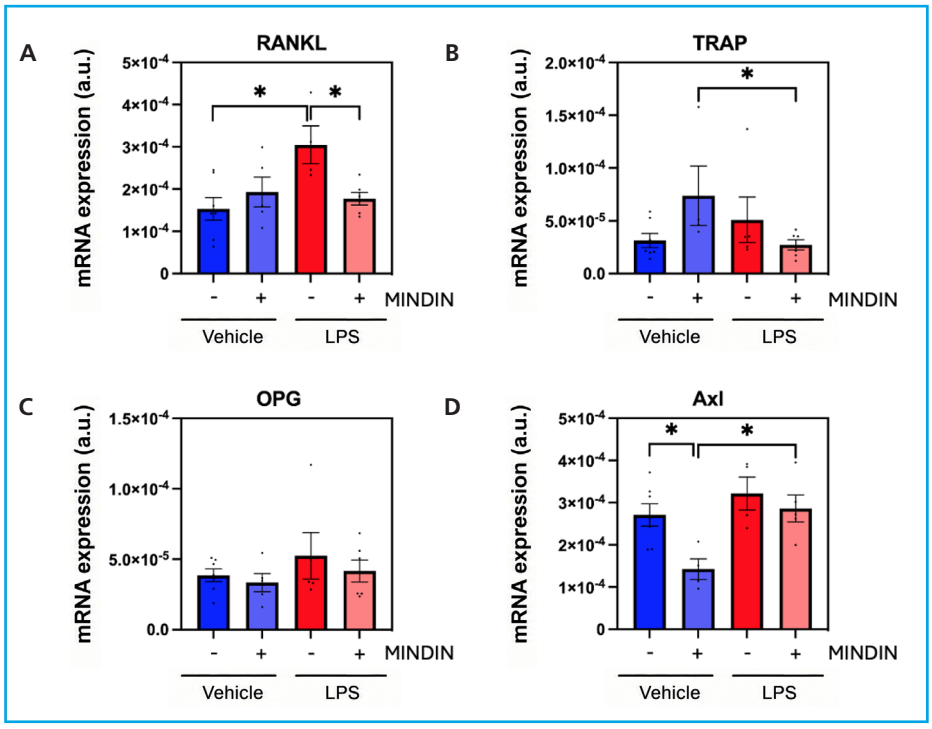

Figure 2. MINDIN induces bone remodeling in ex vivo mouse bones. mRNA expression of (A) RANKL, (B) TRAP, (C) OPG, and (D) Axl in mouse ulnas measured by real-time PCR. After 24 hours in culture, ulnas were stimulated or not stimulated with bacterial LPS and subsequently with or without MINDIN during the final three days. Data are presented as mean ± SEM (n = 7–10 per group). *$p < 0.05$; **$p < 0.01$.

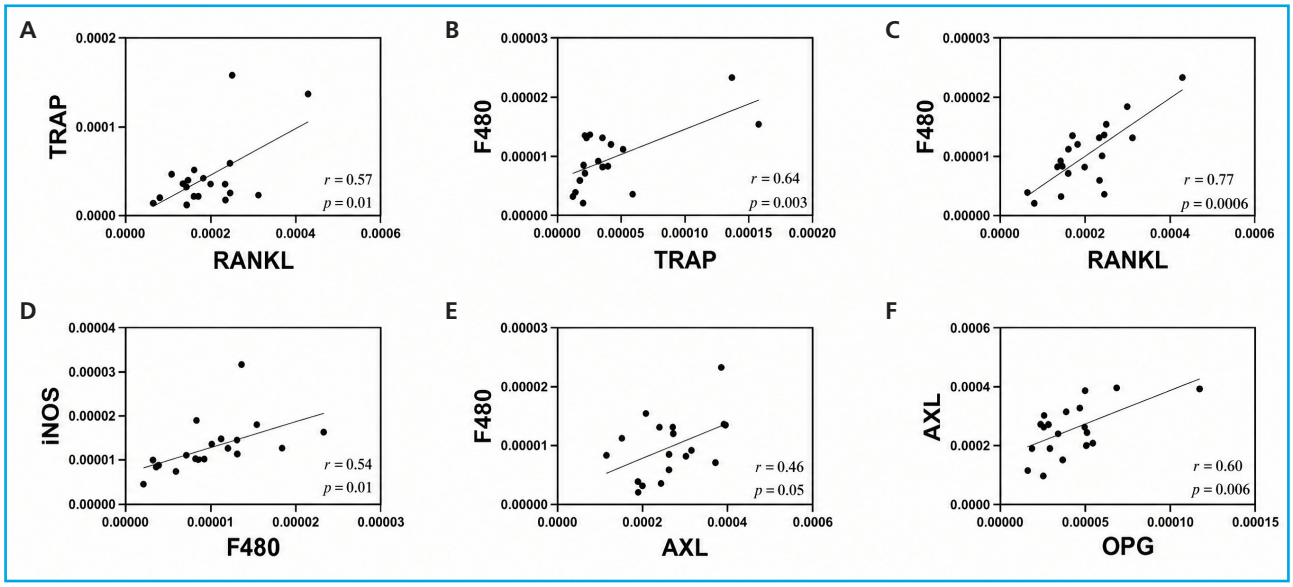

Figure 3. Scatterplots showing linear correlations between mRNA expression of TRAP and RANKL (A), F4/80 and TRAP (B), F4/80 and RANKL (C), iNOS and F4/80 (D), F4/80 and AXL (E), and AXL and OPG (F). The correlation coefficient *(r)* is displayed for each plot and indicates the strength and direction of the linear relationship between the corresponding measured variables. Values close to 1 or –1 indicate a strong linear relationship, whereas values close to 0 indicate a weak or absent linear relationship.

iNOS expression. They also indicate that the modulatory role of MINDIN in bone resorption may depend critically on the pre-existing environment. On the one hand, sequential addition of MINDIN significantly attenuates LPS-induced RANKL expression. On the other, expression of the active osteoclast marker TRAP is significantly lower after sequential stimulation (LPS + MINDIN) than after stimulation with MINDIN alone. Consequently, pharmacological modulation of MINDIN, through small-molecule design or highly specific monoclonal antibodies, emerges as a potential innovative therapeutic target. Drugs targeting this protein

could provide a novel strategy for limiting bone resorption in diseases such as rheumatoid arthritis and osteomyelitis, in which excessive inflammation accelerates bone loss.

Former studies have described the involvement of MINDIN in macrophage activation and the promotion of innate immune responses (32,35,36), particularly through integrin and Toll-like receptor (TLR) signaling pathways (32,35-37). Nevertheless, its role in bone tissue and its interactions with cells of the osteoclast lineage have been little investigated. The present findings provide experimental evidence directly linking MINDIN to the regulation of macrophage polarization and activation of bone remodeling mechanisms, opening new avenues for investigating its function in inflammatory bone diseases such as rheumatoid arthritis, periodontitis, and bone metastases.

At the molecular level, the observed modulation of Axl expression suggests that MINDIN could influence osteoclast plasticity through osteomorph generation, a process recently described as part of osteoclast recycling (19). The ability of MINDIN to induce or suppress AXL expression according to the inflammatory context (direct versus sequential stimulation) may represent an adaptive mechanism for regulating bone resorption during chronic inflammation. Under basal, noninflammatory conditions, MINDIN significantly reduces AXL expression compared with untreated cultures. Nevertheless, this effect disappears under inflammatory conditions (LPS), when MINDIN does not significantly alter AXL levels. These findings may indicate that, under normal physiological conditions, MINDIN acts as a biological brake on AXL expression, reducing the osteomorph reservoir in healthy tissue. Conversely, in a setting of severe LPS-induced inflammation, the inflammatory stimulus may completely override or saturate this regulatory effect of MINDIN on the marker.

Of note, the correlation analysis revealed relevant interactions between inflammatory mediators and bone remodeling markers, providing important insight into the molecular mechanisms underlying osteomyelitis. The correlations suggest coordinated macrophage recruitment and immune infiltration, together with osteoclast activation.

One distinctive finding is that MINDIN reduces expression of the osteoclast-activating factor RANKL when administered after LPS. This coincides with significantly higher expression of the osteomorph marker AXL in the sequentially stimulated group than in cultures exposed to MINDIN alone under basal conditions (without LPS). Because osteomorphs are described as a quiescent population generated by osteoclast fission, the results suggest that MINDIN modulates this cell reservoir only under basal physiological conditions, reducing AXL expression in the absence of inflammation. In contrast, under LPS-induced inflammation, this regulatory effect disappears completely, suggesting that inflammation may abolish MINDIN's ability to influence the quiescence or recycling of these cells.

CONCLUSIONS

Overall, these results position MINDIN as a potentially key mediator in osteoimmunology whose functional role depends on the inflammatory milieu of the tissue. Investigation of MINDIN may have implications not only for understanding bone pathophysiology but also for developing therapeutic strategies aimed at modulating macrophage and osteoclast activity in inflammatory and osteolytic diseases. Nevertheless, gain- and loss-of-function studies will be required to confirm the underlying molecular mechanisms.

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Original

Role of FGF23 in calcium–phosphate metabolism after spinal cord injury

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Abstract

Introduction and objective: after a spinal cord injury (SCI) there is a marked increase in bone remodeling with significant changes in calcium metabolism. However, few studies have addressed phosphate homeostasis in this clinical situation. The aim of this study is to assess circulating FGF23 levels in individuals with recent SCI (< 6 months) and to analyse their correlation with phosphate metabolism, bone turnover markers, and bone mass.

Methods: patients with a recent complete motor SCI (ASIA A) (< 6 months) and circulating FGF23 levels of were included. Bone metabolism parameters (calcium, phosphate, PTH and 25-OHD), bone turnover markers (P1NP, bone AP, osteocalcin, CTx), bone mediators (sclerostin, Dkk-1) and BMD were assessed in all patients at baseline and at 6 months. The results were compared with a control group.

Results: 28 individuals with complete SCI, and a mean age of 32 ± 13 years were included at 104 ± 34 days of SCI onset. 54 % had paraplegia. 18 patients were assessed at 6 months of follow-up. Patients with SCI showed a pronounced disturbance in phosphate and calcium metabolism with hyperphosphatemia, decreases in PTH values and significant increase in bone turnover markers. The values of FGF23 were higher than in the control group, and were correlated with phosphate values. At 6 months, FGF23 evolution showed large variability, with a positive correlation with creatinine at 6 months of follow-up. Changes in FGF23 were positively correlated with sclerostin values at baseline. No correlations were observed with other biochemical or BMD parameters (or with glomerular filtrate).

Conclusions: after a SCI there is a significant increase in FGF23 values, probably as a compensatory mechanism due to the hyperphosphatemia observed in this clinical condition in relation to a high bone turnover status, although a potential association with osteocyte damage cannot be excluded. Furthermore, changes in FGF23 were associated with baseline sclerostin levels.

Keywords:
Spinal cord injury. Bone metabolism. Phosphate. FGF23. Bone turnover markers.

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INTRODUCTION

Spinal cord injury (SCI) causes a marked increase in bone remodeling, leading to rapid bone loss of approximately 20 % in the first year after injury (1). Although several studies have examined disturbances in calcium metabolism in patients with recent SCI, including hypercalcemia, hypercalciuria, and the possible development of kidney stones, nephrocalcinosis, and long-term renal impairment (2-4), few studies have investigated phosphate disturbances in these patients.

Furthermore, several processes in patients with SCI may disrupt phosphate metabolism, including increased bone turnover and suppression of parathyroid hormone (PTH) during the early stages of SCI (5), marked bone loss (with osteocyte apoptosis and morphological changes under unloading conditions), neurogenic bowel and/or hormonal dysregulation (3), and renal impairment developing in later stages of SCI.

Moreover, the osteocyte plays an important role by modulating the synthesis of specific mediators of bone remodeling in response to mechanical loading or its absence, thereby regulating bone metabolism (6). Several studies have evaluated Dickkopf-1 (Dkk-1) and sclerostin in individuals with SCI (1), but no data are available on the role of fibroblast growth factor 23 (FGF23) in bone metabolism following SCI.

FGF23 is a hormone produced primarily by osteocytes that regulates phosphate and vitamin D metabolism (7). Its synthesis is controlled by multiple factors, including post-translational mechanisms (glycosylation, phosphorylation, and proteolysis) and extracellular influences such as oxidative stress, inflammation, and renal injury (7,8). Nevertheless, recent evidence indicates increased FGF23 expression in conditions that induce osteocyte apoptosis, such as oxidative stress, an effect that estradiol may attenuate (9). FGF23 has also been identified as an early marker and predictor of chronic kidney disease progression and associated with cardiovascular complications and osteoporosis (10,11). Thus, FGF23 may contribute to osteoporosis in certain clinical settings. Nevertheless, available findings concerning its role in phosphate metabolism and bone mass regulation under unloading conditions remain contradictory.

This study aimed to characterize FGF23 in individuals with recent SCI (< 6 months) and examine its relationships with calcium-phosphate metabolism, bone remodeling markers and mediators, and bone mass.

PATIENTS AND METHODS

STUDY POPULATION

This prospective study included patients with recent traumatic SCI (< 6 months) and complete motor impairment (AIS grade A or B), recruited at the *Instituto de Neurorrehabilitación Guttmann* between August 2010 and January 2012 and subsequently followed in the Department of Rheumatology at Hospital Clínic Barcelona and Hospital Universitari Germans Trias i Pujol, Badalona (Barcelona, Catalonia, Spain). The present analysis included patients whose circulating FGF23 levels had been measured at enrollment. Results were compared with those of a healthy control group matched for age and sex.

The study inclusion criteria were age older than 18 years, absence of diseases affecting bone metabolism (Paget disease, inflammatory joint disease, endocrine disorders [including hyperparathyroidism and hypercortisolism], malabsorption syndromes, malignant tumors, transplantation, pregnancy, or breastfeeding), and/or no previous antiosteoporotic drugs.

At baseline, a targeted past medical history was obtained to assess osteoporosis risk factors. SCI-related information was also collected, including cause, neurological level, injury severity according to the American Spinal Injury Association Impairment Scale (AIS), spasticity, and associated complications. Laboratory investigations, measurements of bone remodeling markers and mediators (sclerostin and Dkk-1) and FGF23, and densitometry were performed at baseline and every six months throughout the first 12 months of follow-up, and subsequently every 6 or 12 months in routine clinical practice.

The study was approved by the ethics committees of *Hospital Clínic de Barcelona* and Instituto de Neurorrehabilitación Guttmann and complied with the relevant guidelines for research involving human participants. All patients provided written informed consent.

LABORATORY MEASUREMENTS

Blood was collected from all patients between 8:00 and 10:00 a.m. after an overnight fast. Plasma FGF23 was measured using a second-generation enzyme immunoassay (Human FGF23 C-Terminal, Immutopics, San Clemente, CA, United States; normal value < 130 RU/mL), which quantifies the C-terminal portion of intact biologically active FGF23 and its inactive C-terminal fragment, with intra-assay and inter-assay coefficients of variation of 3 % and 5 %–7 %, respectively. Calcium, phosphate, and creatinine were measured by standard laboratory techniques. Serum 25-hydroxyvitamin D (25-OHD) and PTH were measured using automated chemiluminescence assays (Liaison, Diasorin, and Advia Centaur XP, Siemens, respectively). Bone formation markers included bone-specific alkaline phosphatase (bone ALP; IDS, Vitro), osteocalcin (OC; radioimmunoassay, Elsa-Osteo-Cis, Gif-sur-Yvette, France), and procollagen type I N-terminal propeptide (P1NP; automated Cobas e411 assay, Roche). The bone resorption marker was the C-terminal

telopeptide of type I collagen (CTx; automated Cobas e411 assay, Roche). Serum sclerostin and DKK1, mediators of bone remodeling, were measured by ELISA (Biomedica, Austria), with intra-assay coefficients of variation of 4 %–6 % and 7 %–8 %, respectively, and inter-assay coefficients of 5 %–7 % and 9 %–12 %, respectively. Estimated glomerular filtration rate was calculated using the MDRD/CKD-EPI equations with creatinine, age, and sex. Results were compared with those of healthy age- and sex-matched controls.

BONE MINERAL DENSITY

Bone mineral density (BMD) of the lumbar spine, proximal femur (femoral neck and total hip), and lower limbs was measured in all patients by dual-energy X-ray absorptiometry (DXA; Lunar Prodigy, Radiation Corporation, Madison, WI, United States). Densitometric risk categories (normal BMD, osteopenia, and osteoporosis) were defined according to World Health Organization (WHO) criteria. Bone mass was not measured at the distal femur or proximal tibia because this technique had not been validated when participants were enrolled.

STATISTICAL ANALYSIS

Results are expressed as mean ± standard deviation (SD). Differences in continuous variables were assessed using the nonparametric Mann–Whitney U test and differences in proportions using Fisher's exact test. Paired variables were compared using the Wilcoxon test. Associations between variables were assessed using Pearson's correlation coefficient. p values < 0.05 were considered statistically significant. Statistical analyses were performed using SPSS version 18.0 (Chicago, IL, USA).

RESULTS

FGF23 levels were available for 28 of the 42 patients with SCI recruited into the study (1) and for 18 controls.

The clinical characteristics of the patients with SCI are presented in table I. Median age was 31 years (range, 18–67 years) and median time since SCI was 96 days (range, 49–175 days). All had complete motor SCI, most with complete sensory loss (AIS A); 46 % had tetraplegia, and most had spastic SCI (75 %). All used wheelchairs for mobility. The leading cause of SCI was a road traffic accident, followed by falls from a height and direct axial trauma.

Table I. Clinical characteristics of patients with recent SCI included in this study

	Patients with SCI (n = 28)
Men (n)	27
Osteoporosis risk factors	
Daily calcium intake before SCI (mg/day)	480 ± 304
History of kidney stones (%)	7.1
Current smoking (%)	28.6
Daily alcohol use (%)	7.1
Family history of femoral fracture (%)	21.4
Family history of osteoporosis (%)	7.1
SCI characteristics	
Time since SCI (days)	104 ± 34
Paraplegia / tetraplegia (n)	15:13
Baseline AIS grade (n)	26 A: 2 B
Spasticity (%)	75
Causes of SCI	
- Traffic accident	50
- Fall from height	14.3
- Diving into shallow water	10.7
- Sports accident	7.1
- Direct axial trauma	14.3
- Other	3.6

SCI, spinal cord injury; AIS, American Spinal Injury Association Impairment Scale.

Individuals with recent SCI showed marked alterations in several parameters of calcium–phosphate metabolism (Table II). Specifically, serum phosphate levels were significantly higher, whereas PTH and creatinine levels were lower than in the control group. Conversely, estimated glomerular filtration rate (MDRD/CKD-EPI) was higher than in controls. Notably, 25-OHD levels were similar in both groups. In addition, individuals with recent SCI had significantly higher levels of bone turnover markers (total alkaline phosphatase, bone-specific alkaline phosphatase, osteocalcin, P1NP, and CTx) than controls.

FGF23 concentrations were higher in patients with SCI than in controls (83.5 vs 63.9 RU/mL). No significant laboratory, anthropometric, or densitometric differences were observed between patients with SCI whose FGF23 levels were above or below the mean. Only 4 patients had baseline FGF23 levels above 130 RU/mL (the assay's upper reference limit). No differences were found between patients with elevated versus normal FGF23.

Baseline FGF23 was positively correlated with body weight (r = 0.413, p = 0.029) and lower-limb BMD (r = 0.381, p = 0.045). After adjustment for age and BMI, FGF23 was positively correlated with serum phosphate (r = 0.738, p = 0.037). In controls, FGF23 did not correlate with laboratory or anthropometric variables.

Table II. Comparison of FGF23, calcium-phosphate metabolism, and bone remodeling parameters between patients with SCI and controls

	Patients with SCI *n* = 28	Controls *n* = 18	*p* value
Age (years)	32 ± 13	35 ± 11	0.265
Men (%)	96	100	*n.s.*
BMI (kg/m^2)	22.4 ± 3.8	23.8 ± 2.6	0.137
FGF23 (RU/mL)	83.5 ± 38.7	63.9 ± 31.5	0.027
Calcium-phosphate metabolism parameters			
Creatinine (mg/dL)	0.62 ± 0.16	0.95 ± 0.1	< 0.001
Glomerular filtration rate (MDR / CKD-EPI)	134 ± 20	103 ± 13	< 0.001
Phosphate (mg/dL)	4.43 ± 0.46	3.49 ± 0.52	< 0.001
Calcium (mg/dL)	9.54 ± 0.423	9.43 ± 0.42	0.227
Total ALP (mg/dL)	223 ± 76	139 ± 32	< 0.001
PTH (pg/mL)	17.46 ± 9.89	48.25 ± 16.56	< 0.001
25-OHD (ng/mL)	22.1± 9.3	23.2 ± 7.5	0.574
Bone turnover markers			
Bone ALP (ng/mL)	15.7 ± 7.8	11.2 ± 2.4	0.021
OC (ng/mL)	27.2 ± 9.7	20.3 ± 7.6	0.028
P1NP (ng/mL)	187 ± 136	51 ± 21	< 0.001
CTx (ng/mL)	1.49 ± 0.55	0.53 ± 0.23	< 0.001
Bone remodeling mediators			
Dkk-1	63.0 ± 16.7	42.2 ± 13.6	< 0.001
Sclerostin	40.3 ± 14.4	36.0 ± 10.5	0.437

SCI, spinal cord injury; BMI, body mass index; FGF23, fibroblast growth factor 23; total ALP, total alkaline phosphatase; PTH, parathyroid hormone; 25-OHD, 25-hydroxyvitamin D; bone ALP, bone-specific alkaline phosphatase; OC, osteocalcin; P1NP, procollagen type I N-terminal propeptide; CTx, C-terminal telopeptide of type I collagen; Dkk-1, Dickkopf-1.

Eighteen of the 28 patients with SCI (64 %) had FGF23 measurements available at the six-month follow-up. Mean FGF23 at six months was 65.1 ± 25.8 RU/mL (range, 26–122 RU/mL), representing a percentage change of –1.4 ± 55 % and considerable interindividual variability. Both baseline and 6-month FGF23 levels correlated positively with creatinine at 6 months ($r = 0.479$, $p = 0.038$ and $r = 0.494$, $p = 0.031$, respectively), but not with estimated glomerular filtration rate. Furthermore, changes in FGF23 correlated positively with baseline sclerostin ($r = 0.616$, $p = 0.005$), including after adjustment for age and BMI ($r = 0.804$, $p = 0.016$). No other significant correlations were identified.

DISCUSSION

The findings show higher FGF23 and phosphate levels in patients with recent SCI than in controls. Given their preserved renal function in the early phase after injury, the positive correlation between these parameters suggests that the increase in FGF23 is a counterregulatory response to hyperphosphatemia resulting from increased bone turnover. FGF23 was not correlated with renal function, although a positive correlation with lower-limb bone mass was observed. Changes in FGF23 varied considerably between patients but correlated positively with baseline sclerostin. This is the first study to examine FGF23 behavior in this clinical setting of disuse osteoporosis and its associations with calcium-phosphate metabolism and bone mass.

The FGF23–Klotho axis has a central role in regulating mineral metabolism, particularly phosphate and calcium homeostasis (12). FGF23, secreted primarily by osteocytes and osteoblasts, acts on the kidneys to decrease tubular phosphate reabsorption (TRP) and the tubular maximum for phosphate reabsorption normalized to glomerular filtration rate (TmP/GFR), thereby increasing renal phosphate excretion. FGF23 also decreases production of 1,25-dihydroxyvitamin D (calcitriol) by inhibiting renal 1α-hydroxylase and upregulating 24-hydroxylase. These actions reduce intestinal absorption of phosphate and calcium (12). FGF23 reg-

ulation is multifactorial and involves phosphate concentrations, vitamin D, PTH, and kidney function (12). In chronic kidney disease, circulating FGF23 rises as a compensatory response to maintain phosphate homeostasis, contributing to mineral and bone disorders (12). In the present study, FGF23 did not correlate with kidney function estimated by glomerular filtration rate. Its biological activity requires Klotho, a transmembrane protein acting as a coreceptor in the kidney and other tissues. Interaction of FGF23 with Klotho activates FGF signaling and modulates expression of genes involved in mineral metabolism. Soluble Klotho may also serve as a coreceptor in tissues lacking transmembrane Klotho, extending FGF23 action beyond the kidney. More recently, Klotho has emerged as an important regulator of healthy aging, with new studies broadening its potential therapeutic applications (13).

Of note, this is the first report examining circulating FGF23 in patients with SCI. Studies of FGF23 in other unloading settings have yielded conflicting findings and have used different assays and techniques to quantify FGF23 (intact hormone, C-terminal assays, proteomics, and/or microRNA quantification). Higher, lower, and normal FGF23 values have all been reported under different unloading conditions (14-16). FGF23 RNA may increase under certain gravitational unloading conditions, depending on duration, associated bone turnover, and counteracting weight-bearing exercise (15). Nevertheless, other animal studies of unloading have found no significant changes in plasma FGF23 (16). Human studies in this field have examined astronauts and individuals undergoing bed rest. In a study of healthy participants undergoing 21 days of bed rest with extensive proteomic and metabolic profiling, FGF23 was identified as an aging/frailty marker, although its relationship to phosphate metabolism was not evaluated (17). Similarly, Mastrandrea et al. (14). found increased intact FGF23 after 14 days of bed rest. In astronauts exposed to microgravity, phosphate tended toward the upper limit of normal and tubular phosphate reabsorption changed, but FGF23 assessed by proteomic methods did not change (18).

Furthermore the literature on Klotho is inconsistent. Liu et al. (19). reported increased Klotho expression in adipose tissue in an animal model of SCI, although their study addressed lipid rather than phosphate metabolism. Other studies under unloading conditions have reported lower Klotho levels (16). Alterations in Klotho expression have also been described in other spinal cord disorders, including multiple sclerosis (20), in a study examining its potential neuroprotective role.

Patients with recent SCI exhibit substantial disturbances in calcium metabolism, including hypercalcemia and hypercalciuria, attributed to accelerated disuse-related bone turnover (1-3). They also have lower creatinine levels without impaired glomerular filtration, potentially reflecting the marked loss of muscle mass associated with SCI (1). Traumatic SCI also frequently coexists with long-bone fractures and surgical procedures that may contribute to calcium disturbances. Nevertheless, data on phosphate metabolism in this setting are sparse. Hyperphosphatemia in early SCI was previously identified by the same research group (1,21). More recently, Kumar et al. (22). also reported higher circulating phosphate in recent than in long-standing SCI. Phosphaturia and 24-hour urinary creatinine excretion were not measured in the present study; therefore, tubular phosphate reabsorption measures (TRP and TmP/GFR) were unavailable. Other investigators have assessed urinary phosphate excretion after SCI. Naftchi et al. (23), in 20 individuals with recent SCI monitored weekly for 16 weeks, reported hyperphosphaturia limited to the first 2 weeks, while hypercalciuria and elevated urinary markers of bone resorption (hydroxyproline) persisted longer. Moreover, Ohry et al. (24). described hyperphosphaturia in patients with recent SCI (2–74 weeks after injury). Conversely, Kannisto et al. (25). found no abnormalities of phosphate metabolism in individuals with SCI of a mean duration of 13 years. Collectively, these findings suggest that hyperphosphatemia and hyperphosphaturia occur in the first weeks after SCI, probably reflecting the markedly increased bone turnover during that period. Bone remodeling increases particularly during the first year after SCI (1-3), although up to 30 % of individuals may still have elevated turnover after 10 years (2).

Of note, in the acute phase of SCI, *PTH and calcitriol synthesis* are suppressed, an effect attributed to hypercalcemia and increased bone turnover (5). Elevated PTH and calcitriol ordinarily stimulate FGF23 synthesis. Renal function was preserved in the study population. These findings suggest that the rise in FGF23 represents a compensatory response to hyperphosphatemia resulting from the marked increase in bone remodeling during the acute phase of SCI.

Similarly, changes in FGF23 in these patients were correlated with baseline sclerostin. The relationship between FGF23 and sclerostin has been reported previously, and both mediators are produced by osteocytes and regulate bone metabolism. Ito et al. (26). demonstrated that sclerostin directly stimulates FGF23 synthesis in osteocytes in animal models and human bone cultures. Patients with SCI in the present study had higher FGF23 and Dkk-1, but no significant correlation was observed between these mediators. Carrillo-López et al. (27). showed that FGF23 in the presence of soluble Klotho induces Dkk1 expression in osteoblasts, inhibiting Wnt/β-catenin signaling. This suggests that FGF23 modulates osteoblast activity through local mediators such as Dkk1, contributing to altered bone remodeling in chronic kidney disease, where close relationships between circulating FGF23, sclerostin, and bone turnover have also been described (28). Recent evi-

dence also indicates that FGF23 stimulates osteoclast formation and survival, suggesting a regulatory role in bone resorption (29). Accordingly, an additional contribution of FGF23 to the markedly increased bone turnover after SCI cannot be excluded.

The main limitations are the lack of 24-hour urine measurements of tubular phosphate reabsorption and loss to follow-up. Nevertheless, this is the first study of FGF23 and phosphate metabolism in this clinical setting and provides new insights into its pathophysiology. It also represents one of the largest prospective SCI cohorts published to date, with substantial enrollment and comprehensive characterization of calcium-phosphate metabolism. The C-terminal FGF23 assay is another limitation because it detects intact hormone and degradation fragments. Although renal function was preserved, an intact FGF23 assay might have provided a more specific estimate of the biologically active hormone (7,8,10).

In summary, patients with recent complete SCI have elevated circulating phosphate and FGF23. Increased phosphate may be related to accelerated bone remodeling during the acute phase of SCI. The association between FGF23 and sclerostin suggests an additional role for FGF23 in bone remodeling regulation and warrants further study. These findings identify disturbances in phosphate metabolism early after SCI and characterize FGF23 behavior in this clinical setting for the first time.

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Original

Treatment persistence in osteoporosis among patients with type 2 diabetes *mellitus* — A population-based study

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Abstract

Introduction: in patients with type 2 diabetes *mellitus* (T2DM), fracture risk is often underestimated, and the prescription of osteoporosis drugs (ODs) is lower compared with non-diabetic individuals. In addition, persistence with ODs is generally low.

Objective: to assess whether there are differences in persistence with ODs between patients with T2DM and non-diabetic individuals.

Methods: population-based cohort study. Individuals who initiated an OD between 2012 and 2016 were selected from the SIDIAP database. Participants were followed until death, discontinuation or switching of the OD, or the end of the study (31 December 2018). Persistence was calculated as the ratio of the number of defined daily doses (NDDD) without a treatment gap longer than 3 or 6 months. Non-parametric methods were used to detect differences in persistence.

Results: of the 45,020 patients who initiated an OD, 6,072 had T2DM. Oral bisphosphonates were prescribed in 80.6 % of cases, followed by denosumab (9.7 %), with a similar distribution between diabetic and non-diabetic patients. Considering a 3-month gap for all ODs, persistence during the first year was 47.6 %, decreasing to 31.2 % at 2 years and 21.9 % at 3 years, with no differences between patients with T2DM and non-diabetic individuals. Persistence was higher for denosumab.

Conclusions: no significant overall differences in persistence were observed between patients with T2DM and non-diabetic individuals. Persistence with ODs decreased with longer follow-up, regardless of diabetes status.

Keywords:
Osteoporosis. Type 2 diabetes *mellitus*. Persistence. Treatment.

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INTRODUCTION

Osteoporosis is a systemic skeletal disease characterized by low bone mineral mass and deterioration of bone microarchitecture, associated with increased bone fragility and fracture risk (1). In Spain, the estimated prevalence of osteoporosis in 2019 was 5.4 % (22.6 % in women and 6.8 % in men) (2). Between 2019 and 2034, demographic changes are expected to increase the number of fractures in Spain by approximately 29.6 % (2).

Type 2 diabetes *mellitus* (T2DM) is among the most prevalent long-term comorbidities in Western countries, especially among older adults and people with obesity. Its prevalence in Spain was 10.3 % in 2012 and is projected to reach 11.8 % in 2030 and 12.7 % in 2045 (3,4).

T2DM and osteoporosis frequently coexist. Osteoporosis has been shown to manifest differently in people with and without T2DM. Despite having greater bone mass than the general population (5,6), individuals with T2DM have a higher risk of fragility fractures than those without diabetes (7-9), particularly hip fractures (10), even during the first years following diabetes onset (11). Increased bone fragility in T2DM is associated primarily with impaired bone quality rather than reduced bone mineral density (12). A recent meta-analysis comprising 22 studies (more than 13 million individuals, including more than 2 million with T2DM) found a 25 % higher risk among individuals with T2DM than among those without diabetes, with a greater increase in women than in men (13).

Fracture risk is genuinely underestimated in patients with T2DM. Proposed corrective approaches include incorporating the trabecular bone score (14,15) or selecting rheumatoid arthritis as a surrogate for T2DM in the FRAX© tool (16). Furthermore, patients with T2DM are up to 30 % more likely not to receive osteoporosis medication than people without diabetes, further compromising disease management (17).

In developed countries, persistence with drugs for chronic diseases is approximately 50 % (18). Persistence with osteoporosis therapies is also low, at 47 % after 1 year and 26 % after 2 years, although higher persistence has been reported with parenteral therapies (19).

Numerous factors have been associated with poor persistence with osteoporosis treatment, including dosage and route of administration, knowledge of the disease, adverse effects, and the number of concurrent chronic treatments (20,21). The PRISMA study of T2DM prevalence in Spain found that more than half of patients with T2DM were on ≥ 2 drugs (22).

Although no studies have specifically examined persistence with osteoporosis drugs (ODs) among patients with T2DM, lower persistence might be expected because of the greater burden of chronic medication use.

The objective of this study was to determine whether persistence with ODs differs between individuals with T2DM and those without diabetes. Furthermore, differences in persistence according to sex were investigated in an exploratory analysis.

METHODS

POPULATION AND DATA SOURCE

We conducted a population-based retrospective cohort study in the primary healthcare setting in Catalonia. Data were obtained from the SIDIAP database (*Sistema d'Informació per al Desenvolupament de la Investigació en Atenció Primària*), which contains electronic health records entered by more than 1,300 primary care professionals working at Institut Català de la Salut (ICS), the public health care provider with the broadest coverage in Catalonia (approximately 75 % of its population). These records include sociodemographic information; ICD-10-CM codes for recorded diseases and procedures; physical examination findings in structured forms (blood pressure, heart rate, weight, body mass index, etc.); information on smoking and alcohol consumption; primary care laboratory results (biochemistry, hematology, serology, etc.); and pharmacy dispensing records (drugs collected by patients from community pharmacies). The representativeness of SIDIAP relative to the general population of Catalonia has previously been established (23). Earlier SIDIAP studies in patients with T2DM found a prevalence similar to that reported in other Spanish studies (24,25). The database has also been used to investigate the epidemiology of fragility fractures and novel fracture predictors (26,27).

STUDY POPULATION

From all individuals in SIDIAP, a cohort of patients aged 50 years or older was selected who initiated one of the following ODs between January 1, 2012, and December 31, 2016: an oral bisphosphonate, a selective estrogen receptor modulator (SERM), denosumab, or teriparatide. At least 1 year of patient information before treatment initiation was required. Patients who had received an OD in the preceding 12 months or had a history of malignancy, Paget disease, or HIV infection were excluded. Follow-up continued until the end of the study (December 31, 2018), switching to another OD, or death, whichever occurred first.

Data were collected on age, sex, body mass index, smoking status (current, former, or never smoker), alcohol consumption (none, low risk, or harmful consumption according to the number of weekly alcohol units), Charlson Comorbidity Index, fractures and falls before OD initiation, and use of calcium and/or vitamin D supplements. ODs were identified using the World Health Organization Anatomical Therapeutic Chemical (ATC) classification system. Medications were grouped as bisphosphonates (alendronate, risedronate, and ibandronate), SERMs (raloxifene and bazedoxifene), teriparatide, and denosumab.

STATISTICAL METHODS

The study population was characterized using univariate descriptive analyses. Means and standard deviations were calculated for continuous variables, and absolute frequencies and percentages for categorical variables.

Persistence was calculated as the ratio of the number of defined daily doses (NDDD) to treatment duration, measured from the first to the last pharmacy dispensing without a gap longer than three months. Persistence was also assessed using a 6-month gap. Nonparametric methods were used to identify differences in persistence between individuals with and without T2DM and by OD type. Kaplan–Meier curves were also used to evaluate annual persistence survival (until treatment discontinuation) for each medication overall and separately in participants with and without T2DM.

ETHICAL CONSIDERATIONS

The study complied with national and international ethical standards (Declarations of Helsinki and Tokyo). The protocol was evaluated and approved by the ethics committee of *IDIAP Jordi Gol i Gorina* (code 19/081-P) and was classified by the Spanish Agency of Medicines and Medical Devices (AEMPS) as a postauthorization study using a design other than prospective follow-up (code IJG-TER-2019-01).

Data in the SIDIAP database were pseudonymized, preventing identification of the included individuals. Confidentiality was guaranteed throughout the study in accordance with the Spanish Personal Data Protection Act (Organic Law 15/1999 of December 13) and Royal Decree-Law 5/2018 of July 27 on urgent measures to adapt Spanish law to European data-protection regulations.

RESULTS

A total of 45,020 individuals initiated an OD during the study period; 6,072 had T2DM and 38,948 did not have diabetes. Women accounted for 89.8 % of participants, and mean age was 69 years. Baseline characteristics by diabetes status are shown in table I. Patients with T2DM had a higher comorbidity burden, a greater proportion of previous falls (6.6 % vs. 3.7 %), and a higher prevalence of previous fractures (24 % vs. 14.4 %).

Overall, 80.6 % of the patients received an oral bisphosphonate, 9.7 % denosumab, 6.3 % raloxifene/bazedoxifene, and 3.4 % teriparatide. The distribution was similar in both cohorts except for SERMs, which were used more frequently by individuals without diabetes (Table II).

With a 3-month permissible gap for all ODs, persistence was 47.6 % at 1 year, 31.2 % at 2 years, and 21.9 % at 3 years. With a 6-month gap, persistence was somewhat higher (54.7 %, 38.3 %, and 28.2 %, respectively). No significant differences in overall persistence (all treatments combined) were observed between individuals with and without T2DM (Fig. 1). Persistence declined with increasing treatment duration.

According to sex, persistence was significantly higher in women than in men at every time point examined with either a three- or 6-month gap (Fig. 2).

The same pattern was observed when participants with and without T2DM were compared by sex. With a 3-month gap, persistence among women with T2DM was 47.8 % at 1 year, 31.1 % at 2 years, and 21.26 % at 3 years, versus 43.7 %, 26.9 %, and 18.3 %, respectively, among men ($p < 0.05$). With a 6-month gap, the corresponding rates were 55.4 %, 39.1 %, and 28.7 % among women with T2DM and 48.9 %, 32.8 %, and 23.9 % among men with T2DM ($p < 0.05$). Among women without diabetes, persistence with a 3-month gap was 48.1 %, 31.6 %, and 22.3 % at 1, 2, and 3 years was 43.5 %, 28.1 %, and 19.9 % among men without diabetes ($p < 0.001$). With a 6-month gap, persistence rates among women without diabetes were 55.3 %, 38.8 %, and 28.7 %, significantly higher than those among men without diabetes (48.9 %, 33 %, and 24.3 %).

However, comparisons of overall persistence between individuals with and without T2DM showed no significant sex-related differences.

Among the different therapies, denosumab had the highest persistence overall and in both the T2DM and nondiabetic cohorts, with higher persistence than the other ODs throughout follow-up (Figs. 3 and 4). No OD achieved persistence > 50 % at 2 years.

Table I. Baseline characteristics of all patients and of patients with and without T2DM

		Overall	Patients with T2DM	Patients without diabetes	*p* value
Age (median; IQR)		69 (17)	75 (13)	67 (17)	0.000
Female sex (*n*; %)		40,431 (89.8)	4982 (82.0)	35,449 (91.0)	0.000
Missing BMI (*n*; %)		9281 (20.6)	361 (5.9)	8920 (22.9)	
BMI (median; IQR)	BMI (median; IQR)	26.88 (6.21)	28.91 (6.64)	26.52 (6.01)	0.000
Missing smoking status (*n*; %)		4089 (9.1)	177 (2.9)	3912 (10.0)	
Smoking	Never smoker	31,952 (78.1)	4762 (80.8)	27,190 (77.6)	0.000
	Current smoker	4413 (10.7)	348 (5.9)	4065 (11.6)	0.000
	Former smoker	4566 (11.2)	785 (13.3)	3781 (10.8)	0.000
Missing alcohol use (*n*; %)		7478 (16.6)	420 (6.9)	7058 (18.1)	
Alcohol use	None (*n*; %)	28,865 (76.9)	4668 (82.6)	24,197 (75.9)	0.000
	Low risk (*n*; %)	8360 (22.3)	940 (16.6)	7420 (23.3)	0.000
	Harmful use (*n*; %)	317 (0.8)	44 (0.8)	273 (0.9)	0.000
Comorbidity Charlson index (*n*; %)	None	37,030 (82.3)	2546 (41.9)	34,484 (88.5)	0.000
	Low	4632 (10.3)	1719 (28.3)	2913 (7.5)	0.000
	High	3358 (7.4)	1807 (29.8)	1551 (4.0)	0.000
Previous fractures (*n*; %)		7087 (7.5)	1460 (24.0)	5627 (14.4)	0.000
Previous falls (*n*; %)		1702 (3.8)	399 (6.6)	1303 (3.7)	0.000
Supplement use (*n*; %)	No previous use	18,381 (40.8)	2804 (46.2)	15,577 (40.0)	0.000
	Previous use	26,639 (59.2)	3268 (53.8)	23,371 (60.0)	0.000

IQR, interquartile range; BMI, body mass index; T2DM, type 2 diabetes mellitus.

Table II. Percentages of osteoporosis medication use in patients with T2DM, without diabetes, and overall

	Total		Patients with T2DM		Patients without diabetes	
	n	%	*n*	%	*n*	%
	45,020		6072	13.49	38,948	86.51
Oral bisphosphonates	36,282	80.59	5103	84.04	31,179	80.05
Denosumab	4387	9.74	506	8.33	3881	9.96
SERMs	2841	6.31	165	2.72	2676	6.87
Teriparatide	1510	3.35	298	4.91	1212	3.11

T2DM, type 2 diabetes mellitus; *BF, bisphosphonate; SERM, selective estrogen receptor modulator.*

With a 3-month gap, persistence with denosumab was significantly lower among patients with T2DM than among those without diabetes ($p < 0.05$). Moreover, persistence during the second treatment year was lower among patients with T2DM on SERMs or teriparatide. With a 6-month gap, persistence with denosumab remained lower in T2DM, as did persistence with SERMs during the first treatment year. No significant differences were observed among patients receiving teriparatide (Table III).

DISCUSSION

Overall, persistence with osteoporosis drugs was suboptimal: < 50 % at 1 year, 32 % at 2 years, and 22 % at 3 years, with similar rates among participants with and without T2DM. These findings are consistent with those of an earlier SIDIAP study conducted in 2012, which reported persistence of 47 % at 1 year and 26 % at 2 years (19), and with international studies (28,29). A UK study using the CPRD database reported overall persistence of 43.6 % at 1 year and 36.4 % at 2 years (29).

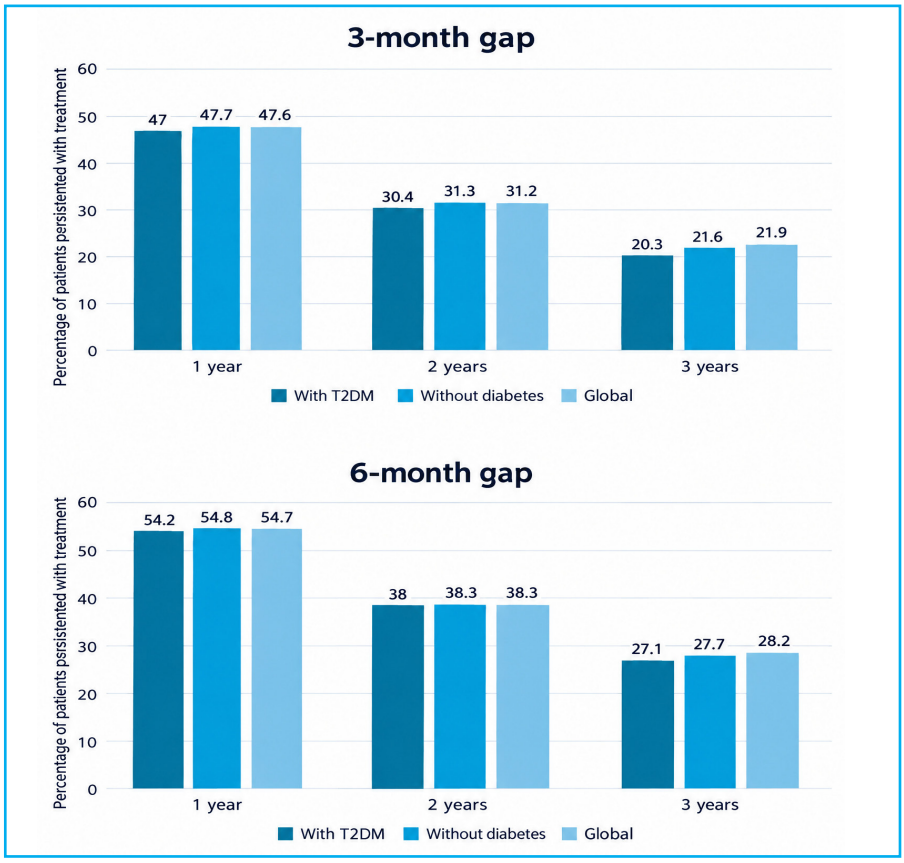

Figure 1. Overall treatment persistence (3- or 6-month gap).

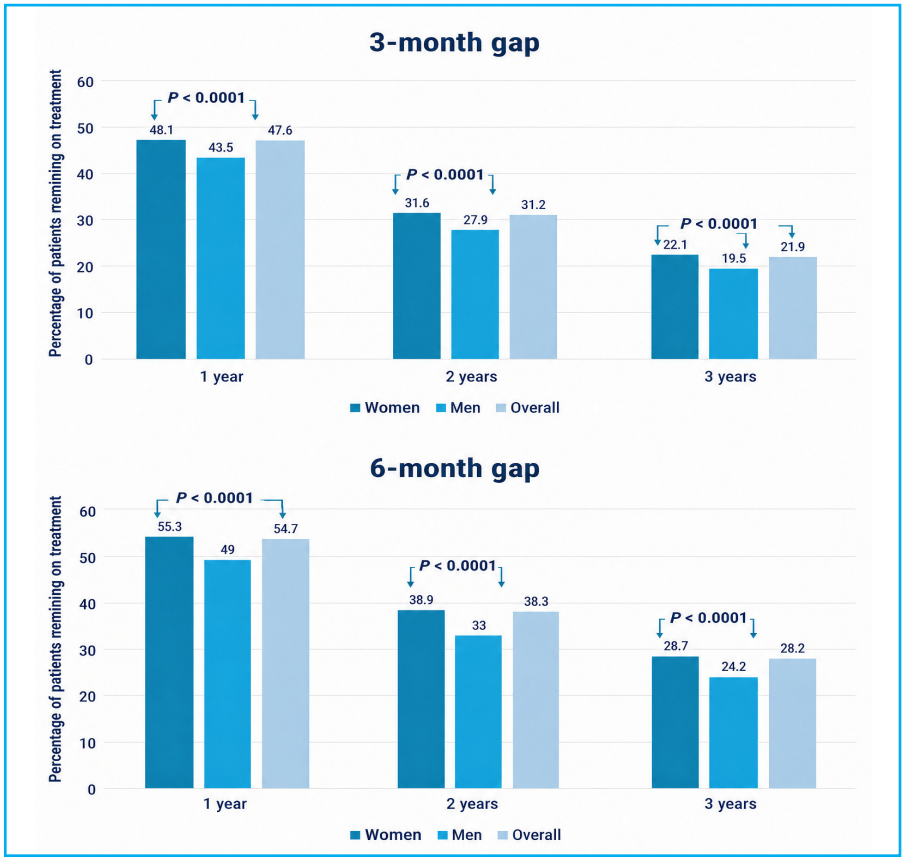

Figure 2. Overall treatment persistence by sex (3- or 6-month gap).

The 2-year persistence was similar to that in the present cohort for oral bisphosphonates but higher for denosumab (64 %). Other studies of postmenopausal women found 2-year denosumab persistence of 62 % in Sweden (30), 50 % in the United States, and 75 % in Canada (31). These differences may partly reflect national reimbursement arrangements, drug prices, and access according to local budgetary policies (32).

Persistence with antiosteoporosis therapy remains a clinical challenge, particularly among patients with comorbidities. A previous SIDIAP study found greater comorbidity among patients with T2DM, potentially affecting persistence adversely. However, a past medical history of fracture might improve awareness of the disease and thus favor adherence (17,33). Approximately one-quarter of individuals with T2DM in the present cohort had previous fractures, versus just > 14 % of

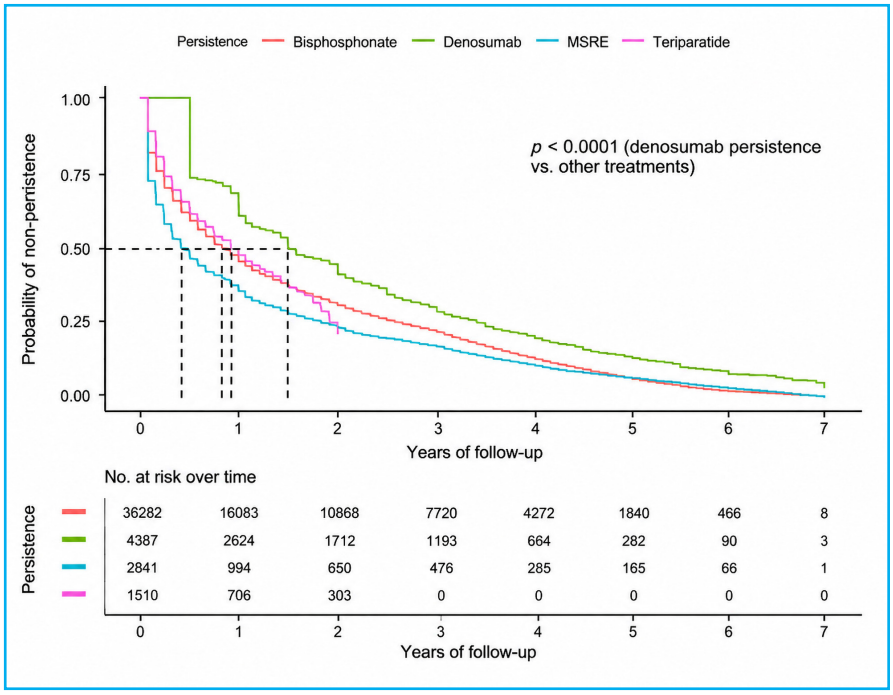

Figure 3. Kaplan–Meier survival curves of annual treatment persistence.

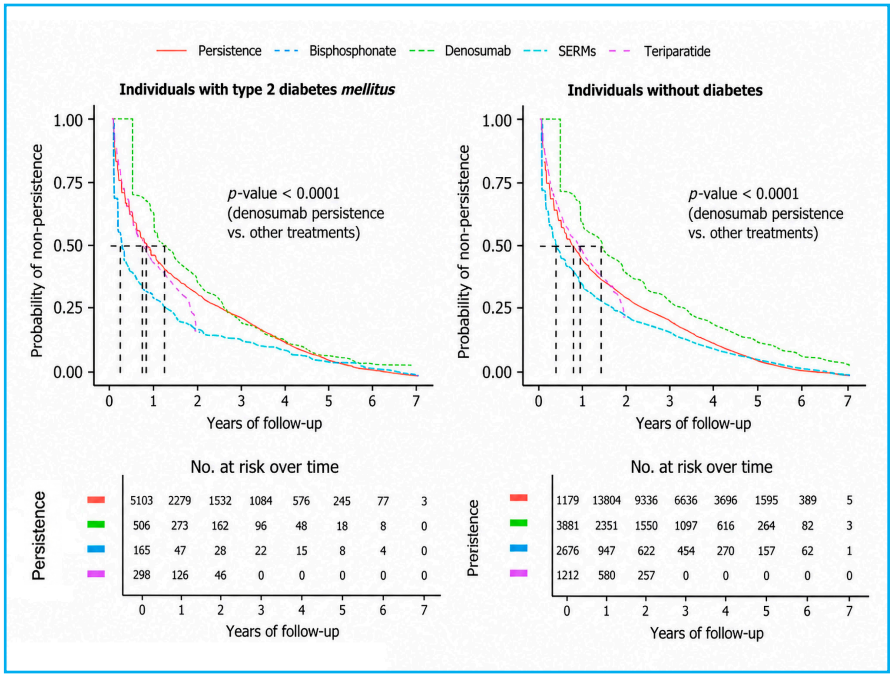

Figure 4. Kaplan–Meier survival curves of annual treatment persistence by therapy and cohort.

Table III. Persistence according to treatment among individuals with and without T2DM (using a 3- or 6-month gap) at 1, 2, and 3 years

		Individuals with T2DM							Individuals without diabetes					
		1 year		2 years		3 years			1 year		2 years		3 years	
	Total *n*	*n*	%	*n*	%	*n*	%	Total *n*	*n*	%	*n*	%	*n*	%
3-month gap														
Overall	*6072*	2856	47.0	1844	30.4	1235	20.3	*38,948*	18,583	47.7	12,193	31.3	8422	21.6
OB	*5103*	2366	46.4	1584	31.0	1114	21.8	*31,179*	14,402	46.2	9604	30.8	6826	21.9
Denosumab	*506*	306	60.5*	176	34.8*	98	19.4*	*3881*	2569	66.2*	1634	42.1*	1133	29.2*
SERMs	*165*	51	30.9	28	17.0*	23	13.9	*2676*	1000	37.4	646	24.1*	463	17.3
TPD	*298*	133	44.6	56	18.8*			*1212*	612	50.5	309	25.5*		
6-month gap														
Overall	*6072*	3293	54.2	2308	38.0	1646	27.1	*38,948*	21,330	54.8	14,923	38.3	10,800	27.7
OB	*5103*	2759	54.1	1987	38.9	1485	29.1	*31,179*	16,683	53.5	11,820	37.9	8767	28.1
Denosumab	*506*	320	63.2*	207	40.9*	131	25.9*	*3881*	2772	71.4*	1916	49.4*	1418	36.5*
SERMs	*165*	62	37.6*	37	22.4*	30	18.2	*2676*	1219	45.6*	814	30.4*	615	23.0
TPD	*298*	152	51.0	77	25.8			*1212*	656	54.1	373	30.8		

T2DM, type 2 diabetes mellitus; *OB, oral bisphosphonate; SERM, selective estrogen receptor modulator; TPD, teriparatide.* *p < *0.05 (patients with T2DM vs patients without diabetes).*

those without diabetes. Furthermore, the prevalence of previous falls was also almost twice as high among patients with T2DM. Both factors may contribute to greater disease awareness and thereby improve treatment persistence.

The absence of differences in persistence with ODs considered collectively between patients with and without T2DM suggests that other factors, including health care system support, patient education, and interdisciplinary collaboration, highlighted in an earlier systematic review of strategies to improve persistence (20), may help explain the findings. Healthc are professionals have also been shown to overestimate their patients' actual adherence to osteoporosis drugs (34). A consensus statement from the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO) and the International Osteoporosis Foundation (IOF) recommends understanding the problem, measuring adherence, identifying the causes of nonadherence, and addressing them (21).

When individual medications were considered, persistence with denosumab was lower at every time point among patients with T2DM than among those without diabetes.

Overall, most studies of persistence with osteoporosis drugs in this setting have assessed short-term persistence; few have followed patients for > 2 years (19,35,36). The present study assessed 3-year persistence (excluding teriparatide because of its limited treatment duration) and found an overall rate of 21.8 %. Another study evaluating 36-month persistence reported rates of 38 % for denosumab and 17 % for oral bisphosphonates (37).

Most studies examining persistence with different osteoporosis treatments have focused on postmenopausal women, and little evidence is available on sex-related differences. In the present exploratory analysis, women consistently had significantly higher persistence than men in all settings and periods examined. One possible explanation is lower awareness and understanding of osteoporosis and its consequences among men, negatively affecting adherence and persistence.

Denosumab was associated with the greatest persistence overall and in both diabetes cohorts, exceeding that of the other ODs throughout follow-up. This finding is consistent with former studies reporting better short- and long-term persistence with injectable than with oral treatments (19,28,38). Less frequent administration schedules, such as denosumab, have been associated with better adherence than daily treatments such as teriparatide (28), as seen in the present study. Conversely, among patients on oral bisphosphonates, 1-year persistence was lower with monthly ibandronate (approximately 40 %) than with weekly or daily alendronate (> 47 %) (19,39). More frequent oral administration might help establish a regular routine and reduce forgotten doses (39).

A literature review concluded that parenteral therapies such as denosumab improve adherence, especially in patients with comorbidities due to simpler dosing and a lower GI burden vs oral bisphosphonates (40).

LIMITATIONS AND STRENGTHS

This study was primarily descriptive and therefore cannot establish causality or evaluate direct drug effects. Its purpose was to observe and describe associations between different treatments and the outcomes analyzed. Confounding by indication is possible—for example, more severe disease among denosumab or teriparatide users than among alendronate users—despite efforts to account for baseline differences.

The absence of key markers of disease severity, such as bone mineral density, and the finding that drugs with less convenient schedules (such as daily teriparatide injections) showed better persistence than weekly oral alendronate in some analyses suggest that residual confounding may partly explain the observed results.

Other important limitations should be considered. First, treatment dates were based on pharmacy dispensing, which does not always correspond to actual administration. Nevertheless, any resulting misclassification would be expected to be random and would therefore tend to bias comparative persistence estimates toward the null. To minimize this limitation, three- and 6-month gaps were assessed. Second, treatment discontinuation or switching could not be attributed to a physician's vs a patient's decision because both were recorded identically in the database.

Similarly, because the data came from primary care, persistence with zoledronic acid could not be assessed and no comparisons with that drug could be made. All oral bisphosphonates were analyzed together, precluding assessment of differences between daily, weekly, and monthly regimens. An earlier SIDIAP persistence study using data from 2007–2011 found similar rates with weekly oral bisphosphonates (31 %–39 %) and monthly preparations (35 %–40 %), with the highest rates for monthly risedronate (40 %) and weekly alendronate (39.9 %). Much lower rates were reported with daily preparations (7.7 %–14.1 %) (35). These findings suggest that factors beyond dosing frequency—including administration route, adverse effects, and dosing instructions—may account for the poorer persistence with oral bisphosphonates compared with other agents.

Finally, the data cover 2012–2018 and may not reflect current persistence patterns. Nevertheless, overall persistence was similar to that reported for the same population in earlier periods (2007–2011 and 2012) (19,35), suggesting a longstanding problem. Investigating persistence after the coronavirus pandemic would be valuable given its effects on the management and monitoring of chronic diseases.

Conversely, important strengths include the large sample size, which enabled detection of statistically significant differences even when small. In addition, unlike many previous studies, persistence was assessed for up to 36 months, except for teriparatide.

In conclusion, persistence with ODs is low in both individuals with and without T2DM, with higher persistence for parenteral than for oral therapies. Women consistently showed higher persistence than men in all periods analyzed. No significant overall differences in persistence were observed between the T2DM and nondiabetic cohorts. Persistence decreased with longer follow-up. Strategies are needed to identify nonpersistence and its associated factors and to improve treatment continuation. Current proposals include improving disease knowledge and promoting shared decision-making by actively involving patients in prescribing decisions.

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Efficacy of teriparatide combination therapies on bone mineral density in postmenopausal osteoporosis: a network meta-analysis

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Abstract

Introduction: teriparatide (TPTD) is an effective anabolic agent for osteoporosis; however, its comparative efficacy when combined with antiresorptive drugs or other interventions remains uncertain. This network meta-analysis (NMA) aimed to compare the effects of various TPTD combination therapies and monotherapies on bone mineral density (BMD) in patients with postmenopausal osteoporosis.

Methods: Bayesian random-effects models were used. Primary endpoints were mean differences (MDs) in percentage change in BMD at the lumbar spine, femoral neck, and total hip. We estimated MDs with 95 %CrIs and ranked treatments using SUCRA probabilities.

Results: a total of 10 studies were included in this analysis. No combination therapy demonstrated statistically significant improvements in mean BMD across skeletal sites. At the lumbar spine, denosumab (DEN) + TPTD showed the largest estimated effect (MD, 3.12; 95 %CrI, -1.65 to 7.83). For the total hip, zoledronic acid (ZOL) + TPTD yielded the highest estimated mean change (MD, 6.75; 95 %CrI, -1.65 to 15.10). At the femoral neck, DEN + TPTD had the greatest estimated effect (MD, 3.71; 95 %CrI, -3.08 to 10.5). SUCRA rankings indicated DEN + TPTD as the most effective treatment for the lumbar spine (79.32) and femoral neck (76.45), whereas ZOL + TPTD ranked highest for the total hip (83.77).

Conclusions: this NMA indicates that TPTD combination therapies may offer potential benefits in improving BMD, with DEN + TPTD and ZOL + TPTD ranking highest across different skeletal sites. However, the absence of statistically significant differences underscores the need for further studies.

Keywords:
Combination therapy.
Osteoporosis.
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INTRODUCTION

Postmenopausal osteoporosis (PMO) is clinically characterized by low bone mineral density (BMD), microarchitectural deterioration, and increased susceptibility to fragility fractures due to estrogen deficiency (1). It remains a major global health burden, as postmenopausal women (PMW) experience a 15.17-fold higher mortality rate, a 5.84-fold greater burden in disability-adjusted life years (DALYs), and a 6.29-fold greater burden in years lived with disability (YLDs) compared with premenopausal populations, underscoring the urgent need for more effective therapeutic strategies (2). Although antiresorptive agents remain widely used as first-line therapies, their predominant suppression of bone resorption may be insufficient to rapidly restore skeletal mass and strength in women at very high fracture risk, prompting greater reliance on bone-forming approaches and optimized treatment sequencing (3). Teriparatide (TPTD), a recombinant human parathyroid hormone (PTH) 1-34, is a prototypical anabolic agent with well-established antifracture efficacy in severe PMO, including significant reductions in vertebral and nonvertebral fractures (4). It was the first anabolic therapy approved by the US Food and Drug Administration (FDA) for osteoporosis treatment (5).

An ideal antiosteoporosis agent would theoretically both suppress bone resorption and stimulate new bone formation; however, neither currently available antiresorptive therapies nor PTH analogs achieve this dual action. Consequently, combining anabolic and antiresorptive therapies has been proposed as a practical alternative, based on the rationale that stimulating bone formation while simultaneously inhibiting resorption could yield greater gains in bone mass and strength than either approach alone (6,7). However, the 2020 guidelines of the American Association of Clinical Endocrinologists and the American College of Endocrinology state that, until the effects of combination therapy on fracture risk are better understood, the concomitant use of multiple osteoporosis agents is generally not recommended for osteoporosis treatment (8). Moreover, when anabolic agents are used, current guidelines recommend transitioning to sequential therapy with an antiresorptive agent, such as a bisphosphonate or denosumab, after discontinuation of the anabolic course to preserve gains in BMD and reduce the risk of subsequent fractures (8,9).

Therefore, given the limited available data and the lack of comprehensive head-to-head comparisons among TPTD combination therapies and other interventions, a network meta-analysis (NMA) is warranted to rank therapeutic strategies according to their efficacy on BMD outcomes, while also emphasizing the need for further evidence regarding the clinical effectiveness of TPTD-based combination approaches.

METHODS

This systematic review and NMA were conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement. The study protocol was prospectively registered in the International Prospective Register of Systematic Reviews (PROSPERO; CRD420251185687) on 10 November 2025.

SEARCH STRATEGY

A comprehensive literature search was conducted across multiple electronic databases, including Cochrane, MEDLINE, and ClinicalTrials.gov. The search was independently performed by HN, SA, and OW using predefined keywords and Medical Subject Headings (MeSH), including "Teriparatide," "Parathyroid Hormone," "Combination Therapy," "Osteoporosis," and "Postmenopausal Osteoporosis" (Supplementary material: https://www.revistadeosteoporosisymetabolismomineral.com/files/526/ADMA1-00115-02.pdf). The search strategy was developed according to the PICOTS-SD framework as follows:

- Population: adults (≥ 18 years) with PMO.
- Intervention: TPTD-based combination therapy.
- Comparator: TPTD monotherapy, other active monotherapies, or alternative TPTD-containing combination regimens.
- Outcomes: mean change in BMD at the lumbar spine, total hip, and femoral neck.
- Time: no restrictions applied.
- Setting: all relevant clinical settings.
- Study design: randomized controlled trials (RCTs).

ELIGIBILITY CRITERIA

Types of studies

Only RCTs were eligible for inclusion. Observational studies, case series, case reports, narrative reviews, systematic reviews, book chapters, editorials, commentaries, as well as in vitro and in silico studies, were excluded.

Outcomes of interest

The primary endpoint of this study was to compare and rank the efficacy of TPTD-based combination therapies relative to TPTD monotherapy and other treatment strategies in improving BMD at the femoral neck, total hip, and lumbar spine.

Study selection

Studies were identified through a structured screening process. After duplicate records were removed using Zotero (version 7.0.15; Corporation for Digital Scholarship, Virginia, USA) on 10 November 2025, HN, SA, and OW independently screened titles and abstracts for relevance. Full-text articles of potentially eligible studies were subsequently assessed according to predefined inclusion criteria. Reference lists of included studies were manually reviewed to identify additional eligible reports. Final study inclusion was determined by reviewer consensus.

Data collection process

Data were extracted using a standardized form by 2 reviewers (HN and SA). Extracted variables included study characteristics, sample size, participant age, intervention details, daily treatment regimens, and follow-up duration.

Summary measures

Changes in BMD were analyzed as continuous outcomes using mean differences (MDs) as the summary effect measure.

Risk of bias assessment

Two reviewers (OW and IB) independently evaluated study quality using the Cochrane Risk of Bias tool, version 2 (RoB 2).10 Disagreements were resolved through consensus.

Synthesis of results and statistical analysis

For studies reporting outcomes only in graphical form, numerical values were extracted using WebPlotDigitizer (version 4.8; Autometris LLC; https://apps.automeris.io/wpd4/). A Bayesian NMA was conducted using the MetaInsight platform (version 6.4.0; NIHR Complex Reviews Support Unit; https://crsu.shinyapps.io/MetaInsight/) accessed on 20 November 2025.11 Results were summarized using forest plots, with between-study heterogeneity addressed through a random-effects model. Inconsistency and sensitivity analyses were also conducted. Treatment rankings were generated using the surface under the cumulative ranking curve (SUCRA), with higher values indicating greater efficacy. Studies identified as having a high risk of bias, as well as poorly fitting studies based on leverage plots falling outside the threshold line at $c = 3$, were excluded when present. Inconsistency was further assessed using the node-splitting model.

RESULTS

STUDY SELECTION

The study selection process is summarized in figure 1. Following an initial retrieval of 717 records from the database search, screening based on predefined criteria resulted in 35 articles undergoing full-text assessment. Of these, exclusions were made for mismatched interventions ($n = 13$), inappropriate comparators ($n = 8$), and outcomes not aligned with the study objectives ($n = 4$). Consequently, 10 studies fulfilled all inclusion criteria and were incorporated into the NMA for data synthesis.

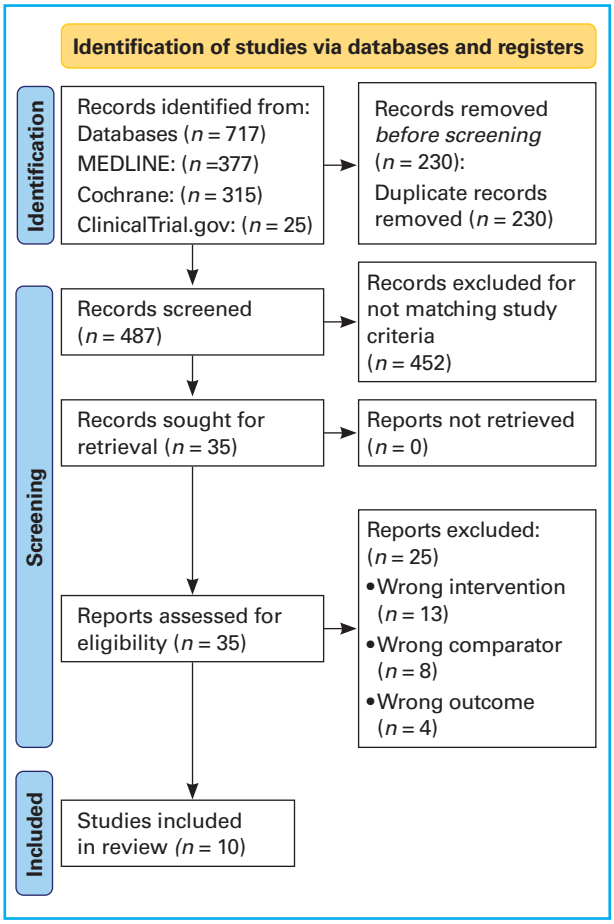

Figure 1. PRISMA flowchart diagram.

CHARACTERISTICS OF THE INCLUDED STUDIES

A total of 1459 participants from 10 RCTs were included. The studies enrolled PMW with osteoporosis from the United States, Europe, China, and multinational cohorts. Sample sizes ranged from 33 to more than 412 participants, with mean ages predominantly in the sixth to seventh decades of life. All trials evaluated TPTD-based combination therapies versus TPTD monotherapy, with some including additional active comparators such as alendronate (ALD), raloxifene (RAL), zoledronic acid (ZOL), denosumab (DEN), or nonpharmacological interventions (pulsed electromagnetic fields [PEMFs] or whole-body vibration [WBV]). TPTD (20-100 µg/day) was most frequently combined with antiresorptive agents, and calcium and vitamin D supplementation was routinely provided. Follow-up periods ranged from 6 to 30 months, most commonly 12 months. Overall, study quality was acceptable, with several trials at low risk of bias and the remainder showing some concerns. Further details are shown in table I.

NETWORK META-ANALYSIS RESULTS

Figure 2 and figure 3 illustrate the network and forest plots for BMD changes at the lumbar spine, total hip, and femoral neck. No intervention showed a statistically significant difference in mean BMD change across these skeletal sites. At the lumbar spine, the largest estimated effect was observed with DEN + TPTD (MD, 3.12; 95 %CrI, -1.65 to 7.83), followed by PEMF + TPTD (MD, 2.48; 95 %CrI, -3.83 to 8.72). For the total hip, ZOL + TPTD demonstrated the highest estimated mean change (MD, 6.75; 95 %CrI, -1.65 to 15.1), with DEN + TPTD ranking second (MD, 4.27; 95 %CrI, -4.32 to 12.7). At the femoral neck, DEN + TPTD showed the greatest estimated effect (MD, 3.71; 95 %CrI, -3.08 to 10.5), followed by ZOL + TPTD (MD, 3.17; 95 %CrI, -3.56 to 9.92). SUCRA-based ranking indicated that DEN + TPTD had the highest probability of being the most effective intervention for lumbar spine BMD (SUCRA, 79.32) and femoral neck BMD (SUCRA, 76.45), whereas ZOL + TPTD ranked highest for total hip BMD (SUCRA, 83.77). Detailed SUCRA rankings are presented in table II and figure 4.

SENSITIVITY AND INCONSISTENCY ANALYSES

No studies were identified as having a high risk of bias or as poorly fitting according to the leverage plots. Inconsistency assessments using node-splitting analyses likewise revealed no significant findings, except at the femoral neck. A significant local inconsistency emerged between RAL + TPTD and ALD + TPTD (p = 0.0043). While direct evidence indicated a significant benefit of RAL + TPTD, indirect evidence pointed in the opposite direction. This discrepancy resulted in a non-significant overall network estimate at the femoral neck. Further details are provided in the supplementary material (https://www.revistadeosteoporosisymetabolismomineral.com/files/526/ADMA1-00115-02.pdf).

PUBLICATION BIAS

Publication bias was evaluated qualitatively using funnel plots and quantitatively using Egger regression tests. In this analysis, lumbar spine, total hip, and femoral neck plots appeared asymmetrical; however, Egger regression tests showed no significant bias, with p = 0.2121, p = 0.9783, and p = 0.4635 for the lumbar spine, total hip, and femoral neck, respectively, as illustrated in the supplementary material (https://www.revistadeosteoporosisymetabolismomineral.com/files/526/ADMA1-00115-02.pdf). Such discrepancies may reflect small-study effects and heterogeneity, particularly given the limited number of included studies. Because funnel plots are less reliable with small sample sizes, the observed asymmetry is more likely attributable to heterogeneity.

DISCUSSION

The main strength of this study is that it represents the first comprehensive NMA comparing the effectiveness of various TPTD combination therapies, including both pharmacological and nonpharmacological interventions, in patients with PMO. Although the comparative analysis revealed no statistically significant differences in increasing BMD across interventions, this study demonstrated that several TPTD-based combination therapies consistently showed larger estimated mean changes in BMD vs other interventions in PMO.

In particular, the combination of DEN + TPTD demonstrated the greatest estimated effects at the lumbar spine and femoral neck, while ranking second at the total hip. The direction of effect observed in this study is consistent with a meta-analysis by Sun et al. (2022), which reported that combination therapy with TPTD + DEN in PMO was associated with greater BMD gains than monotherapy, including statistically significant mean percentage increases of approximately 2.91 % at the lumbar spine and 3.19 % at the hip (12). The overall beneficial effect may be attributed to DEN's ability to fully inhibit the proresorptive effects of TPTD through suppression of the RANKL pathway, while still allowing TPTD-induced stimulation of bone formation via activation of parathyroid hormone receptors. This combination thereby establishes a dynamic balance between bone formation and resorption (13).

Table I. Study characteristics

No.	Study ID	Participants (n)	Age	Intervention	Basic treatment/day	Follow-up (months)	RoB 2
1	Deal 2005 (24)	I: RAL + TPTD: 69 C: TPTD: 68	I: 66.6 ± 7.5 C: 66.1 ± 7.8	I: RAL 60 mg/day orally + TPTD 20 µg/day IM C: TPTD 20 µg/day IM	Calcium 1000 mg Vitamin D 400-800 IU/day	6	Low
2	Finkelstein 2010 (25)	I: ALD + TPTD: 20 C: TPTD: 20 ALD: 29	I: 62 ± 7 C: 65 ± 7 ALD: 64 ± 6	I: ALD 10 mg/day orally + TPTD 100 µg/day IM C: TPTD 56.5 µg/day IM ALD 10 mg/day orally	Calcium 1000-1200 mg Vitamin D 400 IU	30	Some concerns
3	Cosman 2011 (14)	I: ZOL + TPTD: 137 C: TPTD: 138 ZOL: 137	I: 65 ± 8.8 C: 63.8 ± 9.1 ZOL: 66.1 ± 9.0	I: ZOL 5 mg/year IV infusion + TPTD 20 µg/day IM C: TPTD 20 µg/day IM ZOL 5 mg/year IV infusion	Calcium 1000-1200 mg Vitamin D 400-800 IU	12	Some concerns
4	Muschitz 2013 (26)	I: ALD + TPTD: 41 RAL + TPTD: 37 C: TPTD: 47	I: 71.6 ± 8.5 C: 71.7 ± 9.3	I: ALD 70 mg/week orally + TPTD 20 µg/day IM RAL 60 mg/day orally + TPTD 20 µg/day IM C: TPTD 20 µg/day IM	Calcium 1000 mg Vitamin D 800 IU	18	Some concerns
5	Tsai 2013 (27)	I: DEN + TPTD: 30 C: TPTD: 29 DEN: 33	I: 65.9 C: 65.5 ± 7.9	I: DEN 60 mg/6 months + TPTD 20 µg/day IM C: TPTD 20 µg/day IM	Calcium 1200 mg Vitamin D 400 IU	12	Low
6	Muschitz 2014 (The CONFORS Study) (28)	I: ALD + TPTD: 41 RAL + TPTD: 37 C: TPTD: 47	I: 72.4 ± 9.1 70.5 ± 8.4 C: 72.8 ± 8.9	I: ALD 70 mg/week orally + TPTD 20 µg/day IM RAL 60 mg/day orally + TPTD 20 µg/day IM C: TPTD 20 µg/day IM	Calcium Vitamin D	12	Some concerns
7	Leder 2014 (The DATA Extension Study) (29)	I: DEN + TPTD: 24 C: TPTD: 28 DEN: 31	I: 65.9 ± 9.0 C: 65.5 ± 7.9 DEN: 66.3 ± 8.3	I: DEN 60 mg/6 months + TPTD 20 µg/day IM C: TPTD 20 µg/day IM DEN 60 mg/6 months	Calcium 1200 mg Vitamin D 20 ng/mL	24	Some concerns
8	Xuan 2025 (30)	I: PEMF + TPTD: 15 C: TPTD: 113 PEMFs: 108	I: 65.04 ± 7.10 C:65.23 ± 7.07 PEMFs: 65.37 ± 7.38	I: PEMF for 5 months followed 1 month later by TPTD 20 µg/day IM C: TPTD 20 µg/day IM PEMF monotherapy	Calcium 600 mg Vitamin D 125 IU	18	Some concerns
9	Jepsen 2019 (PaVOS study) (31)	I: WBV + TPTD: 15 C: TPTD: 18	I: 69 ± 5 C: 69 ± 8	I: WBV training protocol consisting of 12 minutes of training with a WB-V:rest ratio of 1:1 minute, including 6 minutes of vibration, 3 days/week with 1 day between sessions + TPTD 20 µg/day IM C: TPTD 20 µg/day IM	Calcium 800 mg Vitamin D 400-800 IU	12	Some concerns
10	Wei 2021 (32)	I: ZOL + TPTD: 48 C: TPTD: 48	I/C:64.31 ± 2.56	I: ZOL 5 mg/day IV + TPTD 20 µg/day IM C: TPTD 20 µg/day IM	Calcium carbonate 600 mg Vitamin D 125 IU	6	Some concerns

ALD: alendronate; DEN: denosumab; PEMFs: pulsed electromagnetic fields; RAL: raloxifene; RID: risedronate; TPTD: teriparatide; WBV: whole-body vibration; ZOL: zoledronic acid.

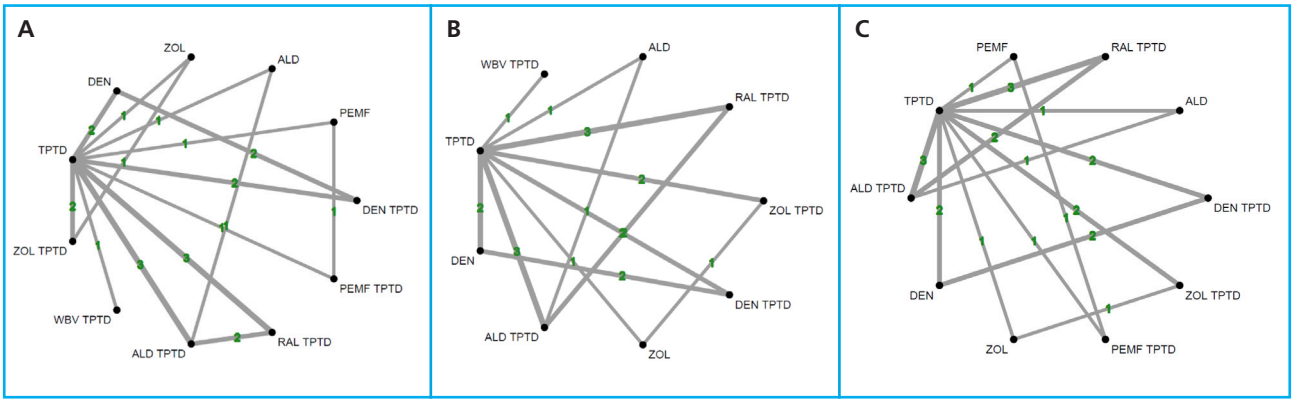

Figure 2. Network plots for BMD at the: (A) lumbar spine; (B) total hip; and (C) femoral neck.

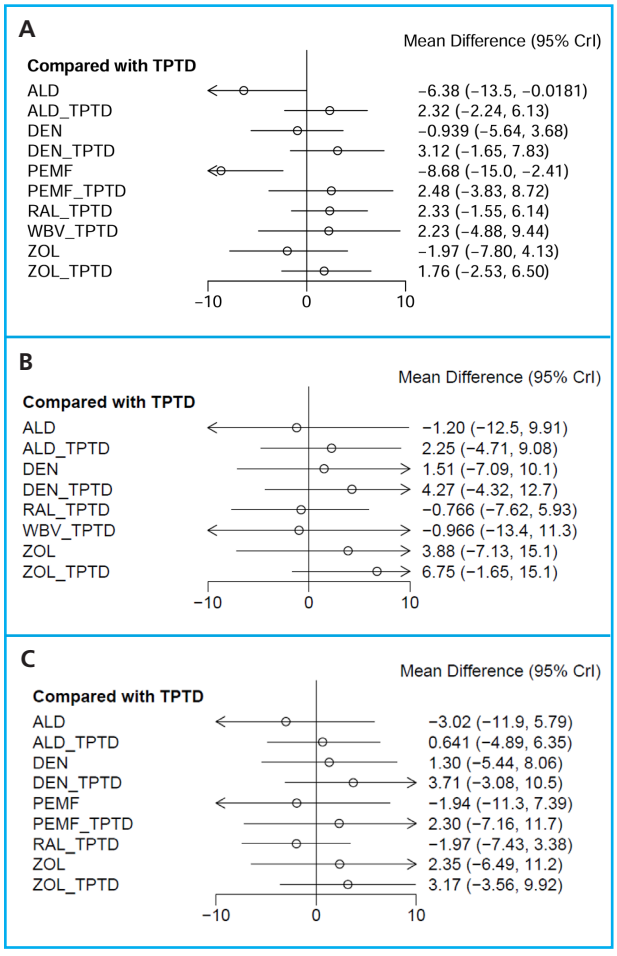

Figure 3. Forest plots of the NMA for: (A) lumbar spine; (B) total hip; and (C) femoral neck.

Table II. SUCRA rankings

Treatment	Lumbar spine	Total hip	Femoral neck
ALD	10.57	30.85	22.27
ALD + TPTD	70.97	56.13	50.89
DEN	35.85	48.08	55.05
DEN + TPTD	79.32	69.70	76.45
PEMF	3.98	N/A	29.41
PEMF + TPTD	71.59	N/A	63.47
RAL + TPTD	71.56	29.83	24.88
TPTD	42.97	34.34	42.46
WBV + TPTD	67.81	33.50	N/A
ZOL	29.75	63.81	63.14
ZOL + TPTD	65.61	83.77	71.97

At the total hip, the combination of ZOL + TPTD produced the greatest estimated improvement in BMD. Whereas TPTD exerts a distinctive adverse effect on cortical bone by increasing cortical porosity, the addition of ZOL appears to mitigate this remodeling process by reinforcing cortical structure, thereby enhancing hip BMD. Nevertheless, in the clinical trial setting, the absence of sustained BMD gains during the final 6 months may reflect renewed cortical remodeling and increasing cortical porosity as the antiresorptive effect of ZOL diminishes (14).

Furthermore, combining TPTD with nonpharmacological therapies (PEMF/WBV) was also associated with improvements in lumbar spine and femoral neck BMD, with outcomes comparable to those achieved with TPTD combined with antiresorptive agents. However, no improvement was observed in total hip BMD. WBV delivered through a vibration platform transmits mechanical signals across the skeletal muscle system via centrifugal conduction from the lower limbs. This stimulation triggers widespread neuromuscular activation and contributes to the regulation of bone metabolism. Its benefits appear even greater when paired with pharmacological treatments such as TPTD, in which synergistic effects have been reported (15). A meta-analysis also found that the use of PEMF to-

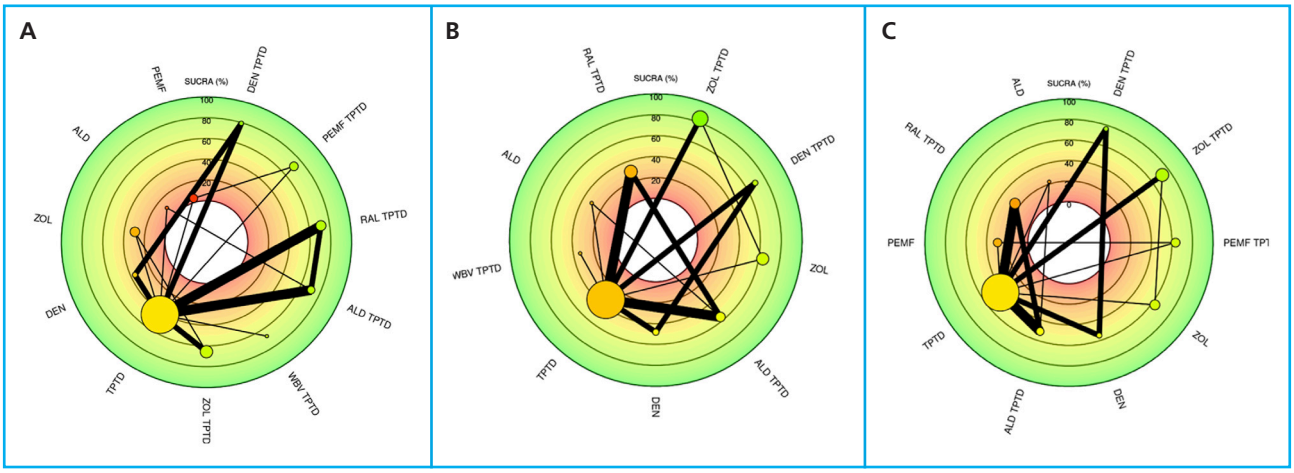

Figure 4. SUCRA plots of the NMA for: (A) lumbar spine; (B) total hip; and (C) femoral neck.

gether with standard medications resulted in significant increases in lumbar spine and femoral BMD (16). PEMFs enhance osteoblast activity, inhibit osteoclast formation, and modulate bone marrow stromal cells and osteocytes, thereby preserving bone mass and strength (17).

Emerging evidence from previous meta-analyses suggests potential benefits of combination therapy. A meta-analysis by Lou et al. (2019) demonstrated that combination regimens were superior to monotherapy, whereas more recent data from Jin et al. (2025) indicated that the DEN + TPTD combination may be clinically preferable for BMD enhancement (6,13). Consistent with these findings, our NMA identified a trend favoring combination therapy for BMD improvement, with the highest rank probabilities observed for DEN + TPTD and ZOL + TPTD.

A key limitation of this study is that, although several TPTD-based combination strategies achieved high SUCRA rankings, the corresponding credible intervals were wide and consistently crossed the null effect. This finding reflects substantial uncertainty in the estimated treatment effects and indicates that these rankings should be interpreted cautiously. The observed imprecision is likely attributable to small sample sizes, the limited number of included studies, heterogeneity in interventions and follow-up durations, and reliance on indirect comparisons inherent to the NMA framework. A local inconsistency at the femoral neck was also identified, further indicating that the rankings should be interpreted with caution. Consequently, although relative treatment rankings provide insight into potential comparative performance, the absence of statistically significant differences highlights the need for larger, adequately powered RCTs to more precisely define the effects of combination therapies on site-specific BMD outcomes.

This study focused exclusively on TPTD-based combination strategies and did not address other anabolic agents, such as abaloparatide (ABL) or romosozumab (ROMO). Evidence regarding ROMO in combination therapy for PMO remains limited. Meta-analyses have suggested that ROMO demonstrates superior efficacy compared with TPTD (18). This monoclonal antibody targets sclerostin and acts through a dual mechanism, promoting bone formation while simultaneously reducing bone resorption (19). Observational data indicate that the addition of ROMO to ongoing DEN therapy produced significant gains in lumbar spine BMD (20). Preliminary analyses further suggest that ROMO combined with DEN resulted in greater improvements in BMD and required fewer injections compared with DEN plus TPTD (21). Meta-analyses evaluating ABL have demonstrated beneficial effects on BMD and a lower risk of adverse events compared with TPTD (22). However, clinical trials investigating ABL in combination therapy remain limited and are still ongoing, including NCT04467983 (23). Future studies comprehensively comparing combination therapies for PMO are warranted to better define optimal treatment strategies. In addition, comparisons between sequential TPTD therapy and combination therapy have not yet been adequately evaluated, highlighting the need for future direct head-to-head trials.

CONCLUSIONS

This NMA highlights a favorable trend for TPTD combination therapies in improving BMD, with DEN + TPTD and ZOL + TPTD emerging as leading candidates in relative treatment rankings. However, the absence of statistically significant differences underscores the need for larger, adequately powered clinical trials.

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Mechanisms of action and implications of vitamin D in cardiovascular disease and diabetes — A mini-review

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Abstract

Vitamin D (Vit D), traditionally recognised for its role in calcium–phosphate homeostasis and bone metabolism, is now known to exert pleiotropic hormonal effects mediated by the widespread expression of the vitamin D receptor (VDR). Growing evidence suggests that Vit D contributes to the regulation of cardiovascular function, glucose metabolism, insulin sensitivity, and inflammatory pathways. This review summarises the principal molecular and cellular mechanisms through which Vit D may influence cardiovascular disease, diabetes, and metabolic syndrome, drawing on experimental, epidemiological, and clinical evidence. A narrative synthesis of mechanistic findings from basic research was combined with evidence from observational cohorts and randomised controlled trials assessing vitamin D status or supplementation. Vit D influences cardiovascular function through several mechanisms, including enhanced endothelial nitric oxide production, reduced oxidative stress, inhibition of endothelial adhesion molecules, modulation of immune responses, suppression of the renin–angiotensin–aldosterone system (RAAS), and regulation of vascular calcification. In glucose and lipid metabolism, Vit D exerts direct effects on insulin secretion by pancreatic β-cells, improves peripheral insulin sensitivity through GLUT4 translocation and mitochondrial biogenesis, modulates adipokines such as adiponectin, and attenuates chronic low-grade inflammation by inhibiting NF-κB signalling and promoting regulatory immune phenotypes. Vit D also contributes to RAAS regulation and the epigenetic control of genes involved in energy homeostasis. Maintaining adequate Vit D status may contribute to integrated prevention strategies for cardiovascular.

Keywords:
Vitamin D.
Cardiovascular disease. Diabetes.
Metabolic syndrome.

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INTRODUCTION

Vitamin D (Vit D), or more precisely its precursor ergosterol, is thought to have been among the earliest biologically relevant molecules produced in primordial phytoplankton following exposure to sunlight. Over time, various higher animal species began to use Vit D to regulate essential physiological functions. However, it was not until approximately 1822 that the hypothesis emerged that sunlight might influence rickets, a bone disease characterised by skeletal deformities in children (1). In the early 20th century, exposure to sunlight was conclusively shown to prevent or treat rickets (1).

In the 1930s, researchers discovered that ultraviolet irradiation of ergosterol derived from certain algae and yeasts could produce vitamin D_2 (ergocalciferol), which subsequently began to be used to fortify foods (2). By contrast, although Goldblatt and Soames described the production of vitamin D_3 (cholecalciferol) through ultraviolet irradiation of skin sterol precursors in 1923 (3), its industrial production was developed later, around the late 1950s and early 1960s, through the conversion of cholesterol derived from lanolin into 7-dehydrocholesterol, followed by ultraviolet irradiation. During the second half of the 1960s, the metabolism of vitamin D and the structure of calcitriol, its biologically active hormonal form, were elucidated.

In subsequent decades, research into the pharmacological effects of vitamin D expanded considerably, encompassing not only its role in skeletal integrity and fracture prevention but also its actions on numerous organs expressing VDR. Particular attention has been paid to its effects on systems of major public health importance, including the cardiovascular system, glucose metabolism and diabetes, and the immune system.

This review aims to provide a concise overview of the principal mechanisms of action underlying numerous clinical investigations into the potential benefits and limitations of Vit D in modulating major extraskeletal diseases affecting large populations. Specifically, we summarise the biological properties through which Vit D may contribute to the prevention and treatment of selected pathological conditions.

A literature search was conducted in PubMed, Scopus, and Web of Science. Articles published during the preceding 5 years were considered, together with selected landmark studies of historical relevance. The search strategy included the following terms: "vitamin D, cardiovascular disease, endothelial mechanisms, inflammation, RAAS (renin–angiotensin–aldosterone system), lipids, adipokines, insulin, β-cells, VCAM-1, ICAM-1, and E-selectin". Eligible studies provided evidence or novel insights that extended existing knowledge. Studies that did not provide sufficiently convincing or relevant evidence were excluded.

MOLECULAR AND CELLULAR MECHANISMS IN CARDIOVASCULAR DISEASE

Vit D, traditionally regarded as a regulator of calcium–phosphate homeostasis and bone metabolism, is now recognised as a pleiotropic steroid hormone with systemic effects, including cardiovascular actions. Its biologically active metabolite, 1,25-dihydroxyvitamin D_3 (calcitriol), exerts genomic and nongenomic effects by binding to the nuclear vitamin D receptor (VDR), which is expressed in numerous extraskeletal cells, including endothelial cells, cardiomyocytes, vascular smooth muscle cells, and immune cells. In recent years, substantial epidemiological and experimental evidence has suggested an association between Vit D deficiency and an increased risk of cardiovascular disease (CVD), including atherosclerosis, hypertension, and heart failure (4,5). Although the causal nature of this association remains debated, several molecular mechanisms may support a pathophysiological role for vitamin D in regulating vascular function and preventing arterial sclerosis (6).

MODULATION OF ENDOTHELIAL FUNCTION

The effects of vitamin D on endothelial function are supported by the expression of VDR and the enzyme 1α-hydroxylase (CYP27B1) in endothelial cells, enabling local calcitriol production. This facilitates autocrine and paracrine regulation of vascular function by increasing endothelial nitric oxide synthase (eNOS) expression and, consequently, nitric oxide (NO) production, which promotes endothelium-dependent vasodilation (7,8). Furthermore, vitamin D enhances the activity of antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase, thereby reducing oxidative stress (9,10). Under pathological conditions, oxidative stress resulting from excessive production of reactive oxygen species (ROS) promotes NO degradation and suppresses its synthesis, reducing NO bioavailability.

In addition to these protective mechanisms, vitamin D inhibits the expression of endothelial adhesion molecules, including VCAM-1, ICAM-1, and E-selectin, thereby limiting monocyte recruitment into the vascular wall. These effects help preserve endothelial function and counteract early atherogenic processes. Experimental studies have shown that vitamin D_3 inhibits platelet adhesion and the expression of VCAM-1 and MT1-MMP in human endothelial cells, suggesting potential therapeutic relevance in early atherosclerotic disease (11,12).

ANTI-INFLAMMATORY AND IMMUNOMODULATORY EFFECTS

Chronic inflammation is central to the progression of atherosclerosis. Clinical studies have demonstrated

that innate and adaptive immune responses can either promote or regulate atherosclerotic processes. Modulation of inflammation may therefore represent a therapeutic approach to atherosclerotic disease, and calcitriol influences immune responses by acting on several immune cell populations (13).

Several studies indicate that vitamin D regulates vascular tone, prevents fibrosis, and modulates vascular hypertrophy by inhibiting the transcription factor NF-κB. This suppresses the production of proinflammatory cytokines, including IL-6, TNF-α, and IL-1β, while promoting the synthesis of anti-inflammatory cytokines such as IL-10 (14). Vitamin D also inhibits monocyte and macrophage recruitment and modulates cholesterol uptake and esterification. In addition, it promotes autophagy of lipid droplets in macrophages, enhances cholesterol efflux, and regulates macrophage polarisation.

Experimental findings further suggest that vitamin D directly inhibits the formation, adhesion, and migration of lipid-laden monocytes, thereby exerting antiatherosclerotic effects.

Furthermore, inflammation is modulated through changes in T-cell polarisation, with reduced differentiation towards Th1 and Th17 phenotypes and increased regulatory T-cell (Treg) activity. These changes may attenuate vascular inflammation and endothelial injury (15,16).

INTERACTION WITH THE RENIN–ANGIOTENSIN–ALDOSTERONE SYSTEM (RAAS)

The potential effects of vitamin D on blood pressure regulation arise partly from its endocrine role in the renin–angiotensin–aldosterone system (RAAS). Clinical studies have shown that low vitamin D levels are associated with increased plasma renin activity, higher angiotensin II concentrations, and enhanced RAAS activity within vascular tissues (17). Calcitriol suppresses renin gene transcription, reducing angiotensin II concentrations and RAAS activation. The resulting attenuation of vasoconstriction and adrenergic overactivation may improve blood pressure regulation, reduce oxidative stress and vascular inflammation, and protect against myocardial hypertrophy and fibrosis. These mechanisms may contribute to protection against hypertension and its cardiovascular complications (18,19).

REGULATION OF MINERAL METABOLISM AND VASCULAR CALCIFICATION

One of the principal physiological functions of vitamin D is to promote intestinal calcium absorption. Vitamin D is therefore essential for maintaining normal mineral and bone metabolism. It stimulates intestinal calcium and phosphate absorption, regulates bone turnover, and suppresses parathyroid hormone (PTH) secretion through the endocrine activity of calcitriol.

Vitamin D consequently influences calcium–phosphate homeostasis and the processes underlying vascular calcification. Severe vitamin D deficiency, particularly in patients with renal impairment or secondary hyperparathyroidism, can disrupt calcium and phosphate metabolism. Vitamin D deficiency increases PTH secretion and may lead to secondary hyperparathyroidism, with disturbances in circulating calcium and phosphate concentrations that can favour mineral deposition in arterial walls (20,21). Nevertheless, sustained hyperphosphataemia does not invariably result in vascular calcification.

Epidemiological studies have associated vitamin D deficiency with arterial stiffness, hypertension, and endothelial dysfunction in both patients with chronic kidney disease and otherwise healthy individuals. Experimental evidence has also demonstrated that vascular smooth muscle cells can undergo osteoblast-like phenotypic transformation.

Conversely, calcitriol regulates the expression of anticalcification proteins, including matrix Gla protein (MGP) and osteopontin, and may inhibit ectopic mineralisation, potentially reducing arterial stiffness and slowing the progression of arteriosclerosis (22-24).

INDIRECT METABOLIC EFFECTS

Moreover, vitamin D influences metabolic factors associated with cardiovascular risk. Experimental evidence suggests that low circulating vitamin D concentrations may contribute to increased white adipose tissue accumulation, insulin resistance (IR), and dyslipidaemia (25). Conversely, vitamin D may improve insulin sensitivity by increasing insulin receptor expression and enhancing intracellular signalling. It may also attenuate adipose tissue inflammation and reduce the release of proatherogenic adipokines.

Associations between low vitamin D concentrations and adverse lipid profiles have been reported in children, adolescents, and adults. Adequate vitamin D intake may improve lipid parameters by lowering triglyceride concentrations and increasing high-density lipoprotein cholesterol levels. These effects could indirectly contribute to reducing overall cardiovascular risk.

RECENT EXPERIMENTAL AND CLINICAL EVIDENCE

Animal models lacking VDR or enzymes involved in vitamin D synthesis develop left ventricular hypertro-

phy, increased renin concentrations, and vascular calcification, supporting a possible direct pathophysiological role (26). Large observational cohorts have shown that serum 25-hydroxyvitamin D [25(OH)D] concentrations below 20 ng/mL are associated with an increased risk of major cardiovascular events (27,28). However, randomised controlled trials, including VITAL, ViDA, and DO-HEALTH, have not consistently demonstrated significant reductions in cardiovascular mortality following vitamin D supplementation, although modest benefits have been suggested in individuals with severe deficiency (less than 15 ng/mL) or in selected subgroups (29-32).

The VITAL trial (VITamin D and OmegA-3 TriaL), a randomised, double-blind clinical trial, enrolled approximately 25,000 participants older than 50 years who received 2000 IU of vitamin D daily for 5 years. Most participants had adequate vitamin D concentrations at baseline. As reported by Zhu et al., the study did not provide conclusive evidence supporting vitamin D supplementation for the prevention of cancer or fragility fractures.

The ViDA study enrolled more than 5000 participants aged 50-84 years who received 100,000 IU of vitamin D monthly for more than 3 years. No significant effects were observed on cardiovascular events, respiratory infections, nonvertebral fractures, falls, or cancer incidence. However, in participants with low baseline vitamin D concentrations (≤ 30 nmol/L), improvements in bone mineral density (BMD) and arterial function were reported by Scragg et al.

Finally, a post hoc analysis of the DO-HEALTH trial included 777 participants who received 2000 IU of vitamin D daily for 3 years. Participants who also received omega-3 supplementation and participated in a structured exercise programme showed modest attenuation of biological ageing, assessed using the PhenoAge methodology, according to Bischoff-Ferrari et al.

Taken together, these findings suggest that vitamin D acts primarily as a modulatory factor rather than a direct therapeutic intervention. Maintaining sufficient serum 25(OH)D concentrations to ensure adequate synthesis of $1,25(OH)_2D$ therefore appears to be a reasonable strategy within comprehensive cardiovascular prevention (33).

CELLULAR MECHANISMS IN GLUCOSE AND INSULIN METABOLISM

The widespread distribution of vitamin D receptors in organs and tissues involved in glucose metabolism suggests that adequate circulating vitamin D concentrations may influence the development and progression of insulin resistance, diabetes, and metabolic syndrome. This hypothesis is supported by the expression of the nuclear vitamin D receptor (VDR) in pancreatic β-cells, hepatocytes, adipocytes, skeletal muscle cells, and immune cells. Calcitriol binds to VDR, which subsequently forms a heterodimer with the retinoid X receptor (RXR), regulating the expression of numerous genes involved in metabolism, inflammation, and insulin sensitivity.

DIRECT EFFECTS ON PANCREATIC β-CELLS

The abundant expression of VDR in pancreatic β-cells suggests that vitamin D may directly regulate their function. Some studies have shown that vitamin D insufficiency alters calcium channel activity, reduces insulin synthesis and secretion, and increases insulin resistance (34,35). The potential contribution of vitamin D deficiency to insulin resistance is further supported by associations between insulin resistance and polymorphisms in both the VDR gene and the vitamin D-binding protein (DBP) gene (36). Pancreatic β-cells express not only VDR but also 1α-hydroxylase (CYP27B1), enabling local activation of 25(OH)D and subsequent nuclear effects of calcitriol that enhance insulin gene (*INS*) transcription and glucose-stimulated insulin secretion. Calcitriol also stabilises intracellular calcium concentrations, which are essential for insulin granule exocytosis, and protects β-cells against oxidative stress and apoptosis induced by inflammatory cytokines, including IL-1β, TNF-α, and IFN-γ.

EFFECTS ON PERIPHERAL INSULIN SENSITIVITY

Skeletal muscle and adipose tissue are targets of VDR-mediated signalling. Calcitriol regulates GLUT4 translocation to the cell membrane, thereby improving cellular glucose uptake (37).

Numerous studies have shown that excessive fat accumulation contributes substantially to skeletal muscle mitochondrial dysfunction and metabolic complications (4,38). Vitamin D may reduce lipid accumulation, increase mitochondrial DNA (mtDNA) content, enhance ATP production, and upregulate genes involved in mitochondrial biogenesis (PGC-1α, NRF1, and TFAM) and fatty acid β-oxidation. Overall, vitamin D may attenuate lipid accumulation and mitochondrial dysfunction while promoting oxidative metabolism and mitochondrial biogenesis, as demonstrated in cellular and animal models (39).

Vitamin D deficiency in obese mice increases lipogenesis, as reflected by increased expression of PPARγ,

SREBP1c, and FAS, and reduces markers of β-oxidation, including PPARα, thereby aggravating hepatic steatosis. These findings suggest that adequate vitamin D status may support hepatic fatty acid oxidation. Another study (40) reported that adequate vitamin D concentrations reduce oxidative stress and improve mitochondrial and endocrine function, potentially lowering the risk of autoimmunity, infections, metabolic disorders, and impaired DNA repair. Vitamin D also has antioxidant properties that contribute to maintaining balanced mitochondrial activity and preventing protein oxidation, lipid peroxidation, and DNA damage (41).

Although these findings are promising, much of the evidence derives from cellular or animal models, and not all studies have directly examined the entire sequence from gene expression to mitochondrial function and reduced lipotoxicity. Tissue- and context-specific differences, including diet and the degree of vitamin D deficiency, must also be considered. Nevertheless, available findings support the hypothesis that vitamin D promotes the expression of genes involved in β-oxidation and mitochondrial biogenesis, potentially improving lipid metabolism and reducing oxidative stress (42).

Despite some inconsistent findings, large studies suggest that the beneficial effects of vitamin D on glycaemic and lipid parameters may be mediated partly by adiponectin. A systematic review of vitamin D supplementation found that increases in adiponectin concentrations were generally significant, particularly in patients with diabetes, and more pronounced with daily vitamin D administration (43). Vitamin D may also modulate leptin and resistin, both of which are associated with insulin resistance, and reduce hepatic insulin resistance and local inflammation mediated by cytokines (4,44-46).

IMMUNOMODULATORY AND ANTI-INFLAMMATORY MECHANISMS

Chronic low-grade inflammation is a hallmark of both type 2 diabetes and metabolic syndrome. Calcitriol inhibits the NF-κB signalling pathway, reducing the production of proinflammatory cytokines, including IL-1β, IL-6, and TNF-α, by dendritic cells and macrophages while promoting macrophage polarisation towards the anti-inflammatory M2 phenotype. These effects are reinforced by reduced differentiation of proinflammatory Th1 and Th17 lymphocytes and increased differentiation of regulatory T cells (47,48). Such mechanisms may be particularly relevant in type 1 diabetes, in which autoimmune destruction of pancreatic β-cells could potentially be attenuated by adequate vitamin D availability.

INTEGRATED ENDOCRINE–METABOLIC MECHANISMS

Numerous studies have demonstrated associations between TNF-α concentrations, obesity, and type 2 diabetes mellitus (49). Increased TNF-α expression in adipose tissue has also been associated with insulin resistance in humans and animal models. TNF-α can regulate angiotensinogen (AGT) expression in hepatocytes, suggesting a potential mechanism through which insulin may increase AGT expression and subsequent angiotensin II production (50). This provides a possible mechanistic explanation for hypertension associated with obesity.

By inhibiting renin gene expression, calcitriol reduces systemic and tissue RAAS activation, potentially attenuating adipocyte hypertrophy, inflammation, and insulin resistance associated with excessive angiotensin II activity. The VDR/RXR complex binds to thousands of genomic sites containing vitamin D response elements (VDREs), modulating the expression of genes involved in glucose homeostasis, inflammation, mitochondrial biogenesis, and lipid oxidation (51-53).

LIMITATIONS

This mini-review considered *in vitro* studies, animal models, observational studies, randomised controlled trials (RCTs), and genetic and molecular investigations, which provide different levels of evidence (Table I).

Furthermore, observational studies often include participants without vitamin D deficiency, whereas randomised controlled trials may have insufficient fol-

Table I. Types of evidence, principal findings, and limitations

Evidence	Main findings	Limitations
In vitro studies	VDRE, GLUT4, and PGC-1α signalling	Limited clinical transferability
Animal models	Metabolic and cardiovascular effects	Species-specific differences
Observational studies	Association between vitamin D deficiency and cardiovascular disease	Residual confounding
Randomised controlled trials	Limited or inconsistent benefits	Heterogeneous populations

low-up to capture relevant clinical outcomes. In some cases, vitamin D supplementation may also have been administered at suboptimal doses. Finally, although several biologically plausible mechanisms have been identified, their existence alone does not establish causality or demonstrate clinically meaningful benefits.

DISCUSSION

Vitamin D, through its hormonal actions mediated by VDR, exerts vascular, immunomodulatory, and metabolic effects that may contribute to cardiovascular protection by improving endothelial function, modulating RAAS activity, regulating vascular calcification, and optimising metabolic homeostasis. Nevertheless, despite the substantial pathophysiological rationale, clinical findings remain variable and inconclusive. Vitamin D may act primarily as a permissive factor supporting normal cardiovascular function rather than as a direct therapeutic agent. Correction of vitamin D deficiency remains an important consideration in cardiovascular prevention, particularly in individuals at high risk or with documented deficiency.

The scientific literature indicates that vitamin D, in its active form calcitriol, acts as a pleiotropic modulator of glucose and lipid metabolism through direct effects on pancreatic β-cells and insulin-sensitive tissues, together with indirect effects mediated by immune and inflammatory pathways, RAAS regulation, and epigenetic control of genes involved in energy homeostasis. Adequate vitamin D status may help preserve β-cell function and insulin sensitivity, attenuate chronic inflammation, and maintain balanced lipid metabolism. Vitamin D may therefore have a protective or adjunctive role in the prevention or management of diabetes and metabolic syndrome.

Although several observational studies have reported associations between low vitamin D intake or low serum 25(OH)D concentrations and incident type 2 diabetes, randomised controlled trials have yielded inconsistent results (54). More recent population-based studies (55) have provided evidence of an association between higher vitamin D concentrations and a lower risk of insulin resistance. Nevertheless, contradictory findings remain common, highlighting the need to consider differences in population characteristics, age, adiposity, particularly hepatic fat accumulation, oxidative stress, PTH concentrations, and immune system function. These factors may influence both clinical outcomes and calcitriol availability.

Overall, experimental and clinical evidence suggests that vitamin D may have a predominantly preventive rather than therapeutic role in diabetes and metabolic syndrome, with potentially greater effects in predisposed individuals and those with low baseline vitamin D concentrations. Because commonly used vitamin D doses are based primarily on experience with bone metabolism, higher doses might be hypothesised to be necessary when targeting extraskeletal conditions. However, such an approach requires further investigation. Although renal calcitriol synthesis is regulated by several factors, including PTH, FGF23, and $1,25(OH)_2D$ itself, these regulatory mechanisms do not eliminate the potential risk of toxicity from excessive vitamin D supplementation (56).

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Revisión

Osteocyte signaling hierarchies: integrating mechanotransduction with systemic regulation

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Abstract

Background and aim: osteocytes are emerging as hierarchical regulators of skeletal and systemic physiology. This review examines the integration of mechanotransduction pathways with endocrine and paracrine signalling.

Methods: a comprehensive narrative review of the literature published between 2016 and 2024 was conducted using major biomedical databases.

Results: osteocytes coordinate bone remodelling through sclerostin-mediated inhibition of Wnt signalling and RANKL-mediated bone resorption, while secreting FGF23 to regulate mineral metabolism. Mechanosensing involves fluid shear stress, integrin adhesion complexes, cytoskeletal remodelling, and *PIEZO1* channel activation. Increasing recognition of osteocyte heterogeneity challenges simplified models of signalling dominance. Fundamental questions remain regarding signal prioritisation and spatial coordination.

Conclusions: osteocytes constitute central signalling hubs that link mechanical inputs to skeletal and systemic responses. Emerging spatial and functional technologies will improve mechanistic understanding and inform the development of novel therapies.

Keywords:
Osteocyte.
Mechanotransduction.
Endocrine signalling.
Bone remodelling.
FGF23.

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INTRODUCTION

The historical emphasis on osteoblast and osteoclast biology overshadowed osteocytes, which were regarded as terminally embedded and largely inactive cells (1). This perception has since changed substantially. Contemporary studies establish osteocytes as key regulators of bone remodelling and systemic mineral homeostasis (2).

Through the secretion of sclerostin and receptor activator of nuclear factor-κB ligand (RANKL), osteocytes regulate both bone formation and resorption (3). Their endocrine activity, particularly the secretion of fibroblast growth factor 23 (FGF23), underscores their systemic influence (4). Apoptotic signalling further establishes osteocytes as initiators of targeted remodelling responses (5).

Nevertheless, the precise architecture of osteocyte mechanosensing and the spatial organisation of osteocyte signalling remain debated (6). Addressing these uncertainties is essential for translating osteocyte biology into clinical interventions.

This review examines current evidence on osteocyte signalling hierarchies and outlines priorities for future research.

METHODS

This narrative review was conducted to provide a comprehensive and critical synthesis of contemporary literature on osteocyte signalling. The narrative approach enabled the integration of mechanistic, preclinical, and clinical evidence while highlighting areas of uncertainty and emerging consensus. Rather than performing a quantitative meta-analysis, the review aimed to develop an integrated conceptual framework informed by the available evidence.

A structured literature search was conducted in MEDLINE via Ovid, PubMed, Embase, Cochrane CENTRAL, and Google Scholar. The search period was restricted to January 2016 through December 2024 to capture recent advances in osteocyte biology. Search terms included combinations of "osteocyte" with "signalling", "mechanotransduction", "communication", "RANKL", "sclerostin", and "FGF23", together with "bone remodelling", "bone metabolism", or "osteoporosis". Search strategies were adapted to each database, and additional studies were identified through manual screening of reference lists.

Eligible studies included *in vitro* and *in vivo* preclinical investigations examining osteocyte signalling mechanisms; longitudinal cohort studies and randomised controlled trials evaluating therapies targeting osteocyte pathways, such as antisclerostin antibodies; and relevant systematic reviews and meta-analyses. Non-English-language articles, non-peer-reviewed publications, and reports not directly addressing osteocyte signalling or bone physiology were excluded.

Titles and abstracts were screened for relevance, followed by full-text assessment of selected articles. Data extraction focused on signalling pathways, experimental models, and principal findings, which were synthesised thematically to develop an integrated conceptual framework.

Because this was a narrative review, no formal methodological quality assessment or risk-of-bias evaluation was performed, potentially introducing selection bias. The predominance of murine and *in vitro* models may limit the direct applicability of the findings to human physiology. Furthermore, *in vitro* systems may not fully reproduce the mechanical and biochemical complexity of the osteocyte lacunar–canalicular network. These limitations were considered when interpreting the findings.

MOLECULAR AND CELLULAR MECHANISMS OF OSTEOCYTE SIGNALLING

This section describes the molecular and cellular mechanisms that enable osteocytes to function as mechanosensory and signalling hubs. Particular emphasis is placed on structural organisation, mechanosensory systems, intracellular signal transduction, effector responses, and cell fate decisions. Integration at the tissue and systemic levels is discussed in subsequent sections.

THE LACUNAR–CANALICULAR NETWORK: STRUCTURAL BASIS FOR COMMUNICATION

Osteocytes reside within lacunae and extend 50-100 dendritic processes through micrometre-sized canaliculi, forming a dense, interconnected cellular network throughout mineralised bone (7). This lacunar–canalicular network (LCN) functions as a three-dimensional communication system in which interstitial fluid flow generates shear stress that is converted into biochemical signals, establishing osteocytes as major mechanosensors within the skeleton (8).

The structural integrity of this network depends on an actin-rich cytoskeleton that stabilises dendritic projections (9). Gap junctions composed predominantly of connexin 43 (Cx43) enable direct intercellular communication and rapid signal propagation (10). Cx43 also forms hemichannels that connect osteocytes with the extracellular environment and regulate the release of paracrine factors (11). Osteocyte-specific deletion of Cx43 increases apoptosis, produces empty lacunae in cortical bone, and attenuates anabolic responses to mechanical loading (12) (Table I).

Table I. Major osteocyte-derived signalling molecules and their actions

Molecule	Primary action	Target cells	Key references	Physiological context	Disease relevance
Sclerostin	Inhibits Wnt/β-catenin signalling	Osteoblasts	(3,22)	Mechanical unloading, ageing	Osteoporosis (target of romosozumab)
RANKL	Promotes osteoclast differentiation	Osteoclast precursors	(1,39,40)	Targeted remodelling, microdamage	Osteoporosis, inflammatory bone loss
FGF23	Regulates phosphate homeostasis	Kidney (proximal tubule)	(4,28,29)	Phosphate/vitamin D balance	CKD-MBD, hypophosphataemic rickets
PGE_2	Promotes bone formation	Osteoblasts	(21)	Response to mechanical loading	Mechanotransduction
Wnt ligands	Promote bone formation and cell survival	Osteoblasts	(22)	Coupling, anabolic windows	Bone formation
OPG	Decoy receptor for RANKL	Osteoclast precursors	(39)	Regulation of RANKL activity	Bone protection
DKK1	Wnt antagonist	Osteoblasts	(35)	Inhibition of bone formation	Diabetes, myeloma
EVs	Interorgan communication, miRNA transfer	Multiple distant tissues	(26,27)	Systemic signalling	Alzheimer's disease, osteoarthritis
NO	Vasodilation, signalling mediator	Endothelium, osteoblasts	(21)	Acute response to loading	Mechanotransduction
TGF-β	Coupling factor	Osteoblasts	(47)	Matrix-derived signals	Remodelling balance
PTHrP	Local calcium regulation	Osteocytes, osteoblasts	(20,43)	Perilacunar remodelling	Lactation, increased calcium demand

RANKL: receptor activator of nuclear factor-κB ligand; FGF23: fibroblast growth factor 23; PGE_2: prostaglandin E_2; OPG: osteoprotegerin; DKK1: Dickkopf-related protein 1; EVs: extracellular vesicles; NO: nitric oxide; TGF-β: transforming growth factor β; PTHrP: parathyroid hormone-related protein; CKD-MBD: chronic kidney disease–mineral and bone disorder.

Cx43 interacts with integrins at dendritic attachment sites, suggesting the existence of an integrated mechanotransduction complex (13). Super-resolution imaging has demonstrated colocalisation of β3 integrin with pannexin 1, purinergic receptor P2X7 (P2X7R), and the calcium voltage-gated channel subunit alpha1 H (CaV3.2) along dendritic processes, structures that are absent from the cell body (14). Mechanical stimulation of dendrites triggers directional calcium influx through integrin αVβ3-dependent mechanisms (9), supporting the concept that dendritic processes function as specialised mechanosensory domains.

MOLECULAR MECHANOSENSORS: PRIMARY CILIA, INTEGRINS, AND ION CHANNELS

Osteocytes transduce fluid shear stress through several mechanosensitive systems operating in parallel, including primary cilia, integrin-based adhesions, ion channels, and selected G protein-coupled receptors. The primary cilium, a microtubule-based projection extending from the cell body, bends in response to fluid flow and activates signalling pathways involving polycystin-1 (PC1) (15).

Integrins anchor osteocytes to the canalicular matrix. Integrin β1 is enriched at the cell body, whereas integrin β3 is predominantly localised to dendritic puncta (14), reflecting spatial specialisation. Among mechanosensitive ion channels, PIEZO1 mediates mechanically induced calcium influx in osteocytes (16). Pharmacological activation of *PIEZO1* reproduces loading-induced calcium responses and suppresses sclerostin expression, whereas *PIEZO1* knockdown attenuates fluid flow-induced signalling (17). Conditional deletion of PIEZO1 markedly reduces trabecular bone mass and cortical thickness and severely impairs anabolic responses to mechanical loading (18).

Transient receptor potential vanilloid 4 (TRPV4) localises to discrete sites along osteocyte processes near integrin αVβ3 attachment sites (14). The balance between depolarising cation influx and hyperpolarising potassium efflux probably determines activation thresholds (19). Certain G protein-coupled receptors (GPCRs) also function as direct mechanosensors; fluid shear stress

induces conformational changes in the parathyroid hormone type 1 receptor (PTH1R) independently of ligand binding (20) (Table I).

In addition to these established mechanosensors, caveolae—flask-shaped invaginations of the plasma membrane enriched in caveolin proteins—have been implicated in mechanoprotection and signalling in various cell types. In osteocytes, caveolin-1 localises to the plasma membrane and may participate in integrin signalling and membrane trafficking. However, direct evidence supporting caveolae as primary osteocyte mechanosensors remains limited compared with the evidence for *PIEZO1* or primary cilia (5). Further investigation is needed to establish whether caveolae contribute to the hierarchical organisation of osteocyte mechanosensing.

Together, these pathways indicate that osteocytes possess a distributed, context-dependent mechanosensory apparatus rather than a single dominant sensor.

INTRACELLULAR SIGNAL TRANSDUCTION: FROM MEMBRANE TO NUCLEUS

Mechanical stimulation rapidly activates second-messenger systems. Nitric oxide (NO) production increases within minutes through activation of endothelial nitric oxide synthase and is required for subsequent prostaglandin E_2 (PGE_2) synthesis and sclerostin downregulation (21,22).

Mechanical stimuli also regulate nuclear responses through Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ), which shuttle between the cytoplasm and nucleus. Osteocyte-specific deletion of both *Yap*and *Taz* results in low bone mass, impaired mechanical properties, disorganised collagen architecture, and reduced mechanosensitivity (23). The linker of nucleoskeleton and cytoskeleton (LINC) complex connects the actin cytoskeleton to the nuclear lamina, enabling direct transmission of mechanical forces to the nucleus (24).

EFFECTOR OUTPUTS: SCLEROSTIN, RANKL, AND OTHER SIGNALLING MOLECULES

Sclerostin, encoded by the *SOST* gene and secreted primarily by mature osteocytes, inhibits bone formation by antagonising Wnt/β-catenin signalling. Mechanical loading suppresses sclerostin expression, thereby permitting anabolic activity (22). Brief axial loading induces localised and transient reductions in sclerostin expression in osteocytes located within regions of high mechanical strain (3).

Osteocytes are a major source of RANKL, a cytokine essential for osteoclast differentiation. Osteocyte-specific deletion of RANKL results in severe osteopetrosis, failure of tooth eruption, and absence of trabecular osteoclasts (25). Following fatigue-induced microdamage, surviving osteocytes surrounding apoptotic cells upregulate RANKL and related mediators, recruiting osteoclasts to damaged sites (26).

Mechanotransduction is closely linked to metabolic status. Osteocytes reside in a relatively hypoxic microenvironment and depend largely on glycolytic metabolism (27). Their secretome includes soluble mediators and extracellular vesicles (EVs) capable of traversing the LCN and entering the systemic circulation. EVs derived from young mice improve cognitive function in mouse models of Alzheimer's disease and mediate bone–cartilage communication in osteoarthritis (28). Moreover, osteolineage cells can transfer functional mitochondria to myeloid cells through tunnelling nanotubes and EVs, revealing an additional mechanism of intercellular coordination (29) (Table I).

Osteocytes produce additional signalling molecules involved in bone remodelling, including Wnt ligands that promote bone formation and osteoprotegerin (OPG), which acts as a decoy receptor for RANKL. The balance between these mediators determines the overall remodelling response (Table I).

CELL FATE DECISIONS: APOPTOSIS AND SENESCENCE AS SIGNALLING EVENTS

Osteocyte apoptosis contributes to bone loss in conditions such as disuse, microdamage, glucocorticoid excess, sex steroid deficiency, and ageing (3). Mechanical stimulation promotes osteocyte survival through pathways involving integrins, Src kinase, focal adhesion kinase (FAK), and extracellular signal-regulated kinase (ERK) (30). Sex steroids preserve osteocyte viability through rapid activation of phosphoinositide 3-kinase (PI3K) and Src/Shc/ERK signalling via nongenomic mechanisms (31). Bisphosphonates promote osteocyte survival through ERK activation and Cx43-dependent signalling, independently of gap junction formation (5). Conversely, glucocorticoid-induced apoptosis involves activation of proline-rich tyrosine kinase 2 (PYK2) and disruption of FAK-mediated survival signalling (32).

The penumbra model provides an explanation for targeted remodelling: apoptotic osteocytes identify damaged sites, while adjacent viable osteocytes increase RANKL expression to initiate localised resorption (23). This spatial organisation helps confine resorption to damaged bone while preserving healthy tissue.

Cellular senescence represents a distinct cell fate characterised by stable cell-cycle arrest and acquisition of

a senescence-associated secretory phenotype (SASP), comprising inflammatory cytokines, proteases, and growth factors (33). Pharmacological elimination of senescent cells or suppression of their secretory activity prevents age-related bone loss and attenuates radiation-induced skeletal damage in mice (28).

Unlike apoptosis, which provides a spatially targeted signal for remodelling, senescence exerts broader, chronic effects on the bone microenvironment through sustained release of SASP-associated factors.

OSTEOCYTES AS SYSTEMIC REGULATORS

Although the preceding section focused on molecular mechanisms within individual osteocytes, their functions extend far beyond the bone microenvironment. This section examines how osteocyte-derived signals reach distant organs and contribute to whole-body physiology and disease.

ENDOCRINE FUNCTIONS: FGF23 AND MINERAL METABOLISM

Osteocytes function as endocrine regulators beyond the bone microenvironment. They produce FGF23, a hormone that regulates phosphate homeostasis and vitamin D metabolism (29). Emerging evidence suggests that FGF23 expression also responds to mechanical stimuli. Fluid shear stress suppresses FGF23 transcription in osteocyte-like cells, whereas mechanical unloading increases FGF23 concentrations, suggesting that mechanosensory systems, including primary cilia and *PIEZO1* channels, may directly regulate endocrine activity. However, the precise molecular links between individual mechanosensors and FGF23 transcription remain incompletely defined and represent an important area for future investigation.

Excess FGF23 causes hereditary hypophosphataemic rickets and tumour-induced osteomalacia, whereas FGF23 deficiency results in hyperphosphataemia and ectopic calcification (34). In chronic kidney disease, sustained elevation of FGF23 contributes to chronic kidney disease–mineral and bone disorder (CKD-MBD) and is associated with increased cardiovascular mortality (35,36) (Table I).

INTERORGAN COMMUNICATION: EXTRACELLULAR VESICLES AND BEYOND

Osteocyte-derived sclerostin also influences systemic metabolism, affecting adipose tissue and glucose homeostasis (31). Peroxisome proliferator-activated receptor gamma (PPARγ) signalling in osteocytes regulates sclerostin expression and mediates bone deterioration associated with diabetes (32).

Bone–muscle communication is bidirectional: conditioned media from osteocyte-like cells accelerate myogenesis, whereas exercise-induced muscle-derived factors protect osteocytes against apoptosis (33). Circulating osteocyte-derived EVs further extend this influence to distant organs, including the brain, where EVs from young mice enhance cognitive function in models of Alzheimer's disease (26). These findings establish osteocytes as components of a broader interorgan communication network.

OSTEOCYTE SIGNALLING IN DISEASE STATES

Dysregulation of osteocyte signalling pathways contributes directly to skeletal and systemic diseases, illustrating the physiological importance of the mechanisms described above.

Osteoporosis and oestrogen deficiency

Oestrogen deficiency increases sclerostin expression, epigenetic alterations reduce osteogenic activity, FGF23 concentrations increase, and RANKL expression rises, collectively favouring bone resorption over formation (29) (Table I). Romosozumab, a monoclonal antibody targeting sclerostin, represents a therapeutic application of osteocyte biology and has demonstrated efficacy in postmenopausal osteoporosis (34).

Chronic kidney disease–mineral and bone disorder (CKD-MBD)

CKD-MBD illustrates the systemic consequences of dysregulated osteocyte signalling. Phosphate retention promotes sustained FGF23 secretion, which can become maladaptive and contribute to cardiovascular complications and mortality (34,36).

Diabetes and skeletal fragility

In diabetes, hyperglycaemia and advanced glycation end-products (AGEs) impair osteocyte function and increase the expression of sclerostin and Dickkopf-re-

lated protein 1 (DKK1), contributing to skeletal fragility (35). Osteocytic PPARγ signalling mediates some of these effects (32).

Local inflammation: periodontal disease

Periodontal disease provides another example of osteocyte-mediated pathological signalling. Bacterial infection activates the Toll-like receptor (TLR)/myeloid differentiation primary response 88 (MYD88) pathway in alveolar osteocytes, resulting in RANKL upregulation and localised bone resorption (36) (Table II).

Having described the molecular mechanisms through which osteocytes detect and transmit signals (section "Molecular and cellular mechanisms of osteocyte signalling") and their systemic effects (section "Osteocytes as systemic regulators"), we next examine how these pathways are coordinated at the tissue level to regulate bone remodelling, the process through which the skeleton maintains its structural integrity and adapts to physiological demands.

OSTEOCYTE COORDINATION OF BONE REMODELLING

The molecular mechanisms described in the section "Molecular and cellular mechanisms of osteocyte signalling" operate within individual osteocytes, but skeletal homeostasis requires the coordinated activity of thousands of these cells throughout the bone matrix. This section examines how osteocyte networks integrate local and systemic signals to generate coordinated remodelling responses that align skeletal adaptation with physiological demands.

THE REMODELLING CYCLE: OSTEOCYTES AS CONDUCTORS

Bone remodelling replaces old or damaged bone through tightly coupled osteoclast-mediated resorption followed by osteoblast-mediated formation. Osteocytes regulate this sequence with spatial and temporal precision (37). In cortical bone, remodelling occurs within basic multicellular units (BMUs),

Table II. Alterations in osteocyte signalling in bone diseases

Disease	Key osteocyte changes	Molecular mediators	Key references	Therapeutic implications
Postmenopausal osteoporosis	↑ Sclerostin, ↑ RANKL, ↑ apoptosis	Oestrogen deficiency, ↑ RANKL/OPG ratio	(1,29,33)	Antisclerostin antibodies (romosozumab)
CKD-MBD	↑ FGF23 (maladaptive), ↓ Klotho	Phosphate retention, ↑ FGF23	(4,28,36)	FGF23 blockade (investigational)
Type 2 diabetes	↑ Sclerostin, ↑ DKK1, ↓ viability	Hyperglycaemia, AGEs, PPARγ signalling	(31,32,35)	PPARγ modulation, antisclerostin therapy
Glucocorticoid-induced osteoporosis	↑ Apoptosis, ↑ RANKL, ↓ Cx43	PYK2 activation, ↓ survival signalling	(5,44,55)	PYK2 inhibition (preclinical)
Ageing	Senescence, SASP, network deterioration	p16/p21, JAK–STAT, ↓ autophagy	(48,49,52)	Senolytics (dasatinib + quercetin), senomorphics
Periodontal disease	↑ RANKL, TLR/MYD88 activation	Bacterial LPS, local inflammation	(35)	Local anti-RANKL strategies
Disuse osteoporosis	↑ Sclerostin, ↑ RANKL, ↑ apoptosis	Mechanical unloading, ↓ NO/PGE_2	(3,21,41)	Mechanical loading mimetics
Cancer-associated bone disease	↑ Sclerostin, ↑ RANKL/OPG ratio	Tumour-derived factors, microenvironment	(22,46)	Antisclerostin therapy, bone-targeted agents
Osteogenesis imperfecta	Altered LCN, abnormal signalling	Collagen mutations, secondary effects	(7,8)	Unclear; further investigation needed
Lactation	↑ Perilacunar remodelling	↑ PTHrP, ↑ calcium demand	(43,45)	Targets for calcium mobilisation

CKD-MBD: chronic kidney disease–mineral and bone disorder; AGEs: advanced glycation end-products; PPARγ: peroxisome proliferator-activated receptor gamma; Cx43: connexin 43; SASP: senescence-associated secretory phenotype; JAK–STAT: Janus kinase–signal transducer and activator of transcription; TLR: Toll-like receptor; MYD88: myeloid differentiation primary response 88; LPS: lipopolysaccharide; LCN: lacunar–canalicular network; NO: nitric oxide; PGE_2: prostaglandin E_2; PTHrP: parathyroid hormone-related protein.

whereas on trabecular surfaces, it proceeds through coordinated groups of cells. The LCN provides the structural framework through which osteocytes direct remodelling towards specific regions requiring repair (38).

SPATIAL ORGANISATION OF TARGETED REMODELLING

As described in the section "Cell fate decisions: apoptosis and senescence as signalling events", osteocyte apoptosis provides a spatially precise signal for initiating remodelling. The penumbra model explains this process: apoptotic osteocytes identify damaged sites, whereas adjacent viable osteocytes increase the expression of RANKL and chemotactic factors, selectively recruiting osteoclasts to compromised bone matrix (39,40). Resorption is therefore concentrated in damaged regions and is followed by coupled bone formation to restore structural integrity (41).

This spatial precision depends on the architecture of the LCN described in the section "The lacunar–canalicular network: structural basis for communication", which enables rapid communication between apoptotic and neighbouring cells while limiting signalling to unaffected regions.

MECHANICAL INTEGRATION AT THE TISSUE LEVEL

Building on the mechanotransduction pathways described in the sections "Molecular mechanosensors: primary cilia, integrins, and ion channels" and "Intracellular signal transduction: from membrane to nucleus", mechanical loading suppresses bone resorption and stimulates formation through osteocyte-dependent mechanisms at the tissue level.

Osteocytes distinguish between loading and unloading, dynamic and static forces, and differences in strain magnitude (41). Brief loading episodes suppress sclerostin expression for several hours, creating transient anabolic windows, whereas sustained unloading increases sclerostin and RANKL expression, promoting disuse-related bone loss (34).

In vivo imaging studies indicate that osteocyte calcium responses encode strain magnitude through an increase in the proportion of activated cells rather than the amplitude of individual responses, supporting a binary recruitment model (42). This population-level response converts individual cellular mechanosensitivity into graded tissue-level adaptation.

The integration of osteocyte signalling across multiple scales, from molecular sensors to systemic effects, is illustrated in figure 1.

HORMONAL INTEGRATION: PTH, OESTROGEN, AND GLUCOCORTICOIDS

Osteocytes integrate endocrine and mechanical signals to fine-tune bone remodelling. Parathyroid hormone (PTH) exerts context-dependent effects: intermittent exposure promotes bone formation, whereas sustained elevation favours bone resorption (42). These effects are mediated through PTH1R signalling in osteocytes, which regulates both sclerostin and RANKL expression. Intermittent PTH exposure suppresses sclerostin and promotes bone formation, whereas continuous exposure increases RANKL expression and enhances resorption (43).

Oestrogen preserves bone mass partly by maintaining osteocyte viability (section "Cell fate decisions: apoptosis and senescence as signalling events") and limiting RANKL expression (39). Conversely, glucocorticoids induce osteocyte apoptosis, increase sclerostin and RANKL expression, and suppress perilacunar remodelling, contributing to glucocorticoid-induced osteoporosis (44).

PERILACUNAR REMODELLING: A SPECIALISED OSTEOCYTE FUNCTION

In addition to regulating bone surface remodelling, osteocytes modify the matrix immediately surrounding their lacunae through a process termed perilacunar remodelling or osteocytic osteolysis (45). This process involves the expression of matrix-degrading enzymes, including cathepsin K, MMP13, and MMP14, and is regulated by PTH, PTH-related protein (PTHrP), and mechanical unloading (43). During lactation, osteocytic osteolysis mobilises calcium for milk production. Mechanical unloading similarly expands the lacunar–canalicular system through localised matrix resorption (46). This mechanism enables rapid mineral mobilisation independently of conventional BMU activity.

COUPLING FORMATION TO RESORPTION

The overall skeletal response depends on coupling between bone resorption and formation. Osteocytes influence this balance through both matrix-derived and cell-derived signals. Growth factors released from resorbed bone matrix, including transforming growth

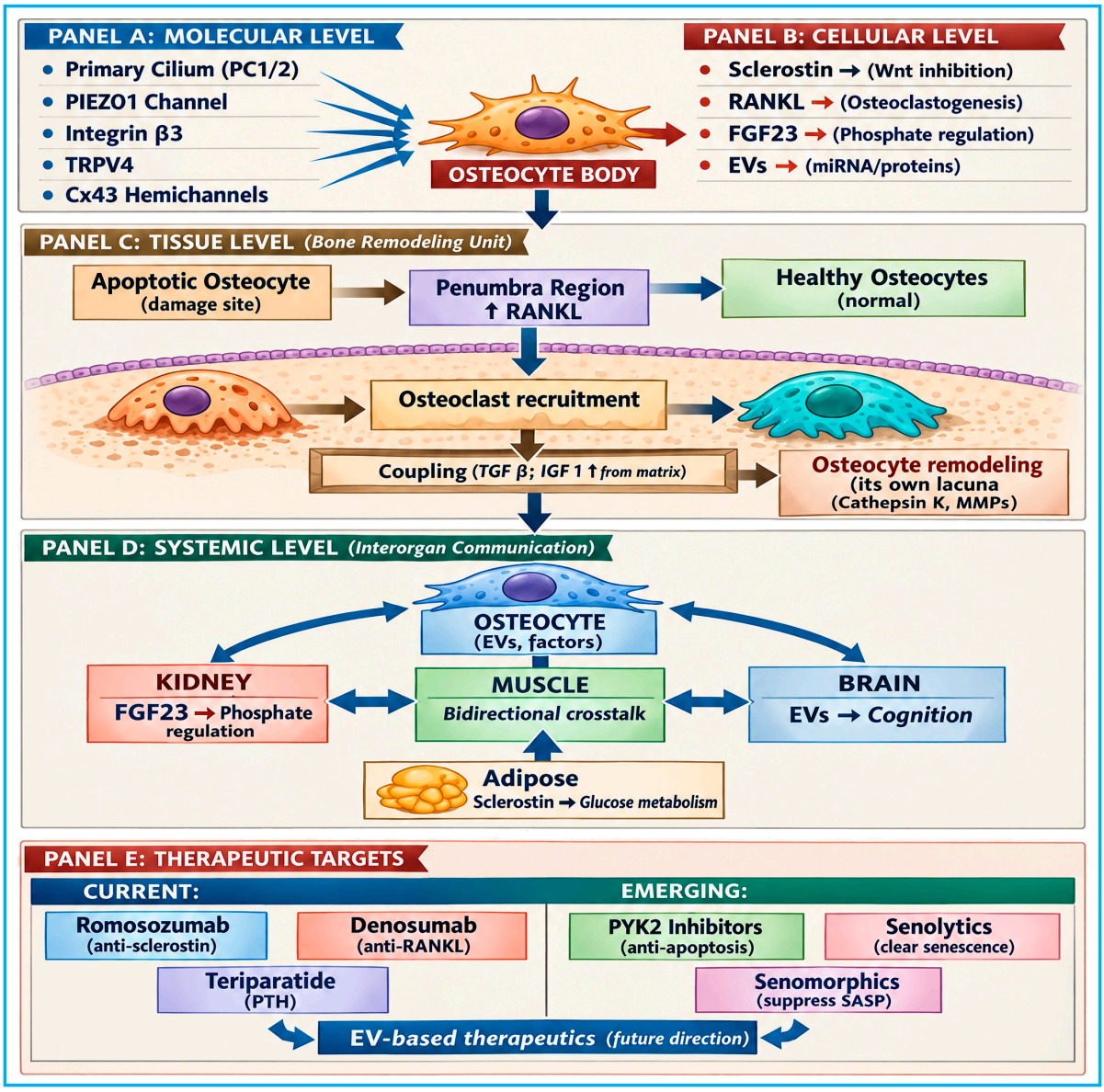

Figure 1. The osteocyte as a multiscale signalling hub. Osteocytes integrate mechanical and biochemical signals across multiple levels of organisation. Panel A illustrates molecular mechanosensors, including the primary cilium, PIEZO1 ion channels, integrin β3 attachment sites, TRPV4 channels, and connexin 43 (Cx43) hemichannels. Panel B shows cellular effector outputs: sclerostin (inhibition of Wnt signalling), RANKL (promotion of osteoclastogenesis), FGF23 (regulation of phosphate metabolism), and extracellular vesicles (EVs) carrying bioactive cargo. Panel C illustrates tissue-level coordination of bone remodelling: apoptotic osteocytes identify sites of damage, adjacent cells within the penumbra upregulate RANKL to recruit osteoclasts, and coupling factors released from the resorbed matrix recruit osteoblasts for bone formation. The inset illustrates perilacunar remodelling, whereby osteocytes locally degrade and replace the surrounding matrix. Panel D illustrates systemic interorgan communication through FGF23 (kidney), EVs (brain), and sclerostin (adipose tissue and skeletal muscle). Panel E summarises current and emerging therapeutic strategies targeting osteocyte signalling pathways. Together, these levels illustrate the central role of osteocytes in integrating skeletal and systemic physiology.

factor β (TGF-β), insulin-like growth factor 1 (IGF-1), and bone morphogenetic proteins (BMPs), promote osteoblast recruitment and differentiation (47). Osteocytes also contribute by producing coupling factors such as Wnt ligands and cardiotrophin-1, supporting osteoblast activity at remodelling sites (48).

AGE-RELATED REMODELLING DYSREGULATION

Ageing disrupts osteocyte-mediated coordination through two distinct but related cell fates described in the section "Cell fate decisions: apoptosis and senes-

cence as signalling events": apoptosis and senescence. Apoptosis provides targeted signals for focal remodelling, but excessive apoptosis with ageing depletes the osteocyte network. Simultaneously, the accumulation of senescent osteocytes creates broader dysfunction through SASP-associated factors that favour resorption and suppress formation (33,49). Together with structural deterioration of the LCN and an increased number of empty lacunae, these changes shift the remodelling balance towards net bone loss with ageing (50).

FUTURE DIRECTIONS: TOWARDS AN INTEGRATED OSTEOCYTE BIOLOGY

Osteocyte research is progressing from descriptive cell biology towards integrated, systems-level investigation. Having examined molecular mechanisms (section "Molecular and cellular mechanisms of osteocyte signalling"), systemic regulation (section "Osteocytes as systemic regulators"), and tissue-level remodelling (section "Osteocyte coordination of bone remodelling"), this final section considers how emerging technologies and conceptual frameworks may unify these processes into a coherent model of skeletal and systemic regulation (51,52).

New technologies now enable the investigation of osteocytes at molecular resolution while preserving their anatomical context. The central challenge is to integrate gene regulation, mechanotransduction, metabolism, and endocrine signalling into a coherent physiological framework that explains skeletal adaptation and systemic disease (53).

TECHNOLOGICAL FRONTIERS

Transcriptomic mapping

Single-cell and spatial approaches

Comprehensive transcriptomic profiling has identified more than 1000 genes that are differentially expressed in osteocytes compared with other bone cells. Although most of these genes have no previously characterised skeletal function, they may provide important insights into osteocyte-specific biology. Notably, human homologues of several of these genes are associated with monogenic skeletal disorders and complex traits, highlighting the translational relevance of investigating osteocyte-specific gene networks even when their molecular functions remain unknown (54).

Single-cell RNA sequencing is beginning to reveal osteocyte heterogeneity across maturation states and disease contexts (55). Early osteocytes near bone surfaces express higher levels of osteocalcin, RANKL, and PTHrP, whereas deeply embedded osteocytes preferentially express sclerostin (56). Sequential collagenase/ethylenediaminetetraacetic acid (EDTA) digestion combined with fluorescence-activated cell sorting (FACS) has enabled the isolation of viable osteocytes suitable for transcriptomic analysis, overcoming long-standing technical barriers imposed by the mineralised matrix (57). Integration of single-cell sequencing with spatial transcriptomics and computational modelling is further improving our understanding of osteocyte diversity and lacunar–canalicular organisation (58).

Epigenetic regulation remains poorly characterised. DNA methylation, histone modifications, and chromatin accessibility probably influence osteocyte differentiation and functional plasticity, but systematic profiling remains limited (55). Clarifying these mechanisms will be essential for understanding age-related changes in gene expression and susceptibility to disease.

Advanced imaging

Visualising osteocytes in action: Advances in multiphoton microscopy, tissue clearing, and reporter systems now permit high-resolution imaging of osteocytes within intact bone (55). An important advance has been the development of *in vivo* loading devices compatible with real-time imaging. Using these systems, osteocyte calcium responses have been shown to encode strain magnitude by increasing the proportion of activated cells rather than signal amplitude, supporting a binary recruitment model (59). These approaches bridge *in vitro* mechanotransduction studies and whole-bone physiology, enabling direct observation of osteocyte network behaviour under defined mechanical loading conditions.

Technical challenges remain. Osteocyte isolation requires careful validation to preserve native transcriptional states (60). Imaging fragile structures such as primary cilia within mineralised tissue remains difficult, and improved fixation and imaging protocols are needed to determine their orientation and functional relevance *in vivo* (61).

Organ-on-a-chip and *ex vivo* models

Organ-on-a-chip platforms and live imaging approaches further enable investigation of interactions among osteocytes, osteoblasts, and osteoclasts under mechanical loading (47). Future improvements may clarify the temporal sequence of cellular recruitment during remodelling and how ageing or pharmacological interventions modify activation thresholds.

FUNDAMENTAL UNRESOLVED QUESTIONS

The hierarchy of mechanosensing

Despite substantial progress, fundamental questions concerning the integration of mechanosensory pathways remain. At the cellular level, whether osteocytes function as the dominant mechanosensors *in vivo* or act in concert with other osteolineage cells remains unresolved (62). The relative contributions of fluid shear stress, matrix deformation, and substrate strain, together with the mechanisms through which different sensors integrate these stimuli, remain incompletely understood (63). The molecular basis of primary cilium-mediated mechanosensing, including possible activation of PC1/2 or TRPV4 channels, requires confirmation under physiological loading conditions (15). Advances in cell type-specific genetic tools should help clarify these mechanisms (62).

Metabolic regulation of osteocyte function

Osteocytes depend on tightly regulated glucose and amino acid metabolism to maintain survival within a hypoxic microenvironment (55). Inhibition of glucose transporter 1 reduces RANKL and osteocalcin expression, linking cellular energy metabolism to bone remodelling capacity (64). Disruption of von Hippel–Lindau protein function enhances hypoxia-inducible factor 1α (HIF-1α) signalling and increases bone mass, highlighting the relationship between oxygen sensing and skeletal homeostasis (65). Taurine production has emerged as a modulator of sclerostin and Wnt signalling (66). Age-associated energy deficits and circadian disruption further compromise osteocyte function (67). Understanding how metabolic decline contributes to skeletal fragility remains a research priority.

The senescence–apoptosis continuum

The temporal relationship between osteocyte senescence and apoptosis remains unclear (55,49). Transcriptomic data from aged bone reveal upregulation of innate immune and inflammatory pathways, including Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signalling (68). Whether these changes initiate senescence or occur as secondary consequences remains unresolved. Autocrine feedback loops may modulate osteocyte responsiveness, but this area remains insufficiently investigated (55). Ageing is associated with reduced expression of PTH1R, vitamin D receptor (VDR), and fibroblast growth factor receptors (FGFRs) in cortical bone, potentially attenuating hormonal responsiveness (50). The mechanisms governing receptor expression and sensitivity warrant further investigation.

Osteocyte heterogeneity and spatial organisation

The functional significance of osteocyte heterogeneity across skeletal sites, including cortical versus trabecular bone, and at different distances from the bone surface remains poorly understood. How spatial location determines signalling responses and how this organisation is established and maintained are important unanswered questions.

TRANSLATIONAL PERSPECTIVES

Targeting osteocyte signalling

Current and emerging strategies: Improved genetic tools are required to validate osteocyte-specific therapeutic targets while minimising off-target expression (55). PYK2 inhibition shows promise for preventing glucocorticoid-induced bone loss in preclinical models (53). Bone-targeted modulation of pathways such as Notch may provide skeletal specificity while limiting systemic toxicity (55).

The therapeutic success of agents targeting osteocyte-derived signals highlights the central role of these cells in bone remodelling. Romosozumab demonstrates that inhibition of an osteocyte-derived signal can simultaneously increase bone formation and decrease resorption (51). Denosumab effectively inhibits RANKL-mediated resorption but does not distinguish between cellular sources of RANKL. Whether selective targeting of osteocyte-derived RANKL could offer advantages in preserving bone formation remains an open question (51). Teriparatide promotes bone formation partly by suppressing osteocyte sclerostin expression, illustrating how established therapies also act through osteocyte-mediated pathways (52).

Senolytics and senomorphics

Senolytic therapies, which eliminate senescent cells, and senomorphic agents, which suppress the SASP, have prevented age-related and radiation-induced bone loss in mice (49). However, the extent to which these approaches selectively target osteocytes remains uncertain (69).

Cancer and the osteocyte niche

In oncology, tumour–osteocyte interactions increase sclerostin expression and shift the RANKL/osteoprotegerin (OPG) ratio towards bone resorption (22). An-

tisclerostin therapy may interrupt this cycle, although further investigation is required to determine whether its benefits arise exclusively from modulation of the tumour microenvironment.

Osteocytes as endocrine therapeutic targets

Establishing causal relationships between osteocyte-derived factors, including FGF23 and sclerostin, and systemic metabolic diseases will require interventional studies in humans (55). Emerging evidence that osteocyte-derived EVs influence distant organs highlights the importance of understanding bone as an endocrine regulator of whole-body homeostasis (47). Pathways regulating perilacunar remodelling are being investigated as potential therapeutic targets for conditions requiring rapid calcium mobilisation. Furthermore, recognition of EV-mediated osteocyte communication opens up potential avenues for targeted therapeutic delivery to the osteocyte network, although these applications remain speculative (26).

TOWARDS A QUANTITATIVE SYSTEMS FRAMEWORK

Enhanced Wnt/β-catenin signalling in osteocytes increases mineral apposition in craniofacial bone, whereas excessive activation promotes lacunar mineralisation, illustrating how signalling intensity can determine divergent biological outcomes (70).

Future progress will require quantitative frameworks integrating mechanotransduction, gene regulation, metabolism, and endocrine signalling across skeletal sites. The next phase of osteocyte research should combine single-cell and spatial technologies with functional validation in genetic models and human tissues. Only through this integration can osteocyte-targeted therapies progress from experimental promise to clinical application.

CONCLUSIONS

Osteocytes have emerged as major regulators of skeletal physiology, but their influence extends far beyond bone remodelling. Three overarching principles can be identified from the current literature.

- First, osteocyte signalling operates across multiple scales, from the nanoscale organisation of mechanosensors along dendritic processes (section "Molecular and cellular mechanisms of osteocyte signalling"), through tissue-level coordination of remodelling units (section "Osteocyte coordination of bone remodelling"), to systemic endocrine effects on mineral metabolism and distant organs (section "Osteocytes as systemic regulators").
- Second, osteocyte functions are context-dependent and heterogeneous. The same cell that suppresses bone formation through sclerostin under unloading conditions can promote formation when mechanically stimulated. Meanwhile, neighbouring osteocytes may undergo apoptosis or senescence, thereby influencing the local remodelling environment. This heterogeneity, increasingly revealed by emerging transcriptomic technologies (section "Future directions: towards an integrated osteocyte biology"), challenges the traditional view of osteocytes as a uniform cell population.
- Third, therapeutic targeting of osteocyte pathways has already transformed osteoporosis management, but realising the full potential of these cells will require moving beyond approaches directed at individual molecules. The success of romosozumab demonstrates that modulation of osteocyte-derived signals can simultaneously promote bone formation and inhibit resorption, representing an important advance in osteocyte-targeted treatment.

The next decade will determine whether advances in understanding osteocyte heterogeneity, mechanotransduction hierarchies, and systemic communication can be translated into therapies that prevent age-related bone loss, attenuate cancer treatment-related skeletal damage, and exploit osteocyte-derived EVs for targeted drug delivery.

Meeting these challenges will require the integrated, multiscale approach outlined in this review, recognising osteocytes not as isolated cells but as central components of a dynamic network connecting the skeleton to the entire organism.

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Case Report

Early-onset osteoporosis in a patient with KBG syndrome

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Abstract

Introduction: KBG syndrome, caused by pathogenic variants in *ANKRD11*, is a rare genetic disorder with variable neurological and skeletal involvement. Although skeletal abnormalities are part of its phenotypic spectrum, early-onset osteoporosis with fragility fractures in adults is poorly documented.

Case report: a 40-year-old man presented with a hip fracture after a low-energy fall. Bone densitometry revealed severe osteoporosis (femoral neck Z-score -2.7), with no evidence of secondary causes. The coexistence of craniofacial dysmorphic features, childhood-onset epilepsy, vertebral abnormalities, and borderline cognitive performance prompted genetic evaluation. Whole-exome sequencing identified a heterozygous *de novo* pathogenic variant in *ANKRD11* (c.1285_1286del), confirming the diagnosis of KBG syndrome. Treatment with bisphosphonates and vitamin D was initiated.

Discussion: this case suggests that osteoporosis may represent an underrecognized manifestation of KBG syndrome, with relevant implications for screening and clinical management.

Keywords:
KBG syndrome. Early onset. Osteoporosis. Hip fracture.

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INTRODUCTION

KBG syndrome (OMIM 148050) is a rare genetic disorder caused by pathogenic variants in *ANKRD11* (Ankyrin Repeat Domain Containing 11), a gene encoding a nuclear protein essential for transcriptional regulation, chromatin remodeling, and neurological and skeletal development (1-3). Its characteristic phenotype includes craniofacial dysmorphism, developmental delay, macrodontia, childhood-onset epilepsy, behavioral disturbances, and vertebral and rib abnormalities (1,2). Inheritance follows an autosomal dominant pattern, but most cases result from a *de novo* mutation (4). Although skeletal abnormalities are part of the clinical spectrum, early-onset osteoporosis with fragility fractures is an exceptional manifestation, with few cases reported in the literature. This report describes a young adult with severe osteoporosis and a hip fracture as the presenting manifestation of previously undiagnosed KBG syndrome.

CASE REPORT

A 40-year-old man had no relevant family history or past medical history of exposure to toxic substances. He had childhood-onset epilepsy and received valproate until the age of 9 years. He had subsequently remained seizure-free despite receiving no antiseizure medication. His past medical history included strabismus, thoracic kyphosis requiring bracing during adolescence, and mild cognitive difficulties. He had no endocrine or gastrointestinal disorders and was not receiving any other medications associated with bone loss.

After a fall from standing height, he sustained a subcapital fracture of the left hip that required internal fixation. He was therefore referred to the Bone Metabolism Unit for evaluation of possible osteoporosis. Physical examination revealed macrodontia with enlarged incisors (Fig. 1), a high-arched palate, a prominent median groove of the tongue, lentigines on the back, mild thoracic kyphosis, and micropenis, together with borderline cognitive performance and mild dysarthria. The remainder of the examination was normal, with no lymphadenopathy, goiter, organomegaly, or other abnormalities.

Bone densitometry consistently showed low values: Z-scores were –2.5 at the lumbar spine, –2.7 at the femoral neck, and –1.7 at the total hip (T-scores of –2.6 at the lumbar spine, –2.7 at the femoral neck, and –1.9 at the total hip), with a trabecular bone score (TBS) of 1.228. General laboratory investigations and tests to rule out secondary causes of osteoporosis (renal and liver function, PTH, TSH, 25-hydroxyvitamin D, LH, testosterone, SHBG, serum protein electrophoresis, free light chains, Bence Jones proteinuria, serum calcium, urinary calcium, tryptase, and anti-transglutaminase antibodies) were normal.



Figure 1. Macrodontia with disproportionately wide incisors.

The combination of craniofacial abnormalities, a history of childhood epilepsy, kyphosis, and mild intellectual disability suggested an underlying genetic syndrome. Whole-exome sequencing was requested and identified a heterozygous pathogenic/likely pathogenic variant in ANKRD11 (c.1285_1286del, p.Ser429Glyfs*8). This two-nucleotide deletion causes a frameshift and introduces a premature termination codon, substantially impairing protein function. The variant is absent from the general population but has been submitted twice to the ClinVar database as pathogenic or likely pathogenic. Neither parent carried the variant, indicating that it was presumably *de novo*. Following molecular confirmation of the diagnosis, weekly alendronate and vitamin D supplementation were initiated.

DISCUSSION

KBG syndrome was first described in 1975 (1) and is now recognized as a consequence of *ANKRD11* haploinsufficiency, with a broad skeletal phenotypic spectrum encompassing kyphosis, scoliosis, brachydactyly, and delayed bone age, among other abnormalities (2,3,5). Nevertheless, early-onset osteoporosis and fragility fractures are uncommon in this syndrome and are poorly represented in larger case series (6). Only three cases suggesting a possible association between *ANKRD11* abnormalities and markedly reduced bone mass have been identified in the literature. The first involved a girl with a 16q24.3 microduplication encompassing *ANKRD11* who sustained a femoral fracture after minimal trauma and had severely reduced bone mineral density, with femoral Z-scores as low as –8.4 (7). The second was described in a cohort of 25 genetically confirmed KBG patients, one of whom developed progressive osteopenia that evolved into osteoporosis during adolescence or early adulthood, affecting both axial and peripheral bone density (8). The third came from a recent study of adults with KBG syndrome, in which a patient was diagnosed with osteoporosis at 19 years of age, further supporting the possibility of premature bone loss in this syndrome (9).

The pathophysiology of bone fragility in KBG syndrome remains incompletely understood. Nevertheless, a heterozygous *Ankrd11*-Yoda mouse model exhibited reduced bone mass, impaired mineralization, and vertebral deformities (10). At the molecular level, ANKRD11 acts as a coregulator of RUNX2, a key factor in osteoblast differentiation, modulates p53-dependent pathways, and participates in acetylation and deacetylation complexes such as HDAC3, which are essential for transcription of osteogenic genes (3,11). Moreover, recent studies suggest that haploinsufficiency of this gene may impair chondrocyte maturation in the growth plate, compromising trabecular microarchitecture during early skeletal development (12). This hypothesis is supported by the fact that whole-exome sequencing in this case detected no additional pathogenic variants in genes related to bone metabolism. Nevertheless, this technique has limitations: it cannot exclude variants in noncoding regions or complex structural abnormalities, and interpretation of variants of uncertain significance remains limited.

Overall, this patient represents one of the best-documented cases of early-onset osteoporosis associated with KBG syndrome in adulthood. The absence of secondary causes, the presence of a *de novo* pathogenic variant in *ANKRD11*, and a hip fracture at a young age strengthen the hypothesis that bone fragility may be an underrecognized manifestation of the syndrome, with important implications for patient screening and follow-up.

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